VOLUME 9

FOWLER'S ZOO and WILD ANIMAL MEDICINE

Current Therapy

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A Tribute to Murray E. Fowler

R. ERIC MILLER, DVM, DACZM

This is a sad introduction to write but also one that is celebratory. It is sad because Murray Fowler died in May 2014 and this is the first of nine editions of *Zoo and Wild Animal Medicine*, the book he initiated, without Murray as an editor. This introduction is also celebratory because it summarizes a life lived to the fullest with so many around the world and lived to the fullest with Audrey and his family. Nearly everyone in the zoo and wildlife community has vivid, caring memories of Murray Fowler, so I will humbly try to provide some thoughts in a relatively brief tribute.

Murray's resume is legendary: he was a founding member of the American College of Veterinary Internal Medicine (ACVIM), the American College of Veterinary Toxicology (ACVT), and the American College of Zoological Medicine (ACZM). He was also a member of the American Association of Zoo Veterinarians (AAZV), a President of nearly every organization in our field, and a member of even more, including the European Association of Zoo and Wildlife Veterinarians (EAZWV) and the Brazilian zoo veterinarians (they even call it Groupo Fowler).

Murray wrote or edited more than 25 books—on zoo and wildlife medicine, New World camelids, elephants, toxicology, and wild animal restraint. All were always hands-on and practical texts! And I would note that Audrey, his wife, was a behind-the-scenes hero of many of these books—she was the quiet editor who ensured that the books always included proper grammar and sentence structure!

Murray's achievements are many. For example, in 1976 Murray founded the first residency in zoo and wildlife medicine at the UC-Davis. He contributed much to zoo and wildlife medicine during sabbaticals in Germany, Denmark, San Diego, the United Kingdom, and Uganda. In recognition of these achievements and many others, he received numerous awards. The following are but a few examples:

- The American Association of Zoo Veterinarian's (AAZV) Dolensek Award
- The Association of Zoos and Aquariums (AZA) Marlin Perkins Award
- The British Zoological Veterinary Society's (BZVS) Park Davis Award
- The AAZV's Murray Fowler Scholarship Fund supports the attendance of international zoo and wildlife colleagues at the AAZV's annual meeting
- * The European Association of Zoo and Wildlife Veterinarians (EAZWV) Honorary Membership and
- The Iowa State Alumnus Award.

Yet, Murray was much more than just an author or an editor, or a member of any single group. He was a veterinarian who very much belonged to our worldwide zoo community. The most recent edition of Zoo and Wild Animal Medicine (ZAWAM)—the 8th, which he received just before his death, illustrated that world view. Its 82 chapters include authors from 17 countries on 6 continents.

In another example, when the Iron Curtain separated so many people in Europe, he and Rudolph Ippen from East Germany worked beyond political boundaries to create an international alliance for our profession. I am comforted believing that somewhere Murray and Rudolph are sharing good memories and celebrating the future of our field they did so much to establish.

On a personal level, it was also clear to me how deeply Murray cared for individual zoo and wildlife veterinarians around the world when he and Audrey and my wife and I were able to attend meetings and symposiums together. I heard many times how Murray and Audrey had been welcomed in so many places, and how those experiences led to lifelong friendships that meant so much to them.

A typical sentiment about Murray came from Teresa Fernandes, a veterinarian at the Lisbon Zoo: At her first AAZV meeting in Tulsa, she and a friend from Moscow met Murray—she wrote, "He was kind enough to exchange a few nice words with us and shake hands with us, complete strangers to him. We had come all the way from Europe and yet this moment made it all worth it by itself. Never in my wildest dreams did I think I would be having a private dinner with the 'father' of zoological medicine." I believe that she, like so many zoo and wildlife veterinarians around the world, were part of Murray's "zoo family," a family that he deeply treasured.

I would like to offer one more illustration of his caring nature: Working with him on the 4th edition of ZAWAM was the first time I had edited a textbook. I had never been asked for autographs, but when the requests came in, I signed "Best wishes, Eric"—that was until I saw Murray's autographs. He wrote deep, touching and personal notes because he had taken the time to talk to each recipient and to hear each person's own history and hopes. That

experience resulted in our only negative interaction, as I scolded Murray for not offering me better training! Those autographs also illustrated how much he loved teaching and how much he loved students. In interactions, both brief and years long, Murray always encouraged everyone that he met to be their best and to pursue their dreams. As I wrote this memoriam, another became clear to me—frankly, if you were not a friend of Murray's, you simply had not had a chance to meet him.

I will repeat the acknowledgement I wrote for the 7th edition of ZAWAM: "Dr. Fowler initiated the first edition of Zoo and Wild Animal Medicine in 1978, when few texts existed in the field of zoo and wildlife medicine. In the subsequent 32 years, he has shown an unwavering dedication to the dissemination of this information with seven subsequent volumes of this text—not to mention many other related books authored by him. He has been, and continues to be, a mentor and an inspiration to many in our field, myself included." When he edited that acknowledgement, in typical Murray fashion, his comment was, "Well, that is a bit over the top."

In conclusion, when asked how he would like to be remembered, Murray once said, "I guess I would like to be remembered as a capable veterinarian, with an interest in a broad range of different species of animals, and as a teacher with a desire to share in the written and oral form as much information that I have." That was an understatement.

The word giant is overused. And despite his previous modest statement, Murray truly was a giant—not only in veterinary medicine, but in life. I believe that the best way we can honor Murray is to continue developing our profession of zoo and wildlife medicine in the way I believe he would have wanted. That will require not only committing our best efforts to caring for the animals entrusted to us, but also by being kind to each other and above all, teaching, mentoring, and sharing with both students and our peers in our field. That is the way that Murray will live on in each of us. I owe a personal thank you, and I believe we all owe a collective thank you, to Murray for everything that he did for our profession and for us individually. His memory will live on in the title of this book and in our hearts.

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Preface

In many ways, this is a sad edition of *Zoo and Wild Animal Medicine* (ZAWAM) to edit as it is the first without Murray Fowler. He was truly one of the giants of the field of zoo and wildlife medicine (see tribute in this edition). Appropriately, it will always be called "Fowler's" ZAWAM. At the same time, it is a pleasure to welcome two new co-editors, Paul Calle and Nadine Lamberski.

This 9th edition contains 100 chapters and returns to the current veterinary therapy format featuring focused, specialized topics, which will also be the focus of the 10th edition. The last (8th) edition featured the taxa-based approach, which will also be the focus of the 11th edition.

This volume contains many taxa-based clinical topics, but also additional issues important to zoo and wildlife veterinarians (e.g., leadership, occupational health, education of the public [children] about our profession, sample importation, the role of animal welfare, and decision making, as well as One Health, and emerging diseases).

The challenges for zoo and wildlife medicine, and for the veterinarians who implement it, are worldwide. Therefore, as in previous editions, the authors are an international group and in this edition represent 115+ authors from 21 countries on 6 continents: Argentina, Austria, Australia, Brazil, Canada, Colombia, Denmark, France, Hungary, Germany, Hong Kong, Indonesia, Madagascar, Mexico, Peru, South Africa, Spain, The Netherlands, United Arab Emirates, the United Kingdom, and the United States.

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We also extend our deep and heartfelt thanks to our family and friends who supported us through our editorial endeavors.

Last, but certainly not least, we thank the following consulting editors who submitted potential topics for this edition:

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SECTION 1

General

1

The Role of Veterinary Advisors in Animal Management Plans

JULIA E. NAPIER

Introduction

The Veterinary Advisor is a member of the Veterinary Advisory Group (VAG), which is a subcommittee of the Association of Zoos and Aquariums (AZA) Animal Health Committee (AHC) and was established with three main goals in mind:

1. To act as a support and advisory body for Species Survival Plans (SSP)/Taxon Advisory Groups (TAG) Veterinary Advisors;
2. To act as a source of information for protocols concerning the roles and responsibilities of Veterinary Advisors; and
3. To serve as an informational resource on veterinary issues that may impact conservation programs.

Background

The concept of the VAG originated with the Infectious Disease Committee (IDC) of the American Association of Zoo Veterinarians (AAZV) and the Conservation and Science Department of AZA, formerly the American Association of Zoological Parks and Aquariums (AAZPA) in 1993. The need for such a group was highlighted at the 1992 International Conference on Implications of Infectious Diseases for Captive Propagation and Reintroduction Programs of Threatened Species. This meeting, held in Oakland, California, was sponsored by AZA, AAZV, and the Captive Breeding Specialist Group of the International Union for the Conservation of Nature Species Survival Commission (CBSG/IUCN/SSC).[1] It emphasized the impact of disease on reintroduction projects and highlighted the importance of risk assessment. A general lack of information on (1) incidence, distribution, and risks of disease in captive and wild populations, (2) effective quarantine protocols necessary to prevent disease transmission, and (3) definitive diagnostic tests to detect and monitor disease had resulted in the lack of a working database for informed risk assessment. The notion of a Veterinary Advisor to each SSP/TAG program was put forward as a way to generate and collect this missing information. The program was created in 1994 and has grown considerably since then (Table 1.1). The disparity in numbers from 1994 to 2016[2] provides a wealth of opportunities for zoo veterinarians to contribute their knowledge, time, and energy to a population that needs a Veterinary Advisor in the VAG.

Currently, the subcommittee is composed of SSP/TAG Veterinary Advisors (which includes clinicians and pathologists) and in a few instances, a nutritionist. The VAG Chair is appointed by the Chair of the AZA AHC. The original "Guidelines for Veterinary Advisors to Regional Conservation Plans" was submitted to the AZA Wildlife Conservation and Management Committee (WCMC) and accepted in 1993. These were revised in 1994 and again in 2001 and 2009. Another revision is underway as of the writing of this chapter. The benefits of these guidelines are twofold: (1) they offer the SSP Coordinator a reasonable expectation of the role of a Veterinary Advisor, and (2) they offer the Veterinary Advisor an outline of basic standards that should be met. Clearly, the exact role and responsibilities of the Veterinary Advisor will differ among the various SSP/TAG programs. The list of SSP/TAG Veterinary Advisors is maintained by the VAG Chair and is updated as necessary. This list is posted on the AAZV website.[3] The AAZV site was chosen to encourage use by zoo veterinarians. In addition to the list of advisors, SSP/TAG veterinary and necropsy protocols, and Annual Report Forms are posted as they become available.

Veterinary Advisor Responsibilities

The traditional role of the Veterinary Advisor as stated in the guidelines includes making recommendations for diagnostic testing and evaluations, providing appropriate laboratory information for disease testing in their specific taxa, suggesting therapeutic protocols for treatment of disease and anesthetic protocols for immobilizations, and developing successful quarantine and preventative medicine measures. However, especially in the past 10–15 years, the role of the Veterinary Advisor as well as the clinical veterinarian in a zoological setting has evolved into something much more complex than just practicing medicine, formulating

TABLE 1.1 Comparison of Species Survival Plans, Taxon Advisory Groups, and Veterinary Advisor Numbers From Inception in 1994–2016

	Species Survival Plans	Taxon Advisory Groups	Veterinary Advisor	% Vets to Programs
1994	69	41	82	75
2016	611*	46	132	20

*Now designated as red, yellow, or green, based on the potential long-term sustainability of the population.

quarantine protocols, and detecting and preventing infectious disease.

The terms "animal welfare" or "wellness" are bandied about frequently, and although there are many interpretations, they universally refer to an animal's well-being in the environment they call home, which goes far beyond just treating illness or injury. Through social media and a variety of animal-related programming on television, a more informed public expects animals to thrive, not just survive in a zoological setting. To that end, zoo veterinarians and Veterinary Advisors have a responsibility not just to care for and cultivate these species but to communicate that goal to the animal management staff as well as the public. Collection veterinarians, and more specifically Veterinary Advisors, are viewed as subject matter experts due to their scientific background, for information on a variety of husbandry issues including but not limited to primary causes of morbidity and mortality, preventative medicine measures, behavior abnormalities and stereotypies, breeding management and success, contraception, exhibit design, enrichment and training protocols, nutrition, acceptable housing (i.e., space, flooring, substrate, lighting, humidity, and temperature), euthanasia guidelines, animal movements, zoonotic disease, and legislation that regulates animals in a zoological setting when it comes before state or federal legislatures.

In addition, Veterinary Advisors should provide annual SSP/TAG Veterinary Advisor Annual Report Forms to animal management staff, including facility veterinarians, that provide an overview of the most recent causes of morbidity and mortality, changes in nutrition, numbers of births and deaths in the population, successful immobilization protocols, and updates in necropsy protocols, as well as successes and failures in contraception.

Multiple functions regarding research may be accomplished by Veterinary Advisors in addition to doing projects on their own. Providing guidance to management groups as to whether proposed projects will be a worthwhile investment regarding the potential to benefit the species is a very useful service. They may maintain a list of current and past research projects, create a library of references, establish tissue banks, and provide direction on disease surveillance and monitoring in both the zoo and free-ranging populations.

The Veterinary Advisor has the unique ability not just to advocate for an animal's well-being in every aspect, but they are an invaluable resource to collection veterinarians, to specific managed populations in both captive and free-range settings, and to in situ and ex situ conservation field projects involving their species of expertise. They also benefit students, animal care staff, and veterinarians whose primary focus is not exotic species, as well as human medicine experts who may volunteer their time to the zoo community.

Additional Husbandry and Regulatory Roles

One of the most current contributions Veterinary Advisors are making that is key to all aspects of animal management is their contributions to the AZA Animal Care Manuals. These manuals provide guidelines for animal care and day-to-day husbandry issues for their respective SSP/TAG.

The veterinary portion encompasses a broad range of topics, including transportation guidelines (i.e., acceptable shipping temperatures) as well as those covered by International Air Transport Association (IATA), preshipment preparations, quarantine testing and duration, preventative medicine measures, therapeutic and vaccination protocols, parasite surveillance and treatment recommendations, and immobilization and anesthesia techniques.

In addition, they include successful reproduction strategies, neonate exam and annual exam checklists, necropsy protocols, special needs suggestions for pregnant and geriatric individuals, and information on zoonotic disease and personal protective equipment requirements for a specific species. AZA accreditation standards and regulatory information pertaining to those species that are covered under the United States Department of Agriculture/Animal and Plant Health Inspection Services (USDA-APHIS) Animal Welfare Act are also incorporated into the manuals.

Conclusion

It is the responsibility of the zoo veterinarian to take care of the animals in their collection. Veterinary Advisors, through proactive communication and collaboration, are in a unique position, with their invaluable expertise, to make the job of the collection veterinarian and animal management generally easier, more efficient, and more informed, thus enhancing the welfare and conservation of the extraordinary species they care for both in a zoo setting and in the wild.

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2

Risk Analysis Framework Guidance for Wildlife Health Professionals

DOMINIC A. TRAVIS AND KRISTINE SMITH

Risk Analysis

Jargon and Standards

Risk aversion is a universal behavior, common to humans and animals alike, and is the topic of many diverse fields of study from economics, to mechanical and life sciences.[1] There is no global definition of "risk" or gold standard for its measures. Yet, most every discipline seeks to measure and manage risk, several with fundamentally similar approaches, and others unfortunately implementing very different terminology. Thus, when approaching the measurement and management of risk, one must consider the field of study within which the problem lies.

"*Risk*," at its core, is the potential of losing something of value, weighed against the potential to gain something of value.[1] In the health sciences, "risk" is generally defined as the probability of an adverse (or positive in some cases) event to occur in a defined population over a specified time interval. Risk can be exemplified through the basic equation:

$$\text{Risk} = \text{Likelihood (of an outcome)} \times \text{Consequence (should it occur)}$$

Risk can be characterized or measured in different ways: qualitatively (e.g., characterized as "high," "medium," or "low"), semi-quantitatively (e.g., rated on a scale of 1–5), or quantitatively (assigned a probability factor or percentage). The outcome should be paired with information regarding the level of uncertainty (how sure or unsure one is) surrounding the estimate, as well as full disclosure of the assumptions made during the process.

Over the past half-century, standardized risk analysis methods have increasingly been applied to areas of health, and specifically to infectious diseases. In 1983, the US National Research Council of the National Academy of Sciences (NRC-NAS) standardized the format for the assessment of the effects of hazardous chemicals on human health in what is referred to as the "Red Book."[2] Standard risk analysis methodologies commonly used in animal and human health fields today can be traced back to this publication. Thus several standards exist today that may be applied to environmental, free-ranging wildlife, and zoo collection risk analyses, among a host of others, depending upon the setting.

The US Environmental Protection Agency (EPA) has guidelines available on its website for ecologic risk analysis. By their definition: "this is a process for evaluating how likely it is that the environment may be impacted as a result of exposure to one or more environmental stressors such as chemicals, land change, disease, invasive species, and climate change.[3] The process published by EPA consists of four parts: planning and scoping (gathering background information on relevant policy and research); problem formulation (what, in terms of plants and animals is at risk and needs to be protected); analysis (what plants and animals are exposed, to what degree, and how likely is it to be harmful); and risk characterization (risk estimation and description).[3] Similar methodologies are available from environmental agencies across the globe.

Global Animal Trade and Infectious Disease Risk

In the 1990s, the World Organization for Animal Health (OIE) implemented a standard methodology to be applied globally when assessing infectious disease risks of animals. According to the OIE, risk analysis comprises hazard identification, risk assessment, risk management, and risk communication.[4] Risk is defined in this context as the measure of the probability of the introduction of pathogens or other hazards to animals or animal populations. The hazard identification process seeks to establish which hazards (diseases) are of concern and how they may be introduced. Risk assessment is the process of estimating the probability or likelihood of hazard introduction, as well as the associated implications. The goal of risk management is to reduce both the likelihood and implications of the introduction of the identified hazards. The involvement of all potentially affected parties in the overall process (e.g., problem formulation, pathway and hazard prioritization, data collection and evaluation, result discussion and

dissemination, management option evaluation, etc.) is the goal of risk communication. This is an important, but often overlooked, aspect of the risk analysis continuum and should take place throughout the entire process.

Wildlife Disease Risk and the Wildlife Interface

Since 1992, the Conservation Breeding Specialist Group (CBSG, now the Conservation Planning Specialist Group [CPSG]) of the International Union for Conservation of Nature Species Survival Commission (IUCN-SSC) has been facilitating collaboration between experts in zoo and wildlife veterinary medicine, disease ecology, and population management to develop a set of methods and tools for realistic and rigorous analysis of disease risks in wildlife, and at the wildlife–domestic animal–human interface. In 2010, recognizing that the range of concerns in relation to wildlife disease had broadened well beyond those associated with animal movements, the OIE and IUCN sponsored the publication of the *Manual of Procedures for Wildlife Disease Risk Analysis* and its companion, the *IUCN Guidelines for Wildlife Disease Risk Analysis*.[5,6] The intent of these publications is to assist in the implementation of risk assessment and management when making decisions regarding biodiversity conservation, wildlife health and biosecurity, and domestic animal and public health, when wildlife disease is a critical factor. In an attempt to support interdisciplinary collaboration, encourage informed decision making, align language, and limit confusion, the IUCN adopted the terminology and framework of the OIE in regard to wildlife risk analysis.

Conducting the Process

The standards and applications of risk analysis are laden with jargon, politics, and variability, which often cause unnecessary confusion and frustration. To begin, risk analysis and risk assessment are different. Risk analysis refers to the overall process, with its independent components. Risk assessment is merely a phase of the risk analysis process.

During the introductory period risk analysis, the initial step—and a critical component of risk communication—is called Problem Formulation. During this step, one should do the following:

- Write a general description of the problem, including why there is a need or opportunity for science-based policy or decision making in this case.
  - Illustrate the problem: draw a picture or diagram to visually represent the issue.
- Identify the "standard" you will follow (in the following case study we will use OIE-IUCN Guidelines highlighted above) in order to establish what methodology will be implemented.
- Identify pertinent stakeholders—those that will need to be a part of the process, or who the science-based policy issue or decision may affect.
- Formulate a plan for communicating with and/or inclusion of stakeholders.
- Conduct a background literature and data search on the problem—this often includes both scientific and policy information.
- Create a list of all potential hazards (usually diseases in this case) that can then be prioritized through the hazard identification phase.
- Establish the group's acceptable level of risk—the level at which stakeholders will require management action options. This accepts the basic premise that "zero risk" does not occur, or rarely occurs. Zero potential morbidity or likelihood of disease transmission is often not realistic.

After Problem Formulation, the hazard identification and then the risk assessment phase (including pathways and threats, vulnerability and consequence assessments) are undertaken.

For illustrative purposes, this chapter presents one example of adaptive use of this framework in the form of a qualitative wildlife disease risk analysis exercise. A recent disease risk analysis was undertaken by the University of Minnesota, EcoHealth Alliance, and Food Systems Institute in partnership with the US Department of Homeland Security (DHS). The goal of the umbrella project is to prioritize and characterize the risk that the trade of wildlife and wildlife products poses to the US food and agriculture systems and public health. The implication of this research is to inform US regulating agencies of potential wildlife import risks that may have not been previously considered and to inform potential risk management and ongoing risk communication.

Case Study: Characterization of the Risk (Pathways, Threats, Vulnerabilities, and Consequences) That the Trade of Wildlife and Wildlife Products Poses to the US Food and Agriculture Systems and Public Health

Problem Formulation

Because of the shear scope and volume of this issue, we first conducted an extended "Problem Formulation Exercise" (initial component of risk analysis) to better understand the issue and prioritize the most important import pathways; this was followed by three preliminary qualitative risk assessments (subsequent component of risk analysis). Step one, Problem Formulation, consisted of:

- A summary of 13 years of US Fish and Wildlife Service (USFWS) Law Enforcement Management Information System (LEMIS) data, capturing all declared and undeclared US wildlife importation records[7];
- A multi-phased stakeholder engagement survey to characterize and rank perceptions and priorities surrounding this proposed threat;
- A multidisciplinary stakeholder workshop that reviewed results of the aforementioned data and survey results, and established ranking criteria to prioritize areas of greatest concern for further assessment.

Core partners included advisors from the US Government (DHS, US Geological Survey, Food and Drug

Administration, Centers for Disease Control and Prevention, US Department of Agriculture [USDA]), as well as private agriculture industry representatives, academia, and several relevant nongovernmental organizations. Data on wildlife trade were derived from the "EcoHealth Alliance 'LEMIS' database"—a compilation of over 20 years of the Convention on International Trade in Endangered Species (CITES) and USFWS LEMIS data obtained from the USFWS via a series of requests over multiple years through the Freedom of Information Act. Country disease status and disease standards information were obtained through open access OIE sources such as the World Animal Health Information Database (WAHID).[8] Data regarding US agriculture were obtained through several USDA Agricultural Research Service/Animal and Plant Health Inspection Service (APHIS)/Foreign Agricultural Service portals, as well as through our advisory team led by Dr. Tracey Dutcher (One Health Coordination Office, APHIS).

Over the 13.5 years examined, wildlife imports to the United States included a total of 5,207,420 individually identifiable shipments between January 1, 2000 and August 6, 2013. The number of annually declared wildlife shipments doubled during the period examined, reaching approximately 400,000 declared shipments imported in 2012 alone. These shipments involved a total of 11,033,468,322 individual specimens/animals, plus an additional 977,109,143 kilograms of specimens/animals measured only in weight. The majority of shipments contained mammals (27%), while the majority of total specimens imported were shells (57%) and tropical fish (25%).

Of the more than 11 billion individual wildlife specimens imported, 27.4% were individually recorded as live upon entry (an annual average of 224.9 million [$s = 42.3$ million; median = 231.5 million] live animals plus an additional 1.8 million kilograms of live animals). Aquatic, amphibian, and invertebrate species accounted for approximately 50% of these live shipments, mainly imported by the aquatic and pet industries. Reptile, rodent, and bird species destined for the exotic pet trade made up the majority of remaining live imports.[7]

Based upon these summary findings and stakeholder prioritization, three separate Risk Assessments were performed:

1. Risk of introduction of OIE listed foreign animal diseases (FAD) into US livestock via the global wildlife trade;
2. Risk of introduction of Middle East respiratory syndrome (MERS) into US public health via the international wildlife (camel) trade;
3. Risk of introduction of OIE listed FAD to US aquaculture industry via the importation of live aquatic animals from Asia.

The rest of this chapter will focus on the first case study illustration.

Hazard Identification

Once pathways of risk are established, the questions "What can go wrong?" and "How can it go wrong?" are posed (the core of hazard identification). Usually the discussion is disease based, but it needn't be. Regardless, it is recommended to start with a list of ranking criteria related to the threat or hazard of concern under the conditions being considered. For disease, ranking criteria might consist of:

- Infectivity/transmissibility (ID50 and LD50, Ro)
- Pathogenicity
- Severity such as morbidity, mortality, reproductive effects, immunosuppression
- Presence of competent vectors
- Species susceptibility, risk of crossing species barriers
- Economic impacts on species of concern
- Other ecosystem effects

In this particular analysis, the concern was based upon "policy and economic" criteria, highlighting diseases of international importance (as defined by OIE listing) being introduced into the US live animal agriculture system (i.e., What is the risk of introduction of OIE listed FAD into US livestock via the global wildlife trade?). In this case, the implied priority criterion would be transmissibility or infectiousness ("spreadability") once introduced, and its ability to cross species barriers and cause infection and/or illness. It is important to be very specific about the endpoint of concern because this is the equivalent of a research hypothesis in the risk analysis framework. In this case study, we were interested in confirmation of at least one "case" of FAD introduction as defined by the US FAD investigation guidelines. Each disease of concern should be evaluated via all the ranking criteria and then ranked overall. Often, this is done semi-quantitatively in a spreadsheet, or as a decision tree. In our particular case study, we were given further guidance by our stakeholders to prioritize FADs of ruminant livestock.

The real value of this process is for stakeholders to discuss which criteria make a disease important in a given scenario, before the assessment, in order to again prioritize time and resources on the most crucial issues. Technically, every high priority hazard must be evaluated further in the risk assessment phase—it is easier to evaluate 5 rather than 100 diseases, and potential hazard lists are often that long. In our case study, Rift Valley fever (RVF) ranked the highest priority, both objectively and through the expert elicitation process. The high-risk category also included foot and mouth disease and Crimean-Congo hemorrhagic fever.

Risk Assessment

In the risk assessment phase, one must ask the questions, "How likely is the hazard to occur?" and "What are the consequences if it does occur?" for each priority disease identified in the hazard identification phase. The risk assessment phase involves building a representative model of the process, collecting data and/or expert opinion, and characterizing the outcome in some way. Disease modeling has recently become all but an entire discipline in itself; thus, only the basic premise is highlighted here. A risk assessment model is a simplification of the real world and should help determine the likelihood or probability of adverse health effects associated with hazard exposure. This

may be qualitative or quantitative depending on the needs of the users and the amount and type of data available. Often, it also must be informed by expert opinion in the wildlife community due to lack of hard data. There are scientific methods, such as the Delphi method, among others, for the collection and analysis of expert opinion that may be used to add rigor to this process.[9]

Risk assessment is an iterative process as both models and data are often refined and updated over time. Usually a brief qualitative assessment gives a good indication of general risk, which allows for the collection and assessment of available data and the likelihood of successful quantitative modeling. Quantitative models may be simple, or deterministic, using point estimates that usually don't reflect the range or variability of the data. Stochastic models are used to incorporate uncertainty surrounding point estimates, and involve the need to match the question and available data with the appropriate tool and method—a more expert modeler should be included in the team if this approach is taken. Often, policy makers want quantitative answers where there are no data to support the kind of model that would produce the type of specific advice requested. Providing inaccurate estimations of the limitations of current results is a major pitfall in this process. Thus, communicating this potential mismatch effectively is a large part of risk communication between scientists and policy makers.

In order to build a risk assessment model, the problem definition needs to be specifically refined, just like that of a scientific hypothesis.

- Risk assessment question: What is the risk of introduction of RVF entering the United States and infecting the beef, dairy, and pork industries via trade in live wildlife species based on assessment of trade data collected from 2000 to 2013?

Under the OIE trade paradigm, the risk assessment is divided into three distinct parts: entry risk (threats), exposure risk (vulnerability), and consequences. Entry risk involves all steps in the pathways from countries of origin to the US ports of entry (i.e., incoming threats). Exposure involves any steps following entry in which an imported animal could potentially expose US populations of animals or people (i.e., US vulnerability to incoming threats). Consequences involve the severity of consequences that are likely to occur following exposure of US populations (either animal or human, to incoming threats).

From the initial combination of country disease status and species host-pathogen status, the risk of exportation and ultimate entry of RVF into the United States can be increased or reduced by multiple steps along the trade pathway, which can be summarized as either shipment or quarantine factors that contribute to or mitigate risk. The exposure risk (vulnerability) assessment involves all steps in the pathway following arrival in the port of entry; these steps include transit to US quarantine, US quarantine itself, transit to the final destination, and interactions that may occur at the final destination between imported wildlife and nearby US populations. The consequences portion of the pathway reflects multiple consequence factors including both the health of US populations (morbidity and mortality) as well as the economic results from transmission and disease. Specifically, this case study analysis was concerned with the consequences of exposure to the US cattle and swine industries, as this risk concern was expressly prioritized by our project stakeholders. However, consequences of exposure of additional animal (e.g., small ruminants) or human populations may be considered in future risk assessments.

Data Sources: For this case study, two main datasets were "mined" (i.e., studied) for analysis. First, we combined USFWS LEMIS data from 2000 to 2013 into a standardized dataset and used these data to extract all declared or confiscated live wildlife imports into the United States. Second, data on RVF official country status were obtained from the OIE's WAHID and Handistatus II portals.[8,10]

Approach/Assessment Platform: The general modeling approach applied here for risk assessment follows the qualitative methodology put forth in the IUCN/OIE Guidelines for Wildlife Disease Risk Analysis.[5] The general format for analyzing risk is the following: Risk = Entry Risk + Exposure Risk + Consequences.

Entry Assessment: A total of 53 species were identified as meeting the minimum requirements of a medium-risk species or higher (17 Artiodactyla, 4 Carnivora, 10 Primate, 1 Proboscidea, 3 Perissodactyla, 12 Rodentia, and 6 Bat). Of the 53 identified species, 20 were imported between 2000 and 2013, half of which were Artiodactyla. The top six most imported medium- to very-high-risk species made up 82.9% of the total number of medium- to high-risk individuals imported. These six included the African lion (*Panthera leo*; $n = 246$), springbok (*Antidorcas marsupialis*; $n = 134$), cheetah (*Acinonyx jubatus*; $n = 121$), natal multimammate mouse (*Mastomys natalensis*; $n = 100$), desert warthog (*Phacochoerus aethiopicus*; $n = 48$), and impala (*Aepyceros melampus*; $n = 45$).

Imports were further refined by country status, so there were 381 individuals from 84 shipments from 2000 to 2013 of high- to very-high-source risk. Because quarantine in the source country could not be confirmed to include vector control, and the majority of shipments were made in less than 3 days, it was assumed that little risk was mitigated during these factors. Therefore, entry risk was high- to very-high for $n = 381$ animals entering the United States over a 14-year period (2000–2013). This comprised 11 species, 5 of which were wild ruminants (187 of the 381 imported individuals; 49%). Large carnivores accounted for another 155 of the 381 (40.7%).

Exposure Assessment: In a case where an infectious animal enters the United States and quarantine measures are inadequate (e.g., mosquito exposure, subclinical long-term infectivity, and fomite transmission), the potential spread is high due to the fact that most high-risk imports were comprised of groups rather than individual animal shipments. This increases the potential spread and complexity of trace-back during investigation, should a negative scenario unfold. Quarantine procedures and regulations for wild

ruminants are assumed to significantly reduce exposure risk when properly conducted, but there is great uncertainty surrounding this factor when quarantine occurs at non-USDA facilities, which was found to be a common occurrence. Further, there is quarantine effectiveness uncertainty regarding diseases such as RVF, the pathogenicity variability of which we are still working to understand in various wildlife species.

Some nonruminant species, such as large, wild carnivores, are not required to be quarantined upon entry to the United States; others, such as rodents and nonhuman primates, may be regulated but not screened for RVF. In either case, internal domestic shipments may occur after port of entry—before quarantine—when quarantine is approved for non-USDA facilities. This adds uncertainty to the exposure assessment and may add risk. We found no data to help define potential risk pathways once animals exit the port of entry or quarantine station within the United States. This represents the greatest gap in information for this assessment and presents a major opportunity for innovation that would help assess the risks outlined or exemplified in this assessment. Further, final destination information was not made available for this assessment. Therefore, the best potential proxy for the missing pathway data above was not available. This represents an opportunity to further define risk and fill a major gap in this assessment.

Thus, exposure risk could not be sufficiently categorized with the data available, but exposure risk likely ranged from low to high depending on the taxonomic order of the imports; however, there was not a large number of risky importations relative to the large number of overall wildlife imports during this time period. Overall, many data gaps exist for this portion of the assessment.

Consequence Assessment: Due to the high morbidity and mortality of the disease in cattle, and the potential for catastrophic economic loss in both cattle and swine industries, the risk of RVF imports was considered to be of low to high likelihood, but whatever level of risk, of high consequence.

Risk Management

Risk management is the process of identifying, selecting, and implementing measures that can be applied to reduce the level of risk. Many times these are disease prevention and control strategies, such as vaccination and treatment of individuals or populations, or personal protective measures, such as wearing gloves and masks for humans facing zoonotic diseases. The idea is to rerun the model under different conditions or assumptions to see how the risk changes in response to intervention actions. Sensitivity analysis—the process of examining the impact of the variation in individual model inputs on the model outputs in a quantitative risk assessment—is often performed to accomplish this. Many times, cost is entered into the equation as well in order to conduct cost-benefit analyses of different management options. The result is a very powerful tool for management authorities to analyze not only risk, but also potential costs of decisions associated with high priority pathogens and their impacts. The idea is to provide scientific input to managers or policy makers about the potential costs and benefits of options they are considering, or that stakeholders may suggest.

Risk Communication

It is often said that risk analysis is an "objective" process. The reality is that in wildlife and/or disease risk analyses there are often so few data available that the analyst begins, subconsciously, to substitute value judgments for facts. Indeed, in assessing the consequences of disease introduction, for instance, a degree of subjectivity is almost unavoidable. While this may be less true for laboratory settings, it is more likely when assessing disease or environmental risks in free-ranging wildlife. Risk analyses are seldom truly objective, and for this reason transparency in declaring all assumptions made is essential.[11] Most assessments go through several iterations, with data collection needs (gaps) highlighted, that are then either filled or augmented with gathered expert opinion. It is very important not only to cite the source of all data, but also to estimate the quality of the data as a contribution to an overall assessment of uncertainty surrounding results. Thus, risk analysis, although often policy driven, must be a scientifically honest evaluation of what we know and don't know. Transparency itself is a commitment to open communication.

Communicating Uncertainty: Assumptions are what we make when we are uncertain. Some assumptions are considered big, others small, depending on the lack of data or data quality. In these cases, there is usually a gradient of evidence, such as expert opinion, proven case studies, or even local knowledge. There are formal scientific processes for eliciting expert opinion (such as the Delphi method), and vetted methods to deal with the variability of uncertain data. The goal of this process is to lay the evidence out logically and to examine the accompanying level of confidence in order to make the best use of existing data, fill important gaps, and put responsible bounds around the results. A risk assessment may sometimes be criticized because many assumptions are made. However, these cases communicate the fact that data gaps exist, and great uncertainty is present, which is important to establish formally in many cases, because it allows for discussions regarding how one might collect data for an improved evaluation of risk.

The "Precautionary Principle": In situations where there is significant scientific uncertainty regarding a risk and its consequences, such as a cause-and-effect relationship not being fully established, the "precautionary principle" is often invoked. This principle holds that a more cautious approach should be taken in the face of insufficient information. In many cases, the precautionary approach has a useful protective effect as the initial response to a potential threat with consequence, especially where valuable threatened or endangered species—or the release of infectious diseases—are concerned. On the other hand, too much or unnecessary precaution may prevent vital progress

in the long term. A transparent discussion of this approach is recommended. The risk communication strategy should include both more cautious and less cautious solutions for discussion.

Finally, the risk communication process is essential in helping decision makers to deal with one of the most difficult problems encountered during the risk analysis process; namely, determining what constitutes an "acceptable risk." Zero risk is seldom, if ever, attainable and some degree of risk is unavoidable—this must be stated at the outset. For example, what is the risk of reintroducing great apes into the wild? Can we ever hope to create a situation where there is zero risk of disease introduction? However, in our passion and excitement, we may convince managers and funders to move forward with little regard for potential implications if this is not discussed up front. On the other hand, health experts may unnecessarily throw up arbitrary barriers due to high perceived risk, which is unsupported by a lack of data and/or great uncertainty surrounding adverse outcomes. This discussion intersects with that of the precautionary principle approach. The goal of risk analysis is to decrease gridlock, not create paralysis.

In the example case study provided here, the statement of "Conclusions and Uncertainty" follows:

- Overall, with the USFWS LEMIS data available, we can make confident statements about viable entry pathways and volume of trade. These data are limited to declared shipments and confiscations. We found no way to adequately estimate the illegal wildlife trade.
- By using the OIE WAHID, we can infer source risk by region but have no way to assess the prevalence in wildlife or the specific source of animals beyond the country of origin and the port of export.
- The largest point of uncertainty in the entry assessment surrounds the likelihood that any given animal selected for shipment is adequately represented by the country status from which it came.
- We used country status, as reported by the OIE, as a proxy for source risk. According to experts, there is major uncertainty surrounding self-reported data on many diseases from many countries.
- Surprisingly, for exposure assessment, there is generally less data available from which to estimate risk within the United States than there is from outside US borders. The complete lack of formal data on animal movement within the United States after entry limits our ability to assess the wildlife–livestock interface potential at the endpoint of this trade pathway.
    - The acquisition of "importer" data might help tighten this assessment slightly but won't detail the post-entry transportation methods, the final destination characteristics, and the purposes of imports (i.e., exposure of imported animals to humans or other animals).
- Most of the hazards/diseases prioritized by our stakeholders were high consequence on either economic or population morbidity/mortality scales (as confirmed through stakeholder elicitation above). Many FADs have little evidence-based information for pathogenesis in captive wildlife, or for how transmission may occur across the species barriers. This is another large data gap that affects the consequence assessment and presents further opportunity to support research on both the ecology and pathogenesis of these agents beyond the normal domestic animal realm.

There is evidence that there is some level of risk of RVF transmission to US livestock from the importation of wildlife species. While the number of imports that are most likely to provide a risk of RVF transmission are relatively few compared to the overall large volume of imports, the consequences of a transmission event would be extensive. Because of this risk, we have recommended investment in further areas of research and further risk reduction measures.

Summary

Assessing risk is part of the human (and animal) condition. It is a process by which we learn and change our behavior for a more successful future. Risk analysis that is transparent, logical, and testable is a purposeful method of conducting this conversation, and—ideally—informing decisions. Simply, it is the interface between science and management/policy decision making. It allows for a relatively quick situation analysis for immediate decision needs, as well as planning for better, more informed, decisions in the future through the collection of more/better data or the use of more sophisticated tools. In the end, it is the triage process of science-based management.

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3

Wildlife Technologies

KAREN BAUMAN

Historically many of the technologies used for animals have been categorized as being field, agriculture, zoo, or lab based, likely because most have been adapted from use in humans (medicine), domestic animals, or lab animals (biomedical). However, just as the traditional dichotomy between zoo-based staff and field biologists has blurred in the past decade, so too has the application of technologies for wildlife become more of a continuum. This philosophical shift, coupled with advances in technology, including the miniaturization of microprocessors and integrated data management tools, has led to an exponential growth of technologies that are relevant to veterinarians working with wildlife.[1,2]

This chapter will focus on three types of technologies: (1) biotelemetry and biologging, (2) environmental loggers, and (3) digital imaging. Recent advances in endocrine monitoring, contraception, and diagnostic imaging (radiology, computed tomography, magnetic resonance imaging) are discussed elsewhere in this edition (see Chapters 13, 22, 31, 32), and ultrasound, thermography, and molecular technologies have been reviewed recently in previous editions of *Zoo and Wild Animal Medicine*.[3–5]

Biotelemetry and Biologging

Karesh previously reviewed biotelemetry for wildlife veterinarians and provided a thorough description of the equipment and a basic explanation of the functionality related to animal location (colloquially referred to as telemetry).[6] Although positional estimation of an animal's location using either very high frequency (VHF) radio signal or global position system (GPS) technology has obvious importance to wildlife veterinarians for studies of disease ecology, there are several excellent books and reviews available that cover these aspects of the technology in detail.[1,2,7–9] Discussion of biotelemetry in this chapter will focus on its less known uses for physiologic and behavioral data collection.

Biotelemetry and biologging are techniques that allow remote measurements of physiologic and behavioral data from devices carried by animals.[10–13] Biologging was first developed in the 1940s to study species in marine environments.[13] Biotelemetry, which was originally used to monitor the physiologic responses of pilots, was adapted in the early 1960s as a technique to study small mammal populations.[10,14] The differences between biologging and biotelemetry are primarily the means by which data are received and stored, but researchers also separate the two based on the environments in which they are used.[15] In biotelemetry, the device carried by the animal is a transmitter (sometimes referred to as a tag) that does not store the collected data, but rather continuously transmits it to receiver, which is typically connected to a computer to automate data collection. Most receivers are remotely located (meters to kilometers) from the devices, but some must be located close (centimeters), for example, to read radio frequency identification devices (RFIDs; passive and active types), such as passive integrated transponder (PIT) tags. Biologging traditionally has been used in marine environments where the radio signals that transmit data for biotelemetry do not propagate. Data are recorded and stored on the device carried by the animal (sometimes referred to as archival or store-on-board loggers). The differences between the two technologies are subtle, and within the past decade there have been new hybrid devices, as well as crossover in data retrieval techniques.[2,15] Because there is now overlap between the two technologies, for simplicity in this chapter the term biologging will be used generically to refer to both technologies.

Biologging devices may be equipped with a wide range of sensor types (e.g., heart rate, blood flow, temperature, locomotion).[11,16] Devices come in many forms, with most being custom-made to facilitate them being carried by a diversity of species—from insects to whales (see Cooke et al., 2004 for review by order).[11] This customization ensures that the devices are the correct weight and shape for the animal being monitored, collect data for the specified time period, and will remain affixed to the animal as required. Weight of the device is a tradeoff between the weight of the battery needed to collect the data over the specified time period and the body weight of the animal; it is generally recommended that the devices not exceed 2%–5% of the body weight of the animal.[11,17] However, this "rule of thumb" does not take into account other measures of animal welfare, such as changes to behavior, energetic output (especially

related to increased drag for aquatic and avian species), and discomfort. Animal welfare has been cited as a concern in biologging studies, with authors asserting that devices should be attached in such a way as not to cause, or at least to minimize, detrimental effects to the individual animal.[1,15,18] This may be less of a concern in the future because new battery technology is facilitating miniaturization of devices and some RFIDs are now being equipped with sensors. Biologgers generally range in cost from $100 to $500 per unit, with some very specialized implantable devices costing $1000 or more. Most devices may be reused, although they typically have to be sent back to the company and refurbished. The most significant expense associated with biologging is the purchase of the receiving equipment and software. Albeit expensive, the purchase may be considered a long-term institutional investment in much the same way that purchasing an endoscopic equipment or ultrasound system would be.

Physiologic and behavior biologging sensor-equipped devices can be divided into two attachment categories: external and internal. In most wildlife species, attachment of either type requires anesthesia, but the external devices rarely require surgery. External attachments are less expensive than internal devices equipped with similar sensors and include an array of devices that can be glued onto skin, shells, or feathers; sutured or strapped on (such as halter-type monitors); worn (e.g., collars, ear tags, or bands); and sat upon (eggs). Although unusual in wildlife applications, a halterlike biotelemetry belt has been used to measure heart rate and respiration in wildebeest *(Connochaetes taurinus)*.[19] Other less common applications include the use of acoustic recording devices on collars to record chewing and vocalizations and miniature video cameras attached to the ventral feathers of birds and collars of mammals.[20–23] Accelerometers are now often used in collars and glued-on devices to study behavior, activity levels, or circadian and/or movement patterns.[24–26] Passive- and active-type RFID tags are commonly used in domestic animal species to monitor feeding bouts, feeding and drinking amounts, locomotion, and activity, as well as estrus behavior and general health.[27–29] In elephants, active RFIDs have been used to study social affiliations, activity, and use of exhibit spaces.[29,30]

Most internal devices were developed for human or biomedical research purposes and tend to be more expensive, technologically intensive, and, in some cases, invasive compared with external devices. Internally implantable equipment, such as intraperitoneal and subcutaneous devices, as well as those that are sutured to blood vessels and organs, requires surgical placement. Until recently, core body temperature could be measured only with intraperitoneal devices or devices sutured to blood vessels, but recently miniaturized devices with thermographic sensors have been used to compare intraperitoneal, subcutaneous, and intramuscular temperatures in antelope.[31,32] Rey also placed rabbits with multiple, similarly placed temperature devices and an accelerometer into a respirometer to measure metabolic rate.[33] Subcutaneous devices are used to measure rectal and vaginal temperatures in cattle, and Hoskinson measured cloacal temperatures in lorikeets for comparison with core temperature.[28,34] They can also be used to measure activity and movement.[31] Cardiovascular disease is a common cause of morbidity and mortality in captive great apes, and several authors have begun to use devices to monitor cardiovascular parameters in unanesthetized chimpanzees.[35] Similar devices have been used in domestic animals and to study hibernation effects in bears.[36,37] Lastly, rumen boluses to measure pH may be applicable to wildlife species, as well as the ingestable cameras currently used in humans to study intestinal transit times and pathology.[28,38]

Environmental Loggers

Data about ambient conditions, as well as information about the environment in a habitat, may be useful for addressing research and husbandry questions, as well as providing important information to veterinarians and animal care staff. Environmental loggers differ from biologgers in that they are intended to be used within the environment (e.g., mounted to a wall or a probe inserted in the soil to measure moisture) and not carried by an animal.

Most environmental loggers are used in industry so they are available in a great diversity of sizes and types. Those that may be of interest for monitoring changes that occur in and around an animal's habitat or conditions during animal shipments include: temperature; humidity; light; water flow, level, turbidity, and salinity; barometric pressure; soil moisture; carbon dioxide; acoustic sound pressure and decibels; and wind speed. Environmental loggers can measure a single condition or combinations of up to three variables. It is important to note that not all environmental loggers are battery powered (some require AC power), and all are rated for specific environmental conditions (e.g., indoor only). Most are designed to store data until retrieved for download, but WiFi and mini network capabilities are becoming more common. Because environmental loggers are typically available "off the shelf," they are often less expensive than biologgers, averaging approximately $150 per unit. In most cases they are "standalone," so the only additional cost is for software and potentially a download cable. There are several important factors that must be considered when using environmental data loggers. The logger must be capable of recording data within the range you believe possible, plus an appropriate buffer on either side. For example, a temperature logger with a range of 10–37.7°C (50–100°F) would work well for warm water jellyfish but would likely be unsuitable for cold water jellies, which thrive in water 15°C (59°F) or colder.[39] Another important consideration is the logging interval, which must be selected carefully because it affects battery life and data storage. More frequent sampling results in more data to be stored (and analyzed later!) and drains battery power more quickly than longer sampling intervals. In addition,

even if a logger is capable of recording data at the intervals needed, the acquisition time of the logger must also be considered. One of the most basic considerations for the use of environmental loggers—determining if the logger will record data using the units of measure you need to address your question—also can be the most complex. For some data, such as temperature, this is straightforward (Celsius or Fahrenheit), whereas with other sensor types the choice may be more complicated. For example, light loggers may record data in lumens, candles, lux, light intensity, and/or wavelength, so more background knowledge for interpretation may be required. Likewise, acoustic loggers, although an important and emerging area of investigation to measure animal responses to ambient noise, are the most expensive and complex of the environmental loggers.[40–42] Sound meter and passive acoustic monitoring systems (PAMs) must be calibrated properly or they can provide measures of relative sound only, which may address the need to compare the noise levels from one event with another but would not provide decibel measures that could be analyzed or compared with known standards. Scale must be selected to reflect frequency range (lowest and highest amplitudes) that may be encountered, and this selection must take into consideration vibrations and human-made factors. Most sound meters and PAMs have the option for multiple scales, including the "A scale," which filters for frequencies that are most similar to what a human ear can perceive, and the "C scale," which includes lower frequencies that many nonhuman animal species hear.

Digital Imaging

Technologic options for remote monitoring through still photos and video have grown exponentially in the past decade and are now generally within the price range most zoos can afford. One of the simplest, yet most overlooked, tools for gathering remotely activated images in a zoo setting is the trail camera (also known as a camera trap). These units may be quite useful in zoos to document space use in a novel exhibit, social information about group dominance around a key resource such as an enrichment feeder or shift door, and general behaviors that occur during times of the day when staff members are not around. At this time, camera traps are not particularly useful for very small terrestrial mammals, reptiles, and small, rapidly moving bird species, due to the lag time from detection to recording the image (trigger speed), and increased false detections. Changes allowing decreases in trigger speed and reduced detection failures are being implemented, so newer models may be more useful for smaller animals (see Nazir et al. for details of WiseEye, the next generation of camera trap).[43] The price of camera traps is highly variable and depends on battery life, onboard data storage capacity, and ability to withstand environmental conditions. Additional uses for high-quality digital still images under development include systems that use three-dimensional (3D) serial images for body condition scoring in cattle.[44]

Video Systems

Video camera systems have been used in zoos for research and monitoring, with early systems using analog cameras and time-lapse VCRs.[45] In the past decade the range of available options has increased dramatically and now includes single standalone camera and DVR systems and fully integrated multicamera networked systems using power over ethernet (POE) capabilities. Historically, placing multiple cameras to cover a large space was problematic because precise synchronization of the video feeds and time on each video was difficult, but new multicamera systems make synchronization seamless. Many of the security-based systems use proprietary software, which can be expensive and may also make it challenging to download video in common formats such as MP4 or HD64 for sharing videos with colleagues. Video systems can be used to monitor behavior, including stereotypies, activity, social affiliations, use of space, as well as record locomotion over a pressure plate for gait analysis.[28] Although beyond the scope of this review, video imagery from drones or unmanned aircraft vehicles (UAVs) have proved successful in antipoaching contexts.[46] Moreover, there are reviews of UAVs coupled with thermal imaging to provide accurate information of species abundance and distribution in both marine and terrestrial environment.[47–49]

Conclusions

These technologies are of special interest to wildlife veterinarians because they facilitate the collection of unobtrusive repeated physiologic and behavioral data to address health and welfare questions.[50] In the near future, many of these technologies will be integrated and increased data analytics speed will provide results in real time (or almost), which will be helpful for both animal management and clinical medicine.[1,51]

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4

International Sample Movement: Overview of Convention on International Trade in Endangered Species of Wild Fauna and Flora and Selected National Regulations

CHRIS WALZER

The collection of biological samples is central to wildlife health, conservation, and environmental studies. The acquisition and processing of biological wildlife samples is, in most cases, a prerequisite for establishing a diagnosis. Furthermore, information extracted from biological samples can be instrumental in shaping conclusions and guiding policy. The sampling procedure across disciplines can be broken down into several distinct steps: (1) acquisition of the sample, (2) collection/recording of linked sample metadata, (3) initial storage of the sample, (4) transport of the sample, (5) processing of the sample, (6) final storage of the sample, and (7) sharing the sample and/or information from the sample. All steps in this process are potentially regulated and restricted by national and international legislation and constrained by logistical challenges. Considering these individual steps while referring to the respective legislation will provide a solid framework for a sampling plan. Within these individual steps, various options and constraints can be identified and should be carefully considered at the outset when establishing the sampling protocols. Although this discussion on sampling is pertinent to various fields and types of samples, it will focus on samples from wildlife.

Before samples can be collected, investigators must ascertain that all necessary permits for the actual collection process have been requested and approved. This can include a multitude of permits such as, but not limited to: research permits, access permits, and working permits. The type of sample required most often defines the approaches used. On the one hand, the acquisition of blood samples from live animals in considered an invasive form of collection, requiring in most cases the capture and anesthesia of the targeted species, whereas the collection of, for example, fecal samples can occur noninvasively. Similarly, the collection of hair, urine, feathers, shed skin, saliva, and eggshells can be performed noninvasively and, in many instances, without actually observing the animals.[1] It is important to point out that sampling live and dead wildlife requires a profound understanding of the inherent risks involved to the sampled animal and the investigator. Risk mitigation measures, such as the use of adequate personal protective equipment (PPE), safe anesthetic protocols, and animal welfare legislation are but some of the measures to consider. A large and diverse number of sampling guidelines and recommendations are available and can be used as a basis for developing a specific sampling procedure.[2,3] Certain species, such as nonhuman primates and bats, warrant a heightened appreciation and consideration of PPE measures during sampling and subsequent processing due to the potential exposure to life-threatening pathogens, such as Ebola virus, Cercopithecine herpes-1 (B virus), and rabies.[4,5]

Consistently linking individual samples with their respective unique metadata is an essential prerequisite to guarantee the effective future use of the sample. Only the combination of adequately collected, processed, stored, and annotated samples will allow the generation of meaningful results. Various international initiatives are ongoing to streamline and harmonize sample metadata use (e.g., MIABIS: Minimum Information About BIobank data Sharing) and can be used as a guideline.[6]

Sample storage varies widely in respect to the type of sample collected and the subsequent analysis to be performed.

Feathers used for DNA extraction in a genetic study can simply be stored in dry paper envelopes; sex hormones remain stable when chilled. Although nucleic acid from blood samples is easily captured and stored for years on FTA filter cards (Whatman FTA, Sigma-Aldrich Handels Gmbh, Vienna, Austria), blood samples and tissues investigated for viral RNA must be processed immediately. These samples must be stored in an RNA stabilization solution, which stabilizes and protects cellular RNA (e.g., RNAlater Fischer Scientific—Austria GmbH, Vienna, Austria) and in many cases eliminates the need for liquid nitrogen, depending on the environmental temperature.[1,7] In addition to the type of sample collected, sample storage must be carefully considered because it potentially introduces important additional downstream decision points. An alternative approach to wildlife sampling is to process all samples on site, in-country. This eliminates the international sample export process, greatly speeding up diagnostic turnaround time. In addition, in-country processing facilitates sustainable knowledge and technology transfer and in-country data availability. Novel portable diagnostic systems such as smartphone-powered quantitative polymerase chain reaction (PCR; Biomeme two3, Philadelphia, PA, USA) and nanopore DNA sequencing (MinION, Oxford Nanopore Technologies, Oxford, UK) enable the detection of specific genetic material from pathogens and hosts in remote field settings.[8,9]

Most readers at one stage or another in their career will have witnessed the surprise arrival of inadequately shipped biological samples in unmarked soggy cardboard boxes. Nonetheless, it is evident that once samples are to be transported, a plethora of legislation, rules, and regulations need to be considered and strictly adhered to. Legislation, and more importantly its implementation, can vary widely between nations, so the country-specific rules and regulations should be determined before sample collection. However, for the sake of this chapter, we focus on sample movement into and within the United States and European Union.

At the most basic level, the investigator must ensure that prior to shipment of samples the respective valid export permits from the country of origin and the valid import permits from the receiving country have been obtained. The types of permits required fall into several categories and vary in accordance with the type of sample, species, and mode of transport.

Convention on International Trade in Endangered Species of Wild Fauna and Flora

The Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES) is an international agreement among 183 governments ensuring that the international trade in specimens of wild animals and plants does not threaten their survival. Although scientific biological samples are in the majority of cases not traded, they fully fall under the regulatory terms of this agreement.[10] This includes blood and its derivatives, hair, skin, tissue, and extracted DNA. In contrast, most countries, but not all, consider fecal samples to be wildlife byproducts that are exempt from CITES permitting. The first step in applying for a CITES permit is to ascertain whether the species or subspecies in question is covered by the CITES convention and to determine in which CITES appendix the species or subspecies is regulated (www.cites.org/eng/resources/species.html). Appendix I includes species threatened with extinction, and consequently the trade (and scientific exchange) in specimens from these species is permitted only in exceptional circumstances. Appendix II includes species not necessarily threatened with extinction but in which trade must be controlled to avoid overexploitation. Finally, Appendix III contains species that are protected in at least one country. Depending on the applicable listing, the permitting process varies. For Appendix I species an import permit issued by the management authority of the state of import is required first, whereas for Appendix II species an export permit or reexport certificate issued by the management authority of the state of export or reexport is required initially. A comprehensive and constantly updated overview is available online (https://cites.org/eng/disc/how.php). Although CITES is legally binding in national states, implementation can vary in relation to the specific domestic measures adopted for that purpose. Additional international (EU) and national legislation could regulate and limit sample movement. In the United States, for example, the various regulatory mechanisms for migratory birds must be considered (see: http://www.fws.gov/birds/policies-and-regulations.php; and http://ec.europa.eu/environment/cites/info_permits_en.htm). It is therefore absolutely essential to contact the national management authority of the respective state(s) (e.g., United States: the United States Fish and Wildlife Service [USFWS] in the European Union and most other countries the respective ministries of environment).

Veterinary Import Permits

In addition to CITES and national requirements for the movement of wildlife samples, respective so-called veterinary import permits from the national veterinary and/or agriculture departments must be obtained (United States: US Department of Agriculture's Animal and Plant Health Inspection Service; European Union: respective Ministries of Health and Agriculture). The objective of veterinary import permits is to protect livestock or agriculture from materials that may pose a threat. Veterinary permits are needed for a wide variety of materials derived from animals or source materials that have been exposed to animals. Materials that require a permit include, among others, animal tissues, blood, cells or cell lines, fecal samples, RNA/DNA extracts, hormones, and microorganisms, including bacteria, viruses, protozoa, and fungi. In some countries, veterinary export permits for samples are also required.

Be aware that the veterinary import process can be very dynamic, and requirements will change at short notice as the status of animal diseases reported to the World Organization for Animal Health (OIE) changes. Veterinary requirements for the import of wildlife samples vary widely with respect to the species and the country of origin. In general, the import of ungulate, equid, and bird samples can be extremely problematic, if not impossible. Subsequently, specific restrictions on the type, condition, and approved preservation of the sample will apply. Approved preservation can include, among many others: (1) heating to a certain temperature for a determined time period, (2) immersion in formalin or other preservatives, and (3) irradiation. National implementation and requirements will vary significantly. In the United States, irradiation must be performed under the direct supervision of the National Veterinary Services Laboratory or the Foreign Animal Disease Diagnostic Laboratory (FADDL), Plum Island, NY. In the European Union, respective national entities supervise this process. It is important to be aware of the potential negative effects, such as deterioration or destruction of genetic material, from the use of the prescribed preservatives and irradiation.

In some countries, additional requirements can apply to specific species. For example, in the United States, all samples originating from nonhuman primates entail special restrictions, additional permits, and health reporting requirements from the Department of Health and Human Services and the Centers for Disease Control and Prevention.[11] It is highly recommended to contact the respective authorities well in advance of the planned import to discuss and clarify the import process.

Nagoya Protocol

The Nagoya Protocol on Access to Genetic Resources and the Fair and Equitable Sharing of Benefits Arising From Their Utilization (ABS) to the Convention on Biological Diversity is a supplementary agreement to the Convention on Biological Diversity. The protocol provides a transparent legal framework for the effective implementation of the fair and equitable sharing of benefits arising out of the utilization of genetic resources. The protocol aims to prevent biopiracy (i.e., commercial exploitation of biological compounds or genetic sequences by a technologically advanced country or organization without obtaining consent or providing fair compensation to the source country and peoples). The Nagoya Protocol on ABS was adopted on October 29, 2010 in Nagoya, Japan and came into force on October 12, 2014.[12] Although the previously mentioned permits regulate the movement of physical samples, the ABS applies to genetic resources over which states exercise sovereign rights and to traditional knowledge associated with genetic resources (traditional knowledge). It is important to note that, although some 100 parties have signed and ratified the protocol, numerous countries have, to date, not ratified or signed (of particular note, the United States) the protocol.[13]

The European Union ratified the protocol (Regulation (EU) No. 511/2014) in 2014, and the subsequent Regulation (EU) 2015/1866 came into force in November 2015, laying down detailed rules and best practices in implementing Reg. 511/2014. However, implementation in several member states is currently still lacking. Obligations and implementation vary significantly among the signatories, but using the EU as a guideline, users of genetic material and traditional knowledge must exercise due diligence to ascertain that: (1) the genetic resources and traditional knowledge used have been accessed in accordance with applicable access and benefit-sharing legislation or regulatory requirements and (2) benefits are fairly and equitably shared on mutually agreed terms and in accordance with any applicable legislation or regulatory requirement. To fulfill these obligations, parties must issue a permit or its equivalent at the time of access as evidence that access to genetic resources was based on prior informed consent and that mutually agreed terms were established. The parties must make information on the permit or its equivalent available to the ABS Clearing-House for the constitution of the internationally recognized certificate of compliance. The first internationally recognized certificate of compliance was issued on October 1, 2015 by India's National Biodiversity Authority, the competent national authority under the Nagoya Protocol, granting access to ethnomedicinal knowledge of the Siddi community from Gujarat to a researcher affiliated with the University of Kent in the United Kingdom.[14]

Although implementation of the protocol is still lacking in numerous countries, it is also clear that some countries are strictly adhering to the protocol (e.g., Germany) and that major granting agencies are already requesting an internationally recognized certificate of compliance at the time of grant submission. It is only a matter of time before reputable peer-reviewed journals will also require these certificates prior to publishing results that include genetic data from wildlife.

Packaging and Labeling Samples

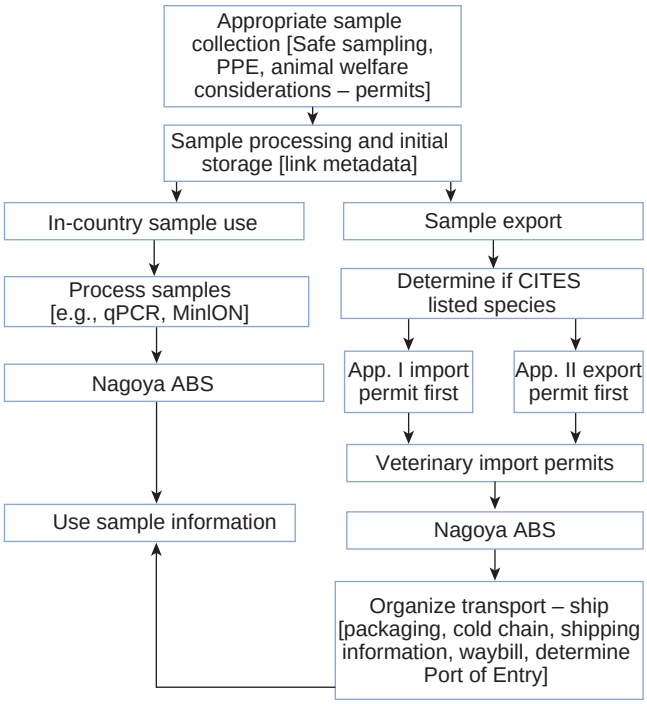
A key requirement for a successful shipment is the correct choice of packaging suitable to the type of sample to be shipped and the conditions that may be encountered along the route. Many transported samples and their respective preservatives and additives constitute a dangerous material (HAZMAT in the United States) that can potentially inflict harm to persons or property and damage to the environment, the means of transport used, or to other goods. The Committee of Experts on the Transport of Dangerous Goods of the United Nations Economic and Social Council (ECOSOC) has developed guidelines that assign a four-digit code (UN number) to the most common dangerous goods. UN 2814 denotes Infectious Substances, Category A, which can cause disease in humans or in both humans and animals, whereas UN 2900 is assigned to Infectious

Substances, Category A, which cause disease only in animals. An excellent online overview document pertaining to packaging and shipping has been compiled within the PREDICT One Health Consortium.[15]

Beyond regulations pertaining to the actual sample, it is important to be cognizant of the fact that additional permits may be required for the preservatives and additives in which the samples are stored (e.g., formalin, alcohol) and when shipping with coolants, dry ice, or liquid nitrogen dry shippers. Depending on mode of transport and the countries involved in the transport, the rules and regulations will vary.[16] Most notably, when shipping cooled samples, it is essential to carefully evaluate each step in the transport process to be able to maintain an intact cold chain. Although an International Air Traffic Association (IATA)-certified nonhazardous liquid nitrogen dry vapor shipper with the supporting documents may easily be checked in at an international airport, it can be equally easily refused by the pilot on a local flight in a remote location, seriously compromising sample integrity.[17]

Shipping and Port of Entry

There are various methods to physically ship samples from the country of origin to the desired destination. In many cases, it is easier to use a commercial shipper to move samples. The shipper will in most cases assist with the necessary Shipper's Declaration and the Waybill. However, commercial companies (e.g., FedEx, DHL) and specifically the respective local offices may have a greater or lesser understanding and competence in appropriate shipment of biological samples. In this author's experience, the greatest problems arise when a commercial shipper ships the samples to a nondesignated port of entry. This will result in the shipment being returned to the country of origin and, in the case of a cold chain transport, to a breech in the cold chain with subsequent sample destruction. The use of a commercial shipper specialized in the transport of biological samples, such as World Courier (http://www.worldcourier.com), appears the most prudent option. World Courier will routinely perform a thorough review of paperwork to ensure that samples will not be rejected, or destroyed, on port of entry. Be aware that such a specialized service engenders significant additional costs. Another option, and in some very remote locations the only option, is to personally carry the appropriately packaged samples back to the desired destination. In these cases, it is essential that you have identified and notified the designated port of entry of your arrival prior to departure from the export site. In most countries and ports of entry, business hours will apply. If you arrive outside of these hours, you must make specific arrangements well before your arrival to guarantee appropriate storage for the samples. In some instances, when arriving from a remote location, it is desirable to have a commercial shipper meet you on arrival to assist in clearing the entry process.



• **Figure 4.1** Simplified workflow for permitting steps in international sample movement.

Conclusions

This chapter summarizes the steps necessary to legally move a sample across international borders. However, it is important to note that this is merely a short summary and does not necessarily consider all regulations necessary in moving samples in all specific instances. Local laws, regulations, and implementation on moving samples will in some instances vary considerably. Furthermore, rules and regulations are prone to change on short notice. Sample movement can be broken down into four distinct but interconnected areas: (1) CITES, Nagoya ABS, other national permitting pertinent to the species, (2) veterinary and agricultural import permitting, (3) packaging, and (4) shipping (Fig. 4.1). It is essential to contact the various responsible agencies that have jurisdiction over biological materials well in advance of the planned sample transport. Similarly, packing and shipping to an appropriate port of entry necessitate planning well in advance of the actual shipping date. Be aware that neglecting to adhere to the various regulations and legislation will potentially incur significant fines and seriously compromise not only the sample transport but also your and your institution's ability to import and move samples in the future.

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5

A Practical Guide for Statistics in Wildlife Studies

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"What is knowledge if you don't use it?"

Dr. Jane Carter, MD, December 2015

Introduction

Information generated as part of scientific research conducted in wildlife species has played a historic and crucial role in generating the knowledge necessary to better understand species behavior, physiology, immunologic responses, epidemiology, and factors affecting animal well-being in different environments, worldwide. Research in wildlife species provides the information needed to assess current practices and to modify and/or implement new animal management procedures to maximize animal health and well-being. In addition to challenges with funding, time, and field logistics inherent in working with wildlife species, researchers are often confronted with complex scenarios that impact study design and statistical analyses in their efforts to promote wildlife health. Because of these difficulties, researchers should carefully plan their research to maximize study validity, relevance, and applicability while minimizing potential bias and using appropriate statistical techniques. Thus in this chapter we provide an overview of key study design features as they relate to the most common statistical analyses in wildlife studies. We have structured this chapter based on common questions (listed as question headings in the text) wildlife researchers face when designing studies and analyzing data. We provide practical answers to these questions and outline different approaches that need to be considered when implementing wildlife studies. An exhaustive, detailed, in-depth, and more technical description (including formulas) of these approaches and statistical methods (as well as other analytic options) may be found elsewhere,[1–5] and researchers are encouraged to access these resources when conducting their work. For the purpose of this chapter, we use terminology that may be generalized and applied to any species and to most study types. The term "outcome" refers to an event, parameter, or measurement of interest (e.g., the presence/absence or prevalence of disease, blood pressure, or temperature readings). The term "factor" will be used to describe animal or sample characteristics for which the investigator is interested in assessing potential associations with the outcome of interest (e.g., age, species, location, facilities, diet, sampling time, and season).

Q1: Where Do I Start?

When planning your study, clearly outline the overall study goal before data collection. This is not only important in prospective studies but also for retrospective studies in which the investigator collects information from preexisting data sources. The study goal is crucial because it will drive the study type, methods, tools needed, and statistical analysis to be conducted to accomplish the study objectives. With this initial information, researchers may plan the necessary field activities, quantity, and type of data to be collected and determine whether the study is feasible based on available funds and logistics. At this planning stage, it is strongly recommended to consult with (or include in the research team) a statistician, biostatistician, or epidemiologist to review the specific aspects and peculiarities of each study. Planning as thoroughly as possible before the field work or data collection commences ensures that key study features are considered and thus avoids the scenario in which investigators find out that there are not enough data collected to evaluate the outcome(s) of interest. Insufficient data collection negatively affects the precision, confidence, and power (when needed) of a study and may introduce bias, which produces inaccurate results. We recommend taking the extra time for careful a priori planning of the study to prevent significant study flaws, rather than trying to overcome major issues during statistical analysis, when it may be too late.

Q2: What Study Type Is Best for Me?

Different study types have different purposes, and all study types have the potential to be relevant if results are

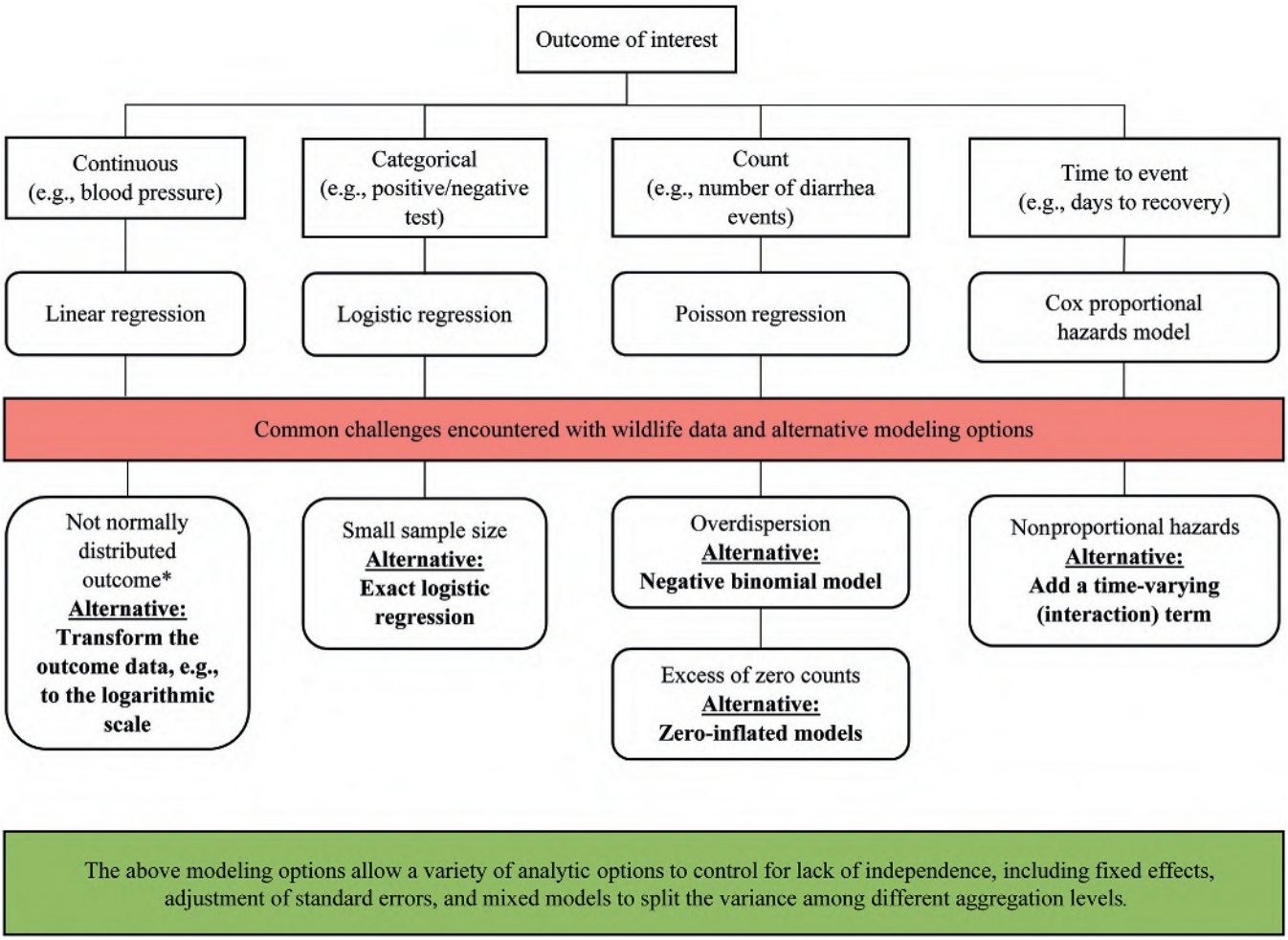
interpreted properly. In simple terms the selection of a study (and analysis to be conducted) depends on the study goal and specific research question. The investigator must be aware of the limitations, strengths, and purpose of each study type and choose a study design that meets the goals of the proposed research. Wildlife researchers could have different goals, such as (1) *describing* an outcome (e.g., health event, clinical case, or animal management in a facility) or (2) *estimating* the prevalence of a disease or the distribution of physiologic parameters from blood samples (e.g., pH or lactate values). In these scenarios, case reports, case series, descriptive reports, or surveys (or census) provide a variety of study options to choose from. When the goal of a study is (3) to *compare* interventions such as drug combinations for immobilization, vaccines, or screening/diagnostic tests, physiologic parameters, or risk factors for a disease among different groups of animals, *analytic studies* including experiments (e.g., laboratory or controlled field trials) or *observational studies* such as cross-sectional, cohort, and case-control studies are common studies used in this field. Keep in mind that cross-sectional studies are not appropriate if the goal is to obtain disease incidence because the outcome and factor are evaluated at a single point in time; case-control studies are not appropriate if the goal is to determine the probability (risk) of disease in a given population, because the investigator selects the "cases"; and cohort studies are not the best option if the outcome of interest is rare, because the investigator would need to wait a considerably long time to identify just a few rare cases in the study population.

Q3: What May Affect the Validity of My Study?

Study validity has been described as the absence of a systematic error (bias) in results.[1] The three general types of biases that may negatively impact the validity of wildlife studies are (1) selection bias, (2) information bias, and (3) confounding bias. A detailed review of validity and bias in veterinary studies may be found in other texts.[1,5] In brief, *selection bias* occurs when the selected study subjects are systematically different from those animals in the target population[5] or, in other words, when animals selected for the study have different characteristics than those animals not selected for the study.[5] One common example occurs when wildlife researchers are restricted to have access only to individuals of certain sex and age, and thus in observational studies this scenario may result in a study population not representative of the source (and target) population to which the investigator desires to extrapolate the study results. *Information bias* occurs when the data recorded on the outcome or factors are inaccurate, for example, when a screening/diagnostic test does not correctly classify the disease or infection status[1] of an individual due to the lack of test sensitivity (causing false negative results) or lack of test specificity (causing false positive results). Similarly, information bias may result when a measuring device does not accurately record the true value of a parameter, for example, a field device or laboratory technique (e.g., lactate, pH, or optical density values from an enzyme-linked immunosorbent assay) that does not provide accurate readings. *Confounding bias* occurs when a factor (known as the confounder) is not included in the analysis and this "missing factor" is associated with **both** the outcome of interest and another factor that the investigator analyzed. Thus, when confounding bias is present, conclusions may be incorrectly drawn regarding associations between an evaluated factor and an outcome, when in fact, another unmeasured factor is influencing the outcome. For example, say an investigator is evaluating the impact of location (geography) on prevalence of a certain disease in a species. Age is known to be associated with prevalence of this disease, and assume that 70% of animals in location A are adults, compared with only 15% of animals in location B. If age is not included in the analysis, then the location results could be affected by confounding bias, where the analysis ignores a factor (age) that is both associated with the outcome (disease prevalence) and another factor (location). There are several ways to prevent confounding bias during the study design and also means to adjust the analysis to control for potential confounding factors described elsewhere.[1,5] In this chapter, common and robust statistical methods to adjust results for potential confounding factors are described in Q9 and Fig. 5.1.

Q4: How Many Animals (or Samples) Do I Need for My Study?

When *estimating* disease prevalence or the average value of a continuous outcome (e.g., blood pressure in mm Hg, lactate values in mM/L), the (1) level of confidence, (2) precision, and (3) expected variability in your data will determine the number of animals/samples required. For example, to conduct a study with 95% confidence to estimate that the disease prevalence is approximately 15% with a precision of 2% will require a larger sample size as compared with the same study with a 6% precision (the variance[1,3] in this case would be the prevalence times [1-prevalence], thus 0.15×0.85). For *analytic* studies when the goal is to compare an outcome between groups, the study should be planned to have at least 80% power. The investigator must clearly indicate the magnitude of the difference to be detected rather than just saying higher or lower. The magnitude of the difference to be detected is key in determining the number of animals/samples needed; the greater the difference to be detected, the fewer animals/samples needed, and vice versa, the smaller the difference to be detected among groups, the larger the sample size needed. Thus a study with 80% power to detect a difference of 5% in disease prevalence between females and males and declared statistically significant will require a considerably larger number of females and males than the same study to detect a difference of 35% between females and males. Equally, a study designed to detect a



• **Figure 5.1** Multivariable analyses options to account for the effect of multiple factors on the outcome of interest. *Technically, the residuals should be normally distributed, but often, a quick assessment can be done by evaluating the distribution of the outcome data.

drop in blood pressure of 60 mm Hg after a drug is applied will require a considerably smaller sample size as compared with a study designed to detect a drop in blood pressure of 20 mm Hg. Ideally, the difference (hypothesis) to be tested in any analytic study should represent a clinically, biologically, or epidemiologically relevant and meaningful difference.

Q5: What Is the Formula or Software I Need to Use for Sample Size and Power Calculations?

Sample size and power formulas for specific types of studies, including estimations, comparisons, or for studies aiming to detect disease, may be found in the recommended reading.[1–5] In addition, most statistical and epidemiologic software (PASS[a], SAS[b], STATA[c], R[d], S-PLUS[e], EpiTools[f], among others) provide user-friendly platforms to perform these calculations allowing, if necessary, for adjustment of sample sizes for small populations, lack of sensitivity and specificity of screening/diagnostic tests, and lack of independence (clustering) when present.

Q6: Is My Study Invalid and/or Irrelevant Because of Small Sample Size?

Absolutely not! For example, even studies with $n = 1$ (case reports) could still be informative and relevant when reporting a disease or pathology not previously (or rarely) reported in a particular species or in a given location.[6,7] Equally, it is important to note that studies with relatively small sample sizes could be relevant[8,9] for a specific field or species if that species is extremely difficult to work with (e.g., endangered species). In these cases, data should be presented using techniques appropriate for studies with

[a]PASS, Power Analysis and Sample Size Software. NCSS, LLC. Kaysville, UT.
[b]SAS software. SAS Institute, Inc. Cary, NC.
[c]Stata statistical software. StataCorp. College Station, TX.
[d]R: a language and environment for statistical computing. R Development Core Team. Vienna, Austria.
[e]TIBCO Spotfire S+. TIBCO Software, Inc. Palo Alto, CA.
[f]EpiTools. Sergeant, ESG, 2017. EpiTools epidemiological calculators. Ausvet Pty Ltd. Available at: http://epitools.ausvet.com.au.

small sample sizes, which include counts (e.g., 2/6) rather than using proportions and 95% confidence intervals, and using the median, quartiles (Q1 and Q3), and ranges as opposed to the mean, standard deviation, and corresponding 95% confidence interval for continuous outcomes. The median is a more stable estimate of central tendency in these scenarios because the mean may be impacted by extreme observations.[3,4] In addition, when comparing groups, it is strongly recommended to use nonparametric statistical tests designed to analyze data arising from studies with small sample sizes or when data do not meet the statistical test assumptions.[3] Of course, what is considered a small sample size has been the subject of some debate, but depending on the statistical test required, some authors[3] consider studies with $n < 20$ or $n < 25$ as candidates for nonparametric analyses, whereas others[2] consider studies with $n < 30$ as candidates for nonparametric analysis. The most important caution to consider in studies with relatively small sample sizes is to avoid generalizing and/or extrapolating results to a larger population if the data in fact are not representative of a larger population. Rather, the investigator should acknowledge the study limitations and discuss the clinical, biological, or epidemiologic relevance of results and highlight the strengths, novelty, and or contribution of their data to the field. In fact, studies with small sample sizes not only may be extremely relevant for gathering data and generating knowledge but may also provide valuable information to investigators interested in further testing additional hypotheses.

What about studies with large sample sizes? It is worth noting that studies with large sample size have a greater precision and power; however, a large sample size in and of itself (e.g., 300 or even >1000 animals) does not guarantee validity and/or relevance just because of sample size. All key aspects of study design are also relevant to studies with large samples, and of particular interest in wildlife species, potential confounding bias must be addressed in the study design and data analysis, as well as proper interpretation of the clinical, epidemiologic, or biological relevance of the obtained results (see Q10).

Q7: How Do I Need to Structure the Data to Be Able to Conduct the Statistical Analysis?

Details regarding good data-recording practices have been described,[1,5,10] and thus here we provide a practical guide to enter data in an electronic format using Microsoft Excel or other spreadsheet software in a way that will facilitate the analysis in most statistical software.[a–e] Examples of spreadsheets with data formats that are suboptimal and optimal for data analysis are shown in Fig. 5.2A and Fig. 5.2B, respectively. Animals/samples should be identified in a column containing only the animal identification information, and individual observations should be entered one per row. Repeated measurements from the same animal (or different tests applied to the same blood sample) may be entered in a separate column indicating different sampling points or different tests (0, 1, 2, 3, etc.). All of the collected information for that particular individual (e.g., age, sex, treatments, blood pressure, diseased, or nondiseased status) should be entered in different columns, with columns containing only information relating to one factor or animal/sample characteristic (you may add as many columns as necessary). Letters or numbers may be used to codify the data, and this greatly facilitates data analysis. For example, if there are different groups in your data set (e.g., two different treatment groups), it is best to include the group as its own column rather than separate sheets for different treatment groups (0, 1 or A, B; see Fig. 5.2B). **Consistency**: Use the same unit (e.g., kilograms or pounds) for all measurements. Record factors using a consistent format (e.g., DD-MM-YYYY for date) in every row. For nonnumerical information, it is best to use the exact same word or phrase rather than multiple versions of the same idea (e.g., "left lateral" rather than "left," "L," "left lateral," and "L lat"). Similarly, make sure that spelling and letter case for names, drugs, etc. are identical across rows. **Conciseness**: Do not enter any unnecessary information in a column. For example, a numerical column (e.g., temperature) should only have numbers, no letters. The units °C or °F may be in the title of the column or in a separated study log document indicating the measurement units for each factor. Do not enter notes/comments in the same column as numbers or codes; make a separate column specifically to add notes if needed. **Clarity**: Give each column a short but logical title. If anything needs more explanation, consider making a separate sheet or study log document with a key. This practice is valuable for making data easily interpretable to others, and for defining more ambiguous variables (e.g., subjective scoring systems or which treatment was assigned to which group) to reduce uncertainty when reviewing data in the future. **Missing values:** If any factor has missing information, leave the cell blank rather than adding not available (N/A) or unknown (UNK), or adding a zero. If needed, make comments in the comments column.

Q8: What Do I Need to Do If the Observations in My Data Set Are Not Independent?

A common characteristic of studies conducted in wildlife species is the lack of independence among observations (data points), such as when researchers collect multiple samples from the same animal at different points (i.e., repeated measurements), when the same sample is measured with different devices/tests, when animals are sampled as part of herds or facilities, or when sampled animals are geographically related. If this level of aggregation could be related to the outcome being investigated, the data points are considered clustered and therefore not independent from one another. When the investigator is confronted with

	A	B	C	D	E	F
1	16-04-13					
2	Day 1	Group 1 Sample 1	ID	Temperature	Weight	Age Category
3		Sparkle CTR1		99.8F	94kg	Juv
4		Prince	ICZ3	100	74 kg	Adult
5		Polly	CNP 4	101.4	108 kg	Ad
6		Fred	IXC8	99.5	88 kg	J
7						
8	April 17, 2013	Group 3	ID	temp	wt	
9	Day 2	April 17 - Dumbo	TKE2	98.6	258 lb	Adult
10		Bean	FPR5 ? Check tattoo	110.9	unk	young
11		NCR3		1003	99kg	juvenile?
12		Bop	MXP6	N/A	105kg	Ad
13						
14	18-04-13	Group 1 Sample 2	ID	Temperature	Weight	Age Category
15		Prince	ICZ3	100.8	74 kg	ad
16		Fred	IXC8	98.9	87 kg	juv

A

	A	B	C	D	E	F	G	H	I	J
1	Date	Day	Group	Name	ID	Temperature	Weight	Age Category	Sample	Notes
2	16-04-13	1	1	Sparkle	CTR1	99.8	94	Juvenile	1	
3	16-04-13	1	1	Prince	ICZ3	100	74	Adult	1	
4	16-04-13	1	1	Polly	CNP4	101.4	108	Adult	1	
5	16-04-13	1	1	Fred	IXC8	99.5	88	Juvenile	1	
6	17-04-13	2	3	Dumbo	TKE2	98.6	117	Adult	1	
7	17-04-13	2	3	Bean	FPR5	110.9	79	Juvenile	1	Check tattoo
8	17-04-13	2	3	Red	NCR3	100.3	99	Juvenile	1	
9	17-04-13	2	3	Bop	MXP6		105	Adult	1	
10	18-04-13	3	1	Prince	ICZ3	100.8	74	Adult	2	
11	18-04-13	3	1	Fred	IXC8	98.9	87	Juvenile	2	

B

• **Figure 5.2** (A) Suboptimal data structure/formatting for statistical analysis. (B) Optimal data structure/formatting for statistical analysis.

• BOX 5.1 Rule of Thumb for Analysis

Proceed to the statistical analysis only after the data set has been checked for consistency, completeness, and accuracy.

this scenario, it is strongly recommended not to ignore it, which could result in overestimating the statistical significance of results by artificially increasing the sample size. Some examples of statistical tests/methods that allow accounting for lack of independence include the *McNemar's* test, repeated measurements analysis of variance *(ANOVA)*, and *paired t-test,*[2–4] as well as the multivariable analysis[1] techniques summarized in Fig. 5.1.

Q9: Which Statistical Test/Method Do I Need to Analyze My Data?

See Box 5.1. Most statistical and epidemiologic books contain self-explanatory flowcharts to guide investigators to select an appropriate statistical test.[2,3] The selection of a statistical test/method largely depends on the type of data of the outcome being measured (e.g., categorical or continuous data). In addition, the number of groups to compare, number of factors being evaluated, and the statistical test assumptions (including data distribution and sample size) play an important role when selecting a statistical test. A standard process to select statistical tests includes (1) specifying the hypothesis to test, (2) describing and displaying the data graphically, and (3) checking the data distribution and the statistical test assumptions. As a practical example, we chose a commonly used statistical test, the Chi-squared test, to describe the approach to be used to select a test. When the outcome of interest is categorical and dichotomous, as often is the case when studying whether animals tested positive or negative to a test, whether animals are sick or healthy, and the investigator is only interested in comparing two groups (e.g., females vs. males), then a standard 2×2 table using the Chi-squared test to compare the proportion of animals having or not having the outcome of interest is appropriate. For this test, the assumptions are that observations are independent and that the expected frequency in each cell of the 2×2 table is at least 5. If the data do not meet these characteristics, then the *Chi-squared* test is invalid and a nonparametric option such as *Fisher's exact* test should be used in scenarios with small sample size or *McNemar's* test when data are not independent (e.g., two screening tests used on the same blood sample). If the investigator is interested in evaluating the impact of multiple factors on the outcome (Box 5.2) (e.g., age, location,

• BOX 5.2 Multivariable Analysis

The vast majority of outcomes studied in wildlife species are determined by multiple factors. Collecting data on as many of these factors as possible allows for a comprehensive statistical analysis. Multivariable models are techniques that include linear, logistic, and Poisson regressions, as well as survival analysis models. These methods are the most commonly used analytic approaches to control for confounding by including potential confounding factors in a model and thus evaluating the combined effect of these factors on the outcome of interest.[1]

time, and sex), then a multivariable approach using *logistic regression* analysis is recommended. Using this approach, results may be adjusted for potential confounding effects, and, if needed, the investigator may control/adjust results for lack of independence between observations to split the variance between different aggregation (clustering) levels.

In the scenario of a ***continuous outcome*** (e.g., blood pressure in mm Hg, or calcium and phosphorus in ppm), the *Student's t-test* (for comparing two groups), *ANOVA* (for two or more groups), and/or linear regression analysis (to evaluate multiple factors) are appropriate. When the assumptions of these tests are not satisfied by the data, nonparametric tests such as the *Wilcoxon rank sum* test, *Kruskal-Wallis* test (nonparametric ANOVA), or transformation of outcome data to the logarithmic scale for linear regression are options to consider.

When the outcome of interest is ***counts*** (e.g., number of diarrhea episodes in a period of time), Poisson regression analysis provides a robust option because count data often (especially when the outcome is rare) have a Poisson distribution, which is characterized by the mean being equal to the variance.[1] When data are not independent, the main assumption of Poisson models (mean = variance) is often violated, and in these scenarios negative binomial models may be used for analysis. In addition, if an excess of animals did not have the outcome (count = 0, no diarrhea) relative to those animals that did have the outcome, zero-inflated models provide a robust option in which the researcher may investigate factors impacting the probability of not having the outcome (0 counts) and factors impacting having a greater number of outcomes among those animals in which the outcome occurs.

When the investigator is interested in the ***time taken*** for an outcome to occur, survival analysis techniques including life tables, Kaplan-Meier curves, and Cox proportional hazards models[1,11–14] may be used, depending on the data distribution and study design.

Q10: Are My Results Statistically Significant?

This is by far one of the most common questions among researchers. It is worth noting that, although not exempt from debate,[15,16] the *P*-value is an important statistic to consider but certainly not the only one. The *P*-value is the probability of making an error (type I error) in which the investigator concludes that there is a significant difference between groups (e.g., a drug reduces blood pressure) when in fact there is not. In general, $P < .05$ (<5% chance of error) is considered statistically significant. Having statistically significant results means that the observed differences are not likely due to chance, and rather, differences in the measured outcome are associated with the factor(s) evaluated in the study. Important study design aspects, such as sample size, power, the magnitude of the difference being evaluated, and the amount of variability in the data, have a direct and strong impact on *P*-values and should be considered when interpreting results. Furthermore, it is imperative to note that a study with a statistically significant result does not necessarily mean that the result is clinically relevant, and vice versa. In studies with small sample size, it is less likely to find statistically significant results; however, findings still may have an important clinical, physiologic, or ecologic implication, especially if the magnitude of the difference identified is biologically important. Hence, although not replacing one for the other, the investigator is encouraged to discuss both the statistical significance and clinical importance of the obtained results.

Q11: How Do I Present and Summarize My Results?

This largely depends on the forum in which the results are being displayed, which may include scientific journals, or poster or oral presentations to a myriad of audiences, including the scientific community, policy makers, or wildlife management personnel. In general, when presenting your results, after clearly outlining the goal of your study, it is recommended to present a detailed *descriptive analysis* showing the distribution of both the outcome and factors of interest. If the outcome or factor data are categorical in nature, presenting proportions with their corresponding 95% confidence intervals are recommended. If the data are continuous and normally distributed, then means, standard deviations, and 95% confidence intervals are appropriate to summarize the data. If sample size is relatively small, use of medians and range (minimum and maximum values), as well as 25th and 75th percentiles, is recommended to summarize and describe the data distribution. When there are different groups of interest (e.g., treatment groups, species, sex, sampling points), the researcher should conduct a stratified analysis of any variables of interest for each group of interest. If the investigator tested a hypothesis, then initially *univariable* results describing associations between the outcome and only one factor of interest (e.g., changes in lactate [outcome] values over time [factor] after immobilization) should be presented. The limitations of descriptive and univariable analyses, such as if factors known to impact the outcome were not considered in the analysis

(potential confounders), should be clearly indicated. When multivariable models are used to analyze the impact of multiple factors in the outcome, researchers must clearly indicate that results are adjusted by the effect of all other factors in the model. Relevant information showing the differences among groups, such as coefficients, odds ratios, count ratios, and hazard ratios, and their associated 95% confidence intervals and *P*-values should be included in tables.

Q12: How Do I Communicate My Results?

When interpreting results, rather than only describing what was found from a statistical point of view (e.g., "we found a statistically significant difference [$P < .001$] among groups"), the investigators are encouraged to perform a final (sometimes difficult) assessment of their study and run a "relevance" test, what we like to call the *"So what?"* test. Ask yourself: what is the meaning of the results obtained? Why should we care? The results should be applied for the intended audience and relevant information beyond just the statistical significance level should be provided to support your conclusions. The *"So what?"* question should be applicable to any type of study, and often, the difficult answer to this question lies embedded in the original goal of the study.

Conclusion

Despite all the known complexities inherent to working with wildlife species, researchers should maximize the value and impact of their work by using available resources to carefully design and implement studies, analyze data, publish scientific manuscripts, and communicate and disseminate findings to different audiences. However, even more importantly, we encourage researchers to actively share and promote the use of the best available scientific evidence with key stakeholders and decision makers at different levels. The validity, relevance, and applicability of results from different types of studies may (and should) play a critical role in informing the decision-making process to promote wildlife health and well-being. After all, ***"What is knowledge if you don't use it?"***[17]

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6

Opportunities to Inspire the Next Generation of Veterinarians

JOHN M. SYKES IV

Introduction

Modern zoological institutions have mission statements that include goals well beyond simple entertainment. For example, the Wildlife Conservation Society (WCS), which operates five zoological parks in New York City, has a mission to "save wildlife and wild places worldwide through science, conservation action, education, and inspiring people to value nature."[1] Zoological veterinarians contribute directly to the science and conservation action goals through professional publications and participation in field projects. Their roles in the education and inspiration aspects are often indirect: they keep animals healthy and happy so that the animals (or other educators) may inspire and educate the visitors. However, there are many opportunities for veterinarians to participate directly in the education and inspiration of our visitors, particularly children. This chapter explores many of these opportunities and outlines one particularly in-depth program for teaching children about zoological medicine.

Booths

One of the simplest ways to reach people directly is to design a tabletop display or activity similar to those seen at street fairs (Fig. 6.1). The table may be staffed by members of the veterinary team who may easily rotate shifts if needed. This type of activity is useful for special events on the grounds of the institution (e.g., Earth Day, special fundraisers) but may also be easily adapted for use off grounds at community fairs, state fairs, or local science, technology, engineering, and mathematics (STEM) events. There are many types of activities that work well for these events that may highlight the veterinary aspect of caring for animals including: skulls and dentistry (see the Little Zoo Vets (LZV) section below for details), comparative anatomy models, darts and how they work, etc. Many affordable microscopes now come with a small display screen on top. There are models that are completely battery operated, allowing for easily portable discussions of comparative hematology (e.g., nucleated red cells), hemoparasites, or ecto- and endoparasites. If power is available, large TV screens are now relatively inexpensive and may play videos without needing to be connected to a computer. These booth events are easily set up and require minimum staff time to prepare or manage.

Mentorship

There are many opportunities to mentor young people interested in veterinary medicine—some of which may involve a significant time investment, but some may be very impactful with only a minimal amount of time. Many institutions participate in year-long mentorship programs for youth. Veterinary staff may often participate in these programs without needing to design or manage the whole program themselves. One example is the WCS's Bridging the Gap program.[2] One component of this program is participation in monthly mentoring sessions with zoological professionals. Veterinary staff are able to participate as one of those professionals and contribute to the larger program. Alternatively, some programs involve a consistent connection with a veterinary staff member with a school or class, but not a one-on-one mentorship. These programs may be as simple as visits by the professional to the school to talk about his or her career or visits by the class to the institution's veterinary hospital for a tour and discussion. There are also opportunities for zoological veterinarians to participate in local community events outside of the institution. Many communities hold regular career fairs, where veterinarians and veterinary technicians are often welcome to participate. Local veterinary organizations may also have programs in which to participate. For example, the Veterinary Medical Association of New York City organizes a high school career exploration program.[3] High school students from around the city are invited to come to four evening programs. Each program consists of at least two presenters from different aspects of veterinary medicine, such as ophthalmology, emergency medicine, equine medicine, dentistry, public health, etc. Students learn about the wide range of careers open to veterinarians, including zoo and wildlife options. These career fairs/exploration programs require minimal

time investment but have the potential to inspire many new young people to pursue veterinary medicine.

Veterinary Windows

Some institutions have designed their veterinary hospitals to allow the public to observe veterinarians working in real-time (Fig. 6.2). These "windows" may be managed in different ways. Some institutions may have scheduled procedures in the window on a regular basis and include the time for the events in their visitor's daily activities schedule. Others choose to use the window for only special events, such as education classes or tours. The degree of interpretation also varies by institution. Some facilities use signage and prerecorded videos to explain the activities occurring. Others may have an educator explaining what is happening in real-time. Veterinary staff participation may be as minimal as simply performing the procedures in the window, or could be as involved as speaking to the public through a microphone before, during, or after the procedure. Regardless of the level of involvement and interpretation, these windows provide the visitors with a glimpse into the high level of medical attention provided to the animals in our care.

• **Figure 6.1** Example of a tabletop display/booth at a special event at the Central Park Zoo. Using prepared skulls, veterinary staff are discussing with visitors the variations in animal dental anatomy and how that affects their diet and veterinary care. (Courtesy Julie Larsen Maher © WCS.)

Public Dissections

One interesting approach to educating zoo visitors about animal anatomy occurs at several Scandinavian zoos including the Copenhagen Zoo in Denmark. This institution conducts regular "public dissections" of clinically healthy animals that have been euthanized (Fig. 6.3). The dissections occur "behind the scenes," but all visitors to the zoo are welcome to attend. Veterinarians are able to discuss comparative anatomy and display actual specimens at these events in order to educate the public about the amazing diversity of biology within the animal world. Feedback from visitors has been overwhelmingly positive, as they appreciate the opportunity to see anatomic features and learn facts about animals that are hard to see or learn in typical zoological programs (personal communication, Mads Bertelsen, 2017) (see Chapter 23).

Education Programming

Most zoological institutions have some form of an education department. These people are generally in charge

• **Figure 6.2** Example of a specially designed window into the veterinary clinic to allow visitors to observe live veterinary procedures at Disney's Animal Kingdom. (Courtesy © Disney's Animal Kingdom.)

• **Figure 6.3** Zoo veterinarian discussing the cardiopulmonary anatomy of a recently euthanized lion with invited members of the public at the Copenhagen Zoo. (Courtesy Frank Rønsholt, Copenhagen Zoo.)

of designing and implementing programming for school groups, on-grounds classes, and outreach to local schools. They may also participate in adult education, teacher education, or volunteer coordination. These staff members often interact with animal care staff, particularly when ambassador animals are involved in programming; however, in practice they rarely interact with the veterinary department. There are many opportunities to increase collaborations between the two groups. Veterinary staff may be guest speakers/teachers in existing education programming, particularly in summer camps or adult education classes. Tours through veterinary hospitals may be included as education offerings either by training educators to give the tours or by scheduling hospital staff to assist. Participation in education programs may also expand into designing and teaching entire programs as described in the next section.

Little Zoo Vets Program

The LZV program at WCS is an example of a complete education offering, which is focused on zoological veterinary medicine. The program was inspired by the Little Vets program[4] offered by Dr. David Bessler in Manhattan, New York. That class offered children in second through fifth grade an opportunity to learn about veterinary medicine through after-school classes taught by Dr. Bessler. His advanced class included specialties, such as shelter medicine and zoo medicine. It was out of these initial zoo medicine classes that the LZV program arose.

The LZV program was designed and is taught by a zoo veterinarian (Dr. Sykes) with assistance from other members of the veterinary staff (technicians, residents) and educators. The program is currently offered through the WCS education department as a paid experience with a maximum enrollment of 20 students per 90-minute class. The class is provided for children aged 8–10 years old at the Central Park Zoo during the school year as an after-school activity, and at the Bronx Zoo during the summer either as a stand-alone class or as an after-camp activity for children attending the Bronx Zoo summer camp. Currently, two sessions consisting of five classes each are offered. At Central Park Zoo, the classes are once per week in the spring and fall, and at the Bronx Zoo the classes are offered daily for 1 week with two sessions offered each summer. The topics for each session are: Session 1: Physical exam—focus on the eyes, upper gastrointestinal tract, blood, darting, and goat exam/graduation; Session 2: Physical exam—focus on the ears and heart, lower gastrointestinal tract, anesthesia, bones, and goat exam/graduation.

Classes include a mixture of discussion and activities (see sample class outline in Table 6.1). Most classes start with a traditional classroom setting: students in seats with the teacher presenting material on the screen using a slide-based presentation (Fig. 6.4). These presentations include photos, videos, animations, and sounds that highlight various aspects of zoological medicine. For example, in a discussion of lameness, videos of lame animals (peacocks) are presented, and the students are asked to determine which leg is unsound. During a discussion of the function of the heart, cardiac sound recordings from various animals (human, porcupine, amazon parrot, and kestrel) are played to practice counting heart rates. The presentation is followed by hands-on activities using live animals, traditional biofacts/biomaterials (skulls, bones, feathers, shells), custom-built models, real veterinary equipment, and some more unusual biomaterials (blood, feces).

Live animal activities are limited to completely non-invasive procedures. Students use ophthalmoscopes, otoscopes, stethoscopes, and are allowed to lightly touch animals where appropriate. The animals that participate in these activities are conditioned to be part of animal ambassador programs commonly used in zoological education programming. They are minimally restrained for these activities and the activity is stopped if any distress is noted. Examples include looking through an ophthalmoscope at a guinea fowl eye (Fig. 6.5), listening to a screech owl's heart (stethoscope is placed on the bird by the instructor), or looking at goat pinnae through an otoscope.

Traditional biomaterials are used in the class, but often in different ways than most educators use them. For example, domestic dog skulls (commercially purchased) are used to practice dental scaling. The teeth of the skulls are painted with a concentrated brown sugar solution and allowed to dry. During the upper gastrointestinal tract (teeth) class, students use hand scalers to clean the teeth, allowing for a discussion about veterinary dentistry (Fig. 6.6).

Custom-built models help to illustrate points that may be difficult using biomaterials or live animals. The most involved model is the bird palpation/bandaging model (Fig. 6.7). This model is used primarily to practice bandage placement. Each limb has three "bones," one of which is broken. Students must determine which bone is broken, and then the best way to bandage that fracture using the principles of immobilizing a joint above and below the fracture. Each model also has a coelom full of foreign objects, and students

TABLE 6.1 Sample Little Zoo Vets Class Outline

Opening: Introduction of instructors; scrub tops are distributed; photos are obtained to make name tag licenses for remaining classes.

Didactic portion: Slide-based discussion
Physical exam: Whole body exam, use 4/5 senses (no taste), outline class for today.
Eyes: Using photos of normal and abnormal animal eyes, snake shed, and store-bought models, the following is covered: eyelids, third eyelids, snake eye caps; cornea and lens, specifically the difference between cornea cloudiness and cataracts; pupillary light reflex (PLR) using videos of a snow leopard with normal PLR, a macaw with voluntary pupillary movement, and an owl with minimal response to light. Discussion of knowing what is normal for different species is important for zoo veterinarians; eye position predator versus prey—view video of peacock walking after predator/prey activity.

Group activity: Predatory/Prey demonstration (field of view and depth perception). One student serves as the "predator" and holds two purple strings in left hand and two green strings in right hand. With an eye patch over the left eye and while staring straight ahead, the other students hold the ends of the purple string taut and move laterally until they are out of the right eye field of vision. Repeat with the patch over the right eye. The field of view range is seen by the separation between the lateral-most students, and the binocular/depth perception field is seen in the overlap between the two string colors. Repeat the activity, this time with two students as the "prey," one for each eye (so that they can be turned back-to-back to position each eye laterally). Follow this up with tossing a ball to each student, first using both eyes open, then with an eye patch on one eye, and compare the difficultly in catching the ball (depth perception). Proceed with discussion of how this knowledge might be important to know as a zoo veterinarian.

Station activities: Small groups at each station will rotate for the remaining class period between: (1) practice using an ophthalmoscope on a model eye (turn on/off, make the light blue, make the light a slit, look at the eye model, and draw what you see); (2) palpation practice: using palpation models, determine what may be in the animal's stomach using only your hands, not your eyes (models consist of large animal prints with holes cut in the abdomen area, and prints are attached to cardboard boxes just behind the holes and various objects are placed within the box)—students can then challenge each other by placing new objects in the boxes for other students to try to guess; (3) body condition scoring: using printed scoring charts and animal photos to give a body condition score to the various animals; and (4) live animal station: look at the outside of the animal's eye using the ophthalmoscope and describe what you see.

Materials list: Name tag supplies; photo release; class roster; camera; plastic eye models; sterilized snake shed; string and eye patches; palpation cut-outs and box of "unknowns"; data sheets for ophthalmoscope practice, palpation, body condition scoring; hand sanitizer; ophthalmoscopes; two live animals (ideally one mammal and one bird) with handlers.

• **Figure 6.4** Example of the initial phase of most Little Zoo Vets classes where students are in a typical classroom lecture-style setting while the veterinary instructor is at the front of the room at the Bronx Zoo. (Courtesy Julie Larsen Maher © WCS.)

• **Figure 6.5** Little Zoo Vets student examining the eye of a guinea fowl using an ophthalmoscope at the Bronx Zoo. (Courtesy Julie Larsen Maher © WCS.)

must practice their palpation skills to determine what those objects are.

Some of the most enjoyable, though logistically difficult, parts of the class involve using real animal biomaterials. During the blood class, students are allowed to make blood smears, stain them, and examine them under the microscope. During the feces class, students perform a fecal flotation test and dissect a fresh elephant fecal bolus (Fig. 6.8). The biomaterials used in the class are materials left over from regular clinical veterinary activities—none are collected for this purpose. Additionally, personal protective equipment is worn and students are monitored closely during these activities. A scrub top is supplied for each student and laundered at the zoo between classes. Each student must

• **Figure 6.6** Little Zoo Vets student scraping "tartar" (brown sugar) from the teeth of a domestic dog skull at the Bronx Zoo. (Courtesy Julie Larsen Maher © WCS.)

• **Figure 6.7** Little Zoo Vets students learning about palpation and bandaging techniques using a custom-built model of a bird at the Bronx Zoo. (Courtesy John Sykes © WCS.)

wear eye protection, a face mask, and exam gloves to participate. The samples used come only from healthy animals with minimal zoonotic potential (e.g., no primate samples). These activities are clearly more complicated to execute but are also highly rewarding for the kids, all of whom may tell the difference between a mammal versus a bird or reptile blood smear at a glance by the end of the class!

Clearly, the goals of these activities are not to perform a complete fundic exam or auscult a grade 1/5 murmur, or even to serve as a recruitment tool for zoo veterinarians. Rather they are to introduce the students to the concepts of how zoological veterinarians approach a physical exam and, more importantly, how amazing the huge variety of creatures are with which we work.

Conclusions

Veterinary participation in education programs serve many purposes, including increasing revenue, enhancing existing programming and the reputation of the institution, and

• **Figure 6.8** Little Zoo Vets student dissecting an elephant fecal bolus at the Bronx Zoo. Note the personal protective equipment being worn: scrub top, eye protection, face mask, and exam gloves. (Courtesy John Sykes © WCS.)

delivering on the institution's mission. For many institutions, some education programming is paid for by the attendees and may serve as a source of revenue. Veterinary-specific programming may increase that revenue for many different purposes, including general operating, veterinary expenses, or special projects. Veterinary education classes also enhance the institution's existing programming. The public is always asking for more contact with the experts who work directly with the animals. Veterinary programming may help to serve that demand and help to drive interest toward other programming. These classes also offer an opportunity to showcase the excellent care being provided to our animals and serve as a clear narrative about the high quality of care that zoo animals receive.

The most important advantage to developing these programs is to help veterinary staff contribute to the mission of their organization by directly educating and inspiring the public. Participating directly in the mission not only helps to accomplish it but also serves to improve job satisfaction. It may be easy for veterinary staff to become insular in their department and lose sight of why they became involved in zoological medicine. Connecting with the public may remind staff of how important their contributions are to the institution and may help to retain their talent in the field.

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7

Strategic Planning for Zoo Veterinary Operations

SCOTT TERRELL

"If you fail to plan, you are planning to fail."

Benjamin Franklin

Why Is Strategic Planning Important?

The zoological veterinary practice is an essential part of the success of any zoological operation. Regardless of whether a particular zoo is a not-for-profit or a for-profit institution, the management of the zoo requires good business practices to ensure the long-term success of the institution. Good business practices allow allocation of resources to essential mission-based objectives such as excellence in animal care (including medical care), conservation, education, and research. To ensure the success of an institution as a whole, all aspects of the institution should strive to make good business decisions and use business management tools to continually enhance the performance of their team(s). Strategic planning is a business management tool that guides an organization/division/team (hereafter referred to as a "team") to increase performance through focusing resources, time, and effort of everyone in the same direction and with the same goals. Strategic planning is also an excellent leadership tool. A well-prepared strategic plan sets clear direction and goals for all members of the team and establishes accountability for success or failure of those goals. It is easy to discount the value of strategic planning or think that it is a tool only for traditional retail businesses or large corporations. However, all organizations and teams need direction and purpose. Lack of direction results in decreased morale, decreased productivity, and increased anxiety because the future is unpredictable and uncertain. A strategic plan provides a buffer against indifference and drives purpose and direction for any organization regardless of its size.

The typical zoo veterinary leader is challenged with applying the strategic planning process in two distinctly different ways. The first is when they have the opportunity to contribute to the leadership and overall strategy of the institution. The second is to ensure that the strategies, priorities, and goals of the veterinary team align with those of the overall institution. Increased understanding of the strategic planning process in both contexts will better position the zoo veterinary leader to influence the overall organization/institution, as well as her or his own team. By aligning the veterinary team priorities with the organization/institutional strategy, the zoo veterinary leader may ensure that the veterinary team provides maximum value to the organization.

What Are the Components of a Strategic Plan?

A strategic plan may be a relatively simple document or a complex process.[1,2,5] Strategic plans may be created for an individual, a small team, or a large organization. The strategic plan is a roadmap for an organization to move from today to an envisioned future. When well done, the strategic plan not only inspires future change and improvement in the organization but also aids in day-to-day decision making/priority setting in the current state and provides a tool for measurement of success or failure.[3] The basic components of a strategic plan are as follows:

- Vision—A vision statement formulates a picture of the impact of your organization or team in the future.[5,6] The vision presents an image of what success will look like if the organization/team achieves its mission. Vision statements should be inspirational and aspirational and should motivate team members toward unity in a common goal (Box 7.1).
- Mission—A mission statement describes your organization or team's purpose and formulates a picture of why your organization or team comes to work and what they do "today."[5–7] The mission statement typically includes a clear statement of purpose, the way(s) in which that purpose will be achieved, and an identification of the ultimate benefit of the purpose (see Box 7.1).
- Strategic objectives—Strategic objectives are long-term, continuous strategic areas that connect your mission to

• BOX 7.1 Examples of Well-Formatted/Written Vision and Mission Statements

- Vision statements
  - **The Nature Conservancy:** Our vision is to leave a sustainable world for future generations.
  - **Goodwill:** Every person has the opportunity to achieve his/her fullest potential and participate in and contribute to all aspects of life.
  - **Teach for America:** One day, all children in this nation will have the opportunity to attain an excellent education.
  - **Make-A-Wish:** Our vision is that people everywhere will share the power of a wish.
  - **San Diego Zoo Global:** We will lead the fight against extinction.
- Mission statements
  - **Patagonia:** Build the best product, cause no unnecessary harm, use business to inspire and implement solutions to the environmental crisis.
  - **Wounded Warrior Project:** To honor and empower wounded warriors.
  - **National Wildlife Federation:** Inspiring Americans to protect wildlife for our children's future.
  - **Make-A-Wish:** We grant the wishes of children with life-threatening medical conditions to enrich the human experience with hope, strength, and joy.
  - **San Diego Zoo Global:** San Diego Zoo Global is committed to saving species worldwide by uniting our expertise in animal care and conservation science with our dedication to inspiring passion for nature.

your vision. Strategic objectives tend to be less specific and more descriptive than priorities and goals.

- Strategic priorities—Strategic priorities are short-term goals (generally 1 year or less to a significant milestone or completion) that convert your strategic objectives into specific performance targets. Strategic priorities represent an opportunity (along with goals later) to set specific timelines, clear measures of success, and accountabilities. In most cases, strategic priorities are led by senior leaders on a given team with front-line leaders and team members accountable for individual goals (next).
- Goals—A number of different terms may be used to describe what I am calling "goals" for this chapter. Other common terms include: tactics, action items, and commitments. The goals are the detailed steps that must be completed to accomplish your strategic priorities. Goals provide the opportunity to assign specific tasks, timelines, measurements of success, and accountabilities to leaders or front-line team members. Goals are best written in the "SMART" format. A well-written goal is **s**pecific, **m**easurable, **a**chievable, assigns **r**esponsibility, and is **t**imely (Box 7.2).[5] A well-written goal should facilitate the development of clear measures of success and accountability.

How to Create a Strategic Plan?

One of the most daunting things about strategic planning is the process itself. Countless books and websites describe

• BOX 7.2 Description of the Components of a SMART Goal

- **Specific** goals identify a clear action or result.
- **Measurable** goals have a measurable end point or change parameter to indicate success.
- **Achievable** goals are self-explanatory; there is no value in setting goals that cannot be accomplished.
- **Responsible** persons or teams should be designated as part of the goal-setting process.
- **Timely** goals set specific timelines for a significant milestone or completion.

Example #1, Strategic priority of increased business efficiency: Reduce expired drug wastage/disposal by 10% (based on cost) year over year in fiscal quarter 1. Responsible person: hospital manager.

Example #2, Strategic priority of environmental stewardship: Increase recycling of hospital waste materials by implementation of three novel recycling streams by end of calendar year. Responsible "person": veterinary technician team led by specific individual.

Example #3, Strategic priority of safety: Reduce OSHA reportable hospital employee injuries by 20% year over year for 2018. Responsible "person": team as a whole led by Veterinary Director.

From Olsen E: *Strategic Planning Kit for Dummies, ed 2,* Hoboken NJ, 2012, Wiley Publishing, Inc.

detailed processes for strategic planning.[1,2,5] The process does not need to be that complex. In fact, depending on the size of the team, the base expectations described previously may be accomplished in as little as a half-day or 1-day work session. A dedicated leader and team may accomplish the strategic planning process and create a useful plan with some readily available resources and a relatively small investment in time and money. The following is a description of the basic steps to create a strategic plan.

Get Support From Leadership

The zoo veterinary leader should have commitment from the Zoo Director or hierarchical leadership that the strategic plan, strategic objectives, and strategic priorities will be supported by the organization. Without that commitment, a team runs the risk of spending time and energy on a plan that cannot be implemented due to a lack of support or resources. This could have a negative effect on the team that far exceeds the absence of a plan. In most cases the goals and priorities of the zoo veterinary team should and will align with the strategic objectives of the larger organization. Truly, the zoo as a whole should be working toward the same vision and mission.

Pick the Team

Strategic planning is a business management tool implemented at the highest levels of leadership within an organization. Although this "top down" approach may work, it is more often best to pull together a diverse group of

team members from all levels of your organization. As with all aspects of business and society, diversity adds strength to an organization or a process. For a veterinary team, it would be prudent to include key partners and stakeholders (zoo operations, animal husbandry, education, etc.) in the strategic planning process, as well as a diverse representation of the skillsets/expertise on the veterinary team itself.

Use of a Facilitator

The use of a trained human resource (HR) facilitator or outside consultant makes the strategic planning process easier but is not mandatory. One of the key roles of the facilitator (whether internal to the team or an external partner/consultant) is to create a safe environment for exchange of ideas and free and open discussion, regardless of position. A facilitator may also help to minimize introduced bias from the "boss" or senior leader in the room. Ideally, a facilitator should have experience with the strategic planning process and some level of familiarity with the team or organization. The facilitator is not the decision maker in the process but does ensure that all aspects of the process are addressed and captured for the decision makers.

Set Aside Time and a Location

If you are going to plan, then plan. Plan to make time and plan to find a location as free from distractions as possible. This is easier said than done in the hectic world of a zoo veterinary practice; however, your team members must know there is commitment to this process. That commitment will not be apparent in an environment of constant interruption or disruption. Depending on the size of your planning team and the state of your existing plan (i.e., starting from scratch vs. reviewing an existing vision and mission), it is reasonable to expected that the strategic planning process will take at least a half day of effort. When starting from scratch with a new team or organization, that time commitment could extend to multiple days. Prework in the form of questions or discussion topics (see strengths, weaknesses, opportunities, and threats [SWOT] analysis later) may expedite the real decision making and increase efficiency in the dedicated strategic planning sessions.

Create, Renew, or Review Your Vision and Mission Statement

Most organizations have some form of an existing vision and mission statement. In those cases the strategic planning team will be focused on reviewing the existing vision and mission or perhaps renewing/reenergizing the statements themselves. If you are starting from scratch in an organization that lacks a vision and mission, the first phase of the strategic planning process is to identify these key components. The key components of vision and mission statements were covered earlier and several examples are listed in Box 7.2. A detailed description of the visioning process and mission statement development is beyond the scope of this chapter; however, there are numerous resources available on the internet and in a variety of books on leadership, strategic planning, and business management.[1,5–8]

Assess Your Overall Team/Environment

Assessment of a team for the strategic planning process may be accomplished via external or internal assessment. External assessments may come in the form of external auditors, peer reviews, or accreditation inspections. Internal assessments rely on the team/team members looking inward and honestly identifying strengths and weaknesses as part of the planning process. It is important to develop strategic objectives that build on team strengths and take advantage of opportunities, while acknowledging and minimizing or overcoming weaknesses and threats. The simplest form of a team assessment would come in the form of a discussion with the members of the strategic planning team to identify basic strengths, opportunities, and external threats. Going beyond a basic discussion, a number of other team/business assessment tools exist.

One of the most common and most practical assessment tools is the SWOT analysis, a tool that may help an organization or team identify internal and external factors that contribute to the success or failure of strategic objectives.[4,5] "SWOT" is an acronym for strengths, weaknesses, opportunities, and threats. The "strengths and weaknesses" descriptors are often used to identify factors internal to the business or team, and the "opportunities and threats" descriptors are used to identify factors external to the business or team.

The individual components of the SWOT analysis are typically defined as:

- Strengths: characteristics of the business or team that give it an advantage over others (internal factors).
- Weaknesses: characteristics of the business or team that place the business or team at a disadvantage compared with others (internal factors).
- Opportunities: elements in the external environment or industry/profession that the business or team could take advantage of (external factors).
- Threats: elements in the external environment or industry/profession that could cause trouble or challenges for the business or team (external factors).

The SWOT analysis process is driven by a set of questions designed to highlight each of the four categories. Some examples of questions that could be used in a zoo veterinary environment are provided in Box 7.3. Answers to the questions may be captured in a 2×2 matrix such as that shown in Fig. 7.1. From this matrix, common themes are identified to help identify strategic objectives and priorities.

Identify Strategic Objectives and Priorities

SWOT analysis (or other similar assessment tools) may be used effectively to build an organizational strategy. Steps necessary to execute the strategy involve identification of

• BOX 7.3 Examples of Questions That Could Be Used to Perform a SWOT Analysis of a Team in a Zoo Veterinary Environment

Strengths

- What do our teams do really well?
- What sets our team apart from other zoological institutions or veterinary practices?
- What do our employees/team members particularly value about us?
- What do our operating partners (animal husbandry team, park operations team, etc.) particularly value about us?
- What do our professional colleagues particularly value about us?
- As you look back on successes over the past years, do you see any patterns or trends?

Weaknesses

- What is our team's Achilles heel?
- Are there any institutional policies or practices that create barriers for us?
- For what do our employees/team members criticize us the most?
- For what do our operating partners criticize us the most?
- What negative perceptions (true or untrue) do our employees/team members/partners believe about us?
- What elements of our business add little or no value?
- Is there a resource or process we lack that negatively impacts our business?

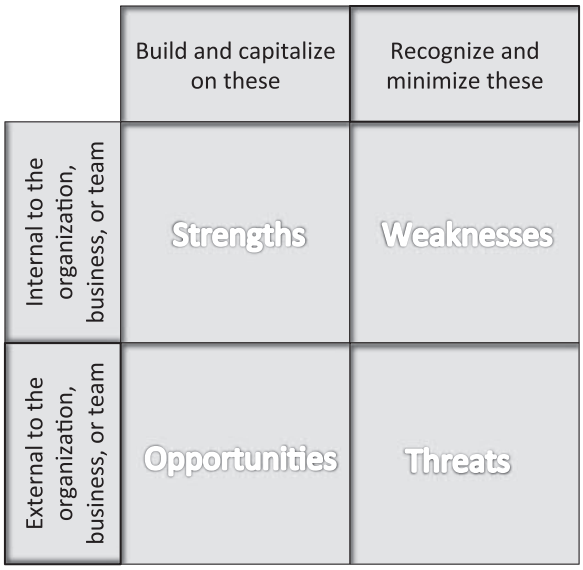
Opportunities

- Are there any new technologies, resources, or processes that we should be utilizing?
- Are there any new products, services, experiences, or opportunities we should be offering?
- Are there any initiatives, external to our existing team/organization that could benefit us?
- Is there anything that our operating partners request of us that we are not offering?
- As we look at other zoological institutions, what do they do better than us?

Threats

- If you were a zoo professional looking for a place to work, would you choose us? Why or why not?
- Are there any initiatives, external to our team/organization, that could harm us?
- For what do our zoo/veterinary colleagues criticize us the most?
- What negative perceptions (true or untrue) do our zoo professional colleagues believe about us?
- Have the needs of the zoo community changed recently in a way that we cannot react to? or influence?
- What is/are the biggest threat(s) to the veterinary profession? The zoological profession?

internal and external factors and selection of the most important factors. From this analysis, one may determine the strategic objectives—those objectives so significant to the overall well-being of the team/organization that they require defined efforts over time. The strategic plan should focus on these objectives in combination with the overall business environment to set specific strategic priorities.



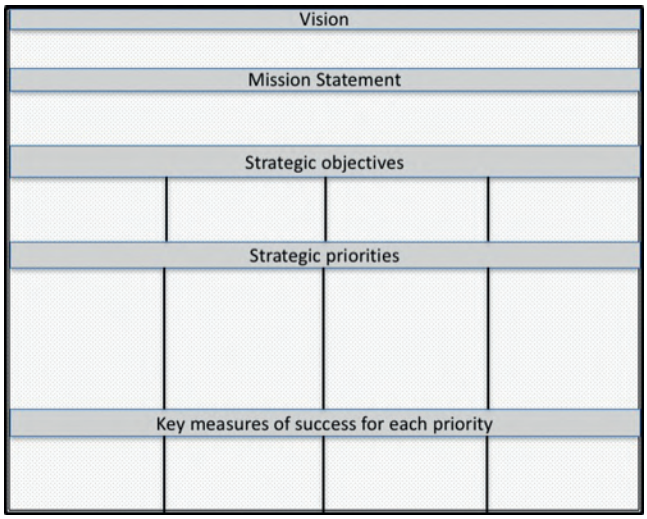
• Figure 7.1 A typical 2 × 2 matrix used to format a SWOT analysis upon which strategic objectives and priorities are set.

In general, strategic objectives and priorities should be designed to leverage strengths of a team/organization and take advantage of opportunities, while recognizing and minimizing weaknesses and threats.

For example, a SWOT analysis of a veterinary/animal care team at Zoo X identifies veterinary expertise and hospital facilities as strengths in the SWOT analysis. At the same time a threat is identified from external "advocacy" groups that seek to misrepresent or downplay the high level of care that animals receive in zoological institutions. This threat has the potential to impact attendance and favorability among zoo patrons. Zoo X identifies animal care and communication as strategic objectives over the long term and develops strategic priorities for the upcoming year to focus on continuous improvement of animal medical care as well as targeted communication of animal care–related stories through social media and local media outlets. Special goals may then be created for each of these priorities by a variety of team members. This is a generic example of the idea of building a strategic plan that capitalizes on the strengths and opportunities of a team/organization while minimizing weaknesses and addressing threats.

Communicate Your Strategic Plan

A strategic plan is useless in the hands of only a few senior leaders. To be truly effective, the strategic plan should be communicated to all levels of an organization and to all team members. As stated earlier, a well-designed and properly communicated strategic plan provides clear direction for a team and may increase employee engagement and effectiveness. The methods of communicating a strategic plan are as varied as there are teams applying the process. It is common for many organizations to use a one-page format to highlight the basic components of their strategic plan. One example of such a format is provided in Fig. 7.2.



• **Figure 7.2** Template for concise capture of strategic planning key components.

Numerous alternative formats exist and may be custom tailored to the needs of your organization or team.

Empower and Expect Leaders and Team Members to Set Goals and Establish Accountability

After your strategic plan is communicated to the team, it is time to start setting goals to accomplish the strategic priorities identified in the strategic plan. Goal setting is a collaborative process between leaders and team members. Identification of specific strategic priorities should make this process easier for both leaders and team members. If goals are set appropriately (e.g., using the SMART model), then there should be a clear chain of accountability for specific goals and priorities. The goals and associated accountability may then be used for periodic or annual performance reviews, performance management, or recognition/reward.

Review in Regular Intervals and Amend as Necessary

To ensure the strategic plan performs as designed, you must hold regularly scheduled formal reviews of the plan and refine as necessary. Ideally, a schedule should be established to review the progress of strategic priorities at least on a quarterly basis, if not more frequently. Establish clear accountability for progress or completion, but be willing to amend goals and priorities in a dynamic environment. It is ridiculous to think that components of the strategic plan will not change in an ever-changing world. Lacking the willingness and agility to change course (when driven by truly significant need) will set the team and plan up for failure. Strategic objectives often remain relatively constant over time, but priorities and goals can and will change. If changes are made to the strategic plan, be sure to communicate those changes across the organization.

Conclusion

A strategic plan may be one of the most important business management tools available to a progressive leader, organization, or team. That same plan may also serve multiple purposes to align work efforts, assist with priority goal setting, motivate individuals, and establish accountability. The components of a strategic plan may be defined with moderate effort through a process that a team of any size may accomplish. In the end, the ultimate goal of any strategic plan for any team is to create an environment where every team member understands the goals, priorities, long-term objectives, mission, and vision for their organization. In the context of a zoo veterinary team, the strategic plan should align the veterinary team with the larger zoological organization, ensure the veterinary team is providing long-term value for the organization, and provide a platform for the veterinary leadership to request support and resource growth at the organizational level. A solid strategic plan sets the framework for team and organizational success into the future.

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8

Organizational Influence: Navigating the Leadership Road for Zoo Veterinarians

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Introduction

Influence is the elusive ability to make an impact on the thinking and actions of others without exerting control. Organizational influence is the power to shape policy and affect organizational planning. It can be the catalyst for making the right things happen by cultivating trusting relationships with others.

Zoo veterinarians who have influence are valuable assets to their organizations. Consider these possible stories about the influence of zoo veterinarians. Dr. A spent years preparing to be a zoo veterinarian. After much personal sacrifice, she became specialty-board-certified in zoological medicine. She is now working as a staff veterinarian in a large zoo. She is disappointed, though, that even with all this training and expertise, she does not have the impact that she had expected. She seems to be treating the same medical problems and has been unable to influence changes to prevent the problems. A colleague recognizes this and initiates a discussion about how to increase her sphere of influence. She did not realize how much her job success and satisfaction would depend on her ability to influence people.

Dr. B is the head of the animal health department at a medium-sized zoo. Her department has been left out of decision making on several animal care issues. She has voiced her disapproval of several curatorial decisions. She believes that the decisions were not in the best interest of individual animals. She recently spoke to the Director to gain some control over these decisions. Despite these efforts, she finds herself isolated and at odds with curators and administrators. She confers with a leadership coach who helps her discover that trying to take control is not working. She looks for a different approach.

Dr. C has a zoo veterinary department under his authority. He prides himself in his ability to be decisive and in his capacity to make things happen. His ambition is to move up the leadership ranks so that he can have a greater influence on animal welfare and wildlife conservation. He has applied for executive positions but without success. Some of the zoo leadership like his can-do style but find he has problems in his department. Employee surveys say that Dr. C takes credit for team successes and tends to blame the team for losses. His staff accuses him of having selfish motives. Dr. C privately thinks that the behavior of his team holds him back from a position of greater influence. During a candid conversation, his boss confronts him with the dysfunctions in his department. Because of these problems, he has lost his credibility as a leader. The conversation helps him understand that his ambitions have taken the focus off the people he depends on the most. He wonders how he can improve his relationship with them.

These three competent veterinarians had trouble exerting the influence they desired. In essence, they were not able to achieve the greater purpose for which they were hired.

Zoo veterinarians add value to their organizations. They function as animal welfare advocates. They also have a regulatory responsibility as attending veterinarians. As such, they provide credibility and legitimacy to their organizations. They have training and daily experience in complex decision making. They are trained to ask questions and obtain a history, and thereby, to look at the whole picture. That, combined with years of science education, gives them valuable skills and abilities that may benefit animal health and welfare. So it is disappointing for zoo veterinarians when they lack influence over organizational decisions and strategic direction.

Organizational influence is more valuable than position, authority, or control. Having positional authority does not guarantee influence. And, remarkably, having influence does not require a position of authority. The opportunity to influence is available to anyone. Influence reflects a person's value to others and a measure of his or her effectiveness. It brings significance to a career. When a person's ability to influence others is high, it benefits the reputation of

the person, the team, the organization, and the whole profession.

Traps and obstacles to becoming influential abound. If veterinarians learn to overcome these barriers, though, they can make the most of their value and serve their organization to a greater degree. If they have influence, they are less inclined to suffer burnout and more likely to have a satisfying career. Studies looking at physician burnout show that having influence and meaning at work are drivers for engagement and prevent burnout.[1] This chapter is a compilation of principles and practices designed to help overcome the obstacles. The ideas come from a study of the leadership literature and my journey, mistakes and all, in leading zoo animal health teams. Further insight has come from interviews with successful zoo leaders. This chapter is not a formula for success. My results have been far from the ideal. Rather, I offer this as one path to follow toward becoming a more influential leader.

People: How Relationships Impact Organizational Influence

The quality of work relationships has a direct impact on organizational influence. Amid the busyness and scope of a zoo veterinarian's job, managing the care of each animal takes priority. However, to gain influence and make a long-term impact, nurturing the many work relationships is crucial. Being a zoo leader means being good at relationship building. The following sections discuss six building blocks that zoo veterinarians may use to form healthy relationships.

Begin With Humility

Successful relationships start with genuine humility as their foundation. Humility connects people through a common human bond.[2] A humble person has a modest and accurate assessment of his or her importance and abilities. Humility requires a high degree of self-awareness and empathy. Being humble is not a sign of weakness; indeed, it requires a good deal of strength and assertiveness. Humble leaders assert themselves on behalf of their team's accomplishments rather than for themselves.[2] Humble leaders have self-confidence but do not project a feeling of self-importance. Humility is power under control.

We trust and enjoy following leaders who are humble and not self-serving. Humble leaders are effective and influential. Jim Collins labels the most successful leaders as Level 5 leaders. He describes these leaders as having both humility and fierce resolve.[3] Zoo veterinarians, in general, have the resolve. Lacking humility, this resolve can be intimidating and objectionable. Veterinarians can be intimidating just because of their position and education. Those who act in a humble manner can counter this tendency and be the welcome exception. Working effectively at the executive level is an example of where humility may be an asset. A zoo veterinarian may think his or her role is to speak for the animals and to represent their departments at the executive level. The executives welcome those who can step away from their singular focus as veterinarians and see the big picture. It takes humility to change focus, listen and learn, and serve the whole organization. Those who do will be in a strong position of influence, which, coincidentally, will benefit the animal focus as well.

Humility is a leadership attribute that one may develop. Several character-based behaviors describe humble leaders. For example, admitting mistakes, managing emotions, being honest, being an effective listener, delegating decision making, and giving recognition are among the behaviors that depict humble leaders.[2] With motivation and practice, anyone can be an authentic and humble leader. A humble leader puts others at ease, which in turn, produces lasting relationships with those they need to influence.

Seek to Serve Others First

Having humility leads to other attributes that help us grow our influence. One such attribute is a willingness to serve others for the greater good.[4] Most people object to the idea that their job is to serve others. Still, having an attitude of service is key to developing organizational influence. Approaching the situation with an attitude of service shows that the intention is to put others' needs ahead of one's own. In other words, a leader's job is to serve, not to be served. Good leaders set direction, then turn the organizational chart upside down. These leaders, in essence, work for their people. They use their position to remove obstacles for getting work done. They put the focus on others and not on themselves.[5]

When putting servant leadership into practice, it is helpful to ask clarifying questions to help direct one's behavior: Whom do I serve? Is my focus on myself or others? How may I help the situation? When things go well, who gets the credit? Am I willing to be vulnerable? When and how do I say "no"? Do those I lead grow as people? Do they, while being served, become more autonomous, more likely themselves to become servants?[4] The answers to these questions help us distinguish an outward (selfless) mindset from an inward (selfish) mindset.[6] The practice of servant leadership has the power to transform both the leader and those who are served so that respectful relationships may flourish.

Put Relationships Above Results

Conventional wisdom says we should direct our best efforts at achieving goals and results. Good animal health and welfare outcomes are, indeed, the results we are after. Veterinarians must also pay attention to business and financial goals. To achieve these desired outcomes, it requires teams of people working together effectively. Teams function best when they make trusting relationships their foundation and priority.[7]

Great leaders can be the model for developing trusting relationships within their teams. To illustrate, imagine an animal health crisis, such as a sudden medical emergency in an elephant. The organization's leadership is often present at the scene. However, the purpose should not be to direct or judge the actions of the animal care teams. Instead, leadership's role is to be present and care for the needs of the people. The attention by the leadership ensures that the animal care team can achieve the best result possible. In one institution, this philosophy has played out in many crisis situations and has become an unspoken rule. "In a crisis, pay attention to the people first." The principle is just as important in daily work as it is during a crisis. Pay attention to relationships first and collective goals and results will follow. Putting people first bolsters team trust, influence, and ultimately the health of the whole organization.

Build Trust

Diversity of abilities and unity of purpose are the twin engines that power success in teams. If diversity is the source of talent on a team, then trust is the glue that holds the team together and creates unity. By serving others with humility, we depend on trust to protect and sustain the relationships. We need the trust of our coworkers to have successful outcomes for the animals under our care. Each of us can think of a person with whom we have a high-trust relationship. Those relationships feel special. Communication is quick and easy. Things get done with little effort. It is enjoyable. The opposite is true with a low-trust relationship. High-trust relationships produce exceptional financial and mission-related results.[8]

Trust engenders confidence, and the lack of trust leads to suspicion. There is a continuum between confidence and suspicion. But a "line of trust" exists between these two states and yields two distinct outcomes. Above the line, communication flows, and assumptions about each other's behavior are good. When conflict arises, we have faith that we can resolve it. Below the line nothing is easy, and doubt prevails. Everything becomes an issue. We lose confidence that we can work out a conflict. We slip above and below the line based on our choices and actions. However, we can build up "relationship equity" over time. In doing so, our position relative to the "line of trust" becomes more stable. Little transgressions then have a lesser effect on the position of our line of trust.

One can understand how to build trust by viewing it as a function of character and competence. In the veterinary context, character is similar to professionalism. Competence is related to technical ability.[9] Character includes our integrity, motives, and intent with people. Our character is who we are. Competence includes our capabilities, skills, results, and track record. Our competence is what we do. We can use this concept in a practical way to build trust. Building trust requires that we make and keep commitments.[8] For example, a veterinarian may commit to helping design a new exhibit. By honoring the team commitment and contributing to the process, that veterinarian demonstrates integrity (character) and capability (competence). Making a commitment creates hope. Trusting relationships arise out of this practice.

Trusting relationships are essential in accomplishing the critical work of zoos and aquariums. We depend on many key stakeholder relationships for animal care. Be smart about trusting others but be willing to do so. "Be" (character) and "do" (competence) in ways to increase trust with others. No relationship is perfect, and everyone betrays trust occasionally. But strive for high-trust relationships. They are the foundation for developing organizational influence.

Honor Your Staff

For obvious reasons, we aim to influence the high-level decision makers in our organizations. However, in doing so, we may forget about the importance of our staff. More than most, the actions of our teams are critical to our success in establishing organizational influence. An excellent support staff understands that their leaders care about their well-being. These leaders honor their team as individuals and as a group. Honor is a status we give to others. It builds them up, raises their dignity, and prepares them for a higher level of performance. Honoring the staff means treating them as if it were your job to serve them rather than vice versa. It is unconditional and separate from accountability and performance (which you still must address).

Honoring those with less authority fosters loyalty and wards off unhealthy conflict. Employee satisfaction surveys usually include opportunities to provide comments. The most troubling comments come from those who report being treated harshly by those in authority. People want to be treated with dignity and respect and not valued just for a function they serve. Veterinarians, because they are viewed as authorities, are in a unique position to turn that around and bolster the status of others. This recognition will give them a reason to be loyal and engage in the greater vision. One way to do this is to give particular credit to staff members for team achievements instead of implying credit for oneself. As an example, one leader that I know has a reputation for honoring his close staff. In a variety of ways, he makes sure they know that their personal well-being is important to him. He advises his teams to do the same by saying "It's how we do things here. It's the people that matter." Honoring your staff is one of the most valuable and underutilized tools for developing organizational influence.

Connect With Purpose

When teams feel appreciated, they become more motivated and may become tremendously influential. Motivating people is a leader's number one job. Strict accountability is one approach used to motivate. Rewards and punishment do have a place in managing people. But good leaders do not rely on them as the primary tools for motivation. These tools create a transactional environment that may

feel like manipulation. Employees lose their desire to be self-motivated, requiring even closer supervision.

Instead, seek to inspire with an emphasis on "why." In other words, work to discover the team's purpose and be good at telling the "why" story. Pay attention to their hearts, that is, the intrinsic motivation. Understand their deepest drivers and relate that to a common purpose, the "why."[10] When people know and believe in the purpose, they motivate themselves and one another. Accountability becomes less of an issue. Our profession and what we do lends itself well to this approach. What we do has a compelling purpose and is motivating in itself. The role of the leader is to remind people of the overarching purpose and how it relates to the job at hand.

Besides the constant reminders of the high-level vision, one must also be ready to share the "why" of the moment. Veterinarians going into procedures are good at telling the "what" and the "how." They are good at telling staff what they are going to do and what they want them to do. They are also used to telling people how they are going to do something and how they want them to assist. But how often do we take the time to explain the "why"? The "why" gives context and meaning to a procedure or activity. Sharing the "why" assures those working with us that we have thought through the procedure. It makes it clear that what we are doing is important, necessary, and part of a greater purpose. The "why" connects the vision to the role of animal health and to the task at hand. It reminds people that together we are making a difference. Sharing the "why" of the moment is another way of honoring and inspiring our staff. An inspired staff is a productive staff, and that enhances influence at all levels.

Practical Steps: Taking Your Influence to the Next Level

Once we appreciate the significance of strong relationships, we are in a good position to adopt specific practices to grow our influence and that of our teams. Good practices should build upon relationships by clarifying roles and expectations for conduct. They should encourage leadership growth in others who can, in turn, become influencers. The following sections provide a few such practices to consider for increasing organizational influence.

Give Up Control to Gain Influence

Most of us are eager to influence decisions that could affect us, but trouble may arise when those involved in decision making have conflicting perspectives. We all want the animals to thrive under our care. Why, then, would conflict occur when making decisions about their care? The answer lies in our human nature. Human beings have a desire for authority and control. This desire works against our ability to influence others, especially when controversial or high-stakes decisions come up.

The good news is that we can overcome our desire for control by adhering to an important principle. Simply stated, it is this: to gain influence, give up control. That is, to obtain long-lasting influence, abandon the desire for authority and control. Yes, this principle is counterintuitive, so most of us would rather disregard it. We often hear that striving for control is the noble thing to do and the way to ensure the right decision is made. But, we work with people. So, if we wrestle with people for control, we will lose valuable influence, trust, and credibility in the long run. Instead, we may choose to respond with humility and respect, treating others as people with needs and desires similar to our own. We can act to serve their needs as well as our own. If we follow this path, our influence in the organization and with other people will soar. Application of this principle may lead to strong, trusting partnerships with synergistic outcomes. In the end, giving up control leads to more influence and better decisions.

Further practical steps flow from this principle. Once a partnership develops, the parties clarify their roles. One party takes on the "Decider" role and the other the "Adviser" role for a given decision or type of decision. The Decider is the responsible party and has the ultimate decision-making authority. Good Deciders seek input from those in the Adviser role; they take full responsibility for outcomes and blame no one if things go wrong. In contrast, the role of the Adviser is to influence the Decider. They may change the minds of others by providing evidence, interpretation, and recommendations. Their manner must be professional and respectful, honoring the Decider's position. Veterinarians often find themselves in this Adviser role. They have specialized knowledge and often uncover the problem first and in the most depth. So, they may be the primary drivers in the decision-making process by initiating dialog and providing their perspective. It is important to remember that neither role is more valuable than the other, and both are necessary for making good decisions. Indeed, successful partnerships are not based on equal and identical functions. Rather they thrive when roles are defined and distinct.[11]

Giving up control to gain influence is a paradox. Even so, it is a powerful servant leadership principle. It is useful for those without positional authority who want to have influence. Giving up the desire for control is a principle worth understanding and applying in our work and home environments. It achieves better results and becomes the right thing to do in serving others.

Create Principle-Based Standards

Having accountability for ourselves and in the teams that we lead shows responsibility. Accountability gives us credibility with others and builds our global influence. But holding ourselves and others responsible is tricky business. Caring more about rules than people invariably leads to opposition to those standards. But how can we keep people accountable and still serve our staff's needs? How can we uphold high standards without damaging relationships? One approach

is to create principle-based standards. Accountability, then, becomes part of the culture. In addition, having the team develop the standards themselves helps make them relevant and accepted. Without such standards, each person tends to do what is right in their own eyes, leading to confusion and conflict.

Working together in our animal health departments, we designed seven principle-based behavior standards to ensure accountability. We wrote them to be consistent with our broader organization's code of conduct.[12] We put the phrases into our words so they would make sense in our work environment. For each standard, we wrote a catchphrase to make the concept more relevant. We also wrote clarifying questions to help identify specific, desirable behaviors.

Standards should reflect the unique nature and values of each workplace to be most effective. Here is an example of the format and wording for one of our standards. Code of Conduct: "Use Your Words Wisely." Our rewording: "Think before you speak." Catchphrase: "My words and nonverbal messages affect those around me and our work environment." Clarifying questions: Am I respectful? Am I phrasing my message in a positive manner and offering solutions? Am I addressing the issue directly with the person involved? Do I express gratitude whenever possible?

The remaining six standards along with their catchphrases were: Take responsibility (By holding myself accountable for my actions, there is no one else to blame), work effectively (When I use my time wisely, the entire workplace benefits), build excellence (Bring out the best in yourself and others), harmonize (I can achieve harmony when I balance my work and personal life), create joy in the workplace to relieve stress (I can help create a pleasant and respectful workplace), and make lasting memories (Create a legacy of learning, innovation, service, and leadership). Again, these are examples based on a particular work environment and would necessarily be different elsewhere.

With agreed-upon, principle-based standards, a common language emerges for acceptable behavior. A culture of shared accountability results. To sustain these practices, then, the leader's job is to provide a venue for repetition and reminders. Some workgroups choose to review a standard at the beginning of each day. A practice such as this gives everyone an opportunity to apply and reinforce the behaviors and develop leadership skills as well.

Develop Leaders

If we want sustained organizational influence, we need to invest in others and help those around us become leaders themselves. Developing leaders is not just for succession planning. It is essential for smooth daily operations and successful outcomes. In such an environment, each worker becomes a leader. Each employee, regardless of rank, takes responsibility and has the authority for his or her area of expertise and duty. Often, though, as the authority figure, we have the need to appear in charge, give directions, and be the source of knowledge. This authoritative approach diminishes the opportunity for others to develop their leadership skills. Even more, it leads to confusion around daily tasks and assignments. Workers abdicate responsibility and avoid making decisions. Errors become more prevalent. In these environments, it is characteristic for employees to use language that shows their dependence and insecurity. Before taking action, they may use weak phrases, such as: "I would like to…" "What should I do about…" " I have no authority to…" "It is not my job." "Tell me what you want me to do."

A simple practice of using empowering language may reduce this dependent behavior. The object is to push decision making to the level where the information is. This method empowers people and develops them as leaders. It transforms insecurities into confidence. It builds trust and reduces errors. Empowering phrases, encouraged and modeled by those in authority, show a leadership intent, and that trust is implicit. Here are some example phrases that signal confidence when taking action: "I intend to…" "I plan on…" "I will…"[13]

Using this approach to empower may play out in a variety of situations. The following is an outline of the process and gives examples showing how this may work. (1) Any staff member, regardless of his or her position in the department, sees a need for action and knows what to do. (2) That staff member tells the responsible party (e.g., his or her boss, veterinarian, peers) that he or she intends to take action. (3) The responsible party acknowledges the intention. He or she may ask clarifying questions and agree to the action or make a change as needed. The roles are crystal clear. Authority and responsibility are in their proper context. Feelings of being micromanaged vanish. Delegation is effortless. This process pushes the authority to act down to the source of information. It eliminates the confusion of who is going to decide to act; it reduces time wasted waiting for someone to order an action; it avoids mistakes; individuals assert themselves; they make full advantage of their knowledge and experience but get the opportunity for others to check their reasoning; it enables employees to grow in their jobs; and they become leaders themselves.

Example 1

Situation: A manager noticed that an area in the hospital needed urgent repair. Stated intention: The manager got a bid for repair. She reported to her supervisor that she intended to institute the repair during the next budget period. She was confident that she would stay within year-end budget, but expenses would be over for the current period. Intention authorized: The supervisor paraphrased the issue to make sure she understood it. She asked questions about the urgency of the repair and then agreed to the action. Result: The action happened without delay by the person with the most information to take decisive action. Other stakeholders were informed and had an opportunity to weigh in on the decision. The manager took personal responsibility. She checked her reasoning with others, which

increased buy-in and decreased the likelihood of mistakes. Everyone's credibility and influence grew. She developed further as a leader.

Example 2

Situation: A technician had the job of tracking long-term medications. He was aware of the dangers of using a flea product on cats, which was designed for dogs. In reviewing the prescriptions, he noticed that a dog housed with a cheetah was being given the dog product. He suspected that the cheetah was being exposed to the wrong product. Stated intention: The technician reported the problem to the veterinarians with confidence and without fear of criticism. He said that he intended to make the change in the long-term medications to the correct product. Intention authorized: One of the veterinarians responded and approved. Result: The technician's response ensured that the animals were treated appropriately. The necessary action happened quickly without drama. The issue did not fall between the cracks waiting for a decision or for someone to take responsibility. Errors and threats to animal health were averted. The veterinarians were humble enough to encourage the technician to develop as a leader. The influence of the whole department grew.

These examples show how simple changes in language change the way people work. The use of empowering phrases signals the intention to take responsibility. Making this work takes courageous leaders who encourage and support decisions made by those closest to the information.

Looking Forward

Being successful along the leadership road requires the skill of influencing others. Organizational influence means developing good quality relationships and applying best practices. As a reminder, do not expect perfection in yourself or others. Achieving strong organizational influence is a long, slow process—and well worth the effort.

Influence is big. It gives meaning and impact to our careers. Influence is a powerful tool, which we can use in working with others to accomplish great things. How we lead and influence others becomes our reputation and a measure of our career success. What do you want your career story to be? Make it intentional. Start writing it now. In the end, that story will likely be about the people you served and influenced along the way. Then look forward and make your story happen.

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9

Contingency Planning for All Hazards and Foreign Animal Disease

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Introduction

Anyone who is involved in the management of non-native or indigenous wildlife understands that there are inherent risks when working with these animals. These include risks from the animals themselves, and external risks such as natural disasters. Incidents such as "big cat" escapes, high-impact flooding events, and infectious disease exposure may severely impact a facility, its staff, its visitors, and its animals.

Following an incident, there is typically increased scrutiny of the facility, and questions arise about the staff's ability to manage collections in the event of a future disaster. In a 24-hour news cycle, the media is able to seize upon a story and, aided by social media, repeat it again and again until it soon "goes viral." Consider the 2016 shooting of a great ape in response to a breach of an exhibit. This incident was a topic of conversation for months, and a cell phone recording of the event has since been viewed millions of times. The incident resulted in protests, petitions, memes, and news stories. Despite a sound explanation that this action was taken to save the life of a child, it still proved to be extremely challenging to manage the perception of the public, versus the expertise of the animal's keepers. This event highlights the increasing public scrutiny on facilities that manage these animals, and their ability to plan for emergencies.

There are a wide variety of facilities that own, breed, and/or exhibit non-native or indigenous wildlife species as their mission, their hobby, or their business. This includes accredited and nonaccredited zoological collections and aquariums, drive-through animal parks, exotic game ranches, animal sanctuaries, and private individual owners and/or breeders of these species. There may be multiple authorities and statutes that govern their activities, depending on the type of species cared for, the state in which the facility is located, and the intended use of the animals. Each individual facility must know which statutes, laws, or agencies have jurisdictional authority with regard to the species in their care.

See Table 9.1 for a quick reference list for acronyms used in this chapter.

What Is Contingency Planning? And Why Is It Needed?

Contingency planning is the development of plans and procedures that may be implemented when normal operating procedures are disrupted due to incidents or events. Adverse media attention is certainly not the only reason that contingency planning should be a priority in animal management. For example, it is very important to have a contingency plan for dangerous animal escapes or unintended contact situations between the animals and the public or staff. It is paramount to avoid injury or loss of life of any persons or animals in such situations. For facilities that house endangered species, lack of planning for an incident could mean a significant loss to the species gene pool. The health and welfare of these animals during an incident should motivate facilities to begin the planning process and bring it to a purposeful status. As a bonus, planning may also increase the efficiency of response and the safety for responders.

Contingency Planning Principles

If a facility has yet to begin development of contingency plans, whether for a natural disaster, animal escape, or foreign animal disease, there are planning principles recommended by the Federal Emergency Management Agency (FEMA) Course IS230c: Fundamentals of Emergency Management[1] that provide a strong foundation. Adapted for exotic animal and other wildlife facilities, these principles are listed below.

- Planning must be community based, representing the whole population and its needs. A facility should recognize that they are part of the larger, interconnected community.
- Planning must include the participation of various stakeholders and subject matter experts in the community.

TABLE 9.1 Acronym List

Acronym	Definition
CAP	Control Access Point: Designated sites where various levels of biosecurity and protocols change to protect collection and or staff
CPG	Comprehensive Preparedness Guide: Documents developed by Federal Emergency Management Agency to assist with contingency planning
EM	Emergency management: A professional discipline that prepares, prevents, mitigates, responds to, and assists in recovery from disasters
FAD	Foreign animal diseases: Diseases not normally found in the United States. Detection triggers intense response action and often results in trade restrictions on agricultural commodities
FADPReP	Guidance developed by US regulatory officials that outlines national strategies for the control of foreign animal diseases
FMD	Foot-and-mouth disease
ICS	Incident Command System: A structure used by animal health regulatory officials to organize a disease response
LOS	Line of Separation: A structural or functional division between areas for defining varying levels of biosecurity
NAHEMS	National Animal Health Emergency Management System: Series of guidance documents for the management of infectious diseases
SAHO	State Animal Health Officials: Also known as State Veterinarians in the United States
THIRA	Threat and Hazard Identification and Risk Assessment: Standardized process used to assess the likelihood of incidents
USDA	US Department of Agriculture: One of its many missions is the prevention of and the response to foreign animal diseases

Facilities may find value in joining or participating in local response planning groups led by Local Emergency Management.

- There are proven processes for the development of contingency plans.
  - Planning starts by considering all threats and hazards.
  - Plans should be flexible and scalable. This means that planning considers, for example, one antelope escape but can expand for the escape of a dozen.
  - Plans should clearly identify goals and objectives.
  - Planning does not need to start from scratch.
  - Planning identifies tactics and tasks needed to fulfill objectives.
  - Effective plans outline what to do and why to do it. Roles and responsibilities are outlined.

A fundamental truth in planning is that the planning *process* is more important than the written plan itself. The relationships and partnerships forged with the local response community during the planning process may determine the success or failure of plans during a catastrophic event.

Contingency Planning Step 1: Form a Collaborative Team

Regardless of whether your facility has been included in local planning efforts, it is the responsibility of facility owners or operators to include community emergency management (EM) officials on your plan development team. Without this integration, you may NOT be adequately prepared to respond to many incidents. Local EM efforts are maximized when businesses within the community participate in the planning and capability building process. EM professionals may provide knowledge of future plans to mitigate the impact of a particular hazard; for example, a new levee planned in the next 3 years to minimize flooding. They have knowledge of local resources and may serve as a conduit to them if needed in a response.

Optimal planning team composition will vary depending on many factors, such as size, location, species, and site-specific risks. Ask other facilities with robust plans about their planning team composition, and the value that diverse backgrounds added to their planning process. A veterinary professional should be on the team. This involvement is imperative for a number of reasons; the facility veterinarian may educate EM professionals about the collection and specific risks. They will be critical in explaining the capabilities of the facility's animal health paraprofessionals, and will serve as the conduit to State Animal Health Officials (SAHOs) and other regulatory personnel when a foreign animal disease (FAD) or zoonotic disease is suspected. Facility managers will be key participants, providing an understanding of the facility footprint and infrastructure; additional planning team members may include keepers and curators.

As a part of the planning process, first responders must be included; local police, fire departments, and emergency medical services should be involved in plan development or review. SAHOs must also be included or consulted, especially when planning for an FAD event. Their expertise

will be needed to explain the roles and responsibilities of the facility, the State Animal Health agency and the US Department of Agriculture (USDA) in FAD outbreaks. It is wise to discuss the resources (trained responders and equipment) available at the state level and facility. If your state government has an Animal Health Emergency Management coordinator, you may find that to be a good contact as well.

As the team begins its work, it is highly recommended to discuss the use of the Incident Command System (ICS). ICS is an integrated, organizational tool that is flexible and scalable and has been in use by EM, fire fighters, and State-USDA disease Incident Management Teams as a framework to organize responses to incidents. It is recommended that development of your plans consider the use of the ICS framework in order for the facility to fully integrate into the coordinated response. Basic ICS training is free and available on the FEMA Emergency Management Institute's website.[2] Training may also be available directly through your local responders.

The facility planning team should consider appointing a leader who may assign tasks, develop deadlines, and engage the assistance of other subject matter experts whose input may be needed as plans are developed.

Contingency Planning Step 2: Understand the Situation

The philosophy of "all hazards" planning encourages a thorough risk assessment; only when all risks are considered and prioritized can the development of plans and procedures for incident prevention, mitigation, response, and recovery begin. Facilities are encouraged to understand the basic principles of Threat and Hazard Identification and Risk Assessment (THIRA), which relies on "whole community" input to determine risk, and therefore, to plan development. More specifics about the THIRA process may be found in the Department of Homeland Security guidance document Threat and Hazard Identification and Risk Assessment Guide, Comprehensive Preparedness Guide[3] (CPG) 201. The team's risk assessment should use any results of the community THIRA, in addition to *risks unique to your facility.* This is an opportunity to bring specific and unique needs to the attention of EM, first responders, and the rest of the planning team. In *Fowler's Zoo and Wild Animal Medicine: Current Therapy, Volume 7*, Dr. Mark Lloyd provided some excellent examples of risks that should be considered in the risk assessment process.[4] Additional information about non-native or indigenous wildlife risk assessment is available on the Zoo and Aquarium All Hazards Preparedness, Response and Recovery (ZAHP Fusion Center) website.[5] There are many ways to conduct a risk assessment; use the expertise of your planning team members to come up with the best method for your facility.

When dealing with native and non-native wildlife, the facility must include the assessment of risk from FADs. Foot-and-mouth disease (FMD) is a threat that Federal and State animal health professionals have invested significant resources in prevention, mitigation, response, and recovery planning efforts. FMD has been used as a disease model because of its highly contagious nature to multiple species and the agricultural trade implications of detection. To begin the risk assessment thought process for FMD, consider the following equation:

Hazard + Likelihood + Vulnerability + Consequence = Risk

Discuss the following with the team during the risk assessment process: (1) Do you know what FMD is? (Hazard). (2) Is the disease common or likely to emerge? (Likelihood). (3) Does the facility have animals that can contract FMD or serve as amplifiers or reservoirs of disease? (Vulnerability). (4) How would the detection of FMD in the facility or community impact business operations at the facility, state agriculture, or national agricultural levels? (Consequence). Upon completion of a thorough risk assessment, it is recommended to begin planning for "high likelihood and/or high consequence" events. FMD may be a low likelihood event, but the risk assessment process should include this disease if the facility manages susceptible species, as the consequence likely would be devastating.

Contingency Planning Step 3: Determine Goals and Objectives

It is critical to discuss and determine the goals and objectives of contingency plans *before* the writing begins; this provides focus for the plan. One goal of a facility in the face of a nearby FMD outbreak would surely be to prevent FMD from infecting their collection. If that goal failed, then contingency planning should address the way forward to facility continuity and recovery as soon as possible. Goals of a facility plan may be prioritized based upon its mission and may include:

- **Preservation:** Food animal production systems have the ability to replace animals as a part of the production model if a depopulation strategy is used and receive back the costs of depopulated animals as determined by agricultural industry indemnity calculators. Non-native and native wildlife may not be easily replaceable, especially for threatened and endangered species. Even if they could be replaced, the cost of depopulated animals may be far more difficult to determine, as there is no indemnity calculator for many species. Before depopulation or euthanasia would be used, there are questions that should be considered: Are certain species regarded differently, based upon their endangered species status, Convention on International Trade in Endangered Species (CITES) classification, etc.? Are certain animals incredibly valuable to species sustainability? What is the economic value of the animal, if that can be determined? For preservation to be a goal, the facility must have a sound plan and demonstrate the capability of implementing the strictest

biosecurity possible to avoid infection to its own animals as well as to any other susceptible animals nearby. It is important to remember that this responsibility will be borne by the facility, to the level acceptable by SAHO and USDA.

- **Animal Movement:** Non-native or indigenous wildlife move daily in the United States, but the numbers are miniscule compared to the staggering number of agricultural livestock moved intra- and interstate. Livestock move according to the ages and use of the animals, so it is not surprising that the urgency to move non-native or indigenous wildlife in the short term is also dependent on the facility's operating model. Zoological parks and sanctuaries may seldom move animals in their collection, and as such, may not initially feel impacts from restrictions on movements of animals and animal products during FAD outbreaks the way a game ranch relying on movement of hides, trophy mounts, and other hunting products might. However, if the restrictions last long enough, movements required for species conservation and breeding, to fulfill agreements between facilities, or to meet dietary requirements of certain animals in the collection could be affected and potentially become a financial disaster for facilities with that business model.
- **Visitation:** In facilities that rely on some form of visitation, the restriction of paying visitors to view or hunt animals in an effort to prevent disease spread could financially devastate the facility (as happened in the United Kingdom during an FMD outbreak). If a contagious FAD outbreak occurs near a facility, visitation could be temporarily restricted. If a goal is to resume visitation as soon as possible, plans must be written with that goal in mind.

As plans for FMD are being developed, facilities should include quantifiable goals whenever possible; with preservation in mind, a quantifiable goal may be: "The goal for Acme Zoological Park is to prevent FMD infection in their Nigerian Giraffe." This goal will require development of biosecurity objectives to protect the endangered giraffe. These objectives will drive discussion of strategies and tactics that will become part of the written plan. To ensure the greatest possibility of success, every goal included in the plan should have buy-in from the owners, operators, appropriate governing officials, and staff who will be implementing the plan.

Contingency Planning Step 4: Plan Development

This phase of planning is critical in identifying what elements should be included in your all-hazards plan. Consider the following three plan components: (1) the basic plan, (2) any supporting annexes, and (3) hazard-specific annexes (such as an FMD annex).[1] A basic plan should include introductory material validating the plan and process. This may include the plan, purpose, and scope, and the signatures for plan approval. Other elements of this section may include planning assumptions, such as recognition of the SAHO's authority in the management of FAD events. Supporting annexes cover topics organized by agency or function. For example, a facility may wish to develop an Emergency Electrical Outage Annex after having identified that damage to the electrical grid or supply lines could be caused by any number of hazards identified in the risk assessment (ice storms, tornadoes, rolling blackouts). Additional supporting annexes may be considered for emergency communication, shelter-in-place plans for people and animals, and evacuation plans for people and animals. Disease response plans are ideally suited for a hazard- or incident-specific annex because of the unique circumstances posed by a FAD.

Development of Foot-and-Mouth Disease Incident Annex Using Secure Zoo Strategy

The development of an FMD annex to a facility plan will be challenging, but there is help available to assist with this process. Agricultural industry groups have developed tools known as "Secure" plans to support business continuity for the industry as a whole, as well as for individual facilities/farms. Plans currently under development include Secure Poultry, Secure Milk, Secure Beef, and Secure Pork.[6] Each plan discusses specific details, challenges, and concerns about preparedness, response, and most notably, recovery from catastrophic disease events. In an effort to provide similar "Secure" tools to facilities with exotic or indigenous wildlife, a Secure Zoo Strategy project has been launched.

Secure Zoo Strategy[7] development is currently under the guidance of the ZAHP Fusion Center. It should be noted that Secure Zoo Strategy material is not just for facilities that identify themselves as zoos. There is broad applicability to anyone managing FMD susceptible species. Subject matter experts, including SAHOs, virologists, epidemiologists, and representatives from agriculture and other sectors have contributed to the development of Secure Zoo tools. Like the other "Secure" plans, guidance comes from FAD Preparedness and Response Plans (FADPReP).[8] These planning documents along with the *Foot-and-Mouth Disease Response Plan: The Red Book*[9] (draft September 2014) are available on the USDA FADPReP website. Secure Zoo Strategy (like other Secure plans) is a work-in-progress; however, these tools are designed to help facilities working with their SAHO to draft their own FMD hazard-specific disease annex. Facility owners and operators must recognize that the responsibility lies with them to protect their animals as much as possible.

Secure Zoo Strategy proposes a series of steps to guide a facility through the planning process for prevention and response to an outbreak of FMD. Secure Zoo Strategy encourages the planning team to understand the goals, objectives, and terms discussed in the current FMD Red Book to ensure the facility, SAHOs, and USDA are all using

TABLE 9.2 Premises Designations*

Premises	Definition	Zone
Infected Premises	Premises with a presumptive positive or a confirmed positive case that exists based on laboratory results, compatible clinical signs, FMD, case definition, and international standards	Infected Zone
Contact Premises	Premises with susceptible animals that may have been exposed or potentially exposed to FMD, either directly or indirectly, including but not limited to exposure to animals, animal products, fomites, or people from Infected Premises	Infected Zone, Buffer Zone
Suspect Premises	Premises under investigation due to the presence of susceptible animals reported to have clinical signs compatible with FMD. This is intended to be a short-term premises designation	Infected, Buffer, Surveillance, or Vaccination Zone
At-Risk Premises	Premises that have susceptible animals, but none of which have clinical signs compatible with FMD. Premises objectively demonstrate that it is not an Infected Premises, Contact Premises, or Suspect Premises. At-Risk Premises seek to move susceptible animals or products within the Control Area by permit. Only At-Risk Premises are eligible to become Monitored Premises	Infected Zone, Buffer Zone
Monitored Premises	Premises objectively demonstrate that it is not an Infected Premises, Contact Premises, or Suspect Premises. Only At-Risk Premises are eligible to become Monitored Premises. Monitored Premises meet a set of defined criteria in seeking to move susceptible animals or products out of the Control Area by permit	Infected Zone, Buffer Zone
Free Premises	Premises outside of a Control Area and not a Contact or Suspect Premises	Surveillance Zone, Free Area
Vaccinated Premises	Premises where emergency vaccination has been performed. This may be a secondary premises designation	Containment Vaccination Zone, Protection Vaccination Zone

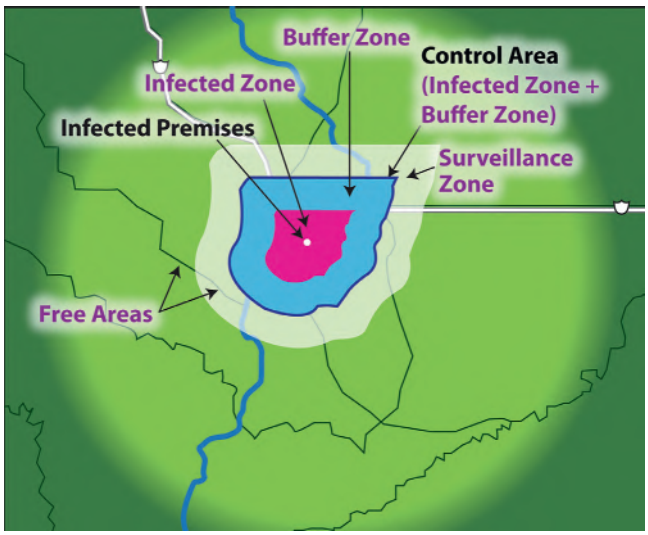
*This summarizes the premises designations that would be used in an FMD outbreak response.
FMD, Foot-and-mouth disease.
Reproduced from United States Department of Agriculture Animal and Plant Health Inspection Service. FADPReP Foot-and-Mouth Disease Response Plan: The Red Book. Available at: https://www.aphis.usda.gov/animal_health/emergency_management/downloads/fmd_responseplan.pdf. Accessed January 2017.

consistent terminology in the planning effort. Obtaining a copy of the State's animal disease incident annex (if available) may provide valuable insight into the goals and objectives of the State's plan. How robust the State Animal Response planning efforts are will likely be reflected by the size of the food animal industries located within that state. One of the first steps of the strategy is to adopt and understand how Premises Designations (Table 9.2) will be used to coordinate individual facility response.

The planning team should discuss the implications of each Premises Designation that may apply to their facility if an outbreak were to occur nearby. These implications will include additional responsibilities, such as increased biosecurity and surveillance, and restrictions to animal movement and visitation. Discuss this in context with the facility's goals (e.g., preservation of the Nigerian giraffe, eventual animal movements, and visitation). Your planning partners should understand the importance of a goal to the facility, the species, and the facility mission.

As mentioned, an essential component of planning for any FAD event will be the development of a Biosecurity Program. Secure Zoo includes guidance for development of a robust facility Biosecurity Program, which could be implemented for numerous disease concerns. The development of a sound program is important, but the ability to consistently implement it is critical. A Biosecurity Program will need to identify both Structural Biosecurity elements, such as fences, walls, or other physical barriers that prevent possible contact with diseased animals, as well as Operational Biosecurity elements, which consist of personnel protocols, equipment use and disinfection, work flow considerations, and other protocols to prevent the spread of disease. Using the concept of increasing Levels of Biosecurity, a facility may identify places in the plan where specified events would act as "triggers" indicating the need to increase biosecurity activities, thus moving the response to the next level. Secure Zoo Strategy recommends this concept based on the current Zoning strategies available in the Red Book,[9] which have been developed based on the proximity to confirmed infected premises.

Using USDA zoning classifications (Fig. 9.1), Secure Zoo provides suggested Performance Standards, which is an extensive list of response elements for possible inclusion in the various biosecurity levels. These Performance Standards are adopted from the other "Secure" programs and are adapted to address the specific needs of facilities



• **Figure 9.1** Visual Representation of Zoning Classifications Infected zone perimeter should be at least 3 km (~1.86 miles) beyond perimeters of presumptive or confirmed infected premises. Buffer zone perimeter should be at least 7 km (~4.35 miles) beyond the perimeter of the infected zone. Control area perimeter should be at least 10 km (~6.21 miles) beyond the perimeter of the closest infected premises. Surveillance zone (SZ) width should be at least 10 km (~6.21 miles), but may be much larger. Zones will be redefined as the outbreak continues. (United States Department of Agriculture Animal and Plant Health Inspection Service. FADPReP Foot-and-Mouth Disease Response Plan: The Red Book. Available at: https://www.aphis.usda.gov/animal_health/emergency_management/downloads/fmd_responseplan.pdf Accessed January 2017; This graphic is provided courtesy of: USDA; Graphic illustration provided by: Dani Ausen, Center for Food Security and Public Health, Iowa State University.)

that manage wildlife. Brief descriptions of the Biosecurity Levels from Secure Zoo are as follows:

Level 1: Preventive Biosecurity: This is biosecurity designed to prevent the introduction of a disease at facilities located outside of the Control Area during the heightened threat or occurrence of an outbreak.

Level 2: Control Area Biosecurity: This enhanced biosecurity is recommended to be used at those facilities located in the Control Area (At-Risk and Monitored Premises).

Level 3: Quarantined/Infected Premises Biosecurity: This is the strictest level of biosecurity and is intended to contain/prevent spread from an outbreak or potential outbreak at the following premises: Infected, Contact, or Suspect Premises within the Control Area.

Terms used in Secure Zoo Strategy are consistent with terminology used in other "Secure" planning efforts. Lines of Separation (LOS) is a concept that seeks to delineate areas within the facility allowing access only to attending staff and equipment that have undergone the strictest biosecurity protocols implemented at designated entry/exits known as Controlled Access Points (CAPs). CAPs may be located at the facility entrance, or exhibit entrance or exits, and include specific biosecurity protocols that must be implemented. In Biosecurity Level 1, a CAP may require clean boots, dedicated clothing, and gloves or other Personal Protective Equipment, along with a footbath. At Level 3, a CAP at a quarantined or infected premise may require Tyvek suits, dedicated footwear, dedicated equipment that cannot leave the area, dedicated personnel, showers upon exit, and other tactics to further minimize the spread of the virus to the lowest possible levels. The LOS/CAP planning concepts allow facilities to customize their protocols for individual exhibits or susceptible species. To facilitate use of the LOS/CAP concepts more fully, Secure Zoo Strategy has developed a mapping tool to help planning teams visualize their facility layout/footprint, utilizing Google Earth with a standard legend. Using an overhead image of the facility, LOS and CAP customization may be added:

Perimeter Fence: The SAHO will want to know where perimeter fences are located, and which could act as an LOS, a barrier between the collection and outside wildlife or people. The penetrability of the fence will dictate how effective it may be to keep out diseased animals. Facilities should designate all access points on the perimeter: gates, fences, etc., to determine which may be closed during a disease outbreak, and establish what biosecurity protocols would be needed during different premises designations.

Isolation Areas: These areas are delineated to show where the tightest, most protective biosecurity can be applied to protect susceptible animals. In our giraffe example, the Acme Zoo has an indoor holding barn where Nigerian giraffes are held during winter months, which is suitable for longer-term holding. Plans may include designating this building as an isolation area, with the strictest biosecurity employed at the only entrance to the barn, and designated as the Isolation Controlled Access Point. In considering the designation of Isolation Areas, questions should be asked, such as: Are there "open air" exhibits or pastures located well inside the facility that could be designated as potential isolation areas? While air movement cannot be controlled, can the area be secured from any possible wildlife interaction, while practicing the highest level of biosecurity possible for staff that service these areas?

Visitation Areas: It will be important to delineate areas where visitors may enter without adding risk of disease exposure to susceptible animals. If visitation is a significant revenue generator, this must be discussed during the early identification of the goals and objectives of plans. It is very possible that closure of the facility may be necessary at some time during an outbreak. Providing a map for the planning team and SAHO about visitor flow may lead to the development of different strategies to resume visitation more quickly, which may mean the avoidance of fiscal collapse.

In addition to the development of a Biosecurity Program, FMD planning should consider challenges to disease surveillance in the facility. Unlike domesticated agricultural species with familiar restraint techniques and validated

testing methodology, exotic species and indigenous wildlife pose very different challenges.

Building upon the momentum from existing "Secure" plans, Secure Zoo Strategy supports novel surveillance strategies for monitoring animals for disease. Consider the recent advances in testing methodologies being used in other species. For example, rather than testing individual animals in dairies, research has provided a test that may use a small sample of milk from a dairy farm's bulk milk tank to determine herd negativity, rather than testing individual animals in a milking herd. The swine industry is exploring the use of the saliva "rope test" to detect the FMD virus on ropes that swine chew on in grouped production settings to avoid having to test individual animals. (J. Tickel, North Carolina Department of Agriculture and Consumer Services Emergency Programs, personal communication, January 2017.) Discuss with SAHOs the possible surveillance strategies that would apply to your facility during the planning process; it is best to understand challenges before a disease outbreak. For facilities that manage native and non-native species, the use of domestic sentinel animals should be considered. A domestic species of low conservation value (e.g., a tame steer), which is in contact with valuable susceptible exotic species via fence line exposure or a shared water source, may be easily sampled daily. An agreement with the SAHO may allow for sentinels to serve as a crucial part of the collections surveillance program and, if negative, confer a negative status for the exotic animals in close proximity.

Basic facility operations will also need to be discussed during the planning process. This should include topics such as mortality management, disposal, and decontamination. Mortality management is an important discussion to have when planning for an FMD event. Facility owners and operators should discuss with their SAHO their "priority" animals for preservation, and determine what might be done to avoid depopulation. Be prepared to explain why these animals are high priority, and be prepared to demonstrate the facility's capability of managing these animals should they become exposed or break with disease. This prioritization for preservation pre-event is also helpful information should vaccination be used as a control option. It is possible that vaccination could be employed to halt disease spread once the specifics of an FMD outbreak are known, but initially it would be in limited supply. A facility should recognize that within its collection, domestic, susceptible livestock or indigenous wildlife of low conservation value may need to be depopulated if they are not being used as sentinels.

Disposal and decontamination discussions should also occur during the planning process. Plans will need to be flexible and consider as many options as possible, as the tactics that would be employed in a given facility would depend on many variables. Widely used guidelines for disposal and decontamination may be found in the National Animal Health Emergency Management System's (NAHEMS) Cleaning and Disinfection[10] and Disposal[11] documents available on the FADPReP website. While these documents provide an excellent scientific approach to disposal and decontamination strategies, the extraordinary effort involved in carrying out these activities reinforces why it is important to prevent a facility from becoming infected in the first place. The management of an infected facility will not be easy; it may take weeks or months and will be extremely expensive. Invest time increasing facility disease resiliency; make your facility less vulnerable to infection by continuous improvement in biosecurity in standard operating procedures, and be prepared to enhance those biosecurity measures in the case of an outbreak as discussed previously (Levels 1 to 3 biosecurity).

Contingency Planning Process Step 5: Plan Preparation, Review, and Approval

This step involves the final writing and organization of the plan itself. Once drafted, any planning process should identify who has the authority to review and approve the plan, and lay out a schedule for a future plan review. Do not forget that the staff required to execute the plan should, at a minimum, be involved in the approval process. Staff may recognize that elements of a plan may not be achievable at the operational level either in the short or especially the long term, necessitating the need for plan adjustments. Not specifically mentioned in the planning process, but critical to success, is the need for training the staff on their individual roles and responsibilities to execute the plan. Once plans are developed, work with your SAHO to explore training and exercise opportunities that test your plan in a no-risk, no-fault environment. Consider the development of a tabletop exercise to test specific elements of your plan; conduct training and drills on the different types of CAPs, and/or perform donning and doffing of personal protective equipment, set up footbaths, and try using them (and keeping them clean) for a day and then a week. With the help of your SAHO, consider participating in state level exercises, where a scenario includes an exotic animal facility in a Control Area, or as an Infected Premises. Most states with large agricultural animal populations develop these exercises periodically. Find out how your facility might be included.

Conclusion

All-hazards contingency planning should be a priority for any facility that manages non-native or indigenous wildlife. Planning efforts protect not only the collection, but also the staff, the public, and the agricultural interests, depending on the hazard. The success of the planning process will depend on the planning team itself. Carefully consider what subject matter experts may assist with risk assessment, plan development, and response. Veterinarians will be important planning team members; they will be crucial to develop plans to prevent disease and should be the point of contact for SAHOs, USDA, and other regulatory agencies.

Facilities need not "reinvent the wheel" when it comes to contingency planning. Leverage the expertise of your planning partners to assist with the steps in the process.

No one caring for exotic or indigenous wildlife wants to think about the possibility of an FMD outbreak in the United States, but it is critical to consider. The impact on the animals and staff will be incredible, regardless of the facility's mission. While this may seem to be a "low likelihood" event, every owner and operator should realize that high consequences mean recovery from FMD detection could be a long-term process.

In addition to the USDA and applicable State planning guidance, Secure Zoo Strategy is available to help facilities align themselves (where appropriate) with other "Secure" planning efforts. While facility objectives may differ from agricultural animals destined for the dinner table, there are similar goals of continuity and recovery. Secure Zoo Strategy recognizes that additional goals of preservation, animal transportation, and visitation must be considered in planning efforts for wildlife facilities, and that everyone on the planning team must recognize these goals and work toward individual plans to resume normal operations as soon as possible.

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10

Veterinary Occupational Health and Safety in the Zoo and Wildlife Setting

ELIZABETH E. HAMMOND

Introduction

Occupational safety protects employees in the work environment. The governing agencies and regulations may vary by country. In the United States, occupational safety is governed by the Occupational Safety and Health Administration (OSHA), which was created in 1970 and is a division of the Department of Labor.[1] In Canada, the governing body for occupational safety is the Canadian Centre for Occupational Health and Safety. In Europe, the OSHA equivalent is EU-OSHA. The International Labor Organization is a division of the United Nations and provides information on labor laws in various countries. The US OSHA guidelines will be used as an example in this chapter.

Employers and employees are responsible for monitoring, reporting, and preventing accidents, illness, and death in the workplace. Prevention of zoonotic diseases is one important aspect of the occupational health program. Because zoo and wildlife veterinarians are knowledgeable about zoonotic diseases, they are often asked to consult on zoonotic diseases and how they relate to occupational health. The goal of this chapter is to bridge the gap between employee occupational health, worker safety in a veterinary setting (most references are for small animal practices), and nonhuman primate (NHP) zoonotic disease prevention programs, as well as provide references for occupational health and safety.

The employer is responsible for initiating the Occupational Health and Safety Program (OHSP). A core group of individuals, led by the safety coordinator, should be assigned to implement and review the OHSP on a routine basis. This group may include the facility director or manager, the veterinarian, the consulting occupational health physician, human resources (HR) representative, attorney, etc. A designated safety officer should be identified as the primary contact for employees in the case of a work injury or hazard exposure.

Because of the complexity and dynamic nature of the OSHA laws, it may be helpful to consult a company that specializes in this area. The HR department is often intimately involved with the execution of the OHSP due to informed consent of employee rights, employer responsibilities, and knowledge of how to ensure proper documentation. Records should be maintained and easily accessible by responsible staff and include information such as: current employee emergency contact information, employee records of vaccinations, rabies virus antibody titers, intradermal tuberculin skin test results, occupational exposure, and injury incidents. Because this information is confidential, it must be kept in a secure location.[2]

Components of an OHSP include employer commitment and employee involvement, worksite analysis and risk assessment, hazard prevention and control, and safety health training.[2] Risk assessment includes anticipating potential hazards, performing site inspections, reporting findings, and investigating accidents and near misses. A safety committee led by the safety coordinator is an effective way to implement the OHSP and ensure participation of stakeholders. Appropriate facility design reduces work hazards. When this is not possible, control measures, such as personal protective equipment (PPE), should be used. PPE should be considered an additional necessary line of defense and a requirement in certain situations. This information should be outlined in protocols made available to employees.

Employee training is a cornerstone of the OHSP. The facility should have a mechanism for training staff at the time of initial employment, yearly re-training of existing staff, and training of staff that are transferred to a new department. This should be tailored to the individual facility and may include online resources as well as posters. These actions are important to create a culture of a safe work environment. Documentation of training is another key component of the OHSP and should be readily available if requested by an OHSA inspector. Some components of the OHSP as required by OSHA include the development of safety rules, including but not limited to placing signage indicating hazards, directions on proper hand washing techniques, ensuring employees wear appropriate clothing and footwear, ladder safety, needle stick prevention, and correct lifting to prevent back injury.[2] Employees also have

responsibilities under OSHA, which include reading posted work safety guidelines, complying with OHSA standards, abiding by employer safety and health rules/regulations, and reporting hazardous conditions and work-related injuries promptly.

A system of reporting injuries should be established so that employees know whom to contact (e.g., designated safety officer) in the event of an injury and to ensure proper documentation and review of the incident. Any company with 10 or more employees must record any on-the-job illnesses and injuries for the calendar year using the OSHA 300 form as per OSHA regulations.[3] A summary of work-related injuries and illnesses (OSHA 300A Log) should be posted conspicuously at the work site from February 1 to April 30 yearly. The safety officer or designee must notify OSHA within eight hours of a work-related death and within 24 hours for in-patient hospitalization, amputation, or loss of an eye.[4] The OSHA phone number is 1-800-321-OSHA (6742).

Veterinary medicine has inherent safety risks, which may be minimized with appropriate measures. According to a 2011 report, veterinary services ranked 15th in incidence rates for nonfatal occupational injuries and illnesses.[5] As such, the American Veterinary Medical Association (AVMA) Professional Liability Insurance Trust (PLIT) has published the Veterinary Safety Manual (VSM) to address general safety in the veterinary workplace.[2] Many of the topics are applicable to zoo and wildlife veterinary medicine, but additional issues are specific to this field.[6,7] Topics to be covered in this chapter include chemical hazards (hazard recognition and communication, controlled substances), physical hazards (sharps, ergonomics, gas cylinders), radiation safety, infectious agents (infection control, bloodborne pathogens, NHP considerations), and prevention. Detailed information on additional topics (animal handling and restraint, workplace violence, fire and life safety, laser safety) may be found in the references.[2]

Hazard communication covers the hazardous properties of chemicals in the workplace. OSHA requirements include keeping a list of hazardous chemicals and maintaining Safety Data Sheets (SDS, previously known as Material Safety Data Sheets [MSDS]). These sheets contain information regarding the hazards of the substance, first aid measures if exposure occurs, and proper storing and handling of the material. The SDSs should be readily available to employees and periodically reviewed for relevancy. Obsolete files should be kept for a minimum of 30 years.[2] Training of employees is an integral part of the hazard communication plan. For example, it should include information on what is considered an exposure, proper work procedure (e.g., skin and eye protection when working with chlorine bleach), and response to accidental exposure (e.g., spillage of mercury in a thermometer).[8] In the United States, proper container labeling according to OSHA's Globally Harmonized System is of utmost importance, especially when chemicals are transferred to a different container. It is also important to know what chemicals cannot be housed together. For instance, chlorine bleach must be kept separate from ammonia and acids to prevent the development of noxious gases.[9] Exposure to commonly used chemicals occurs frequently and may lead to adverse reactions. Proper PPE and eye wash stations should be readily available. Insecticides may be a risk to staff, and employers should ensure appropriate PPE is available (e.g., fit tested respirators). In addition to proper storage and handling, proper disposal of hazardous material must be followed. In the United States, disposal of these agents may be regulated by the state Environmental Protection Agency, so it is important to research all federal, state, and local laws.

Because of their potential for abuse, controlled substances are included in the OHSP. In the United States, controlled substances are regulated by the Drug Enforcement Agency (DEA); controlled substances are divided into five categories or schedules, but this may vary internationally. Schedule 1 consists of drugs used for research purposes only, and they have a high level of potential abuse. Schedule 2 to 5 are more commonly used by practicing veterinarians. Schedule 2 drugs, such as morphine, carfentanil, etorphine (M-99), and thiafentanil (A-3080), are more highly regulated than schedule 3 to 5 drugs and have more restrictions on their storage, use, and ordering. A drug register to track the use of controlled drugs must be kept and available for inspection. In the United States, an inventory of controlled substances should be performed every two years and maintained on site for an additional two years.[2] Schedule 2 records must be kept separate from schedule 3 to 5. In general, controlled substances must be locked in a secure cabinet with access restricted to a minimal number of employees. If a combination lock is used, the combination should be changed whenever staff leave employment.

An additional consideration for zoo and wildlife veterinarians is the hazard of working with potent narcotics, including carfentanil, M-99, and A-3080. Carfentanil is 10,000 times more potent than morphine, and a small volume can immobilize an elephant.[10] Although these drugs can be dangerous to humans, antagonists are available. Human antagonists (reversals) should always be readily available. When working with potent narcotics, the veterinarian should wear appropriate PPE to protect mucous membranes and compromised skin because opioids can be absorbed at these sites. It is essential to avoid a needle stick when working with potent narcotics, so recapping should be avoided. A Human Narcotic Safety Protocol should be written in conjunction with local human emergency personnel that may respond to a potent narcotic exposure.[11] It is important to forge ties with the local emergency responders and emergency department physicians to alert them to the potential for an extreme narcotic exposure. All staff working around these drugs should be trained on the protocol, which should be regularly reviewed. Risks associated with potent opioids and other immobilization drugs are addressed in more detail in Chapter 27.

Radiology with attendant radiation is commonly used in veterinary medicine, and its regulation may vary among

states or countries. Radiograph equipment vendors may be a good resource for regulations and safe working practices. Routine inspection of equipment and registration of x-ray generators must be done and may vary according to local laws. Radiation detection (dosimetry) badges must be worn by staff during all radiation procedures. Dosimetry reports are considered medical records and may not be released without written consent. Dosimetry badges are evaluated quarterly for the level of exposure to radiation using the "ALARA" notification levels (ALARA = **A**s **L**ow **A**s **R**easonably **A**chievable) in the United States.[2] As per OSHA, the following regulations should be followed: individuals under 18 years of age are not permitted to work with radiograph equipment, leaded aprons with thyroid protection greater than or equal to 0.25 mm lead and hand protection greater than or equal to 0.5 mm lead must be made available.[2] The operator must stand at least 6 feet away unless the animal must be held by the operator, and users should avoid the direct beam of radiation. A record of all staff training should be kept, and annual refresher safety training should be performed.

Infection control is another component of the OHSP, and handwashing is the most important way to prevent the spread of disease. Hands should be washed with soap for a minimum of 15 seconds. Warm water may increase compliance, but cold water is equally effective. Soap should be dispensed from a disposable container because refillable soap dispensers may harbor infectious organisms.[12] Hands-free faucets, soap, and towel dispensers may be advantageous. When running water is not available, 60%–95% ethyl alcohol or isopropyl alcohol-based hand sanitizers are very effective once the gross debris has been removed. However, they do not eliminate bacterial spores (e.g., clostridial spores) or cryptosporidium and are not as effective against nonenveloped viruses (e.g., parvovirus).[13] Fingernails should be kept short, and jewelry should not be worn in order to maximize hand hygiene. Eating, drinking, and smoking in laboratory or animal areas should not be allowed, and employees should be offered a separate area for these activities. Dedicated work clothes and shoes are an important part of disease prevention in the veterinary setting. Work clothes should be laundered at the work site using hot water and drying at a high temperature. This is especially important when working with infectious agents and NHPs.

Bloodborne pathogen (BBP) safety of the OHSP was designed to protect employees from human immunodeficiency virus (HIV) and hepatitis B, and it relates to anyone who may reasonably come into contact with human bodily fluids during the course of his or her work duties. Janitorial workers and first aid responders fall into this category. Because NHPs may also carry zoonotic infectious diseases, zoo and wildlife veterinarians and support staff who work with these species may be exposed to BBP.[14] Identifying potential exposure to BBP and instituting control measures are the first steps in developing this aspect of the OHSP. Providing appropriate PPE (e.g., safety glasses, disposable, liquid-proof gloves) and training are critical to minimize risks. For any first responders, single-use mouth protection shields should be provided for mouth-to-mouth resuscitation. Waste material soaked in human or NHP blood or other potentially infectious material (e.g., tissue) is regulated waste, and it should be placed in a red biohazard bag and removed by an appropriate disposal company. Contaminated surfaces should be cleaned and sanitized. If exposure to infectious material is suspected, the affected area should be washed with soap and water immediately, and the supervisor and safety officer should be notified. The employee may be directed to a physician for evaluation or treatment.

Prevention of hepatitis B is another important aspect of the BBP OHSP. In the United States, employees recognized at potential risk for exposure to human bodily fluids while performing their work duties should be offered the hepatitis B vaccine within 10 days of employment at no cost to them as per OSHA.[2] A declination form should be signed by the employee if he or she opts out of the vaccination and should be kept in the employee's file.[15] In addition, OSHA requires the employer to keep a confidential record of the employee's date of hepatitis B vaccination and a copy of any potential exposure to any infectious material, treatment, and medical procedures. These records must be kept for the entire length of an employee's employment plus 30 years.[2] Additional information on hepatitis B vaccination is found in Table 10.1.

Staff training on zoonotic diseases should also be documented in the employee's record. For employees who potentially have access to animals in a zoo or wildlife setting, it is recommended that they have a pre-employment physical exam, intradermal skin test for tuberculosis, fecal examination, and blood drawn so that a 5 mL serum sample (divided into two aliquots) may be stored at −20°C.[6] However, privacy laws and expenses associated with storing medical samples may discourage facilities from collecting this information. It is best to consult with the HR department, the institution's attorney, the employee's union (where applicable), and a physician prior to establishing a serum bank on site.[16] A list of recommended vaccinations for employees may be found in Table 10.1. In addition to rabies and tetanus prophylaxis, employees working with NHPs should have proof of measles, hepatitis A and B, and poliomyelitis prophylaxis.[16]

Although many zoonotic diseases are relevant to the zoo and wildlife veterinarian, this chapter is limited to several important diseases. Rabies is a potentially fatal disease caused by viruses in the genus Lyssavirus. Any mammal may potentially transmit the virus, although certain animals, such as bats, are known reservoirs for the disease. Pre-exposure rabies prophylaxis is recommended for any personnel working with a potentially rabid animal. In the United States, most states require that mammal bites be reported to the health department and animal control, although this may vary by state and local laws. Prompt treatment of a bite wound and post-exposure treatment greatly increase the chances of survival of a victim of a rabid animal bite.

TABLE 10.1 Recommended Vaccination and Testing for Employees in a Zoo and Wildlife Setting*

Disease	Vaccination	Testing	Frequency	Who
Tuberculosis	BCG, Not routinely performed in United States, may interfere with intradermal skin test	Intradermal skin test	Every 6–12 months; if test positive, evaluation by physician to ensure noninfectious (thoracic radiographs)	Anyone in contact with nonhuman primates or involved in the preparation of their food; any other staff person (e.g., landscaping, maintenance) who accesses a nonhuman primate enclosure; include students/volunteers/interns
Rabies	Series of 3 injections	Serum collection to measure antibody response to pre-exposure vaccination	Test titers every 2 years; booster as needed	Anyone who may work with a mammal with potential rabies virus exposure (wildlife, unvaccinated animals, bats, etc.)
Tetanus	Common childhood vaccine	n/a	Every 10 years, or at discretion of physician (usually booster after 5 years when an injury occurs)	Anyone
Influenza	Changes yearly based on circulating strain	n/a	Yearly	Anyone over the age of 6 months, especially healthcare providers and veterinary personnel
Hepatitis B	3–4 injections given over 6 months	Usually not necessary	After receiving the full series, boosters are generally not needed	Anyone working around humans or animals with positive Hep B status or anyone who may come into contact with human bodily fluids during the routine course of his or her work (e.g., first aid responders, janitorial workers), offer within 10 days of hire; declination letter as needed

*Additional vaccinations may be required when working with nonhuman primates.
BCG, Bacillus Calmette–Guérin.

Psittacosis is the disease caused by the *Chlamydia psittaci* bacterial organism. It may be treated with antibiotics, and human cases are rarely fatal.[17] OSHA recommendations to protect workers from contracting psittacosis through inhalation include providing appropriate respiratory protection (e.g., high efficiency particulate air [HEPA] filter), establishing a respirator program and a psittacosis training and prevention program, and ensuring adequate ventilation in the work area.[18] This program should educate staff on the clinical signs associated with psittacosis in avian species. Keepers should immediately report to their supervisor any indications that a bird may be infected, which may include oculonasal discharge, conjunctivitis, and weight loss. However, many birds may be inapparent carriers. In addition, if an employee is showing flu-like symptoms (fever, malaise, headache), the employee should alert the physician to the fact that he or she works with birds.[17] Confirmed work-related cases of psittacosis must be reported to OSHA, the state health department, and the National Centers for Disease Control and Prevention in the United States.[18] The list of reportable diseases should be reviewed in other countries.

Methicillin-resistant Staphylococcus aureus (MRSA) is a potentially highly resistant version of a relatively ubiquitous organism, and many humans are carriers. This organism should be suspected in any nonhealing wound. If an animal with a wound is determined to have MRSA based on culture and sensitivity, it is best to alert all involved in the care of that animal.[19] Appropriate PPE should be instituted based on the facility and potential exposure. As a general rule, dedicated boots, gowns, and disposable gloves should be worn around the MRSA-positive animal. In addition, any caretakers with nonhealing wounds should alert their physician to the fact that they have been caring for an animal with MRSA. For shared computers, keyboard covers that are easily disinfected may reduce the risk of spreading MRSA or other infectious organisms in the workplace.

Toxoplasmosis is a disease caused by the protozoan *Toxoplasma gondii*. Felids are the definitive hosts for this pathogen, which is found worldwide. In the immunocompetent person, infection may be subclinical, but immunocompromised people may be at risk of developing severe disease. Pregnant women should avoid exposure to *T. gondii* because it can cause serious birth defects in the fetus. Serologic titers should be performed on women of childbearing age to determine serologic status. Pregnant women working around felids should avoid exposure to feline feces or soil contaminated with feline feces.[16] Job reassignment should be considered in those workers considered high risk.

Reptiles and amphibians are often carriers for *Salmonella* sp. Therefore, anyone working with these animals should be informed of the potential risks of this disease and instructed on precautions that should be taken to prevent it. Birds and mammals may also carry salmonella, so any animal in contact with the public should also be screened for salmonella on a regular basis.[20] Hand-washing after handling any of these animals is the best way to prevent disease.

Because humans and NHPs share many diseases, guidelines for disease prevention from the human health care sector may be applicable when working with NHPs. Identification of risks may depend on the history, health, and species of the NHP in the collection, facility design, among other factors. There are varying levels of exposure to NHPs. For instance, cleaning a contaminated cage may expose the worker to aerosolization and direct contact of potential pathogens, whereas maintaining a greater than one meter distance from an NHP carries a lower risk. When working with NHPs, minimum PPE should include a face mask, gloves, long sleeves, and pants, although additional precautions should be taken when working with macaque species.[21] Coveralls or similar outer protective wear and dedicated boots are also recommended. For more direct contact with NHPs and their bodily fluids, such as performing dentistry on an anesthetized animal or cleaning enclosures, face shields and hair covering should also be worn. In hot climates, the use of PPE may cause the wearer to overheat; providing spots to cool down and maintaining hydration may prevent heat sickness in employees. Anyone showing signs of illness, such as upper respiratory infection, cold sores, or gastrointestinal upset, should avoid working with NHPs until clinical signs have resolved. Clothing worn in NHP areas should not be worn outside of that area and ideally should be laundered on site. Use of foot baths with appropriate disinfectants is encouraged to minimize transfer of pathogens from NHP enclosures.

Herpes B virus (*Herpesvirus simiae*, *Macacine herpesvirus*, *Cercopithecine herpesvirus* 1) is often fatal in humans. It is common in macaques, who are inapparent carriers. Limiting exposure, safe handling procedures, and more extensive PPE, such as eye protection, should be worn when working with macaques or animals known to be infected with herpes B. Prompt treatment of bites or scratches is essential. A bite/injury protocol should be available in all primate areas.[22] It is advisable to discuss in advance primate bite protocols with the hospital staff that may be asked to address them. Educating staff about the risks of the disease and how to prevent it are paramount. Other viruses of NHPs, such as STLV, SIV, GaLV, may present potential zoonotic risks.[14,22]

In addition to exposure to BBP, injuries associated with "sharps" (needles, scalpel blades, broken glass vials, etc.) should be included in the OHSP. Safety measures should be taken when using sharp objects, and they should be placed in an appropriate sharps container immediately after use. Needles should not be recapped but immediately disposed of in an appropriate sharps container. However, if recapping is necessary, the one-hand recap method should be used. With this method, one hand is used to scoop up the cap over the needle, and the cap is depressed over the needle by pressing it against a hard object, such as the wall. Needles with a recapping device are commonly used in human medicine and may be a good, safe option in veterinary medicine. Needle caps should never be placed in the mouth.

Gross necropsies are an integral part of preventative medicine and zoo and wildlife health programs, but they present a potential risk of pathogen exposure and physical injury. At a minimum, latex gloves, protective outwear (e.g., coveralls or apron), eye protection, and face mask should be worn. Veterinarians performing necropsies may be exposed to formaldehyde, which is an inhalant hazard and carcinogen.[23] Knife injuries during necropsies are common; 87% of respondents in a survey of zoo veterinarians reported a knife wound during a necropsy and half of those wounds required medical treatment.[7] Measures to prevent knife wounds, such as the use of washable, cut-proof gloves, should be considered where practical. When power tools are used, it is important to wear appropriate eye protection and a respirator. Training on power tool or other heavy equipment use should be mandatory and documented. In addition, injuries often happen when workers are fatigued, so it is important to remind workers to slow down.

The OHSP also covers ergonomics, which prevents work-related musculoskeletal disorders in employees. Employers should provide proper computer working stations to prevent back or wrist strain, eye fatigue, and repetitive motion injuries.[2] In addition, employees should be reminded of safe lifting practices to reduce back injury. A 1998 survey of zoo veterinarians revealed that over half of respondents reported a work-related back injury.[7]

Many veterinary clinics use compressed gas cylinders, which can be very dangerous if mishandled. The manufacturer's instructions should be followed. The cylinders should be stored in an upright position and secured in place, and a protective cap should cover the valve when not in use. The cylinders should not be stored in a warm environment, exposed to corrosive substances, or transported with the regulator attached.[2]

Additional occupational hazards for zoo and wildlife veterinarians include venomous reptiles and dangerous animals (e.g., elephants), but a detailed discussion is beyond the scope of this chapter.[11,24] With proper training and documentation, occupational hazards for the zoo and wildlife veterinarian may be minimized, and the veterinarian can be a great resource for the facility's OHSP.

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11

Research Study Design

NICOLA DI GIROLAMO AND CHRISTOPH MANS

Introduction

A research study is typically composed of several phases, including design, performance, analysis, and reporting. Careful study design is the first critical step to ensure the validity of obtained results (Fig. 11.1). Depending on the type of clinical question to be answered, different types of study designs will be appropriate. Clinical questions can be divided in the following categories: (1) evaluation of effectiveness of interventions, (2) identification of underlying disease etiologies, (3) evaluation of diagnostic test accuracy, and (4) evaluation of prognostic indicators. Researchers should invest significant time in planning the research study and in drafting a comprehensive protocol. Study protocols are considered the single-most important quality control tool for observational and experimental studies, and besides being important for the research group to stimulate proper planning of the study, their public access is fundamental for limitation of publication bias (i.e., bias in scientific literature caused by lack of publication of negative findings,[1] and for evaluation of changes of outcomes occurred during the study[2]).

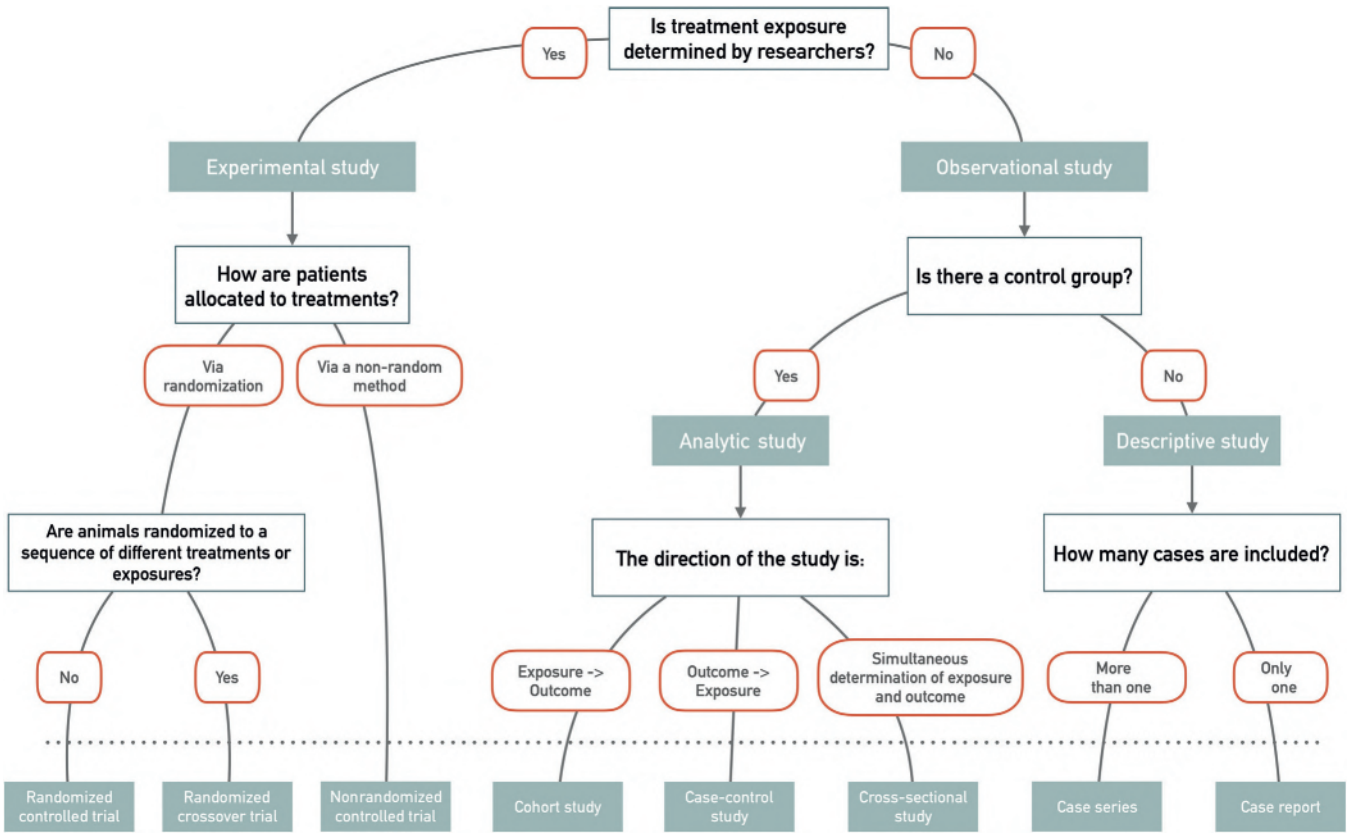
Study Designs for Evaluation of the Effectiveness of Interventions

Randomized Controlled Trials

Randomized controlled trials (RCTs) are considered to have the highest potential to provide the highest-quality evidence for assessing medical interventions.[3] RCTs, defined as studies designed with patients allocated randomly either to an intervention group or a control group, enable avoiding most of the selection bias that occurs in observational studies of interventions, if correctly executed.[4,5] Pragmatic RCTs include a real, patient-based population. Each patient who fulfills certain inclusion criteria is randomly assigned to one of the treatments, typically the novel treatment versus the standard treatment (defined "standard-of-care trials" or "best-available-therapy trials"), or the novel treatment versus a placebo (i.e., "placebo-controlled trials"). In a zoological setting, this would mean that animals that develop a certain disease are randomized to treatments based on a random sequence. Randomized studies may also be performed on healthy individuals in a collection (e.g., for testing a novel diet), and in such case the same methodologic indications apply. Other methods of allocation to a certain treatment (e.g., alternation, allocation based on birth date, allocation based on time of admission) are known to be related to bias, including the overestimation of the effect of the treatment.[6,7] Ideally the caregivers are blinded to the treatment groups. In some instances, this is not possible (e.g., in randomized trials of surgical interventions). In such cases, it is still critical that the outcome assessors are blinded to the treatment groups. In veterinary medicine, randomized trials are frequently performed in experimental settings rather than in clinical settings. The conclusions of such trials in experimental settings are likely to have good internal validity, but the results of these studies may not apply to the real population seen in clinical practice. Although randomized trials are ideal to evaluate interventions, they may be subject to different degree of bias that affect the underlying results.[8]

Crossover Trials

Crossover trials are studies in which animals receive different treatments during different time periods. Typically, each patient receives all the treatments investigated in the study (complete crossover). Crossover trials are especially common in experimental settings and provide additional statistical power with similar, or even smaller, sample sizes. The treatment sequence should be randomized (e.g., A-B-C vs. B-A-C vs. C-A-B, etc.) and all treatment sequences presented equally (balanced design). In addition, at each time point (i.e., day of experiment) the outcomes should be evaluated in all the trial arms to reduce potential bias given by evaluation of outcomes at different time points. Sufficient time between the different treatments is essential to avoid any "carry-over" effect (e.g., due to drug effects or changes in physiologic status due to restraint, blood collection, or other factors). A control group should be included, in particular if the outcomes are challenging to measure or can be affected by various factors (surgery, restraint,



• **Figure 11.1** Simplified algorithm for the determination of the study design of a research study.

anesthesia, etc.) not directly related to the tested intervention. Caregivers should be blinded to the intervention (e.g., drug) administered at each time point to avoid a possible differential care of the animals. Outcome assessors should be blinded to the intervention administered at each time point to avoid biased assessments.

Nonrandomized Studies

Nonrandomized studies provide limited evidence of the effects of interventions. They are the main source of evidence on the intended effects of many organizational or public health interventions and on interventions that cannot ethically be randomized. A large class of nonrandomized studies includes those in which allocation occurs based on predefined groups or on clinical decisions, such as those of the treating clinician, the curators of the animals, or the animal owners. These observational studies are many of the classical epidemiologic designs such as cohort studies, case-control studies, and cross-sectional studies. However, these study designs are better fitted for identification of underlying disease etiologies. Other nonrandomized studies compare a group of animals receiving an intervention with a group of animals of the past that did not receive such intervention. A clear example of such "historically controlled studies" is a study in which rabbits affected by *Encephalitozoon cuniculi* were treated without fenbendazole between 2000 and 2003 and with fenbendazole between 2004 and 2008. The study concluded that the addition of the fenbendazole improved the outcomes of rabbits.[9] However, this study design may be affected by multiple types of bias because a general improvement of the care of the animals could be also responsible for the improved outcomes. In nonrandomized studies, confounding factors need to be addressed by using proper statistical techniques that account for multiple predictors, such as general linear model, generalized linear model, and logistic regression.

Study Designs for Identification of Underlying Disease Etiologies

Cohort Studies

A cohort study is a type of observational study in which a group of animals that share an exposure (e.g., a defining characteristic, or a common event in a selected period) is compared with a group of animals similar in all the other characteristics but that did not have such exposure. Briefly, if the exposed group develops a higher incidence of the outcome than the unexposed, then the exposure is associated with an increased risk of the outcome.[10] The strength of cohort studies is that they enable calculation of incidence rates, relative risks, and attributable risks. The main weakness of cohort study is that for outcomes that are rare or develop over longer time periods, this type of

research design can be slow and expensive. Selection and information bias are a concern with cohort studies.

Case-Control Studies

Case-control studies compare animals with a specific outcome of interest (cases) with animals from the same source population but without that outcome (controls). After an outcome of interest is identified, typically a disease, a retrospective search is performed to identify exposures that could have been responsible for the outcome (e.g., exposure to oncogenic substance, or to an intervention).[11] This design is particularly useful to investigate the etiology of rare diseases. Control animals should be similar to cases in all important factors except for not having the outcome in question (e.g., the disease).[10] To reduce source of bias, selecting controls that are similar to cases for certain criteria (i.e., matching) may be implemented. The selection of an inappropriate control group may severely limit the validity of a case-control study. Another serious source problem of case-control study is recall bias, which occurs when there is a differential recall (i.e., different memory) of an exposure between the group of cases and the group of controls.

Cross-Sectional Studies

Cross-sectional studies are performed to examine the presence or absence of an outcome and the presence or absence of an exposure at a specific point of time. The rates that result from cross-sectional studies are termed prevalence, instead of incidence as in cohort studies. Cross-sectional studies can be performed without the need of follow-up, making them less expensive to perform. The primary limitation of cross-sectional studies is that the temporal link between the outcome and the exposure cannot be determined because both are examined at the same time.[10] For example, in a zoo, reproduction is found to be more commonly impaired in animals with stereotypies. With a cross-sectional study, it is impossible to determine whether the inability to reproduce exacerbates the stereotypies or the contrary.

Study Designs for Evaluation of Diagnostic Techniques

Diagnostic Accuracy Studies

Diagnostic accuracy studies compare the performance of one diagnostic tool (e.g., cytology versus histology for the diagnosis of CANV in reptiles, or CT scan versus radiography to detect pneumonia) with a reference standard. The term "gold standard" is discouraged because every test and assay, no matter how well executed and controlled, carries a nonzero rate of false-positive and false-negative results.[12] A diagnostic accuracy study provides evidence on how well a categorical test correctly identifies or rules out a disease. Thus, after a clinically relevant diagnostic threshold has been established, patients' results can be categorized by the test as true positive, false positive, true negative, and false negative.[13] This information is critical for clinicians and allows for an unbiased evaluation of the diagnostic test results.

Method Comparison Studies

In method comparison studies, two or more diagnostic techniques that provide a numerical outcome are compared. Most diagnostic tools provide numerical outcomes, such as tonometers or glucometers, among others. These studies are aimed to evaluate the agreement between the techniques, and the main outcome should be the mean difference, with 95% limits of agreement that is present between the techniques.[14]

Reference Interval Studies

Reference interval studies aim to determine the estimated distributions of reference values from healthy populations of comparable individuals.[15] Factors that need to be considered when designing or evaluating a reference interval study include: (1) selection of the reference population, (2) preanalytic procedure, such as patient preparation, sample collection and storage, and analytic quality assessment, (3) analytic procedures (e.g., selection of the type of instruments), (4) statistical analysis of reference values, (5) postanalytic procedures, including how the reference intervals are presented, and (6) validation of reference intervals on further population samples.[15]

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SECTION 2

Animal Welfare

12

Overview of Animal Welfare in Zoos

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Introduction to Animal Welfare in Zoos

The mission of zoos and aquariums is broad and emphasizes achievements in areas of animal care, conservation, education, science, and recreation. A critical element of that mission is to constantly strive toward achieving higher standards of welfare for wild animals, for captive animals directly under their stewardship, and for those still free-ranging or under in situ management. Nonhuman animal welfare (from here forward termed "animal welfare") is vital to supporting the goals of zoos and aquariums (from here forward termed "zoos") as modern conservation organizations.[1]

Zoos are united through organizations, including the World Association of Zoos and Aquariums (WAZA), the Association of Zoos and Aquariums (AZA), the European Association of Zoos and Aquaria (EAZA), and other regional zoo organizations, that provide support and channels of communication to maintain welfare and ethical standards; however, a universally accepted definition for animal welfare has not been agreed upon in the zoo or in other professional communities, and several entities have developed their own definitions and descriptions (Table 12.1).[2]

Acceptable welfare standards and best practices remain somewhat vague and elusive; they are the subject of much debate.[3] This may be a result of differing ideas about what animal welfare actually is and the continuum of zoological institutions, from zoos and wildlife parks to game reserves, national parks, and protected areas, thus making the idea of enclosure and captivity fluid and not absolute.[4] This is coupled with the relatively young and extremely broad discipline of zoo animal welfare science grappling with a variety of approaches to the study of welfare, the difficulty of conducting scientific studies in zoos, and a lack of information about many animals, both in captivity and in the wild.[5] Like any science, welfare studies are being continuously advanced, informed, and debated by newer investigations, and are refined as they progress. Emerging research in the area of what constitutes good animal welfare rather than identification of what needs to be avoided may yield constructive information to design best practices.[6]

Despite various definitions, multidimensional aspects, and interdisciplinary principles of animal welfare, it is important to keep in mind that animal welfare belongs to the animal, and that it impacts survival and must be viewed from their perspective. Welfare refers to a characteristic of the individual animal rather than something given to the animal by people.[7] Welfare is about the subjective state of the animal, including the judgment of what is good in the animal's life and what is not good. Because welfare belongs to the individual animal, it will differ between different animals, even when the exact same conditions are provided, and the welfare of the same animal will change as the animal ages. In the case of zoos, animals may have come from a variety of backgrounds, individuals may vary greatly in their previous life experiences, and this may influence their ability to cope with certain challenges. For example, an animal that stays in zoos its whole life requires different skills from an animal that is kept for the future goal of reintroduction into the natural environment or another example of an animal brought into an urban zoo from a semi-free-ranging facility or vice versa.[8,9] By using each animal as its own control, an individual's welfare can be tracked in response to changes in its environment and, thus, an individual's welfare can be measured. The welfare assessment of an animal or a group of animals may result in different conclusions, depending on the goal of keeping an animal.

Moreover, zoos are acutely aware of the public's perception of the well-being of captive animals. Many zoos have a high degree of public approval and acceptance, especially when compared to other institutions caring for captive animals for research or production. At the same time, zoos are under constant criticism from the public because people have complete access to directly observe how the captive animals are behaving. It is the responsibility of the zoo community to retain the public's regard by understanding the changing societal consensus about animals, and by designing zoo environments that meet the needs that the animal itself perceives to be important.[10]

Animal Welfare Assessments in Zoos

Establishing Practical Guidelines for Assessments

Assessing and maintaining welfare for an individual animal or a population of animals is the responsibility of everyone

TABLE 12.1 Various Definitions of Animal Welfare

American College of Animal Welfare[44]	Animal welfare refers to the state of the animal. Assessment of welfare includes consideration of the animal's health, behavior, and biological function
American Veterinary Medical Association[33]	Welfare means overall mental and physical health; protecting an animal's welfare means providing for its mental and physical needs
Association of Zoos and Aquariums[25]	Animal welfare refers to an animal's collective physical, mental, and emotional states over a period of time, and is measured on a continuum from good to poor
World Organization for Animal Health (OIE)[49]	Animal welfare means how an animal is coping with the conditions in which it lives. An animal is in a good state of welfare if (as indicated by scientific evidence) it is healthy, comfortable, well-nourished, safe, able to express innate behavior, and if it is not suffering from unpleasant states, such as pain, fear, and distress

working within a zoo. Everyone has a role to ensure that animals are thriving. An institutional welfare assessment program and welfare committee provide support for the individuals caring for the animals. Additionally, some animal welfare departments have responsibilities for animal training, behavioral husbandry, enrichment, and research.

A proper welfare assessment must draw on multiple disciplines and consider that the perspectives of the human scientists, caretakers, or policymakers often result in different emphases on what constitutes a welfare problem or where efforts should be focused to improve the situation.[11] Each individual evaluating or contributing to a welfare assessment brings his or her own biases and ethical views, which affects how different elements of welfare are weighted in an assessment. When developing a welfare assessment for animals in a zoological institution, there are several over-reaching principles that have been established that can be applied. The following set of recommendations has been extrapolated from animal welfare assessment guidelines for laboratory animals.[12]

1. A team approach allows input from people with different perspectives of the animal's situation. Identification of who is involved with an animal care team will vary. An assessment often receives input from several different individuals, and it works best when people in the team are prepared to work together. It is beneficial when all members of the team are familiar with normal behavior for the species being evaluated and are able to recognize abnormal behaviors.
   a. Animal caregivers—these are the people who observe the animal in the captive environment. This is often the person who has daily interactions with the animal and will notice subtle changes in vitality, activity, behavior, and food intake.
   b. Veterinarians—these are the people with special training to observe and examine the animal's health parameters and should also be familiar with physical attributes specific to the species under evaluation.
   c. Animal curators—these are the people who are trained to observe a variety of animal species in a zoo, and they are knowledgeable about requirements and compliance with guidelines or protocols set by government agencies, professional organizations, and population management plans.
   d. Research investigators—these are the people who may have zoo animals involved in any type of study. The investigators may be collecting specific information that changes over time relative to the animal's welfare. When captive animals are engaged in studies, it is often necessary to have an institutional animal care and use protocol reviewed by the Institutional Animal Care and Use Committee (IACUC) or internal research review panel. This review may initiate a welfare assessment protocol early in the planning of a project.
2. Good communication and records of the observations will aid in reducing the range of variability. Consistency between observers, or at least between the veterinary professionals participating in a welfare assessment of the same animals or facility, improves the follow-through and re-evaluation. There is predictable variation between observers; therefore, having the same people observing the animals for reevaluation is helpful. Furthermore, individual animals may be able to differentiate between the observers and display different behaviors depending on the level of familiarity with the person making observations. When animals are enrolled in studies or research projects, it is important that all team members understand the purpose of the study and the scientific objectives.
3. Identification of which indicators are to be monitored can be challenging, including which behavioral and physiologic parameters are used as indicators of welfare (see Chapter 13). It is important to define and monitor the right types and number of indicators. Common errors include having too many parameters, which prolongs the process and makes it less effective, whereas too few may lead to inaccuracies. Setting the baseline is often the most challenging part of a welfare assessment because it involves agreement as to what may be considered the hypothetical ideal.
4. The record keeping system for welfare information collected should be based on what is appropriate for the institution being evaluated. There are several systems available for recording welfare assessments in zoos, including developing modules within existing electronic medical records systems or independent applications, such as WelfareTrak (Chicago Zoological Society, Brookfield, Illinois), as well as systems utilized in food

or laboratory animal facilities.[13] Record systems often have predetermined lists of factors to be evaluated. These may be set up to answer with simple binary (yes/no) responses or may incorporate numerical or ranking scales from good to bad. Numerical scoring systems provide structure to evaluate clinical signs, physical indicators, and behavioral parameters; however, this system requires a subjective value judgment by each assessor.

5. Frequency and timing of welfare assessments are often determined by the degree of deviation from good welfare. Assessments will vary depending on each situation, and the timing and frequency is often established by the number of animals involved and the allocation of resources to provide effective monitoring. Indicators of poor welfare should be determined first and addressed immediately. After specific thresholds are determined, indicators of good welfare can then be used for monitoring. Signs of poor welfare may be more challenging to detect in some species, which could lead to more frequent assessments in hopes of identifying problems early. The age or condition of illness of an animal will also affect the frequency of health and welfare assessments. With regard to animals involved in a study, the experimental design usually affects the frequency and timing of observations. Signs of illness and pain are important initial considerations that would prompt an immediate corrective action if such signs were unexpected, or unrelated to the research.[12,14]

Animal Welfare Assessment Principles

Methods to perform an animal welfare assessment may be found in textbooks and peer-reviewed publications, although the primary subjects are often laboratory or production animals. Specific recommendations for how to perform welfare assessment of animals living in zoos have only recently emerged in the literature, and several guiding principles or models have been set forth (Table 12.2). An assessment includes an evaluation of both positive and negative welfare states; it is best when measured on a sliding scale from very poor to very good, and should be measured scientifically whenever possible. One approach to organizing a welfare assessment of animals in zoos is to include measurements of two major components, both animal based and resource based.[15]

Resource-based (also termed "design-based" or "inputs") reviews are the most commonly applied evaluation of an animal's welfare assessment. Resource-based measures focus on the animal care and things that are provided to the animals, with natural history taken into account, such as the amount of space, substrate, temperature, diet, and veterinary care. Some welfare resources are measured by being either present or not present, such as providing the correct thermal zone (positive) or being outside of that thermal zone (negative), whereas other parameters are evaluated on a continuum; for example, sufficient food may be provided and the animals are not hungry; however, the diet may not be providing the correct nutritional balance, placing the animals in a malnourished condition or in a state of over-nutrition. An assessment gives consideration to the presentation of the food, the variability in food types, and the feeding schedule, or giving the animals choice and control of food items. Resource-based measures are used in conjunction with information about management practices to help identify the causes of animal welfare problems or indicate potential risk factors.[16] Exhibit design must incorporate species-specific needs and appropriate space allocations, plus provide environmental enrichment features and opportunities for natural locomotion, exercise, and suitable social interactions with other animals in the exhibit. It is often the housing and management conditions for animals maintained by an institution that are scrutinized by the public. Resources are necessary components of welfare, ensuring that conditions are present that provide animals with the potential to experience good welfare, but they do not—in and of themselves—ensure good welfare.[5]

Animal-based (also termed "outcome-based" or "outputs") measures include the physical, behavioral, and mental state of the animals themselves. Because welfare needs to be evaluated from the animals' perspectives, animal-based measures are critical; however, some of these, such as the mental state, can be scientifically challenging to measure. Evaluation of the animal's physical state is usually the area of greatest familiarity for veterinarians because it includes the health of the animal. The physical state assessment includes information provided by the caregiver, such as food and water consumption, feces and urine or urate production, and level of activity. Evaluation includes the objective measurements of a physical examination such as weight, heart rate, respiratory rate, and recovery time after handling. Additional information to assess the physical state of an animal frequently includes diagnostic evaluations, such as the complete blood count (CBC), plasma biochemistry values, fecal evaluation, and urinalysis. Radiographic and additional imaging studies may be included. Physical assessments also include several subjectively scored parameters, such as the animal's degree of responsiveness, body condition score, mobility, and posture.

Many animal welfare studies examine subclinical physiologic changes to determine if an animal is distressed, when the stress response shifts sufficient resources to impair other biological functions.[17] An example would be a bird experiencing stress in a new enclosure that doesn't show outward signs but may become immunocompromised and susceptible to fungal infection. Glucocorticoid measurement using blood, feces, or saliva has been used as an indication of adrenal function and the animal's stress response; however, interpretation of glucococorticoid metabolites concentrations requires caution because it is affected by a long list of variables, not necessarily stressful or distressing, such as diurnal or seasonal rhythms, habituation, diet, sex, and reproduction (see Chapter 13).[18]

Assessment of the behavioral state evaluates whether an animal is engaging in activities typical for the species and not expressing maladaptive behaviors that result in injury or illness. The behavior of captive animals is frequently

TABLE 12.2 Guiding Principles for Assessing Animal Welfare

American Veterinary Medical Association: Eight integrated principles for developing and evaluating animal welfare policies, resolutions, and actions[33]	1. The responsible use of animals for human purposes, such as companionship, food, fiber, recreation, work, education, exhibition, and research conducted for the benefit of both humans and animals, is consistent with the Veterinarian's Oath. 2. Decisions regarding animal care, use, and welfare shall be made by balancing scientific knowledge and professional judgment with consideration of ethical and societal values. 3. Animals must be provided water, food, proper handling, health care, and an environment appropriate to their care and use, with thoughtful consideration for their species-typical biology and behavior. 4. Animals should be cared for in ways that minimize fear, pain, stress, and suffering. 5. Procedures related to animal housing, management, care, and use should be continuously evaluated, and when indicated, refined or replaced. 6. Conservation and management of animal populations should be humane, socially responsible, and scientifically prudent. 7. Animals shall be treated with respect and dignity throughout their lives and, when necessary, provided a humane death. 8. The veterinary profession shall continually strive to improve animal health and welfare through scientific research, education, collaboration, advocacy, and the development of legislation and regulations.
Five Freedoms Model[50]	1. Freedom from hunger and thirst by ready access to fresh water and a diet to maintain full health and vigor 2. Freedom from discomfort by providing appropriate environment including shelter and a comfortable resting area 3. Freedom from pain, injury, or disease by prevention or rapid diagnosis and treatment 4. Freedom to express normal behavior by providing sufficient space, proper facilities, and company of the animal's own kind 5. Freedom from fear and distress by ensuring conditions and treatment that avoid mental suffering
Animal Welfare[51]	1. Need for a suitable environment 2. Need for a suitable diet 3. Need to be able to exhibit normal behavior patterns 4. Need for the company of, or to be apart from, other animals 5. Need to be protected against pain, suffering, injury, and disease
Five Domains Model[1,52]	Animal welfare is divided into physical and functional domains (nutrition, environmental, physical health, behavior) and mental domain (negative and positive experiences). Both internal and external conditions give rise to negative (aversive) and positive (pleasant) subjective experiences, the integrated effects of which give rise to the animal's welfare status.
Maslow's Hierarchy of Needs Pyramid[1]	Animal welfare should be directed toward the highest categories of Maslow's pyramid of wellness and well-being. The bottom of the pyramid includes the critical foundational requirements for survival, including physiologic needs for shelter, water, and hygiene, as understood through experience and science. The middle of the pyramid includes the animals' physical health and safety needs. The top of the pyramid is the most varied and complex welfare-related activities, such as social needs, mental stimulation, and choice. This pyramid, when all parts are achieved, results in an animal retaining and encouraging natural abilities.
Opportunities to Thrive[27,57]	1. Opportunity for a well-balanced diet: fresh water and a suitable, species-specific diet will be provided in a way that ensures full health and vigor, both behaviorally and physically 2. Opportunity to self-maintain: an appropriate environment including shelter and species-specific substrates that encourage opportunities to self-maintain 3. Opportunity for optimal health: providing supportive environments that increase the likelihood of healthy individuals as well as rapid diagnosis and treatment of injury or disease 4. Opportunity to express species-specific behavior: quality spaces and appropriate social groupings will be provided that encourage species-specific behaviors at natural frequencies and of appropriate diversity while meeting social and developmental needs of each species in the collection 5. Opportunities for choice and control: providing conditions in which animals can exercise control and make choices to avoid suffering and distress, and make behavior meaningful

compared to the behavior of their wild counterparts to understand the effects of captivity.[5] The possibility that animals in captivity can perform behaviors that they would normally perform in a more natural environment is an enduring welfare concern, and seemingly a higher priority for zoo animals (never considered tame) than for domesticated animals. Positive welfare exists when an animal performs species-specific behaviors with diversity and frequency seen in the wild, especially those that the animal is highly motivated to perform. The caregivers are often the most familiar with the animal's behaviors and may observe when abnormal behaviors are present, for example, in terms of excessive activity or lack of activity, abnormal vocalizations, excessive grooming or self-mutilation, or even the development of a stereotypy.[12]

Abnormal repetitive behaviors, those without an obvious function, are called stereotypies, and are often behaviors used to assess welfare. Stereotypical behavior has been theorized to be the response of an animal to the presence of abnormal stimuli or lack of stimuli in the captive environment.[19] There are situations in which a high level of abnormal behavior in an animal is associated with enhanced coping abilities. A literature survey found that across species and environments where data were provided, almost 68% of the situations that caused or increased stereotypies also decreased welfare, but also it warned that stereotypies were linked to good or neutral welfare nearly as often as poor and therefore should not be used as a sole index of welfare.[20]

Assessment of mental welfare has also been referred to as an indication of the subjective state of the animal. In most situations, caregivers are unable to directly assess the thoughts and feelings of an animal and therefore must rely on indirect indicators.[12] Evaluation of an animal's mental state may be the most abstract and anthropomorphic part of the welfare assessment because it requires observation and evaluation of the animals' demeanor or emotion. To add to the challenge, there are few objective measures of positive emotions in animals despite widespread physiologic and behavioral measures of negative emotions.[21] Indications of a positive affective state may include facial expressions, paw licks, tongue protrusions, and vocalizations, such as purring in cats or chirping in rats. Unfortunately, these are not markers that can be easily extrapolated across all species.[22] Multiple factors including appropriate husbandry and environment allow for the expression of natural behaviors and satisfactory mental well-being. Enrichment is one method of compensating for compromised conditions in captivity.[12] Enrichment programs try to provide opportunities for animals to express behaviors driven by the positive emotional systems of seeking, play, and caring, and decrease activation of the fear, rage, or panic systems.[23,24]

Animal Welfare Committees in Zoos

With animal health and welfare as a top priority for zoos, formation of an animal welfare committee within a zoo is beneficial to the institution on many levels. One of the primary purposes of a zoo welfare committee is the oversight of welfare assessments. The committee may not be the team tasked with performing the assessment, but may request welfare assessments in response to concerns presented, to identify strengths and areas in need of advancements, to create a system or process to evaluate the welfare, to start scientific evidence-based communications between staff members, and to recommend that improvements be considered, followed by a welfare reevaluation after changes have been implemented. The AZA defines animal welfare as an animal's collective physical, mental, and emotional state over a period of time, which is measured on a continuum from good to poor.[25] For an institution to be AZA accredited, it is required for that institution to "develop and implement a clear and transparent process for identifying, communicating, and addressing animal welfare concerns" in a timely manner without retribution to the staff member or volunteer who raises them.[26] A committee or other process must be developed to address any concerns regarding animal welfare within the institution. The committee or process is supplementary to the normal chain of command within a zoo's animal care department to ensure that the process is not influenced by personal motives or conflicts. If concerns are not being addressed within the standard chain of command, a welfare committee provides an option for matters to be addressed further and with anonymity.[26]

There is no definitive formula on how to initiate and develop an animal welfare committee or process that is standardized and comprehensive for every institution; however, the committee or process should include the following elements: clear communication of the process to staff and volunteers; easy access to the committee by all staff and volunteers; staff with the experience and authority necessary to evaluate submitted observations and implement any necessary changes; and timely feedback to the person submitting the observation.[25,26] An animal welfare committee within a zoo serves in an advisory and nonregulatory role to guide curatorial and executive-level staff in matters of animal welfare. The committee promotes scientific and evidence-based, proactive metrics to develop best practices for animal care and to respond to welfare concerns raised by staff and volunteers to hold the institution accountable for the provision of consistent optimal welfare.[1] In some zoos, the animal welfare committee serves multiple roles, including reviewing research proposals and biomaterials requests, similar to an IACUC, and serves as a forum for welfare discussions.

The zoo animal welfare committee functions best when a small group of members is selected for their leadership skills, and includes staff with current or previous responsibilities for animal welfare, care, and health across departments. The make-up of the committee varies among institutions, but typically includes core internal members consisting of a combination of executive level staff members, curators, keepers, veterinarians, researchers, facilities managers, and other department representatives. Veterinarians should

always be included in the welfare committee given their expertise in medicine, biosecurity, zoonoses management, and postmortem evaluation.[1] Additional internal members consisting of animal care and other department representatives may also be included. Furthermore, zoo animal welfare committees may include one or more community members.[2] External members may consist of a combination of nonemployees who have interest or expertise in animal care and welfare and may include board members, research associates, conservation partners, or local experts from related fields. The committee is led by a chairperson, usually nominated by an executive level staff member, who is responsible for committee organization, documentation, and delegation. Other appointed or nominated positions, such as vice-chair, may also exist, based on the institutional structure. Membership of the committee may be permanent or on a rotational basis. Meetings are generally held quarterly, though subcommittee or task force meetings and ad hoc meetings to address welfare concerns may be held more frequently as needed.

One of the most important functions of the welfare committee is to respond to welfare concerns through a formal complaint driven system that encourages responsible reporting.[27] If a welfare concern is not adequately addressed through the standard chain of command within an animal care department, it may be raised to the welfare committee either through direct communication with one of the committee members or through an anonymous reporting phone line, email account, or online submission form. The system should prompt the reporter to justify the concern and offer potential solutions that are evidence based.[27] Once received, the chair of the animal welfare committee will acknowledge the concern and designate committee members to investigate the complaint through direct observations, conversations with involved staff members, and other necessary means. If a concern cannot be rectified through initial evaluations, an ad hoc animal welfare committee meeting is initiated to investigate further. Following the investigation, and once a consensus is reached on an effective and realistic solution, recommendations will be made to the executive level staff and curators overseeing the animal in question. It is the responsibility of the executive-level staff and curators to put the recommendations into action and to continually work toward a zoo culture that always considers animal welfare in decision-making processes.[27] When a consensus is not able to be reached by the committee, a zoo's executive director or equivalent may be consulted to make a final recommendation. Each step in this process should be thoroughly documented, addressed in a timely manner, and communicated to the initial person filing the complaint. Moreover, animal welfare committees may also consider annual reports or updates to all zoo staff to maintain transparency, although anonymity of the complainants must be maintained, and potentially sensitive details tempered for a general audience. Additional guidance on how best to establish an anonymous reporting system may be found in the Animal Welfare Act Regulations.[28]

As zoos develop their own welfare committees and processes, there are many resources and examples available (Table 12.3). In addition to individual institutions having their own welfare committees, larger management organizations also have welfare committees. The AZA's welfare

TABLE 12.3 Resources for Assessment of Zoo Animal Welfare and Animal Welfare Committee Development

American College of Animal Welfare[44]	www.acaw.org
American Association of Zoo Veterinarians[39]	www.aazv.org
Association of Zoos and Aquariums[25]	www.aza.org
Australian and New Zealand College of Veterinary Scientists—Animal Welfare Chapter[46]	www.anzcvs.org.au
British and Irish Association of Zoos and Aquarium[53]	www.biaza.org.uk
Chicago Zoological Society's Center for the Science of Animal Welfare[30]	www.czs.org
Detroit Zoological Society's Center for Zoo Animal Welfare[31]	www.czaw.org
European Association of Zoos and Aquaria[32]	www.eaza.net
European Association of Zoo and Wildlife Veterinarians[40]	www.eazwv.site-ym.com
European College of Animal Welfare and Behavioral Management[48]	www.ecawbm.com
San Diego Zoo Global Academy: Animal Welfare Course[43]	www.sdzglobalacademy.org
United States Department of Agriculture Animal Welfare Information Center[54]	www.nal.usda.gov
World Association of Zoos and Aquariums[1]	www.waza.org
World Organization for Animal Health (OIE)[49]	www.oie.int
Zoo Aquarium Association[55]	www.zooaquarium.org.au
Zoological Association of America[56]	www.zaa.org

committee mission is to promote good welfare for animals in AZA-accredited zoos, by assisting member institutions in identifying and applying best practices in animal welfare, and through promoting advances in animal science. It achieves its mission by promoting a common understanding of animal welfare in the zoo and aquarium community; assisting zoos and aquariums in identifying and applying best practices in animal welfare; encouraging the development of research projects and assessment tools to advance and monitor animal welfare; educating and engaging AZA zoos in applying assessment tools; and understanding and influencing public perception about animal welfare in AZA zoos.[25,26] The AZA also offers support for and review of animal welfare considerations for in situ, ex situ, and field conservation work.[29] In the United States, there are centers that serve to perform zoo animal welfare research and to disseminate the findings.[30,31] These are supported by AZA and work to identify general needs and support progress across North American zoos and aquariums. EAZA has an animal welfare training officer and has developed an animal welfare working group, both of which are intended to support the initiatives of all EAZA members aimed at reaching high standards of animal welfare.[32] On a global scale, WAZA has developed an Animal Welfare Strategy to provide guidance on how to establish and maintain acceptable animal welfare standards and related best practices. It provides 58 recommendations in nine strategic areas, including: animal welfare and its assessment; monitoring and management of animal welfare; environmental enrichment; exhibit design, breeding programs, and collection planning; conservation welfare; animal welfare research; partnerships in animal welfare; and engagement and interaction with visitors.[1] These resources should be consulted, along with fellow institutions, when developing a new animal welfare committee or process.

Animal Welfare Departments

In addition to animal welfare committees, some zoos employee animal welfare directors within an animal welfare department or institute to oversee animal welfare management, and to serve as a resource for animal care staff within the zoo and for the greater zoo community. This role varies greatly between institutions but focuses on maintaining high standards of animal welfare, as defined by the institution. These departments may include oversight of animal training, behavioral husbandry, enrichment, research review, and technical support. Moreover, these departments may also perform routine welfare assessments for animals in conjunction with the curatorial and veterinary staff, review special events hosted by the zoo and the impact on animal welfare, and organize and conduct prospective studies. Behavioral research through this department is a tool that can measure welfare outputs, and the results aid in the development of a database for making evidence-based decisions about animal care.[27]

Veterinarian's Role in Zoo Animal Welfare

Veterinarians are knowledgeable animal care professionals, who have advanced medical training, scientific understanding, and communication abilities to be animal welfare advocates and leaders within zoos.[34–36] Moreover, veterinarians may work under a legal mandate to have institutional authority to oversee animal care and welfare, as with the Animal Welfare Act enacted by the US Department of Agriculture.[28] It is important to have veterinarians contribute to welfare assessments and participate in welfare committees, in addition to assessing life-long medical needs, guiding hospice and end-of-life decisions, and evaluating and interpreting postmortem information (see Chapter 15). It is the responsibility of the veterinarian to implement existing welfare standards and strive for continued improvements that are informed by medicine, behavior, ecology, and ethics.[37,38] The veterinarian is committed to the role as the animal's advocate but may also have obligations to the institution, caregivers, peers within the profession, the public, and himself or herself. The veterinarian may face challenges when these commitments are conflicting. On a larger scale, zoological veterinarians should be represented as stakeholders in the development of policies aimed at addressing welfare and should contribute to professional organization welfare committees and working groups.[25,34,39,40]

Focused education in animal welfare is expanding through integration into veterinary curriculum and opportunities for continued education in animal welfare exists across all taxa.[38,41–43] The American College of Animal Welfare (ACAW) is a specialty organization focused on offering training in animal welfare and board certification following either a formalized training program or experiential pathway.[44,45] The Australian College of Veterinary Scientists has an Animal Welfare Chapter that offers training and certification.[46] The Royal College of Veterinary Surgeons recognizes animal welfare science, ethics, and law as a specialty subject area and offers credentials in the form of diplomas and certificates.[47] The European College on Animal Welfare and Behavioral Medicine offers a subspecialty in Animal Welfare Science, Ethics, and Law.[48]

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13

Stress and Animal Welfare—Endocrinological Evaluation

CORINNE P. KOZLOWSKI

The selection of appropriate and objective measures to monitor animal welfare is a priority for the zoo community. Glucocorticoid (GC) concentrations are a commonly used physiologic metric for evaluating stress. This chapter presents an overview of the stress response and methods for quantifying GCs from captive wildlife, with considerations for sampling and data interpretation.

Overview of the Stress Response

Stress causes the hypothalamic-pituitary-adrenal (HPA) axis to mediate behavioral and physiologic changes that allow an individual to respond to a perceived challenge. Neurons in the hypothalamus release corticotropin-releasing hormone (CRH), which acts on receptors in the anterior pituitary and stimulates the release of adrenocorticotropic hormone (ACTH) into the bloodstream. ACTH then causes the release of GCs (cortisol in most mammals and fish; corticosterone in amphibians, reptiles, birds, and rodents) from the adrenal cortex. Elevated production of GCs increases energy availability and oxygen intake, enhances sensory function and memory, and decreases pain perception. Blood flow is reduced to organs not needed for movement, and processes not immediately related to survival (e.g. digestion, growth, immune function, and reproduction) are inhibited. These changes are adaptive in the short term but when prolonged, they are associated with negative impacts on the neurologic, cardiovascular, immune, and reproductive systems. Fig. 13.1 illustrates GC patterns associated with acute and chronic stress responses.

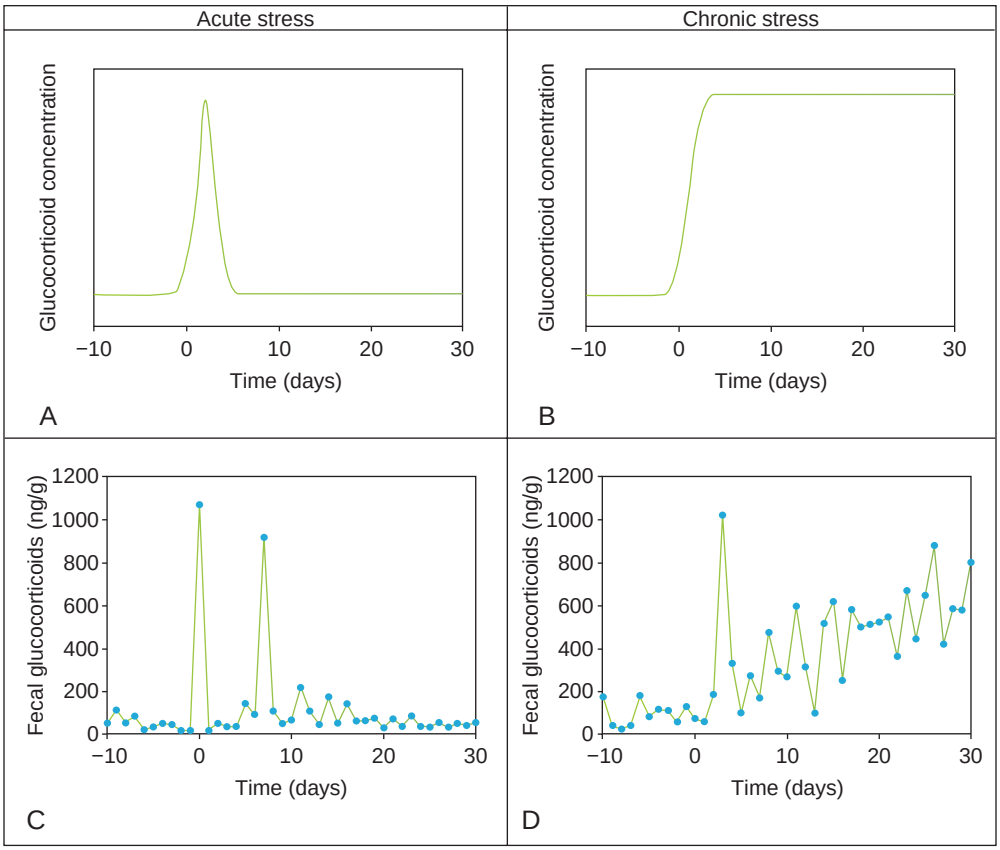
In general, long-term increases in GC production may be evidence of compromised welfare.[1,2] However, adrenal responses also occur during beneficial behaviors that require physical activity, including mating behavior and copulation,[3] play sessions,[4] and responses to environmental enrichment.[5] This may make it challenging to differentiate between adaptive responses and those signaling genuine stress. In addition, individuals of the same species may also differ in their responses as a result of differences in temperament[6] and previous experiences.[1] Therefore, measuring a range of behavioral, health, and physiological responses is recommended to ensure that endocrine data are interpreted correctly.

Types of Samples Used for GC Assessment

GCs may be measured in a variety of sample types (blood, saliva, urine, feces, hair, and feathers), depending on the information required and the feasibility of collection, particularly when repeated sampling over extended periods is necessary. Blood samples are difficult to obtain without inducing GC production, as handling, physical restraint, and collection procedures may elicit an adrenal response.[7] Saliva samples are less invasive, but regular sample collections may require substantial training. Furthermore, blood and saliva samples are point samples that represent only a short duration of time.[7,8] Conclusions based on these samples may be inaccurate as GCs are affected by circadian rhythms and exhibit daily fluctuations.

Monitoring adrenal activity through analysis of fecal or urinary GCs is advantageous because an animal's behavior and adrenal activity are not affected by the noninvasive collection process. Repeated sampling from individuals is possible, allowing for long-term monitoring of hormone changes. Samples may furthermore be collected from socially housed animals without needing to separate individuals. Fecal and urine samples are also not as strongly impacted by pulsatile secretion and circadian rhythms, and instead represent an integrated measure of perceived stressors and resulting adrenal activity. However, knowledge of the lag-time between when a stressor is experienced and the appearance of a signal is required for proper data interpretation. For feces, the lag-time depends on the intestinal transit time from the duodenum to the rectum and varies considerably among species. Values typically range from 6–48 hours.[9] The lag-time is shorter for urine samples, generally ranging from 2–14 hours.[9]

Assessing GC production in hair and feathers, sample types not influenced by circadian rhythms, has the benefit of easy collection and long-term stability. Free GCs diffuse



• **Figure 13.1** Theoretical acute (A) and chronic (B) stress responses, as assessed by glucocorticoid measures. Day 0 indicates the day a stressor was experienced. Acute stress is characterized by a short-term increase in glucocorticoids above an individual's baseline followed by a relatively rapid return to baseline levels, whereas a chronic stress response occurs when concentrations remain elevated above baseline for a significant length of time. The bottom panels present fecal glucocorticoid profiles from two cheetahs, illustrating an acute and chronic stress response. One individual (C) experienced two temporary increases in fecal glucocorticoids after arriving at a new institution. The second individual (D) experienced a prolonged increase in fecal glucocorticoids following transfer to a new habitat. Note that glucocorticoid concentrations in fecal material are typically more variable than blood measures, and proper interpretation requires knowledge of individual baseline values.

into hair through the root, which is supplied by capillaries, and through the highly vascularized base from which feathers originate. While hair grows slowly and is typically collected to assess the impact of stressors over the course of weeks to months,[10] feathers have bands that correspond to daily growth cycles that may be used to provide a record of GC production over several days or weeks.[11] However, little is known about the contribution of glandular secretions (sweat, sebum, or other scents) to the GC content of hair and feathers, and an understanding of the growth rate is required for proper data interpretation.

Validation Procedures

Proper validation involves determining whether sample storage and extraction procedures are appropriate, whether the immunoassay antibodies may detect the metabolites in the samples (for feces and urine), and whether the assay system detects biologically meaningful changes in GC production. A physiologic validation, also known as a challenge test, involves pharmacologically inducing an increase in plasma GC levels through injection of ACTH.[12] Samples collected before and after injection are analyzed to confirm that increased GC concentrations are detected following ACTH administration. An ACTH challenge test may not be possible with endangered or difficult species. A biological validation, serial sampling before and after a known stressful event (e.g., a capture, medical procedure, or transfer), may instead be performed in these situations.[1]

For any validation, it is important to consider the number and ages of individuals tested and to include both sexes, as baseline and peak concentrations of GCs may vary.[13] Females tend to have higher GC concentrations, possibly as a result of differences in the affinity of steroid-binding globulins.[14] Time of year and reproductive status should also be considered, as concentrations for some species are higher during the breeding season,[13] and values for females may be affected by phase of the estrous cycle,[15] pregnancy, or lactation.[12] To minimize these effects, each animal should serve as its own control during a validation, as well as during studies assessing individual welfare.

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14

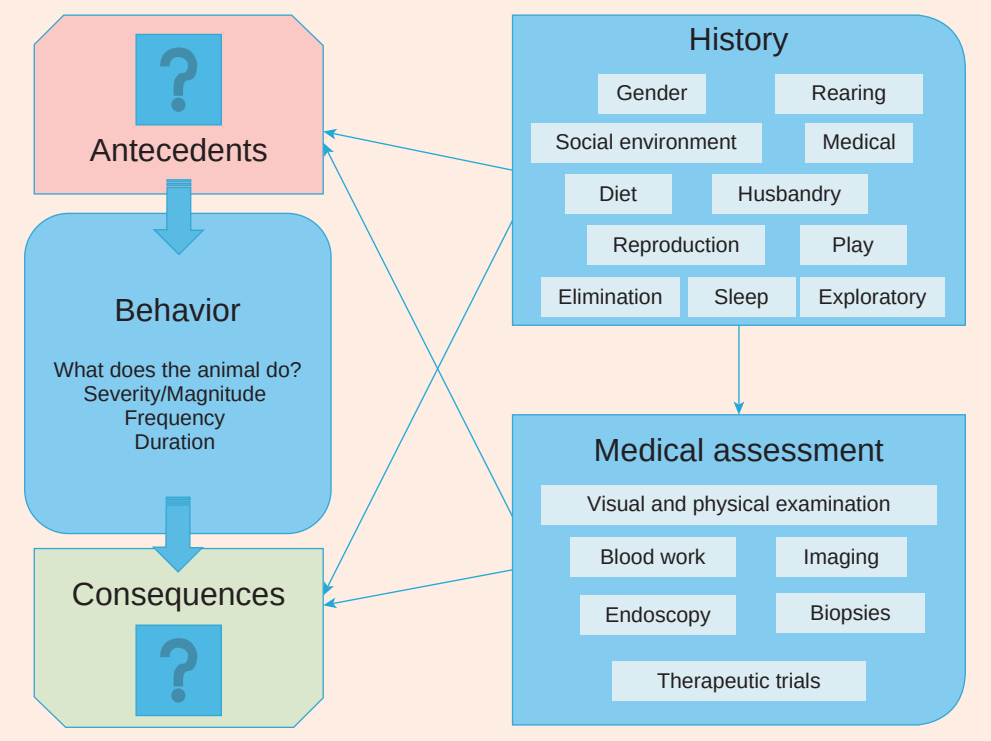
A Systematic Approach in Diagnosing Behavior Problems

MARION RENÉE DESMARCHELIER

One currently accepted definition of behavior states that "behavior is the internally coordinated responses (actions or inactions) of whole living organisms (individuals or groups) to internal and/or external stimuli, excluding responses more easily understood as developmental changes."[1] Behavior is about what the animal *does*, how it acts (or not) in response to the environment. It is not about how an animal *is*, because this would require inferences and anthropomorphic interpretations that might limit the capacity to efficiently solve behavior problems.[2,3] As every living organism constantly displays behaviors, issues with behavior may arise in any species, from invertebrates to great apes, and include a large variety of situations that are often very challenging for zoo veterinarians and managers. From inappropriate vocalization to self-mutilation, veterinarians may be consulted to help solve cases in which the animal is acting in an unexpected manner for the current context. As for any other disorder, it is important to first establish a differential diagnosis list. Unexpected behaviors are often better considered as clinical signs rather than a specific problem entity. Just as weight loss or hematuria may have multiple causes, behaviors such as feather picking or pacing may result from numerous different processes that require tailored treatment regimens. For example, pacing associated with an inappropriate physical environment will be treated differently from pacing associated with gastric pain. Behaviors are the results of complex interactions of genetic, epigenetic (factors affecting gene expression resulting in phenotypes) and environmental factors.[4] However, with a systematic approach, it is generally possible to list a number of potential causes or diagnoses for a given behavior, and solutions to appropriately address the problem. The first important step is the observation and description of the animal's behaviors in a variety of circumstances, including the problematic situation. All behaviors of the animal involved, as well as those of conspecifics or other individuals, are often useful to better understand the social and environmental contexts of the unexpected behavior. Once the problematic behavior has been identified, a functional assessment aiming to identify what happens before (antecedents) and after the behavior (consequences) is central to identifying potential etiologies and solutions. To comprehensively describe these key antecedents and consequences, two further steps are undertaken (Fig. 14.1). Obtaining a thorough, systematic, and detailed history of the case is critically important as more than one factor is generally involved. Additionally, a complete physical examination of the animal should be performed, as underlying medical conditions might be overlooked when assessing behavior problems in zoo animals.

Applied Behavior Analysis

Every behavior has a function.[3] Therefore a detailed description of the problematic behavior is essential. Ideally, the problematic behavior, as well as bouts of normal behavior from the affected individual, should be observed directly. Video recordings may also be used but do not always allow for optimal understanding of the entire situation. The problematic behavior needs to be described accurately using the animal's body language (i.e., the jaguar is licking his tail, his pupils are dilated, he is growling, his body is stiff, etc.) and not labels that include many different interpretations (i.e., the jaguar is stressed, he is aggressive, he does not tolerate this situation, he is territorial, he is dominant, etc.). Many details of the animal's behavior including body language must be collected during this process. Using specific behavior descriptions instead of inferences or human interpretations allows objective measurements and greatly improves communication among observers. In several species, we may differentiate between behaviors of "normal" animals that may be explained or understood within the context of the situation and "abnormal" behaviors in "sick" animals that are more likely associated with a medical condition such as an anxiety disorder. Description of the behavior should include details on frequency and duration as they may have a bearing on the diagnosis. For example, a cockatoo screaming once a day for 30 seconds is more likely to be normal than a cockatoo screaming 50 times a day for 10 minutes at a time. Magnitude or severity



• **Figure 14.1** A systematic approach to behavior problems.

of the behavior should be well documented, as it will be useful for both determining the diagnosis and monitoring the case progression. A problematic behavior will rarely disappear instantly with treatment, but might progressively reduce in frequency, duration, and magnitude/severity. The functional assessment of the problematic situation should also include analysis of what contributes to the behavior. Antecedents include everything functionally related to the behavior that occurs *before* the problematic behavior, such as the context, the potential triggers, and/or environmental stimuli. Antecedents influence the likelihood that a specific behavior will occur, and their identification may help determine the diagnosis.[2] For example, when looking at a circling behavior in a male hippopotamus, the identified antecedents could be the female charging the male when he walks toward the hay. In this example, working on the antecedents by addressing the female's behavior is part of the solution to resolving the male's abnormal behavior. Consequences include what happens immediately *after* the behavior and provide environmental feedback to the animal on what the behavior just performed actually reaped.[3] Consequences influence whether the behavior will be repeated or not in the future. By observing the consequences of a specific behavior, one may generally predict if it will increase or decrease in the future. Continuing the aforementioned hippopotamus example, the consequences of the female behavior (charging the male) are that the male goes away and that she acquires more hay to consume. These are positive and desirable outcomes, making her behavior more likely in the future. Therefore, behavior analysis, through a well-framed functional assessment of the situation, provides essential data to understand the causes of the problematic behavior and often offers a path to the most appropriate intervention and therapeutic plan. To better identify distant and immediate functional antecedents as well as consequences that may be external or internal (such as pain), taking a systematic history and performing a thorough medical assessment are subsequent, crucial steps.

Comprehensive Behavioral History

It is essential to gather basic signalment information, including gender and reproductive status, and a good history to verify the accuracy of information in the medical records. Misidentification of the gender, whether by human or laboratory error, may obviously lead to misinterpretation of behaviors. As few methods have shown 100% accuracy, the gender of nonsexually dimorphic species may be confirmed by combining methods appropriate to the age, species, and condition of the animal. If contraception has been used, details on the surgical technique or chemical protocol administered should be provided. Vasectomy (and its potential failure) versus castration, or the use of contraceptive hormones versus immunocontraceptive agents, will affect both sexual and nonsexual (i.e., social interaction, appetite, activity, etc.) behaviors in very different ways.[5–7] Though still poorly understood in zoo animals, contraceptive agents may have an impact on mood disorders in humans and could be a concern in nonhuman primates.[7,8] Use of temporary or permanent methods to prevent certain

species-typical behaviors, such as onychectomy, wing trimming, or amputation, may have behavioral effects that have been well documented in pet species and should be noted in the history procurement.[9] Previous medical and surgical history should be collected to focus the physical examination and assist in choosing any other appropriate diagnostics. Reviewing keepers' notes may deliver critical information, as some minor, intermittent clinical signs, such as chronic vomiting, might not have been considered as warranting further investigation when initially detected. Wild versus captive birth, rearing methods, and environment are critical factors influencing behavior throughout the life of the animal.[10,11] Human-rearing, maternal separation, and early stress have been shown to negatively, but not always irreversibly, impair the behavior of many different species, from great apes to starlings.[12–24] Early environmental conditions have also been shown to affect reptile and fish behavior.[25,26] As these history factors cannot be altered in adult animals, knowledge of their existence is of little help to solve the observed behavior problem; however, their recognition might enable caretaker staff to make more informed future choices that will aid in the prevention of similar behavior problems. Social environment should be carefully evaluated when assessing an animal for a behavioral issue, as the identified individual may be the victim in a conflict situation. The animal could also be the aggressor, causing severe group stress with subsequent behavioral abnormalities in other individuals. Although individual isolation in a social species may have severe consequences, group housing for solitary species, altering social structures to the needs of captive breeding programs, and retention of offspring with their parents for longer than natural periods may also lead to behavior problems.[11,27–32] An appropriate social structure favors normal behavior in all species, including aquatic species.[33–35] Interactions with other species should be considered, even if only visual or olfactory.[11] The effect of human presence, keepers or visitors, on zoo animals has been studied in various situations, from routine presence around the enclosure to active interactions. The influence on the animal's behavior, whether positive (e.g., through training), neutral, or negative (e.g., by disrupting normal activities) is often very important as it contributes to either chronic stress or daily enrichment.[36–43] Reproductive history, current status, and future breeding plans must be reviewed even when behavior surrounding reproduction is not the main concern. Reproductive failure may be associated with chronic environmental or social stressors and should be considered as another clinical sign.[11,35,44,45] Maternal neglect has been related to chronic stress (i.e., oxytocin inhibited by stress) and hand-rearing (e.g., in felids).[21,22,46] Abnormal behaviors affecting successful breeding are also seen in some anxiety disorders.[47] The current physical environment and husbandry practices should be carefully reviewed due to their primary importance in the development and maintenance of abnormal behaviors in captive animals. Use of more naturalistic and enriched habitats allows the animals to display more species-typical behaviors and may reduce stereotyped and self-directed behaviors in certain cases.[48,49] Appropriate designs, for example with the use of visual barriers and hiding places, or by increasing size and environmental complexity, may help decrease some problem behaviors.[32,50–52] Water quality and the ratio of water to land should be reviewed for aquatic or semi-aquatic species.[35] Subtle behavior changes, such as blepharospasm in case of problems with sterilization systems in marine mammal pools or in aquaria, will often precede more obvious clinical signs.[53] Exposure to natural weather and to appropriate light type and photoperiod also decreases stress in several species.[42,54] Time spent on-exhibit versus off-exhibit and indoors versus outdoors may also shape problematic behaviors.[55,56] Daily routines, from keeper's presence to feeding schedule, should be evaluated in relation to the problem's occurrence. Fixed feeding and public presentation schedules may provoke anticipatory behaviors.[55,57–59] Food types and feeding methods in captivity might interfere with normal behavior, whether related to the feeding location (grazing on the ground versus eating from a high rock), quality (natural browse versus pellets, varying size of food items), quantity (one meal vs. foraging all day), and delivery (in a bowl vs. on the ground).[55,56,60] Feeding methods stimulating natural behaviors such as foraging are less likely to be associated with behavior problems.[61,62] Other factors that need to be investigated when gathering information about a behavior case include elimination, sleep, play, and exploratory behaviors. In many species, elimination behaviors provide useful information on social interactions, territorial use, and possible medical or anxiety disorders. For example, urine-spraying has been linked to anxiety and secondary aggression in felids.[29] Other forms of marking behaviors, such as rubbing their chest on various items, may be relevant in certain situations.[63] Sleep behavior, as well as nocturnal activities, most commonly recorded through video recordings using infrared light, may add a lot to the history by showing if the abnormal behaviors are present or not at night and if the normal sleeping and resting time is disrupted or compatible with normal ranges.[29,32,64] Exploratory behavior, an important part of the activity budget in some species, is often limited in captive settings.[59] Play is another very important part of the normal repertoire in many species and is considered by some authors as an indicator of welfare. Changes in its normal pattern are worth noting.[33,43,65,66] Finally, reviewing the enrichment protocol is important to have a complete picture of the animal's environment. Enrichment is designed to promote species-specific behaviors, should be encouraged for all classes of animals including invertebrates and fish, and has been shown to help decrease abnormal behaviors in some situations.[67–71]

By performing this attentive review of the animal's history, several potential causative factors for the behavioral problem may already be identified. In tandem, ideas for solutions and improvement of the animal's social and physical environments will likely arise from this detailed analysis.

Thorough Physical Examination

With the exception of a few bird species, communication between animals and their caretakers is nonverbal. Hence, only a careful observation of the animal's behavior may help us detect the presence of physical discomfort or mental distress. As veterinarians, the primary role when addressing behavior problems is to eliminate all potential medical causes for the problematic behavior. Studies attempting to find a treatment for a group of behavior problems such as feather picking in parrots or pacing in cats usually fail at finding a solution that works for every case. One of the reasons is that the diagnoses underlying these behaviors and the function of the behavior for each individual may vary. When a bornavirus infection is present and the bird picks at its feathers possibly as a sign of discomfort, no environmental enrichment or antidepressant medication will help decrease the behavior. Some behaviors are well-recognized signs of pain or underlying disease, but others are less commonly recognized (Table 14.1). For instance, fly biting behaviors and licking of surfaces have been associated with gastrointestinal diseases in some dogs.[72] Treatment of the underlying medical condition resulted in resolution of the abnormal behaviors in these dogs. Though not yet well described in the zoo animal literature, it is likely that what is seen in domestic animals is applicable to similar species of wild animals. Some behaviors may seem unrelated to a specific disease but are in fact the manner in which the animal reacts to pain, even if the function of the "abnormal" behavior remains unclear.[72] Examples of behaviors possibly associated with pain include growling, biting, lunging, scratching, licking, feather or hair picking, pacing, and vocalizing.[2,72] So-called stereotypic and compulsive behaviors must also be investigated for signs of underlying medical conditions, as many cases have been successfully treated once a medical diagnosis was established.

TABLE 14.1 Examples of Behaviors Compatible With Physical or Mental Diseases

Behaviors	Systems Affected
Abnormal elimination (rubbing urine on body, etc.)	Urin/Neuro/Endocrino/GI/Psych
Aggressive behaviors (growling, lunging, etc.)	Pain/Neuro/Endocrino/Psych
Circling/pacing/increased motor activity	Pain/Neuro/Repro/GI/Psych
Flank/tail/leg sucking or licking	GI/Dermato/Neuro
Fly biting/head extensions	GI/Neuro/Ophthalmo
Hair plucking/feather picking	Pain/GI/Dermato/Neuro/Psych
Pica	GI/Neuro
Prolonged piloerection	Psych
Restlessness	Pain/Endocrino/Neuro/GI/Psych
Self-mutilations	Pain/Dermato/Neuro/Psych
Star gazing	Neuro/GI/Pain/Psych

Dermato: skin sensitivity, allergies, parasites, etc.; Endocrino: hypo- and hyperthyroidism, diabetes, Cushing, etc.; GI: gastrointestinal, IBD: irritable bowel disease, dental diseases, pancreatitis, etc.; Neuro: congenital, cognitive dysfunction, neoplasia, etc.; Psych: Anxiety disorders, compulsive disorders, etc.; Urin: idiopathic cystitis, infections, etc.; Repro: uterine neoplasia, mastitis, ovarian cysts, etc.

Behavior is likewise influenced by genetic and epigenetic aspects through the expression of different genes at the neuronal level. Interaction of hormones and neurotransmitters is very complex, and as with any other organ, malfunction, whether from birth or acquired later, may occur. Psychiatry in zoo animals is still in its infancy and mainly extrapolates knowledge and experience from human and domestic animal medicine. Psychiatric diagnoses are controversial in most species and will likely remain so due to the inherent complexity of measuring the parameters involved in "mental" (neurochemical) diseases. However, in veterinary medicine, we may refer to anxiety disorders with some imperfect, but objective, assessment tools. Anxiety in veterinary medicine is defined as the anticipation of a future threat or danger (real or imaginary). While fear is appropriate when confronted with real dangers, anxiety becomes a disease if the perceived threat is imaginary and if it impairs the expression of normal behaviors and adaptive responses.[73] Animals with neurotransmitter disorders (anxiety or other "mental" diseases) may behave in what we often interpret as aberrant ways: performing painful or apparently pointless behaviors (i.e., self-mutilations) or attacking individuals that had no prior interaction with them (i.e., redirected aggression). According to human psychiatry, it is possible that these animals perceive a distorted reality that impacts their behavior. In parallel, fear and anxiety significantly interfere with learning processes in the brain, which explains why abnormally fearful animals may not be easily trained, despite possibly normal to superior cognitive skills. Though a rare occurrence, recognizing "mental" diseases in zoo animals may be challenging. These patients might require an appropriate medication to help balance their brain neurochemistry and permit them to learn and function again in a way similar to their conspecifics. Clinical signs suggestive of a "mental" illness such as anxiety disorders in zoo animals might include: startling even at routine noises, not being able to habituate to new objects or new situations, prolonged or frequent piloerection, incapacity to relax and sleep, prolonged recovery after stress, redirected aggression, etc.[73] Normal arousal and vigilance levels highly vary across species, so comparing an individual to its group will help identify if the behavior is atypical.

Finally, the information gathered through the three steps described (applied behavior analysis, history, and physical

examination) must be analyzed simultaneously as many elements are intrinsically related. Stress from the environment may cause gastric ulcers with secondary vomiting and growling at conspecifics. Treating both the gastric ulcers and addressing the environmental concerns is more likely to resolve the abnormal aggressive behaviors than treating one or the other clinical sign. In addition, social status may affect significant changes in the type of immune response in nonhuman primates.[74] Communication between the brain and neurons of the gastrointestinal tract has now been well-described in humans, explaining the long-time observed association between mental and gastrointestinal diseases.[75] From an observer's vantage, behavior is simply the result of the animal interfacing with its environment. Behavior is in fact the consequence of a complex and interconnected web of genes, their level of expression, and resulting physiology, affecting both perception and actions, in a constantly changing environment. Regardless of the approach (ecology, genomics, etc.), animal behavior remains a fascinating topic and needs further objective, nonanthropomorphic studies. There are always individuals behaving differently from the majority. These differences may help define which behaviors are considered "inappropriate" or "abnormal" for a species. Through a systematic approach to these behavior "problems," we will acquire a better understanding of the underlying genetic, epigenetic, and environmental factors that contribute to an animal exhibiting unexpected behaviors. Obtaining a final diagnosis is not always feasible, but as we identify the potential origins of the problem, we will also find viable solutions. Behavior problems are challenging for all including the affected animals, their caregivers, conspecifics (i.e., in cases of redirected aggression), and management dealing with visitor complaints. Addressing behavior problems with an inclusive approach, in cooperation with the veterinary team, keepers, and managers, is therefore an important step in improving animal welfare in zoo animals.

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15

Quality-of-Life Assessment and End-of-Life Planning for Geriatric Zoo Animals

LARRY VOGELNEST AND JESSICA J. TALBOT

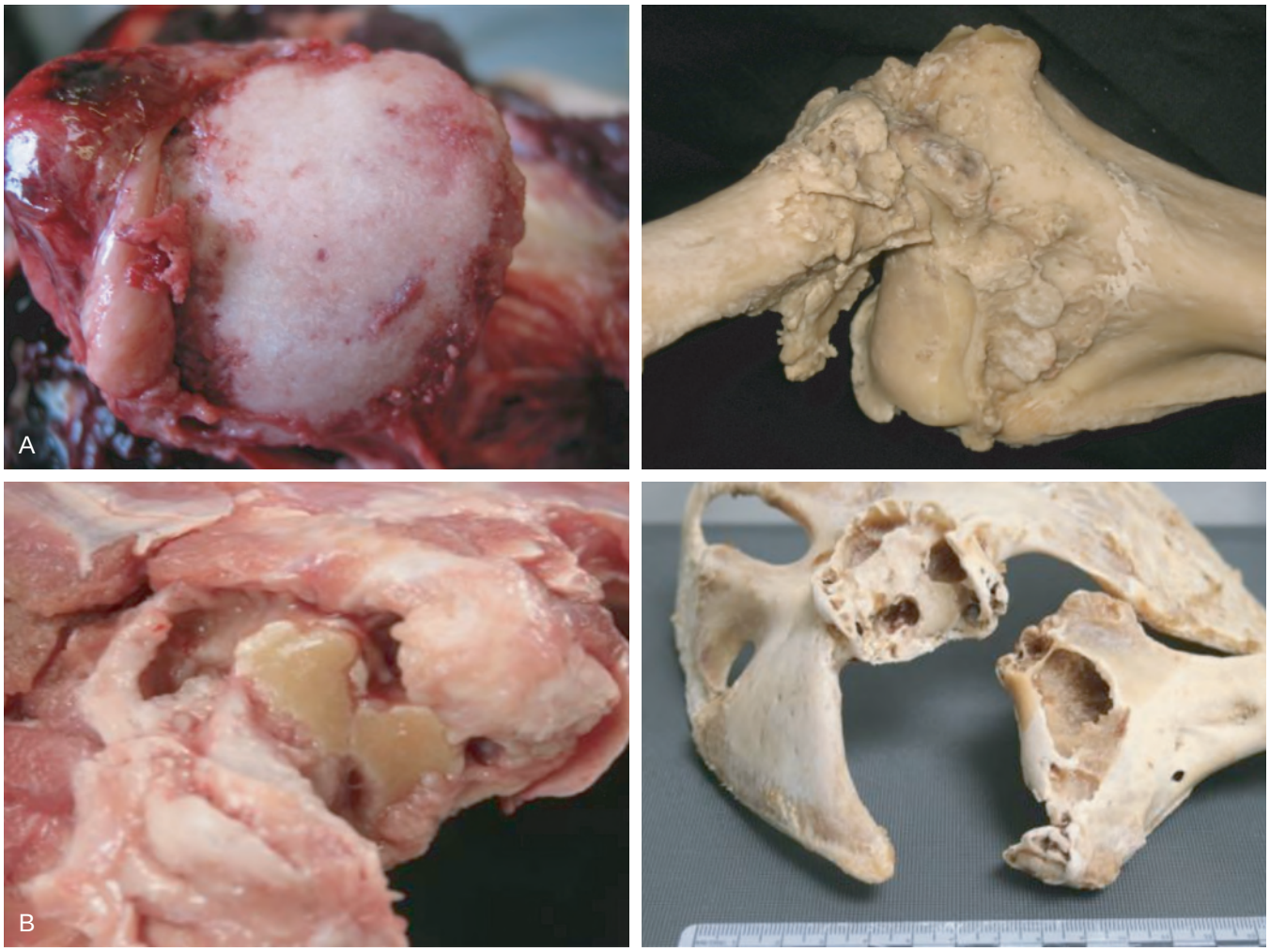
Introduction

Advances in veterinary care, husbandry, and nutrition; lack of predators, trauma, and disease; combined with low stress environments, means that zoo animals are living beyond the average age of their wild counterparts.[1] This has resulted in an increase in the number of animals in our care with age-related health conditions (Table 15.1 and Figs. 15.1 and 15.2).[1–3] These conditions are often painful and incurable and present welfare concerns due to reduced quality of life. Necropsies performed on euthanized aged zoo animals often show advanced musculoskeletal, dental, and organ pathology, indicating that degenerative processes have commenced long before clinical signs become apparent.[4] Recognition of these clinical signs in zoo animals is often difficult due to the cryptic behavior of some species and the desire to mask signs of illness also known as the "preservation response." It is therefore incumbent on those caring for aging zoo animals to implement assessment processes that facilitate early detection of signs associated with age-related degenerative processes.[5] This will then guide appropriate care and direct end-of-life planning to ensure positive welfare outcomes.

Human and companion animal hospice and palliative care models may provide frameworks for measuring quality of life of zoo animals. Human models rely heavily on relief of symptoms, focusing on optimal patient care for the terminally ill to provide a good death.[2,6] This is aided by the use of health-related quality-of-life (HRQoL) assessment tools to measure physical and psychosocial factors in patients (e.g., EuroQol,[7] AQol,[8] Sickness Impact Profile[9]), and genogram models that assess family relationship dynamics for familial and patient support.[10,11] Quality-of-life assessment models have also been used to help companion animal veterinarians formulate end-of-life plans with pet owners (e.g., the HHHHHMM [hurt, hunger, hydration, hygiene, happiness, mobility, more good days than bad] technique,[12] affect-balance model,[13] and the Five Freedoms[14]). In companion animal palliative care, veterinarians educate, support, and guide pet owners to make end-of-life decisions.[2,13,15] This may be adapted for zoo settings, where key stakeholders assume particular roles for decision making (Table 15.2). However, provision of care in zoo animals presents unique challenges. As most zoo animals maintain wild behaviors, the opportunity to provide hospice and palliative care is often limited compared with what may be provided for humans and companion animals. Attempts at treatment, changing environment, providing enrichment, and veterinary intervention are often stressful and, despite good intentions, may in fact negatively impact welfare. We must therefore always accept that good welfare and quality of life, not length of life, are the aim and measure of our animal care.[16,17]

In formulating quality-of-life assessment tools, the psychologic and physical well-being of the animal should be the main focus.[18] Quality-of-life assessment checklists focusing on behavioral characteristics, physical signs, and clinicopathological findings have been developed for zoo animals (Fig. 15.3).[4,19] When assessing aged animals for quality of life, criteria should always be considered in comparison with an animal of the same species in its prime. In addition, response to any medical treatment, curatorial, and logistical imperatives, relevant species-specific criteria (e.g., social vs. solitary species, role and position within a social hierarchy), and population welfare versus individual welfare should be considered and assessed (Fig. 15.3). With this information, key stakeholders may make objective decisions on quality of life that facilitate an individualized welfare-focused end-of-life management plan.

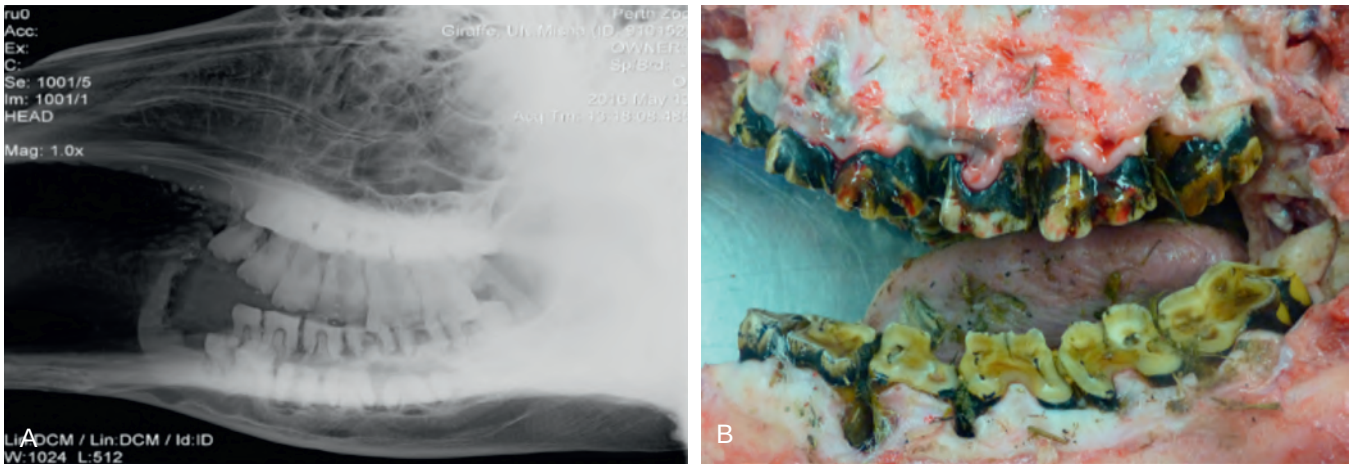
The implementation of these processes also helps defend euthanasia decisions when warranted, by promoting good animal care and welfare. They also educate and assist staff in preparing for an animal's death and euthanasia. It is inevitable, due to the human–animal bond, that zoo staff



• **Figure 15.1** Advanced degenerative joint disease with complete loss of articular cartilage, eburnation, extensive osteophytosis, enthesopathy, and bone cyst formation: (A) Kodiak bears *(Ursus arctos middendorffi)* aged 28.4 years *(left)* and 31.2 years *(right)* at time of euthanasia; and (B) Komodo Dragon *(Varanus komodoensis)* aged 32.8 years at time of euthanasia. (Photo credits: Taronga Zoo.)

TABLE 15.1 Age-Related Health Conditions in Zoo Animals by Body System

Body System	Health Condition
Musculoskeletal	Degenerative joint disease (mono or polyarticular), muscle atrophy, osteophytes, enthesopathy, intervertebral disc disease, degenerative spondyloarthrosis, ankylosing spondylosis[1,26,28,29]
Digestive tract	Oral fistulae, dental attrition, dental fractures and loss, dental abscess, dental resorptive lesions, periodontitis, inflammatory bowel disease, hepatopathy, hepatic lipidosis[1,3,26,28,30]
Urogenital	Renal disease, reproductive senescence, neoplasia, ovarian and uterine cysts, endometrial changes, pyometra[1,3,26,30-32]
Neurological	Dementia,[1] degenerative leukoencephalopathy, degenerative myelopathy[33]
Ophthalmic	Lenticular sclerosis, ocular hypertension, glaucoma, cataracts
Integument	Ulceration, infection
Cardiovascular	Myocardial disease, degenerative valvular disease, cerebrovascular disease[3,28,31,32]
Endocrine	Thyroid hyperplasia, hyperthyroidism[34]
Multi-system	Neoplasia, obesity, chronic inflammation[31,32]



• **Figure 15.2** Radiographs (A) and necropsy images (B) of a northern giraffe *(Giraffa camelopardalis)*, aged 26 years at time of euthanasia, showing advanced periodontal dental disease with attrition.

TABLE 15.2 Suggested Roles for Key Stakeholders Providing Geriatric Zoo Animal End-of-Life Care

Key Stakeholder	Role
Veterinary team	Disease diagnosis Collection of objective clinical data Determining appropriate treatment plan to relieve pain Education of zookeepers on age-related disease and recognition of clinical signs Education of other key stakeholders (media team, zoo management, curators) Perform euthanasia Post-mortem examination
Curators/collection managers	Species/population management to reflect zoo goals Discuss and evaluate veterinary team's aged animal assessment Seek further information from veterinary team as required
Zookeepers	Monitor animals and report behavioral changes and clinical signs Implement treatment and care plans, e.g., administer daily medications
Media team	Collate public interest information Media management

will form emotional attachments with animals in their care.[20] Empathy and compassion for staff and provision of support and counseling are important considerations.[21] Zoo managers and occupational health workers must also recognize the emotional and psychological effect on veterinary teams caring for sick animals and performing euthanasia and implement mechanisms to manage compassion fatigue. This may include professional training on the recognition of signs of compassion fatigue and promoting self-care techniques.[22,23] It is the responsibility of zoo veterinarians to educate other stakeholders on age-associated health conditions, pain recognition, and discomfort, and to assess the need for euthanasia to prevent and relieve suffering.[6,24] Communicating effectively about degenerative and chronic disease early in the end-of-life planning process helps avoid debate and prepares zoo staff for an animal's euthanasia when warranted.[25] For iconic species or individuals, media team involvement in the process may help prepare and inform the wider community of the death of a zoo animal.[26]

Identification of aged animals in a zoo population is crucial to the process of quality-of-life assessment for these animals. Zoos should develop a methodology and database to identify animals approaching or beyond an average or "expected longevity" for the species. "Expected longevity" for a species may be calculated as the age at which 90% of a species' population within regional species studbooks had died.[5] Reported maximum longevities of zoo animals should be interpreted with caution as these are generally "outliers" and do not accurately reflect "normal" longevity for a species. Additionally, age-related changes are likely to start well before an animal approaches maximum longevity. The use of longevity records for a species in the wild is of little use in identifying aged zoo animals as these are often considerably less than longevity of the same species in zoos.

The stage in an animal's life at which quality-of-life assessment commences should be guided by certain triggers. One could argue that this process should start from

Text continued on p. 90

AGED ZOO ANIMAL ASSESSMENT FORM

				Assessment Date:	
Previous Assessment Date:				Frequency:	
Species:		DOB:		Current Age:	
Local ID:		Sex:		House name:	
Microchip #:				Tag/Band #?	

Expected longevity from studbook data:	
Maximum age in the wild:	
Maximum age in captivity:	

Age Assessment – 100% corresponds to expected longevity from studbook data:

< 80%	–2
80 - 100%	–1
101 - 120%	2
> 120%	4

SCORE

ASSESSMENT CRITERIA

PHYSICAL

0 = normal where relevant

Only chronic conditions are considered, i.e. Have persisted for 3 weeks or more

	SCORE		x	SCORE
Body condition score		0 = ideal, 5 = poor (either thin or obese)	2	
Lameness in 1 limb		1 = mild, 5 = severe		
Lameness in > 1 limb		1 = mild, 5 = severe	2	
Stiffness in 1 limb		1 = mild, 5 = severe		
Stiffness in > 1 limb		1 = mild, 5 = severe	2	
Overgrown nails		If present = 3		
Deformity/swelling of joints		1 = mild, 5 = severe	2	
			TOTAL	

	SCORE		x	SCORE
Lethargy		Listless, drowsy, sleepy 1 = mild, 5 = severe	2	
Mobility		Climbing, jumping, standing up on hind legs 1 = reduced, 5 = severe immobility		
Activity		Time spent exploring, foraging, etc 1 = reduced, 5 = very inactive	2	
Weakness		Lack of muscle strength 1 = mild, 5 = severe	2	
			TOTAL	

	SCORE		x	SCORE
Vomiting		1 = rarely, 5 = often	2	
Diarrhoea		1 = rarely, 5 = often		
Constipation		1 = rarely, 5 = often	2	
Reduced appetite		1 = rarely, 5 = often	2	
			TOTAL	

	SCORE		x	SCORE
Exercise intolerance		Gets tired very quickly after exercise 1 = mild, 5 = severe	2	
Coughing		1 = mild, 5 = severe	2	
			TOTAL	

• **Figure 15.3** Example of a quality-of-life or aged animal assessment tool. The criteria are scored based on a comparison with an animal of the same species in its prime.

Discharges	SCORE		x	SCORE
Eyes		1 = mild, 5 = severe	2	
Ears		1 = mild, 5 = severe		
Mouth		1 = mild, 5 = severe		
Nasal		1 = mild, 5 = severe		
Vagina		1 = mild, 5 = severe		
Cloaca		1 = mild, 5 = severe		
Rectum		1 = mild, 5 = severe	2	
Glands		1 = mild, 5 = severe	2	
			TOTAL	

	SCORE		x	SCORE
Dribbles saliva		1 = mild, 5 = severe	2	
Red, swollen gums		1 = mild, 5 = severe		
Broken, worn or lost teeth		1 = few, 5 = many	2	
Difficulty eating		1 = rarely, 5 = often	2	
			TOTAL	

	SCORE		x	SCORE
Loss of vision		1 = mild, 5 = blind	2	
			TOTAL	

	SCORE		x	SCORE
Excessive urination		1 = mild, 5 = severe	2	
Decreased urination		1 = mild, 5 = severe		
Excessive thirst		1 = mild, 5 = severe	2	
			TOTAL	

	SCORE		x	SCORE
Poor coat/skin condition		Dry coat/skin, fur loss 1 = mild, 5 = severe	2	
Skin ulcers, wounds, infections		1 = mild, 5 = severe	2	
			TOTAL	

	SCORE		x	SCORE
Loss of co-ordination		1 = mild, 5 = severe	2	
Urinary incontinence		1 = mild, 5 = severe		
Faecal incontinence		1 = mild, 5 = severe		
Vagueness		1 = mild, 5 = severe		
Reaction Time		1 = slow, 5 = very slow		
			TOTAL	

RADIOGRAPHIC CHANGES PRESENT AT LAST EXAMINATION — Date:

	SCORE		x	SCORE
DJD - single joint		1 = mild, 5 = severe		
DJD - >1 joint		1 = mild, 5 = severe	2	
Ankylosed joint		Single joint = 5		
Ankylosed joint		> 1 joint = 5	2	
Degenerative spondyloarthrosis		1 = mild, 5 = severe		
Ankylosing spondylosis		1 = mild, 5 = severe	2	
Other (specify)				
			TOTAL	

• **Figure 15.3, cont'd**

Continued

CLINICAL ABNORMALITIES AT LAST EXAMINATION			Date:	
	SCORE		x	SCORE
Anaemia		1 = mild, 5 = severe	2	
Renal impairment		1 = mild, 5 = severe		
Liver impairment		1 = mild, 5 = severe		
Neoplasia		1 = mild, 5 = severe		
Reproductive pathology		1 = mild, 5 = severe		
Other (specify)				
			TOTAL	

MEDICATION	SCORE		x	SCORE
Current treatments		1 = nutraceutical alone, 2 = NSAID alone, 3 = nutraceutical + NSAID, 4 = opioid alone, 5 = all of them together	2	
Other (specify)				
Duration of treatment		1 = 3 months, 2 = 3 to 6 months, 3 = 6 to 12 months, 4 = >12 months		
			TOTAL	

PSYCHOLOGICAL	SCORE		x	SCORE
Self directed behaviour		Directing behaviour towards self (licking, scratching, biting) at one area for a prolonged period. Time spent directing behaviour to be rated as 1 = low, 5 = high		
Self injuries behaviour		When behaviour leads to damage rate 1 = minor damage (e.g. Hair loss), 5 = major damage (e.g. Loss of digits)		
Response to stimuli		Stimuli include weather, visitors, enclosure structures 1 = mild impairments, 5 = not responsive		
Spatial awareness		Ability to navigate around enclosure and use all enclosure furnishings 1 = mild impairment, 5 = limited enclosure use.		
Other (Specify)				
			TOTAL	

SOCIAL (only applies to social species)	SCORE		x	SCORE
Changes in hierarchy		Monitored by change in access to food, shelter, grooming opportunities, contact with others (huddling etc); 1 = minimal changes, 5 = significant change		
Target for aggression / ostracised		Monitored by change in access to food, shelter, grooming opportunities, contact with others (huddling etc); 1 = minimal changes, 5 = significant change		
Change in human - animal relationship		The degree to which the animal responds to the presence or cues provided by keepers, negative response lack of response; 1 = minimal change, 5 = significant change.		
Beneficial role within social structure		1 = significant, 5 = not very beneficial		
			TOTAL	

SPECIES SPECIFIC CONSIDERATIONS
Comments:

• **Figure 15.3, cont'd**

CURATORIAL AND LOGISTICAL CONSIDERATIONS	YES	NO
Specimen's condition elicits significant staff and public concern due to deterioration of physical appearance		
Specimen has inappropriate social behaviours for a social species and cannot be housed with conspecifics i.e. Isolated from group.		
Specimen is of low genetic value to the institution and the region.		
Specimen is held off display.		
Institutional over-population exists and may jeopardize the health and well-being of other zoo animals and avenues for the appropriate placement of surplus animals have been exhausted.		
The progress of an approved Species Management Plan or Population Management Plan is prevented or negatively impacted upon by the inability to maintain the appropriate demographic profile of a population OR due to lack of accommodation resulting from facilities being occupied by individual animals no longer required for the collection of the breeding program.		
Is the presence of this animal inhibiting the implementation of a breeding event spatially or socially?		
Opportunity or sensible to transact this animal to alternative institution?		

RESULTS

Total Score:		Previous score:		Date:	
From 1 to 50	Treatment	Previous score:		Date:	
From 51 To 64	Doubtful	Previous score:		Date:	
Over 65	Euthanasia recommended	Previous score:		Date:	

Increase or decrease since last assessment: _____

Increase or decrease since first assessment: _____

NB: All scores are relative – to be compared with previous condition or responses of the animal or to be compared to a specimen of the species in peak condition.

OTHER COMMENTS

CARE PLAN

Medications:	
Enclosure modifications:	
Enrichment:	
Dietary changes:	
Animal watch requirements:	
Other:	
Next health check date:	
Next assessment date:	

ACTIONS

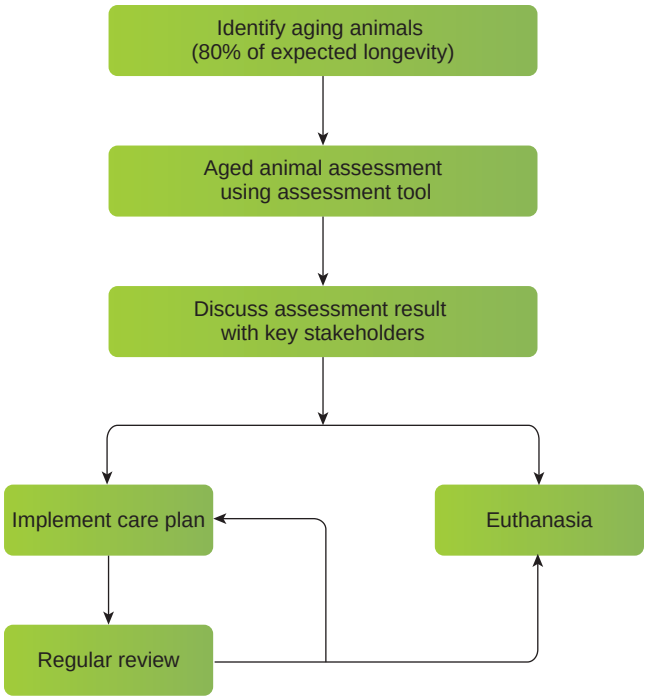
OTHER ACTIONS

Media department to consider sympathetic media releases?	
Curatorial departments to consider implementing acquisitions / dispositions / breeding, etc?	
Visitor interpretations needed?	

ASSESSMENT COMPLETED BY

Senior Keeper	
Veterinarian	
Curator	
Behavioural Biologist	
Other	

• Figure 15.3, cont'd



• **Figure 15.4** Flowchart for the process geriatric animal assessment and management.

birth (welfare assessment tools have been developed for this purpose, e.g., WelfareTrak[27]); however, in relation to aged animals, a useful trigger may be when an animal reaches 80% of expected longevity for the species. Once the process of assessment commences, a physical examination of the animal under anesthesia provides valuable information that feeds into the assessment process. Results of the examination and assessment are documented, and either a care plan is agreed on (including timing of the next clinical examination and assessment), or a decision to euthanize is made (Fig. 15.4).

In conclusion, the implementation of objective processes guided by evidence-based data, utilizing the experience of keeping staff, behavioral biologists, veterinarians, and curators in reaching conclusions, results in positive welfare outcomes for animals in our care. Implementing these processes also fosters a culture of trust, understanding, and collaboration within and beyond the zoologic community.

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SECTION 3

Conservation Medicine

16

Evaluating Camel Health in Kenya—An Example of Conservation Medicine in Action

SHARON L. DEEM

Introduction

The ability to adequately feed the 7.6 billion people that currently inhabit the planet has become increasingly challenging due to climate change and other environmental stressors that are occurring on a global scale and at an ever-accelerating rate.[1] Additionally, as the human demand for animal-based protein continues to escalate, with estimates of a 50% increase during the first 20 years of the 2000s, the need to find ways to feed our species without jeopardizing the survival of wildlife should be a top goal for the conservation medicine initiative[2,3] (see also Chapter 19). We must find solutions to these combined challenges by taking into account the need for proper human nutrition and health, wildlife conservation, and environmental resilience. An example of a holistic conservation medicine program is well demonstrated by our work focused on camel health in the shifting landscape of Northern Kenya. In fact, the changing demographics of people and their livestock in this region may have immediate and long-standing negative implications for sympatric wildlife species. These changes demand a conservation medicine approach.

The semi-arid region of Northern Kenya has experienced great transformations in recent decades caused by changing climate, as semi-arid lands become more arid, and with displaced persons coming into the region from areas of conflict outside of Kenya.[4,5] It is here that the largest refugee camp in the world, Dadaab, which houses an estimated 500,000 people, is found along the border with Somalia.[5] These people are mostly displaced from conflict in their home countries and are, unfortunately, as Muslims and refugees, a marginalized part of Kenyan society.[5] It is these more recent arrivals to Kenya, residing in Dadaab camp, across Northern Kenya, and more widely dispersed throughout Kenya including the capital of Nairobi, that have traditionally used camels as a source of milk and meat. This change in human demographics, combined with climate change, is a driver of demand for camels throughout the region.

Along with this increase in people, the recent increase in drought conditions in the region has compounded negative impacts on human livelihoods. This has led to a switch from a cattle-based economy to one based on camels. Camels provide a strategy for climate change adaptation due to their ability to survive under harsh environmental conditions and as such are a means to improve human livelihoods and climate resilience.[4] The camel is claiming a strong position in the face of East Africa's changing climate.

In Kenya, the growth in the dromedary camel population over the past couple of decades is evident with estimates of 717,500 camels in 2000 increasing to 2.9 million in 2013.[6] During a similar period, cattle numbers in the country decreased by 25.2% because cattle survival has become increasingly difficult in a landscape modified by climate change.[7] Today, Kenya has Africa's third largest dromedary camel population, estimated at 3,091,200 animals; the camel meat and milk industry in Kenya is worth approximately US $11,000,000 annually.[8] This rise in camel production in Kenya is largely due to the increase in droughts, the ability of camels to survive days without water or food, as well as the ability of camels to eat 100% natural forage, all leading to this switch from cattle to camels.[4,6] For example, a study found that 71.5% of the households interviewed in Isiolo County, Northern Kenya, preferred camels over other livestock and cited their endurance to climate factors as the main benefit they gain.[9] Overall land and water footprints for milk production have increased for camels, whereas it has decreased by half for cattle.[10]

This increase in camels has provided protein for the people of Kenya with estimates over a 13-year period (2000–2013) of camel milk production increasing from 335,000 tons to 937,000 tons, and meat production from 15,000 tons to 651,000 tons.[8] These numbers are encouraging for human health because the ability for cattle to survive

in the changing environment has declined; however, this may also indicate three points of concern when viewed in a conservation medicine framework. First, with just one camel milk pasteurization plant in the country, Kenyans have little access to pasteurized milk. It has been estimated that 10% of Kenya's 40 million people drink unpasteurized camel milk.[11] Because raw camel milk is a possible transmission route for many microorganisms, the consumption of unpasteurized camel milk in Kenya may pose a high public health cost in the country.[11–15] Secondly, camels are browsers and have great ability to cover large areas across the landscape, which may lead to competition for food with sympatric wildlife.[16] Lastly, there remains a shortage of veterinary education for camel health and a lack of veterinary support for camel production in the country. As a relatively new large-scale production livestock species in Kenya, and as a species often viewed as most closely linked with marginalized people, veterinary care and biosecurity for camels in Kenya significantly lags behind that for more traditional livestock (e.g., cattle, sheep, goats). This lack of care has potentially devastating implications for productivity losses related to disease-associated morbidity and mortality, as well as an increase in disease transmission between camels, other livestock, wildlife, and humans. All these factors and challenges demand a conservation medicine approach.

What Is a Conservation Medicine Approach and How Do Zoos Fit Within It?

Although a number of definitions have been provided for conservation medicine, one unifying theme may be that it is a strategy that strives to expand transdisciplinary collaborations and communications to improve the health of humans, animals, and the environment.[17] Today, with the push for AZA-accredited zoos to dedicate 3% of their revenue to conservation, the time is right for zoos to be leaders in the this initiative. This should be an easy fit as a core objective of zoo conservation is often to ensure healthy wildlife populations and ecosystems, without compromising the health of humans. Thus, the conservation mission of accredited zoos fits perfectly within a conservation medicine framework.

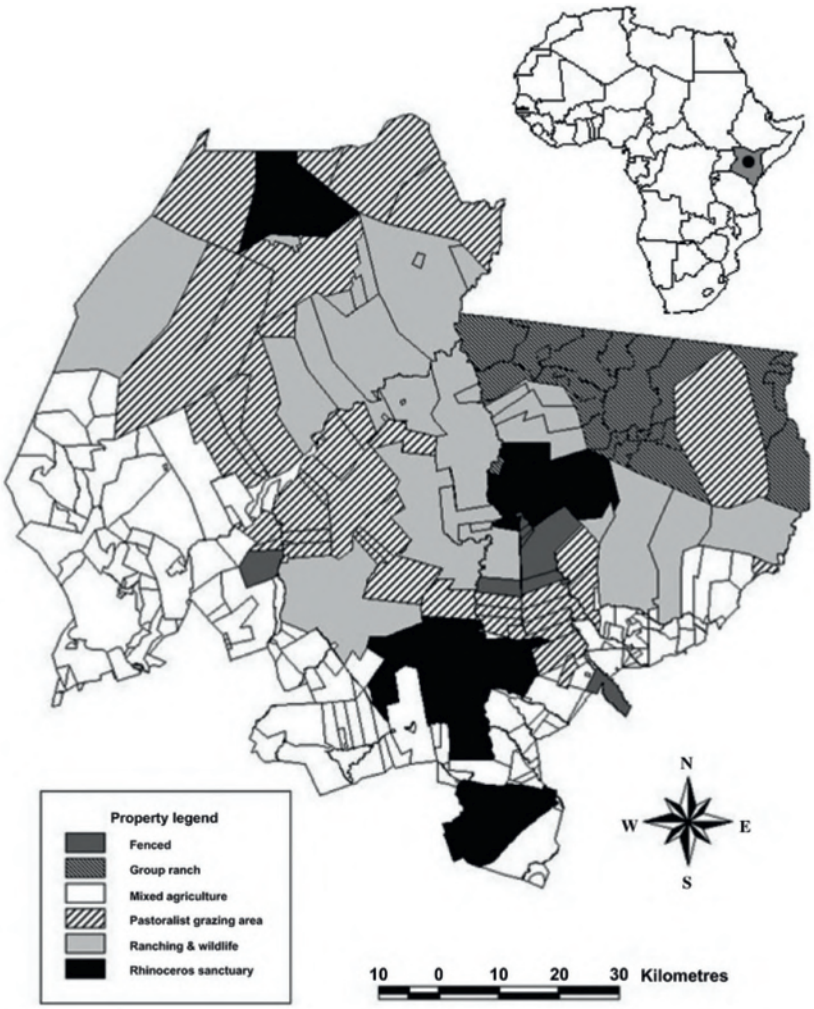
Zoological institutions have an opportunity to play many roles within conservation medicine programs and we may be the advocates that help to ensure species' conservation is considered within these programs for improving human, animal (e.g., domestic and wild), and environmental health.[17–19] Briefly, these roles may include (1) providing healthcare for zoological species, thus ensuring sustainability of biodiversity; (2) conducting studies on diseases of conservation concern; (3) understanding diseases in zoo wildlife as sentinels for emerging diseases of humans and animals in surrounding areas; (4) performing surveillance of diseases in wild animals at the interface of wildlife, domestic animals, and humans; (5) making contributions to the fields of comparative medicine and discoveries in all life forms; and (6) demonstrating the importance of nature for human health.[17–20] This chapter presents a conservation medicine program focusing on camel health in Kenya, which demonstrates roles that zoological veterinarians play to ensure healthy animals and healthy people.

Project Design

In 2012, the Saint Louis Zoo Institute for Conservation Medicine (ICM) was invited to spearhead camel health studies at the Mpala Research Centre (MRC) in Laikipia County, Kenya (Fig. 16.1). The invitation was from colleagues working at MRC and Novus International, an animal nutrition company with headquarters near St. Louis, MO. After researchers embarked on camel nutrition studies, they soon realized that little data existed on the health status of, and disease risks for, camels in Kenya. (This was just before Middle East Respiratory Syndrome [MERS-CoV] was on the international stage with the first detection of MERS-CoV in a 25-year-old student in Jordan.[21] See also chapter in this volume: An Overview of Middle East Respiratory Syndrome in the Middle East.) We at the Saint Louis Zoo immediately accepted this opportunity because the ICM had just launched as a new Zoo department, and one of the Zoo's WildCare Institute conservation centers had been focusing efforts on wildlife conservation in the Horn of Africa for many years prior to this time.

With minimal research, it quickly became evident that a camel conservation medicine program was important because (1) camels had become the "new cow" in Kenya; (2) many diseases of camels may be transmitted to humans, livestock, and wildlife; and (3) the region of Kenya where MRC is located has experienced the largest increase in camel numbers while still holding the highest densities of wildlife in the country, although these numbers are falling significantly as humans and livestock move across the landscape.[9,22]

To get the program underway, we took a multistep approach that included (1) conducting research to better understand the species and region; (2) reaching out to international camel experts and livestock/wildlife and conservation researchers in Kenya; and (3) securing funds and people to make the program a success (Box 16.1). First was the task of gathering literature on dromedary camel health and diseases, and to consider these in a conservation medicine framework. We focused on the diseases that were of most concern for camel productivity and human health, as well as diseases transmissible across the camel-livestock-human-wildlife interface. Dromedary camels may harbor agents with zoonotic potential (e.g., *Coxiella burnetii*, *Brucella* spp., *Toxoplasma gondii*, Rift Valley fever, anthrax) and/or those that may be transmitted only among camels, other livestock and wildlife (e.g., blue tongue, bovine diarrhea virus, *Trypanosoma evansi*).[23–29] It was also clear that some of these diseases (e.g., *Brucella* spp.) are difficult to diagnose in camels.



• **Figure 16.1** Map of Laikipia County, Kenya. (From Browne AS, Fèvre EM, Kinnaird M, et al: Sero-survey of *Coxiella burnetii* (Q fever) in Dromedary Camels (*Camelus dromedarius*) in Laikipia County, Kenya. *Zoonoses Public Health* 2017. doi:10.1111/zph.12337. http://onlinelibrary.wiley.com/doi/10.1111/zph.12337/full#zph12337-fig-0001.)

• BOX 16.1 How to Start a Conservation Medicine Program at the Livestock-Wildlife-Human Interface

1. Do your homework and research *all* aspects of the study; including human, animal, and environmental truths.
2. Reach out to others across disciplines to develop collaborations and partnerships.
3. Find funding streams that will allow you to execute the program.
4. Get the people-power to staff the various parts of the program.
5. Use the program to educate the next generation of conservation medicine practitioners globally.
6. Share the data in the scientific and policy arenas so your science is not just a hobby.
7. Use the program to help others capitalize on your work and/or expand into other directions.
8. Be flexible to modify the program as necessary based on political and biological realities.

It was immediately evident that losses due to infectious diseases in camels impact the economies of local camel herders in Kenya.[30] As seen in similar regions with camel production, we hypothesized that mastitis, with a prevalence estimated at 23%–76% for camels in the region, was also of top concern in Laikipia.[31] Therefore possibly with simple preventive measures we could minimize this camel and human health concern.

The literature review into the study site and how camel production in the region may impact human health, wildlife conservation, and environmental resilience in Laikipia produced a great deal of information on the area with Laikipia County known to have high levels of biodiversity and diverse land-use practices, ranging from pastoralists to commercial ranching, agriculture, habitat conservation, ecotourism, and wildlife research.[22] Furthermore, it was known that the large increase in camels in the region was likely to influence both conservation efforts and land-use dynamics.[22]

After gaining an appreciation for the issues at hand, we needed to determine how to fund the program and what partnerships with other conservation medicine practitioners we could develop. Seed money from Novus International helped get the project started. Subsequently, we were able to secure funds from internal grants at the Saint Louis Zoo, foundations, private donors, and governmental agencies. Collaborations quickly grew in the first year and we continue to have new organizations working with us on camel health issues. These collaborations became increasingly easy to develop following the discovery of MERS-CoV, and the role that camels have in the epidemiology of the disease.[21]

Program Outcomes to Date and Future Plans

Now entering the fifth year of this camel program in Northern Kenya, we have produced a number of scientific and layperson-friendly products to help with camel production, while minimizing human and wildlife health concerns. Early in the study we developed a camel herd health protocol that was shared with local camel herders in the region.[32] Also, after the first field season and with the documentation that mastitis was indeed causing high production losses, we conducted a study to better understand the risk factors associated with mastitis prevalence.[33] Reports were also shared with local camel herders to provide information on the health status of their animals based on complete blood counts, chemistry profiles, and exposure to a number or infectious and parasitic disease-causing agents.

One example of an immediate improvement in camel welfare and productivity was the diagnosis, using simple blood film evaluations, of *T. evansi* in a camel herd that was experiencing high calf mortality and severe morbidity in adults (Fig. 16.2). The herd owner had not been using trypanocide for prevention because trypanosomiasis was thought to not occur in the region. However, in this increasingly interconnected world, with animal movements (e.g., camels from northern Africa and the Middle East into Laikipia County), herders started to appreciate that with the movement of animals comes the movement of all their micro- and macrobiota.

• **Figure 16.2** Adult camel with clinical signs of, and blood film confirmation for, *Trypanosoma evansi*. (Photo credit: Sharon L. Deem.)

We also focused much of our work on *C. burnetii*, because the original literature review indicated that Q fever was an emerging infectious disease (EID) in Kenya, and that during recent years it had been causing significant disease in Kenyans.[34] However, with little known on the epidemiology of the disease in Kenya, we elected to explore the possible role of camels. Our studies have demonstrated a high seroprevalence to Q fever in camels, and the potential role they may serve as reservoirs of the bacteria, with implications for cross-species transmission between livestock, sympatric wildlife, and humans.[35,36]

Rather serendipitously and shortly after we had started working with camels, the emergence of MERS-CoV in the Middle East, with spread to other regions of the world, was a catalyst for the development of this conservation medicine program. The use of samples we had collected and bio-banked in the initial years and just prior to MERS-CoV being on the world stage provided an important source of data for understanding this significant EID in the region.[37]

Lastly, a large part of our camel program in Kenya has been the training of next generation conservation medicine practitioners through involvement in a real-life public health and wildlife conservation program.[19] This has included students from Kenya, the United Kingdom, and the United States. Through this program, we have trained DVM, MSc, and public health students and provided them experiences in which they gain an appreciation for the disease risks associated with changing environmental conditions, protein sources for humans, and the inevitable increase in interactions at the domestic animal/wildlife/human interface.

Future Work

This program is ongoing and we continue to focus on all four core components. These include: (1) improvements in camel husbandry, health, and production including plans for a camel veterinary course at the University of Nairobi College of Veterinary Medicine; (2) Q fever and MERS-CoV epidemiologic studies with emphasis on the role of vectors and use of slaughterhouses for surveillance, respectively; (3) training of conservation medicine practitioners; and (4) continued collaborations across institutions and disciplines.

Developed in 2012 and at a time that few people had camel health on their radar, we now know of the importance of camels in the epidemiology of MERS-CoV and other EIDs (e.g., Q fever) in Kenya.[35–37] Therefore there is an increase in organizations and individuals that are working to improve camel production in Kenya, and we continue to expand partnerships within this program. Only through scientific research, veterinary medical care and public policy for camel production will we be able to mitigate the potential risks to public health, and sympatric wildlife and livestock health, while advancing camel productivity in the region.

We continue as one of the collaborators working to advance camel welfare, health, and productivity; all imperative to help support human health and, as importantly, to be sure that this new form of climate change adaptation and food security does not lead to unchecked negative impacts on the conservation of Kenya's amazing wildlife.

Concluding Thoughts on Conservation Medicine Programs

This work on camel health in Northern Kenya is one example of a transdisciplinary conservation medicine program initiated by a zoo conservation medicine department. The program has already generated the data necessary for better understanding diseases at the camel, livestock, wildlife, and human interface, in the face of changing environments. Zoo staff and veterinarians are often well-versed in this across-taxa approach. Some may question why a zoological institution and zoological veterinarian took the lead on developing a program for a "domestic livestock" species. However, when viewed at the intersection of camel, livestock, wildlife, and human health and disease concerns, this is the type of program zoos should increasingly embrace. Zoological veterinarians are first and foremost veterinarians; we have an ability to partner with public health colleagues, and zoos are working to increase their "fence to field" reach to work for the conservation of wildlife species, while ensuring human livelihoods and health are not compromised.[17,19] As previously stated,[38] one solution to disease and conservation at the wild-domestic animal interface is the implementation of a proactive approach—addressing potential pathogen transmission before a volatile problem occurs. This program did just that.

Charles Darwin is reported to have said that the species that survive are not necessarily the strongest or the most intelligent; rather they are the ones that are the most adaptable to change.[39] This grassroots conservation medicine program, which began prior to camels being on the world stage due to their role in the emergence of MERS-CoV, best demonstrates how the ability to adapt and attend to concerns of the day are in everyone's best interest. A proactive approach to camel health, at a time that camel numbers are growing across the Kenyan landscape, with potential negative impacts for wildlife conservation, is one example of how zoos may lead in the conservation medicine initiative to help address these 21st century challenges. We took an opportunity that at first glance might not have seemed a "good fit" for a zoo conservation department but was shown very early in our work to be an excellent fit.

While working on this chapter, security issues in northern Kenya again escalated due to worsening drought with dire consequences for pastoralists, herders, and ranchers in the region. Food security, and simple human security, will worsen in the short term, if not also the long term. We will have to be ready for these changes. As we strive for improvements in human livelihoods (e.g., poverty alleviation, conflict resolution, food security), it is imperative that the conservation of wildlife species be included in the equation if we are to properly address human, animal, and environmental health, resilience, and ultimately survival.

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17

Disease Risks to Native Wildlife From Zoos and Aquariums

BRUCE RIDEOUT AND CATHERINE HADFIELD

Introduction

Most potential pathogens have co-evolved with one or more hosts and pose a relatively low risk of population-level disease problems in their native hosts as long as a certain level of population health and ecosystem integrity is maintained.[1,2] However, anthropogenic activities are increasingly breaking down the natural ecologic barriers to disease transmission resulting in more frequent opportunities for host switching by pathogens, as well as larger and more sustained wildlife disease outbreaks.[3] If a potential pathogen invades and establishes itself in a new immunologically naïve host species, devastating consequences can follow, as has been seen with agents such as *Batrachochytrium dendrobatidis*, the chytrid fungus of amphibians.

Zoos and aquariums have the potential to increase opportunities for pathogen host switching by bringing into close proximity species that would not otherwise come into contact in an intact ecosystem. This includes not only the risk of pathogen spillover from zoo and aquarium species to native wildlife but also disease amplification by feral and urban-adapted wildlife in our facilities.[4] As conservation organizations, zoos and aquariums have an obligation to take these threats seriously and to establish biosecurity barriers and practices that minimize such risks. However, minimizing the risk of spillover requires an understanding of basic disease ecology as well as the pathogen characteristics that facilitate host switching.[5–7]

Invasion of a pathogen into a new host species or population is a multistep process, and a thorough disease risk analysis would be required to evaluate the risks and identify appropriate mitigation strategies for each step. However, in all scenarios, exposure is the necessary first step. This can occur through a number of avenues, such as direct contact, arthropod vectors, aerosols, water discharge or runoff from animal exhibits, animal waste handling, fomites, and through reintroduction programs. Some potential pathogens are better equipped to take advantage of these exposure opportunities than others. The best invaders will generally be those with a broad host range (i.e., generalist or multi-host pathogens), those that establish reservoirs in the environment or other abundant host species, those with long infectious periods, those that evolve rapidly and thereby adapt to novel hosts, and those transmitted by vectors.

Generalist pathogens are better than specialists at invading and establishing themselves in new hosts simply because the ability to infect multiple host species increases the probability of finding a suitable host when exposure opportunities occur.[7] In contrast, specialist pathogens have lifecycle requirements that generally restrict infection to a single host genus or species, which severely limits the potential for host switching. Examples of generalist pathogens that are adept at host switching include *Toxoplasma gondii*, *Mycobacterium bovis, Mycobacterium tuberculosis*, highly pathogenic avian influenza (HPAI) (see Chapter 38), morbilliviruses, *B. dendrobatidis,* and avian *Plasmodium* species. Generalist pathogens also can have a disproportionate impact on small, vulnerable populations through a phenomenon called apparent competition.[8,9] This occurs when a pathogen that is established in an abundant host spills over into a less abundant host, resulting in greater exposure and greater pathogen burdens in the less abundant host, culminating in more significant disease problems and population impacts.

Pathogens that establish themselves in reservoirs in a new nonnative ecosystem are more effective invaders because the reservoir provides a persistent source of exposure, resulting in constant invasion pressure.[10,11] In addition, reservoirs allow a pathogen to persist even when the alternate host's population size drops below the threshold required to maintain transmission. Without a reservoir, directly transmitted pathogens will generally disappear before their hosts go extinct because transmission cannot be maintained once the host population drops below this threshold. Reservoirs reverse this trend and allow the pathogen to persist until the alternate host becomes extinct. *B. dendrobatidis* and the white-nose syndrome fungus *Pseudogymnoascus destructans* are examples of pathogens with reservoirs that have caused widespread extirpations and extinctions (see Chapter 72).

A long period of infectiousness increases the chance that a pathogen will successfully invade and establish itself

in a new population or species by providing a prolonged transmission opportunity.[12] In other words, there is a lower risk that the pathogen would die out before a transmission opportunity presents itself. The long infectious period of *M. bovis* likely contributed to its successful invasion and establishment in East Africa after spillover from domestic animals.[13]

Pathogens with rapid rates of evolution have greater invasion potential because of their ability to rapidly adapt to novel hosts.[14] The classic example is RNA viruses, which have high mutation rates during replication, as well as the potential for reassortment and recombination events, allowing them to rapidly alter their host range and virulence. With avian influenza viruses, simple point mutations can result in a shift of host range, allowing a switch to transmission between mammalian hosts.[15,16]

Vector-borne pathogens have inherent limitations in host switching potential because of the lifecycle requirements and feeding preferences of their vectors, but those with generalist vectors can have high invasion potential for several reasons. First, vectors act as short-term reservoirs, allowing a pathogen to persist for a period of time in the absence of susceptible hosts, which increases exposure opportunities. Second, vector-borne pathogens have frequency-dependent transmission, which means efficient transmission occurs even in very small, low-density populations. For pathogens with transmission by direct contact, the probability of encountering a susceptible host in a small, low-density population is low, and if exposure occurs, invasion might fail because transmission cannot be sustained. However, with vector-borne pathogens, transmission depends on the frequency of encounters between the vector and host, not the host density, so invasion and transmission occur even in very small, dispersed populations as long as the vector is abundant. This also means that vector-borne pathogens have the potential to drive small populations to extinction, because transmission efficiency is maintained regardless of host population size or density. A classic example of this scenario is the introduction of avian malaria into Hawaii, which has driven a number of native forest birds to extinction.[17,18]

Representative examples of wildlife pathogens with these characteristics are listed in Table 17.1.

In order for any pathogen to become established in a zoo or aquarium and have a subsequent spillover opportunity, it must first gain access to the facility by passing successfully through the quarantine process, or by breaching established biosecurity barriers. The pathogens that are most successful

TABLE 17.1 Representative Wildlife Pathogens With Characteristics That Facilitate Host-Switching and That Have the Potential for Population-Level Impacts

Taxon	Agent	Generalist	Reservoirs	Long Infectiousness	Rapid Evolution	Vectors*
Birds	Circoviruses	X	X	X		
	Bornaviruses	X	X†	X	X	
	HPAI	X	X		X	
	Plasmodium spp.	X	X	X		X
	Mycoplasma gallisepticum	X	X	X		
	West Nile virus	X	X	X	X	X
Mammals	*Mycobacterium tuberculosis* complex	X	X	X		
	Morbilliviruses	X	X		X	
	Sarcoptes scabei	X	X	X		
	Parvoviruses	X	X			
	Mycobacterium paratuberculosis	X	X	X		
	Malignant Catarrhal Fever viruses	X	X	X		
	Treponeme-associated Hoof Disease	X	X	X		
	Toxoplasma gondii	X	X	X		
Reptiles	Ranaviruses	X	X	X		
	Snake Fungal Disease	X	X†	X		
Amphibians	*Batrachochytrium* spp.	X	X	X		
	Ranaviruses	X	X	X		
Fish	*Gyrodactylus* spp.	X	X	X		X*
	Sea Lice (*Caligus* spp.)	X	X	X		
	Koi Herpesvirus		X	X		
	Carp Edema (Pox) Virus		X	X		
	Iridoviruses	X	X	X		

*Vectors: Vectors *or* frequency dependent transmission (X*)
†X: Presumed but still under investigation
HPAI, Highly pathogenic avian influenza.

in making it through the quarantine process are those that have a long incubation period or a carrier state, or that lack effective screening tests, which makes them difficult to detect, or those allowed through quarantine because they are considered inconsequential in their native host. Pathogens with these characteristics should be given particular attention in the risk analysis process described later. The ability of a pathogen to breach established biosecurity barriers is determined more by the care that went into the design of the barriers and the effectiveness of the biosecurity practices at the institution than by the characteristics of the pathogen.

The general biosecurity practices, disease surveillance, and preventive medicine programs of the facility are a foundational component of any effort to mitigate the risk of disease spillover to native wildlife. However, the potential consequences of a spillover event are great enough to warrant more targeted risk analysis and mitigation efforts. One efficient approach is to combine this targeted risk analysis with any new biosecurity planning efforts being initiated, such as those resulting from the increased threat of HPAI and other foreign animal disease incursions. The all-hazards response plans being developed and promoted through the Association of Zoos and Aquariums (https://zahp.aza.org/) are a good model (see Chapter 9). The same types of biosecurity practices that will protect our collection animals from pathogen spillover from native wildlife will also help to mitigate the risk of spillover in the other direction. A biosecurity audit conducted by government animal health regulatory veterinarians is an excellent starting point. The audit will help identify gaps or weaknesses in existing facilities and biosecurity protocols, and the findings can provide foundational information for a subsequent comprehensive risk analysis.

Risk Analysis

The risk analysis (see Chapter 2) should ideally have two components. The first would be a traditional risk analysis focusing on pathogens of concern that have a reasonable probability of being present in the zoo or aquarium. The emphasis should be on pathogens that would be alien to native wildlife, especially those with the high-risk characteristics outlined previously. The success of the risk analysis hinges on assembling a multidisciplinary team with the expertise and institutional knowledge required to identify and evaluate all avenues of risk relevant to the institutional setting. The composition of the risk analysis team will vary depending on the nature of the institution but should include those with expertise in husbandry, management, nutrition, and facility infrastructure, in addition to those with traditional animal health expertise. It can also be very helpful to include stakeholders, such as financial decision makers and government agency representatives responsible for native wildlife management. Even if they are not active participants in the risk analysis process, their input and buy-in is often critical to the success of the effort. The risk analysis process begins with a problem definition, in this case focused broadly on the risk of alien pathogen spillover to native wildlife, and includes the development of a consensus statement on the level of risk that is acceptable. This is followed by the identification of all potential hazards, primarily infectious agents in this case, and an analysis that culminates in a ranking of agents based on the likelihood that a spillover event would occur and a negative population-level impact would follow. It is important to recognize that pathogen spillover is a multistep process, and the probability needs to be weighed at every step. For example, the analysis might consider the estimated probability (usually expressed qualitatively as low, medium, or high) that an agent of concern would be present, that the agent could escape the facility by some avenue, that exposure to native wildlife would occur, that the hosts would be susceptible, that a productive infection would occur, that the pathogen would become established in a population, and that a negative population-level impact would follow. To the extent these steps are independent, the probability of each step needs to be multiplied rather than added in order to establish the cumulative probability. When using qualitative estimates, the key concept to remember is that multiplication results in a reduction in cumulative probability. This can be seen by arbitrarily assigning quantitative estimates at each step, and doing the calculation (e.g., assigning a 50% probability for each step in a seven-step process, as outlined earlier, yields a cumulative probability of $[0.5 \times 0.5 \times 0.5 \times 0.5 \times 0.5 \times 0.5 \times 0.5] = 0.0078$, which is <1%). See Table 17.2 for an example of a cumulative probability analysis for desert tortoises. For those agents that have a cumulative probability of negative population impact that exceeds the established risk tolerance level, mitigation steps would then be developed. A useful tool for conducting such a risk analysis is the International Union for Conservation of Nature (IUCN) Manual of Procedures for Wildlife Disease Risk Analysis,[19] which is available as a free download from the IUCN website (www.iucn.org).

The second component of the risk analysis would be focused on biosecurity for specific transmission pathways rather than specific pathogens. This is important because it has the potential to mitigate the risk of spillover of pathogens that we might not have considered, or that might not have been discovered or characterized yet. The process would be somewhat analogous to what food safety specialists call Hazard Analysis and Critical Control Points (HACCP).[20,21] The idea with this approach would be to map out all of the potential avenues of pathogen escape from a facility and then identify the critical points in each escape or transmission pathway where risk mitigation would be most effective. Particular attention should be paid to situations that result in recurring exposure opportunities, such as areas of water runoff or discharge from enclosures, fecal compost piles, food waste cleanup and disposal practices, fomites, and areas where wildlife or feral animals can come into close proximity with collection animals or enclosures.

Complicating these risk scenarios is the potential role zoos play in altering wildlife disease prevalence and dynamics by

TABLE 17.2 Example of Cumulative Risk Analysis for Mojave Desert Tortoises

Agent or Hazard	Probability: In Source Population	Absence in Destination Population	Translocation Is Only Exposure Avenue	Release and Spread	Establishment	Negative Population Impact	Cumulative Risk	Comments
Mycoplasma agassizii	Very High	Low	Low	Very High	Very High	High D: High Low D: Low	High D: Medium Low D: Low	A remote naïve population may be at high cumulative risk in a high-density scenario and low risk in low density
Mycoplasma testudineum	Very High	Low	Low	Very High	Very High	High D: Medium Low D: Very Low	High D: Low Low D: Very Low	
TeHV-2	High	Low	Low	Very High	Very High	Low	Very Low	Most taxa have multiple endemic herpesviruses

High D, High tortoise population density; *Low D*, low tortoise population density; *TeHV-2*, testudinid herpesvirus 2.
Excerpt from Rideout B, editor: *Transmissible infections and desert tortoise translocation: a comprehensive disease risk analysis*. Report to the US Fish and Wildlife Service, 2015.

inadvertently subsidizing urban-adapted wildlife, such as deer, raccoons, skunks, rodents, opossums, foxes, coyotes, mallards, Canada geese, herons, egrets, and feral cats. Our facilities foster higher densities of these species by providing abundant food and water, reduced predator populations, and complex environments that provide shelter. Because of this, risk mitigation efforts should also incorporate biosecurity practices that minimize the subsidization of urban-adapted wildlife.

Examples of targeted risk mitigation efforts could include the following:

- Minimize the ability of urban-adapted wildlife to obtain animal or human food waste from enclosures or trashcans.
- Employ integrated pest management and vector control practices that keep pests and urban-adapted wildlife at the lowest possible population levels.
- Schedule a site visit with experts in wildlife population control, such as US Department of Agriculture's Animal and Plant Inspection Service's staff in the United States, to get specific recommendations for methods to reduce urban wildlife populations in facilities.
- Eliminate mosquito and other pathogen vector breeding sites.
- Ensure that perimeter fences and facility barriers minimize the transit of urban-adapted wildlife through facilities.
- Minimize the ability of free-ranging aquatic birds to access water features that either have collection birds, or that collect runoff or discharges from exhibits.
- Maintain closed aquatic systems, or ensure that discharged water is effectively treated before it enters a watershed or other area with native species.
- Keep animals destined for reintroduction completely separate from species from other geographic areas and ideally maintain them as close as possible to native habitat and release areas.
- Do not bring species into close proximity that would not have opportunities to interact in the wild, especially species from different continents or divergent geographic regions.
- Ensure that appropriate biosecurity barriers and practices are in place to minimize host-switching opportunities by pathogens.
- Minimize the length of time food waste is left in enclosures, where it could attract native wildlife.

The fact that there are no published reports of direct spillover of significant pathogens from zoos and aquariums to native wildlife suggests that existing biosecurity and preventative medicine programs in zoos and aquariums have been relatively effective. However, the introduction of diseases from nonzoo or aquarium sources to North American wildlife, such as West Nile Virus, illustrates the potential for adverse effects of disease introduction. These risks are real and need to be addressed, particularly for reintroduction programs. Examples of diseases introduced through reintroduction include *B. dendrobatidis* in Mallorcan midwife toads,[22] and whirling disease in rainbow trout.[23] Although there have been no documented population-level impacts on native wildlife from spillover in zoos, there are examples

of pathogen exchange occurring, such as with *Isospora* spp. coccidia in zoos and free-ranging passerines.[24] Continued anthropogenic impacts at the wildlife–domestic animal–human interface will only increase the risk of significant pathogen spillover events in the future.

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18

Feral Cat Dilemma

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Brief Natural History of Cats

In this chapter, we discuss the origin and abundance of domestic cats, the issues surrounding cat overpopulation and loss of wildlife, and the stakeholders involved in the controversy surrounding cat management. We also summarize potential solutions to address the growing populations of cats.

Cats and Human Culture

Cats are believed to be domesticated from the wildcat in the Near East approximately 10,000 years ago. There is evidence for their first link with humans from Cyprus,[1] and cats have been found in drawings of ancient Egyptians where they were worshipped,[2] some images displaying felines with collars. Domestication also occurred in early Chinese civilizations due to cats' useful ability to remove rodents.[2] Domestic cats were originally valued as predators of pests around grain and crop stores and revered as religious figures. Today, cats are cherished as companion animals, welcomed into our homes as family members, and are responsible for regular Internet "sensations." Annual surveys by the American Pet Products Association continue to report that cats are the most popular pet in America and cats have surpassed dogs as the most popular companion animal in most of North America and Europe.[3] Humans often develop strong emotional connections to pet cats, and there is some evidence that pet ownership contributes to short-term improvement in human health,[4] though hypotheses about contributions to mental and physical health remain inconclusive overall.[5] Even if pet ownership may not contribute to a longer, healthier life, any cat owner will provide anecdotal support for the happiness and entertainment provided by their companion on a daily basis.

The Rise of Feral Cats

Domestic cats have long been a common sight in urban and suburban areas throughout the world, and the number of cats is continuously growing. It is expected that a minority of cat-owners in the United States (25%–50%) confine their pets to an indoor lifestyle,[6,7] and this may be one factor contributing to the large numbers of homeless cats today. Homeless domestic cats may be feral (stray, unfriendly, often untamed and unsocialized) or stray, but somewhat tame. Large feral populations of cats may have originated in developed countries as more people migrated to cities from the countrysides in the early 20th century. High-density urban living and the struggle to make ends meet likely led to difficulties keeping pets; this along with lack of sterilization techniques and the prolific nature of cats led to large feral populations in cities by the mid 20th century.[8] Cats may have more than two litters of four or more kittens a year,[9,10] and immigration into areas of sufficient food may be very high.[11] Baker et al.[12] recorded cat densities of 229–523 cats/km^2 in an urban area of the United Kingdom, far higher than native mesopredator densities (averaging 37 animals/km^2 for red foxes) (*Vulpes vulpes*). Others summarized cat density observations and listed over 2000 cats/km^2 in sites in urban Rome, Italy; Jerusalem, Israel; and Ainoshima, Japan.[13] Many Americans support or maintain neighborhood colonies of feral cats,[14] and this is not uncommon in developed nations around the world.

The Controversy

The number of feral domestic cats in the United States is unclear but is thought to be in the tens of millions.[15,16] Such high population estimates have implications for both wildlife and public health,[17,18] and there is broad interest from community groups, nonprofits, and management agencies in reducing cat populations. Biologically effective, yet socially acceptable, management strategies for feral cats are a matter of contention in the United States and in many developed countries abroad.[19] Islands with imminent conservation issues due to cats have used pathogens, poison baiting (Australia, currently), and other lethal control methods. Historically, community management has involved capturing and either socializing for the purpose of adoption or euthanizing unwanted feral cats at local shelters. Citing the lack of success by animal shelters to decrease cat populations, a second strategy growing in popularity, involves trapping cats, sterilizing them and releasing the cats back to the site of capture (Trap-Neuter-Release [TNR]).[14] This control method is considered more humane than

euthanasia and is promoted by advocacy organizations such as: Alley Cat Allies, The Best Friends Animal Society, The American Society for the Prevention of Cruelty to Animals (ASPCA), and The Humane Society of the United States (HSUS). Theoretically, cat colonies should decline over time as neutered members are not reproducing. To date, large cities in 28 US states have adopted TNR as their sanctioned method of cat control though the process remains highly controversial because assessments of these programs have demonstrated little evidence for efficacy in stabilizing populations.[18,20,21] In fact, for TNR programs to result in stabilization or decline, several quantitative assumptions must be met. For example, at least 75% of the population must be sterilized and immigration rate should be zero.[9,22] Both of those assumptions are dependent on social and political *human* factors that are difficult to control. For example, the presence of TNR colonies may encourage "dumping," and feeding stations continuously attract new, unsterilized immigrants.[11]

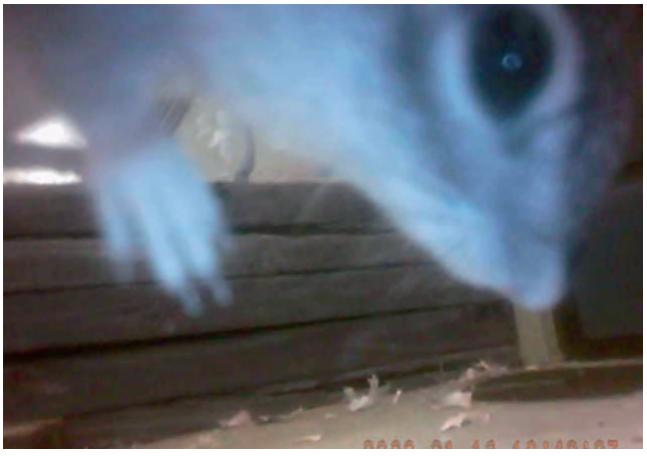
Stakeholders in the Controversy Over Feral Cats

The controversy over management of feral cats seems to stem from positions of two polarizing groups along a spectrum of conviction. Moderate animal welfare organizations that support TNR argue for nonlethal solutions to feral cat overpopulation, and claim domestic cat predation of wildlife is natural and compensatory, whereby predation substitutes for death that would occur naturally.[23] At its most extreme, some cat activists claim domestic cats are adapted to live outdoors and provide biologically inaccurate justifications that cats "play an important role in balancing the local ecosystem."[23] Wildlife advocates argue for cat removal from the environment as cats are an invasive species detrimental to wildlife.[24] Lauber et al.[25] found ethical judgments of those supporting fertility control (TNR) for cats included concern over killing animals to satisfy human interests and protection of the individual cats. In contrast, lethal control is often advocated by people who believe fertility control (TNR) works too slowly,[25] or not at all. Support for TNR has undoubtedly paralleled society's overall movement toward animal welfare values orientations and no-kill shelters. It is worth noting that pro-cat groups spend large sums of money to support TNR and lobby local and state governments to change ordinances to allow TNR activities. For example, PetSmart Charities donated over 20 million dollars toward TNR activities.[26]

Cats Hunt—So What?

Ecologists and conservation biologists are most concerned with the direct impacts of high numbers of feral cats on populations of native wildlife but are also concerned about competition with native predators for food and the spread of zoonotic disease due to interspecific interactions. At the center of the controversy is not that cats hunt, but rather what and how much they hunt and whether it has real implications on prey at the population level. Domestic cats are thought to pose a significant threat to the birds, herpetofauna, and small mammals that they prey upon (Fig. 18.1).[27,28,29] Despite domestication occurring over thousands of years, cats retain the instinct and skill of hunting. Cats have been documented killing a prey item even while eating their favorite food,[30] and Barratt[31] reported the number of prey that cats captured was not influenced by the number of meals provided. Davis[32] observed that domestic cats continued to hunt rats and pigeons during periods of supplemental feeding and that feeding did not decrease hunting. Cats have now been implicated in 63 species extinctions on islands[33] but have also been found to have negative impacts on songbirds in noninsular environments.[6,12] A recent estimate of the broad impact of free-roaming cats on the wildlife of the United States suggests that up to 3.7 billion birds and 20.7 billion mammals fall prey to cats each year.[34] Feral cats are responsible for a greater proportion of the kills than pet cats (Fig. 18.2). This may be related to the increased amount of time feral cats spend outdoors. In fact, we found a significantly higher proportion of stray colony cats to exhibit hunting behavior compared to pet cats studied in Georgia. Sterilization does not appear to

• **Figure 18.1** Select victims of domestic cat attack. (Courtesy DL McRuer, DVM, The Wildlife Center of Virginia, 2015.)



• **Figure 18.2** Still image from Kittycam video of cat carrying captured Eastern chipmunk (Tamias striatus) to its residence. (National Geographic Remote Imaging. Athens, GA, 2011.)

affect a cat's motivation to hunt.[35] Although much effort is devoted to the quantification of how much wildlife cats hunt, the results are unlikely to produce enough evidence to convince many cat activists. The presence of millions of free-roaming cats (both feral and pets) is undeniable. Wildlife faces many threats, some of which are difficult to predict and prevent (e.g., emergent diseases). The impact from invasive species is both predictable and preventable.

Urban and suburban ecosystems serve as habitat to diverse mammals, reptiles, and amphibians as well as resident and migratory songbirds.[6] Suburban environments (e.g., backyards, zoos, parks), contain fragmented islands of natural habitats, surrounded by roads and development that act as barriers to wildlife movement and exert other anthropogenic influences on the health of natural systems (pollution, sediment run-off, loss of plant food sources, bird collisions with windows, etc.). Due to the decline of natural areas and the rapid expansion of developed areas,[36] urban and suburban habitats are critical to the future protection of biodiversity. Suburban backyard habitats may provide valuable resources to native wildlife, but they may become ecologic traps if they harbor non-native predators.

Animal Welfare—From Both Sides

There is also a large group of stakeholders concerned with the welfare of abandoned and feral cats. A few studies report higher disease prevalence among cats living in feral colonies than in owned cats.[37,38] Feral cats are subject to environmental extremes, and various sources of trauma and predation, all of which contribute to high mortality rates,[9,37,39,40] and relatively short life spans.[40] In a study where we outfitted cats with point-of-view cameras, we found free-roaming cats engaged in daily risky behaviors, most commonly: crossing roads, interacting with other cats, drinking from puddles in parking lots, exploring storm drain systems, and entering crawlspaces of buildings.[41] The evidence of the poor quality of life of feral cats has led one animal rights group, People for the Ethical Treatment of Animals (PETA), to agree that euthanasia is the most humane management option for homeless cats[42]; however, others claim feral cats, and especially well-managed cat colonies, may live healthful, relatively long lives (e.g., HSUS, Alley Cat Allies, Best Friends Animal Society). These organizations support the maintenance of feral cat colonies over lethal control.

Whereby cat welfare is well advertised and supported, wildlife victims of cats do not receive as much attention, yet personnel involved in wildlife rescue and rehabilitation regularly treat animals injured by domestic cats. Wildlife rehabilitation continues to gain popularity and National Wildlife Rehabilitators Association (NWRA) membership has grown from approximately 200 to >1800 members since 1984. The exact number of animals rehabilitated is difficult to estimate, but during 2007 alone, NWRA members reported treating over 100,000 animals.[43] Cats were among the top anthropogenic causes for submission of injured animals to a wildlife clinic in Tennessee and resulted in a large percentage of deaths or euthanasia.[44] Cat depredation was the foremost cause for admission of bats to rescue centers in Italy[45] and for juvenile blue-tongue lizards *(Tiliqua scincoides)* in Sydney, Australia.[46] Interactions with cats was the second greatest cause of small-mammal admissions to the Wildlife Center of Virginia over a 10-year period and the second greatest cause of avian mortality at the center.[47] We recently reported that cats contribute substantially to cases presented to wildlife rehabilitation hospitals and even with extensive veterinary intervention, their potential for recovery and release was very slim. We reviewed data collected from 82 wildlife rehabilitation centers throughout North America during a 3.5-year period to determine common causes of admission and found domestic pets to be responsible for 14% of admissions, the second most common identifiable cause of wildlife injury. Greater numbers of birds than reptiles, amphibians, or mammals were admitted for rehabilitation as a result of domestic cat attack, and 78% of these did not survive. The majority of immature individual animals of all species submitted because of cat attacks also died or had to be euthanized because of the severity of injuries.[48] Wildlife rehabilitation data raises awareness of the preventable suffering of individual animals injured by domestic species. One argument proposed by cat activists about the activities of feral cats is that cats have a right to express their natural hunting behavior. The welfare of individual cats receives extensive attention from advocates and many members of the public, whereby the welfare of individual wildlife usually remains unseen.[16]

Public and Wildlife Health Concerns Related to Feral Cats

Cats are reservoirs for numerous pathogens of zoonotic concern, including *Bartonella henselae*, *Salmonella* spp., *Toxocara cati*, tapeworms, hookworms, and *Sarcoptes scabiei.*[49,50] Cats are the definitive host for *Toxoplasma gondii*, which has been increasingly linked to negative neurologic issues in humans, ranging from schizophrenia to cognitive function in children.[51,52] By far, the most serious zoonosis of feral cats is rabies, and people exposed to the virus are often associated with feral cats (i.e., living near or caring for colonies). Outside of cases due to bats, human exposure to rabies is primarily associated with feral cats.[53,54] One study summarizing 10 years of data in South Carolina found that feral cats were more likely to be rabid than stray dogs and the second most common species to expose humans to rabies (behind foxes).[55] Even though many cats in managed TNR colonies receive a rabies vaccination when sterilized, they do not receive boosters and are vulnerable to infection after short-term protection, particularly from other terrestrial rabies vector species (e.g., raccoons) (*Procyon lotor*) that tend to aggregate at feral cat feeding stations.[56] Another serious, but more rare, concern for people interacting with feral cats including caregivers and veterinarians is plague *(Yersinia*

pestis). This bacteria is endemic in the western United States, and cats are responsible for at least one infection every year.[8] Increasing urbanization and its associated high numbers of feral cats lead to further potential for increased contact and transmission of disease between cats and people.[57]

The transmission of pathogens across multiple hosts has been documented in several locations at the urban-wildland interface,[58] and cats may serve as reservoirs for significant pathogens that affect other wildlife, such as Feline Leukemia Virus (FeLV), which was transmitted from feral cats to the endangered Florida panther (*Puma concolor coryi*).[59] Since the discovery that *Toxoplasma gondii* was responsible for mortalities of sea otters (*Enhydra lutris*) in California,[60] the scope and results of studies elucidating the detrimental effects of this introduced pathogen into the marine ecosystem have broadened.[61]

Management Alternatives to Trap-Neuter-Release or Euthanasia

Ultimately, feral cat numbers are unlikely to decline unless multiple strategies are adopted in combination. At the core of that, and depending on the region, a vigorous trap and removal program is likely needed. In addition, strongly enforced, effective licensing, identification, and confinement laws are integral. Continued financial support for animal control agencies and shelters that are tasked to remove, socialize, and adopt animals is also very important. Currently, US and European societies do not allow or support feral dog populations and many propose the same should be true for cats. Lastly and most important is a broad and consistent public education program that encourages responsible pet ownership and promotes (1) the reasons for keeping cats indoors or supervised while outdoors and (2) how to practically achieve this. For example, cat owners need to be shown examples of devices that may be easily used to allow cats to enjoy the outdoors without harming wildlife (e.g., cat runs/aviaries/habitats, flexible cat tunnels, harnesses). In the absence of reproductive feral cats, new cats enter stray populations as "lost pets" or from irresponsible owners who abandon them. Strong enforcement of fines for this behavior and other measures are needed. TNR colonies should be relocated from and should not be established in sensitive habitats where threatened and endangered animal species reside, for example, piping plover (*Charadrius melodus*) shore habitat in Jones Beach, New York.[62]

One of the intermediate solutions that may replace free-roaming TNR colonies is an enclosed colony. An enclosed colony (sometimes termed a "sanctuary") assumes that all of the benefits afforded a managed TNR colony are available for a group of cats that live in an enclosed, typically outdoor space.[63] An enclosed colony would require a colony manager (and the various volunteers that are typically involved in the care of TNR colonies) to provide daily care for the cats, including feeding, medical care, etc. Enclosed colonies would protect the welfare of cats, possibly helping them live a happier, healthier life, as well as help protect wildlife. They may provide a solution, particularly for managing smaller cat colonies, and this option should be explored by more cites and nonprofit organizations. Cats could be trapped, neutered, and then released only within the boundaries of an enclosure where they are safe from vehicles and predators and where wildlife outside the fence is protected. Enclosed colonies would require property, enclosure materials, and structures—a large initial cost; however, many organizations (e.g., Alley Cat Allies), corporations (e.g., PetSmart), and local county and city jurisdictions that donate millions of dollars annually to TNR lobbying and education might contribute to funding them. In addition, TNR programs already utilize dozens of dedicated volunteers[64] who could instead focus their efforts on care and adoption of cats at these facilities. Enclosed sanctuaries may also provide unique opportunities for socializing the tamest of the cats until they are adoptable. Currently, there are few examples of such successful efforts (e.g., Chico Cat Coalition, Chico, California; Blind Cat Rescue and Sanctuary, St. Pauls, North Carolina). Before these enclosures can be promoted as an alternative solution, scientific evaluation of the feasibility of creating and maintaining sanctuaries is recommended. In the end, if we do not come together to create a combination of strategies that all groups can support, cat effects on biodiversity may become so apparent that it may lead to more extreme activities such as the baiting and poisoning exhibited in Australia to protect threatened and endangered species.[65]

The Role of Veterinarians in Feral Cat Management

Public policy decisions in the United States continue to be made based on inadequate information[18] and influenced by loud and passionate advocacy groups. General public attitudes toward cats, experiences with feral cats, and preference for management should be examined across a broader scale. The sociopolitical aspects of feral cat management are the greatest challenge because the highly charged emotions associated with both sides of the issue inhibit progress on actual population reduction. Additional study of public perceptions of feral cats may help local managers make more informed decisions and aid in understanding the growing public debate regarding feral cat management.

Veterinarians who care about wildlife and conservation can and should contribute to resolving the dilemma of feral cat management. First and foremost, do no harm: when asked to participate in a TNR program, consider that TNR is inconsistent with the policies advocated by wildlife conservationists and managers. For example, the Wildlife Society, a 10,000-member strong professional organization that provides board certification for wildlife biologists, and is considered parallel to the American Veterinary Medical Association, strongly opposes TNR.[66] Cats re-released to the environment, particularly when offered supplemental

food and shelter, continue to negatively impact wildlife. Numerous other allied organizations also have strong policy statements against TNR and in support of alternative solutions, including: the American Ornithologists' Union, the International Wildlife Rehabilitation Council, the Association of Avian Veterinarians, the American Association of Wildlife Veterinarians, the National Association of State Public Health Veterinarians, National Wildlife Rehabilitators Association, and many others.[67] Consider disseminating this message to practitioners who are often recruited or hired to participate in TNR under the false assumption that "doing something is better than nothing at all" and explain the intricacies of why this is not true across the board. Become involved in local policy; veterinarians are well-respected members of the community who can have a powerful impact at a town council meeting to determine feral cat management strategies. Become active in educating the public; engage in presentations and panels that involve the general public. Provide cat owners with viable solutions; all of us have friends who allow their cats to roam outdoors. Rather than cringing, provide them with designs of affordable cat runs, inexpensive cat mesh tunnels, training tools for keeping cats on a leash and other means of allowing supervised time outdoors as an alternative to keeping cats only indoors. Engage with others that do not share your views and hold constructive discussions. Remember, all stakeholders agree that the ultimate goal would be to have all cats under the care of a person who cares for them and away from harming wildlife.

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19

The United States Agency for International Development Emerging Pandemic Threats PREDICT Project—Global Detection of Emerging Wildlife Viral Zoonoses

KIRSTEN V.K. GILARDI AND JONNA A.K. MAZET

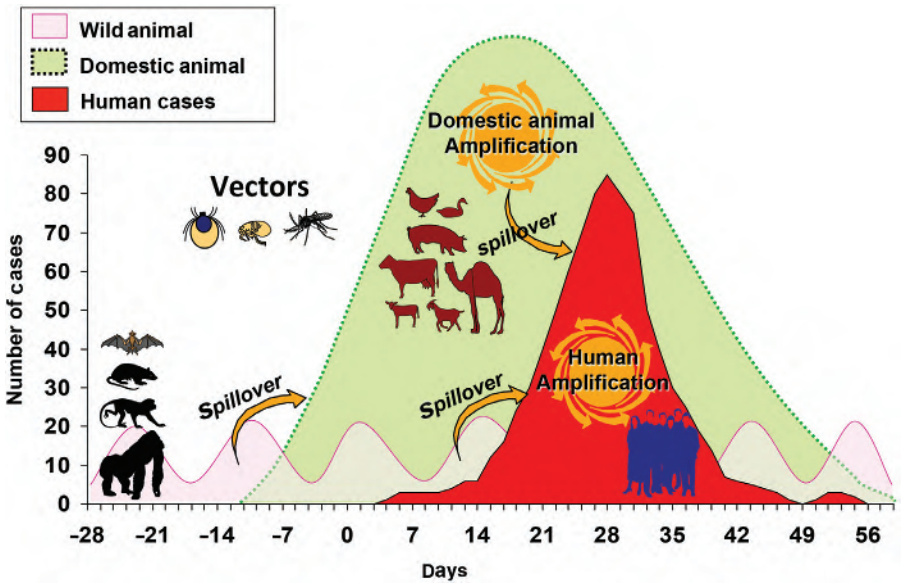
This chapter is based heavily on the more detailed PREDICT-1 Final Report.[31] The authors wish to acknowledge the contributions to the success described herein of the PREDICT Consortium (http://www.vetmed.ucdavis.edu/ohi/predict/publications/Authorship.cfm).

Introduction

The global burden of infectious disease disproportionately impacts developing nations, where human communities have less access to clinical care, clean water, and sanitation. Of particular concern and urgency for global health is the fact that the majority of emerging infectious diseases (EIDs)—diseases caused by previously undescribed pathogens, or by known pathogens that have recently expanded their host and/or geographic range—are of wildlife origin.[1,2] As human population growth and environmental change bring people into contact with wildlife in unprecedented ways and increasing frequency, pathogens carried by wildlife are "spilling over" into domestic animal and human populations.[3] Commonly, these events are occurring in places where a lack of diagnostic testing facilities and planning and infrastructure for outbreak control means that by the time a wildlife zoonotic EID event is recognized, opportunities for effective control measures have passed, resulting in devastating loss of life (Fig. 19.1).

Drivers for wildlife zoonotic disease emergence are biological, ecologic, and behavioral. Viruses, especially RNA viruses, are of greatest of concern, because of their ability to rapidly evolve to gain virulence and adapt to new hosts.[4] Ecologically, certain wildlife taxa are of more concern than others. For example, because bats are an ancient vertebrate taxon that has co-evolved with a diversity of endemic viruses, are highly communal in their biology and behavior, and may migrate long distances, they have proven to be a frequent source of new zoonotic pathogens for people.[5] Nonhuman primates, due to their genetic relatedness to people, have been the source for global pandemic infections like HIV/AIDS and persistent outbreaks of sylvatic yellow fever.[6,7] Direct and indirect contact with rodents, which commonly commingle with people in dwellings worldwide, have also resulted in the emergence of hantavirus and the geographical spread of Chagas Disease (see Chapter 35).[8,9]

Furthermore traditional practices such as hunting lead to human use of wildlife habitat, and human enterprises such as agricultural development and natural resource extraction convert wildlife habitat for human use. These behaviors and activities bring people into close contact with wildlife. As a result, pandemic EIDs such as Ebola virus disease have emerged, particularly in areas of high biodiversity (see Chapter 34).[10,11] Nipah virus, for example, was first reported in Malaysia in 1999, causing pneumonia in domestic swine and encephalitis in swine farmers (see Chapter 40).[12] Once the previously unknown paramyxovirus was isolated from patients, the source of the virus was traced back to Pteropid fruit bats that were roosting in trees in swine facilities that had been constructed in newly deforested habitats. Similarly, severe acute respiratory syndrome (SARS) emerged in November 2002 in southern China, causing flu-like illness in people and causing mortality in 10% of cases.[13] By July 2003, SARS had been detected in 37 countries. A novel coronavirus was isolated from patients and also detected in wild palm civets (*Paradoxurus hermaphroditus*) being sold



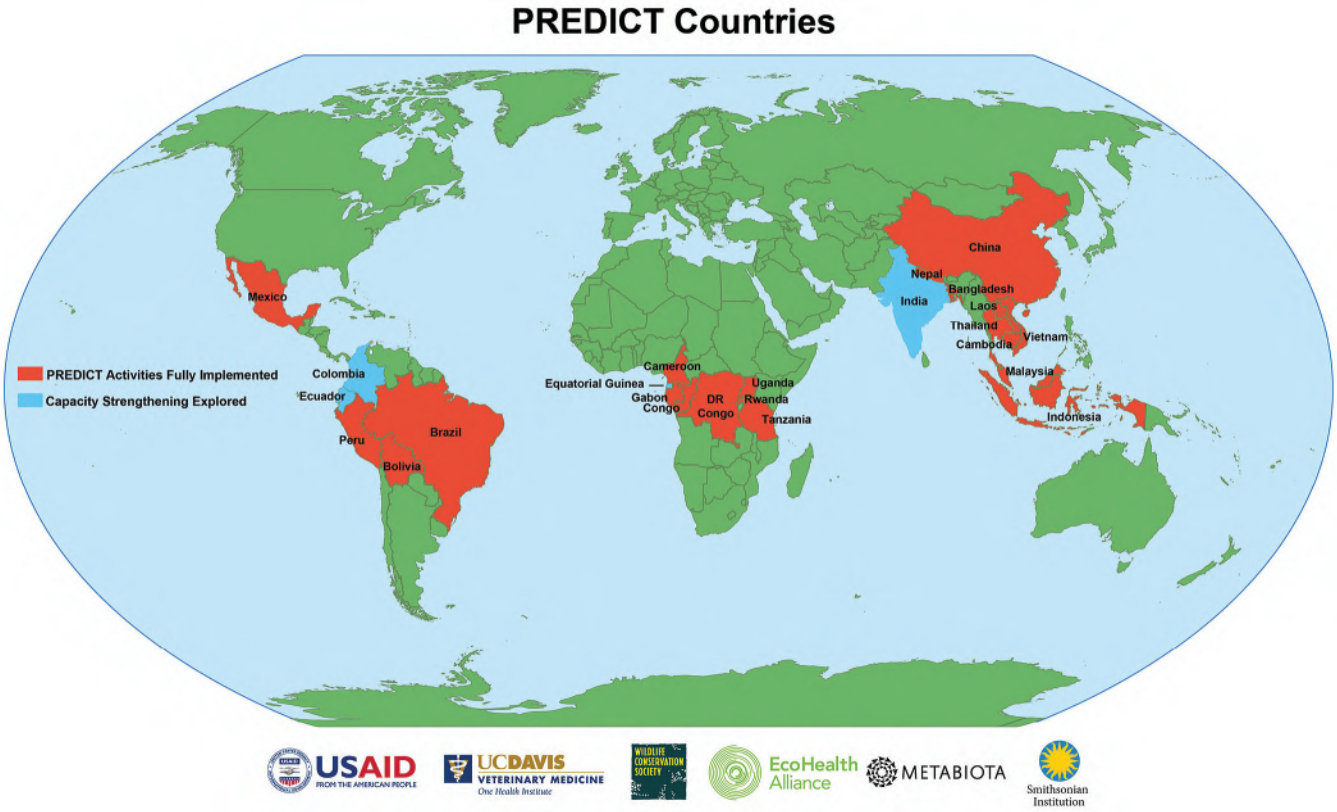
• **Figure 19.1** Wildlife Viral Pathogen Spillover Transmission of emerging zoonotic pathogens from wildlife populations *(pink)* into livestock *(green)* and/or people *(red)* may lead to devastating outbreaks. (Modified from Karesh WB, Dobson A, Lloyd-Smith JO, et al: The ecology of zoonoses: natural and unnatural histories. *Lancet* 380:1936–1943, 2012.)

live in markets for human consumption. This unfortunately triggered large-scale extermination of civets in a misguided attempt to curb the outbreak, as it was later determined that SARS-like coronaviruses (Co-V) were endemic in wild bats, with civets and humans as spillover hosts.[14]

United States Agency for International Development Emerging Pandemic Threats PREDICT Project (2009–2014)

The US Agency for International Development (USAID) has been working globally to improve both our collective understanding of the risk for transmission of pathogens from wildlife to humans, and to increase the capacity in developing countries for effective response and containment. Expanding upon its substantial investments in building worldwide capacity for avian influenza surveillance and control, USAID launched the Emerging Pandemic Threats (EPT) program in 2009 with the goal of strengthening capacities in developing countries to prevent, detect, and control all emergent wildlife zoonotic diseases (not just avian influenza). The EPT program was designed to be proactive and preemptive in its approach to wildlife zoonoses: to increase our understanding of the ecologic, viral, and behavioral drivers of wildlife virus spillover; train people in biodiversity hotspots around the world to detect and control zoonoses and disease emergence; and build capacity among government ministries for outbreak response and mitigation.[15]

Of several core projects that comprised the first 5-year phase of the EPT (2009–2014), PREDICT's mandate was to build the evidence base for zoonotic disease emergence from wildlife sources that would inform capacity-building for EID mitigation, control, and prevention. Led by the University of California, Davis, One Health Institute (in the School of Veterinary Medicine), PREDICT was initially implemented in 20 countries in Africa, South and Southeast Asia, and Latin America by a consortium of organizations including EcoHealth Alliance, Metabiota, the Smithsonian Institution, and the Wildlife Conservation Society (Fig. 19.2). PREDICT's objectives were to: (1) conduct wildlife surveillance and virus discovery in EID "hotspots" characterized by high wildlife diversity and increasing human pressure on natural resources; (2) characterize high-risk human-animal interfaces, behaviors, and drivers of pathogen spillover from wildlife to people; (3) improve virus detection and discovery by developing laboratory and disease outbreak response capacities; (4) optimize predictive models for zoonotic disease emergence and spread; and (5) deploy cutting-edge information management and communication tools to advance a more integrated, global approach to sharing data from zoonotic virus surveillance. As well, the PREDICT Consortium was wholly committed to limiting potential harm to wildlife populations by implementing live-capture protocols only and by expressing a conservation ethic in all activities, especially communications with stakeholders. For example, when discussing bats as hosts of potential zoonoses, PREDICT teams always coupled these messages with an explanation of the importance of bats to ecosystems, agriculture, and biodiversity. Also, in order to discourage bat depopulation, messages emphasized that killing bats would likely result in increased risk for viral transmission due to dispersal of disturbed populations and increases in reproductive rates to compensate for mortality.



• **Figure 19.2** PREDICT Consortium and Countries from 2009 to 2014, a consortium comprised of the University of California, Davis, EcoHealth Alliance, Metabiota, Wildlife Conservation Society, and Smithsonian Institution implemented the USAID Emerging Pandemic Threats PREDICT project in 20 countries in Africa, South and Southeast Asia, and Latin America. (From PREDICT Consortium: *Reducing pandemic risk, promoting global health*. One Health Institute, University of California, Davis, December 2014. http://www.vetmed.ucdavis.edu/ohi/local_resources/pdfs/chapters/6_predict_virus_detection_discovery.pdf)

PREDICT Approach

Wildlife Surveillance at Human-Wildlife Interfaces

While most large-scale animal disease surveillance programs have targeted known or expected pathogens and have been conducted in areas of known disease occurrence, PREDICT's approach had to achieve the early detection of unknown yet potentially emergent viruses. Therefore, a risk-based approach to viral surveillance was utilized. PREDICT country-based teams worked with governmental and nongovernmental partners to identify sites where human activities were resulting in a high level of contact between wildlife and people. Sampling also took place at sites where there were extensive anthropogenic impacts on habitats and landscapes (e.g., not in pristine habitats) adjacent to human communities deemed vulnerable to zoonoses due to a lack of infrastructure for quality food storage, safe hygiene, or accessible health care. Field personnel then conducted sampling specifically at these high-risk human-wildlife interfaces and focused their sampling on the specific taxa—primates, rodents, and bats—for which there was a preponderance of scientific evidence for their role in zoonotic disease emergence.[16] Wild animals were live-caught with traps, nets, or remote immobilization techniques, according to established protocols approved by the Institutional Animal Care and Use Committee (IACUC) for safe and humane capture, and were released post sampling. Standard morphometric measurements and photographs were obtained to aid species identification. Whole blood, mucosal (oral, nasal, rectal, genital) swabs, feces, and urine (when feasible) were collected from each animal, placed in a variety of media (lysis buffer, viral transport media), and then transferred within hours to −80°C freezers or liquid nitrogen dewars for safe storage until testing. PREDICT also developed and utilized techniques for noninvasive sampling of wildlife for application in circumstances that made safe capture of wildlife difficult or impossible.[17] At these sites, qualitative data were collected on the nature and extent of these human-wildlife interactions, and on the presence of domestic animals, water sources, etc.

Virus Detection and Discovery

PREDICT applied consensus polymerase chain reaction (cPCR) and high-throughput sequencing (HTS) tools to detect and describe DNA and RNA viruses present in

wildlife samples. This approach allowed for the detection of viruses presumably present at low levels in the populations surveyed, while also enabling a broad search for known and new viruses in the range of zoonotic viral families, including alphaviruses, arenaviruses, astroviruses, bunyaviruses (including hantaviruses), coronaviruses, filoviruses, flaviviruses, herpesviruses, orthomyxoviruses, paramyxoviruses, poxviruses, reoviruses, retroviruses, and rhabdoviruses. The use of cPCR was appropriate technology for use by PREDICT laboratories around the world, as it was relatively inexpensive and easy to implement in resource-limited countries. Samples identified by PCR were further cloned and sequenced, enabling discernment between previously described and new viruses.

Information Management and Reporting

In order to handle the enormous quantity of wildlife surveillance, site characterization, and laboratory testing data generated by all 20 countries, PREDICT developed an in-house on-line platform for secure, internal, standardized and centralized data collection, collation, and management. Policies on data ownership, governance, and release were developed in collaboration with host governments and tailored to each country. Once laboratory test results were verified and interpreted by the global viral discovery team, test results reports were shared exclusively with host country governments, and any findings of potential concern were discussed with governments first. Once host governments had an adequate period of time to receive and consider the potential implications of PREDICT findings, data were made publically accessible through the public data-sharing and visualization platform, HealthMap.[18]

PREDICT Results

PREDICT is likely the most comprehensive wildlife viral detection and zoonotic disease capacity development program in the world to date. It achieved major advances in understanding wildlife viruses and the factors that contribute to their spillover into human populations on a global scale and in building capacity in less developed countries for the rapid detection and control of EIDs (Fig. 19.3). Through PREDICT, more than 2500 people, including government personnel, veterinarians, students, physicians, laboratory technicians, field biologists, and hunters, were trained in biosafety and PPE, surveillance methods, laboratory techniques, and disease outbreak investigation. PREDICT worked with more than 32 diagnostic laboratories around the world to institute low-cost methodologies for conducting PCR assays for rapid detection of viruses in wildlife samples. By humanely sampling more than 56,000 nonhuman primates, bats, rodents, and other wildlife at high-risk human-wildlife interfaces, PREDICT detected 984 unique viruses in wild animals and people, 815 of which were novel. This effort more than doubled the number of known mammalian viruses in the world.

Novel Viruses (Table 19.1)

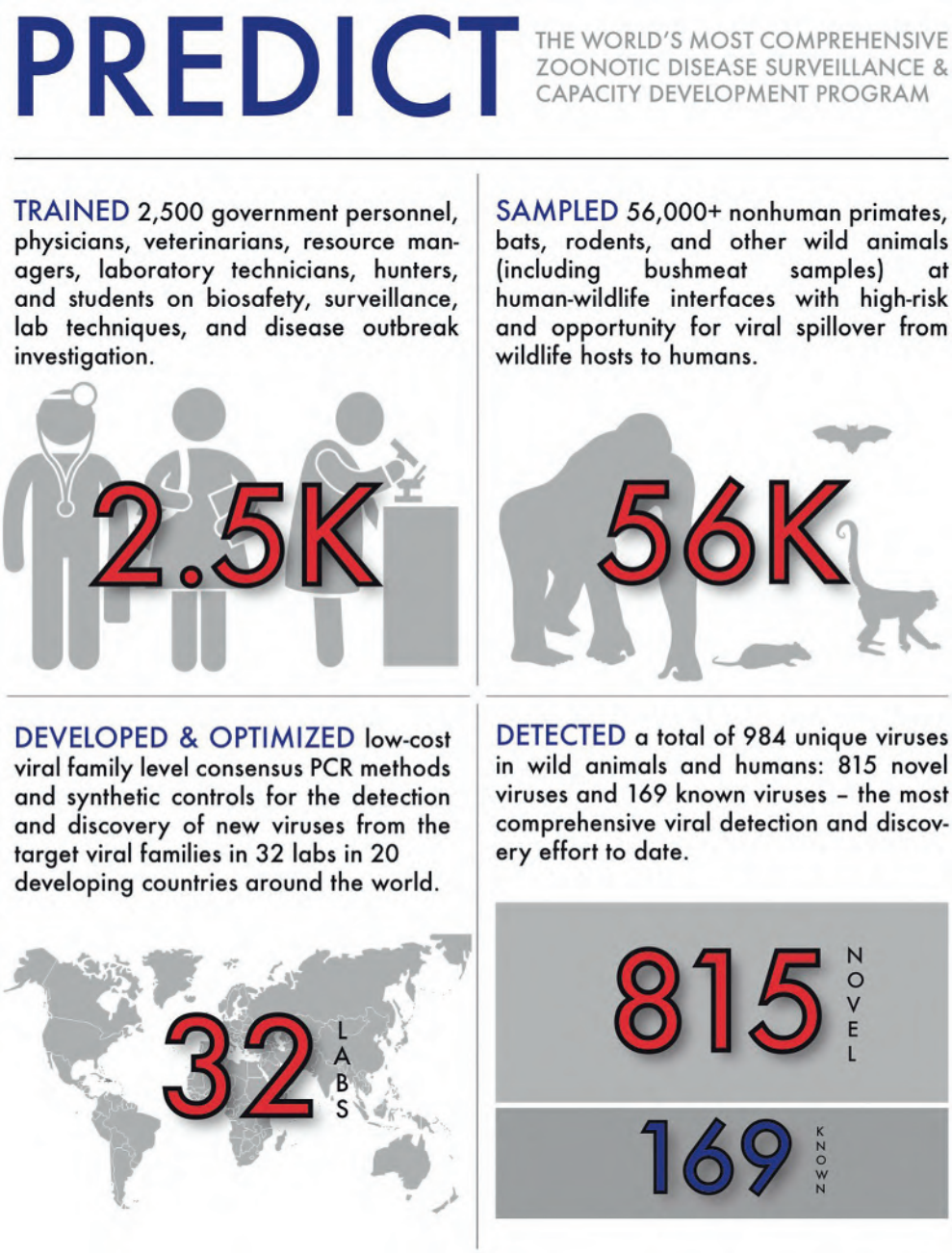
Among hundreds of viral discoveries, many have implications as potential sources of pandemics of wildlife origin. For example, PREDICT effectively doubled the known number of viruses in the family Coronaviridae that includes SARS and Middle Eastern Respiratory Syndrome (MERS) (see Chapter 42). Novel retroviruses and paramyxoviruses were also detected, including the detection of novel henipaviruses (the same viral family that contains Nipah and Hendra viruses) and filovirus exposure in bats.[19–24] As well, PREDICT researchers found strong evidence for western lowland gorillas *(Gorilla gorilla gorilla)* as the nonhuman primate reservoir for Human T-lymphotropic virus in western Africa, and discovered a new simian immunodeficiency virus strain in a naturally infected chimpanzee *(Pan troglodytes troglodytes)* with AIDS-like symptoms.[25,26] Hepatitis B virus (HBV) was found to be circulating among gorillas and chimpanzees and among subspecies of chimpanzees, refuting the previously held assumption that HBV genotypes are host-specific, with implications for the potential for spillover in this group of viruses.[27] Furthermore, PREDICT documented anthropozoonoses—human to primate transmission of viruses—including a human metapneumovirus that caused fatal respiratory disease in wild mountain gorillas *(Gorilla beringei beringei)* and human herpes simplex-1 virus that caused classic stomatitis in confiscated eastern lowland gorillas *(Gorilla beringei graueri)*.[28,29]

New Models for Emergence

PREDICT built upon the original "hotspots" model to further assess patterns of wildlife disease emergence in time and space, confirming that the risk of EIDs is highest in areas of high mammalian biodiversity, and finding that mammal diversity, land use type, and land use change are the most important factors for predicting wildlife EIDs.[3,30] As well, PREDICT searched all published data available through 2010 to identify animal hosts, human activities, and high-risk disease transmission interfaces implicated in zoonotic virus spillover. Network analyses were used to examine these data for transmission pathways, viral traits, host species characteristics, taxonomic ranges, and geographic distributions of zoonotic viruses, to provide fresh insight on the virus characteristics and conditions that pose a risk for future wildlife EIDs.[16]

Outbreak Response

In addition to conducting wildlife viral discovery, PREDICT provided support to governments and institutions during 23 zoonotic disease outbreaks in 10 countries between 2010 and 2014, most of which were impacting human populations. For example, PREDICT collected bat samples and integrated wildlife and human response teams to help respond to a Nipah virus outbreak in Bangladesh and participated in several Ebolavirus outbreak response



• **Figure 19.3** PREDICT Results The first 5-year phase of the USAID Emerging Pandemic Threats PREDICT was the most comprehensive zoonotic disease surveillance and capacity-building effort ever undertaken; it trained 2500 people, built capacity in 32 laboratories, humanely sampled more than 56,000 wild animals, and detected 984 viruses, the majority of which were new discoveries. (From PREDICT Consortium: *Reducing pandemic risk, promoting global health*. One Health Institute, University of California, Davis, December 2014. http://www.vetmed.ucdavis.edu/ohi/local_resources/pdfs/chapters/6_predict_virus_detection_discovery.pdf)

efforts in Uganda by collecting samples from wildlife and domestic livestock and conducting surveys in Ebola-affected communities to better understand how people were coming into contact with wildlife. In 2012, PREDICT provided post-mortem and field investigation support to diagnose a yellow fever outbreak causing mortality in red howler monkeys in Peru, in time for the government to mount a vaccination program to prevent spread to local human communities. During the devastating 2014–2015 West Africa Ebolavirus outbreak, PREDICT achieved the early detection of an Ebola virus causing an outbreak in the Equateur Province, Democratic Republic of Congo, which arose independently of the West Africa outbreak. Rapid and accurate detection of the virus facilitated early control procedures by the government, which effectively contained the outbreak before it could spread.

TABLE 19.1 PREDICT Viral Discovery

Viral Family	Novel Bat	Known Bat	Novel Primate	Known Primate	Novel Rodent/ Shrew	Known Rodent/Shrew	Novel Human	Known Human
Adenovirus	53	3	6	4	32	1	1	3
Astrovirus	153	33	19	3	31	1	0	1
Coronavirus	61	30	3	0	6	0	0	2
Dependovirus	0	0	11	0	0	0	0	0
Flavivirus	3	0	0	1	0	0	0	2
Hantavirus	3	1	0	0	0	2	0	1
Herpesvirus	46	0	48	25	43	6	0	5
Orbivirus	1	0	1	0	0	0	0	0
Paramyxovirus	63	7	0	2	11	2	0	3
Polyomavirus	27	1	4	3	8	0	0	1
Arenavirus	0	0	0	0	2	2	0	0
Rhabdovirus	19	0	2	0	7	0	1	0
Bocavirus	1	0	0	0	0	0	0	0
Enterovirus	0	2	1	3	0	0	0	0
Retrovirus	0	0	5	4	2	0	0	5
Alphavirus	0	0	4	7	0	0	0	1
Poxvirus	0	0	0	1	0	0	0	0
Influenza	0	0	0	1	1	0	0	0
Mononegavirales	0	2	0	0	0	1	0	5
Papillomavirus	0	0	1	0	0	0	0	0
Picobirnavirus	0	0	120	0	0	0	0	0
Picornavirus	0	0	4	0	0	0	0	0
Picornavirales	0	0	4	0	0	0	0	0
Phlebovirus	1	0	0	0	0	0	0	0
Rotavirus	0	0	1	0	0	0	0	0
Anellovirus	0	0	0	0	0	0	1	1
Hepadnavirus	0	0	0	0	0	0	0	1

Note numbers of viruses do not total to 984 as viruses have been found in more than one wildlife host taxa.
From PREDICT Consortium: *Reducing pandemic risk, promoting global health*. One Health Institute, University of California, Davis, December 2014. http://www.vetmed.ucdavis.edu/ohi/local_resources/pdfs/chapters/6_predict_virus_detection_discovery.pdf.

Next Steps: PREDICT 2014–2019

The first phase of PREDICT realized significant advances in our knowledge of the global wildlife virome and the human activities and land use changes that put people at greater risk for spillover infections from wildlife viruses.[31] PREDICT served as a real-world proof of concept of the relevance and appropriateness of addressing disease risk at the human-animal-environment or One Health interface and achieved it with a conservation ethos.[32] Presentations to government partners and interactions with communities about PREDICT created unique opportunities to allay fears about or animosity toward wildlife and to talk about the intrinsic value of wildlife populations, intact habitats, and biodiversity.

In its current phase (2014–2019), PREDICT has embarked upon an even larger scope with a more intense focus on the dynamics of zoonotic viruses in wildlife, people, and livestock (primarily influenza, filovirus, paramyxovirus, and coronavirus) and the human behaviors that drive their

spillover, amplification, and spread. Working with new governmental and nongovernmental organization partners in 30 countries, the team is documenting viral sharing among diverse hosts and targeting even more intense surveillance at high-risk pathways for viral transmission to identify the social and ecological drivers of pathogen emergence. In particular, PREDICT is addressing the risk of wildlife sold as food and medicine in markets in Asia and Africa, exploring both the magnitude of the conservation and disease risks, as well as the palatability of specific interventions to motivate behavior change. Ultimately, the goal is to provide essential information for helping less developed nations strengthen their capacities for epidemic prevention, thereby contributing to pandemic prevention and improving global health security.

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20

Renewable Energy: Effects on Wildlife

PEREGRINE L. WOLFF AND MICHAEL R. SCHIRMACHER

Introduction

Renewable energy provides several environmental benefits compared to generating electricity from fossil fuels including: (1) a decrease in greenhouse gasses and their related impact on environmental degradation associated with climate change, (2) national economic and energy security, and (3) an increase in a country's economic productivity due to an increase in energy production proficiency. However, these are not without environmental consequences.[1] The impacts on wildlife include direct mortality from the installation and operation of an energy plant as well as indirect mortality from habitat fragmentation and loss with resulting decreases in biodiversity. When assessing the impact of renewable energy systems on wildlife, stakeholders should attempt to understand the ecologic consequences and identify the factors that should be addressed in an environmental cost-benefit analysis, prior to large-scale installation.

This chapter will use examples in the state of Nevada for a review of the regulatory process by which renewable energy plants are brought online and regulated with regard to wildlife impact in the United States. In addition, we will review the known impact on wildlife of the most well-studied forms of renewable energy, on and offshore wind, and thermal and photovoltaic (PV) solar. We will also summarize current and proposed mitigation efforts, as well as outline opportunities that zoo and wildlife veterinarians may follow to contribute to mitigating the effects on renewable energy's impact on wildlife.

The US Environmental Protection Agency (EPA) defines renewable energy sources as "green power" if they provide a "high benefit" to the environment by reducing emissions over fossil-fuel-based electricity sources. These green power sources include solar (thermal and PV), wind (on and offshore), geothermal, biogas (methane), eligible biomass, and low-impact hydroelectric sources. In 2014, renewable energy contributed approximately 22.8% of global electricity, and it is predicted that this percentage will increase annually as concerns for climate change and energy security grow, as renewable energy sources improve their cost competitiveness, as countries and states enact policies that set time-based goals for the amount of energy derived from renewable sources, and as demand continues in developing economies for access to modern energy.[2]

Renewable energy projects, in particular wind energy, have gained attention due to direct and indirect mortalities on individual wildlife species or taxa, especially if threatened or endangered species are involved, or if mortality is large enough to have population-level impacts. However, the expected rapid expansion of renewable energy globally, particularly with the conversion of large amounts of land to energy production ("energy sprawl") with associated habitat loss, fragmentation, and potential decrease in biodiversity, is of greater concern.[3] Terrestrial wind, solar PV, and bioenergy (utilizing the rapidly growing, temperate, perennial grass, giant miscanthus [*Miscanthus* x *giganteus*]) are predicted to be the three fastest growing forms of renewable energy, and that land use allocated for renewable energy will conflict with areas identified as high priority for biodiversity conservation.[4]

Bringing Renewable Energy Online in the United States: A Primer

Utility-scale renewable energy facilities generate a large amount of electricity, which is then transmitted to many down-line users via transmission to the grid. The development and operation of these facilities are subject to state and federal fish and wildlife regulations. Each state has its own statutes, regulations, policies, and permitting process for renewable energy development. Thus every state fish and wildlife agency implements its own wildlife regulations in coordination with its federal partners, such as the US Fish and Wildlife Service (USFWS) and the Bureau of Land Management (BLM). State and federal collaboration is imperative for ensuring that North American fish and wildlife and their habitats are conserved and managed in the public trust. To that end, fish and wildlife professionals recognize that renewable energy is a means to lessen US reliance on fossil fuels and remain attentive in seeing that reasonable measures are in place to reduce the impact of renewable energy development on the wildlife resource.

When developing impact minimization measures and the required permits necessary for a new project, agency personnel consider project type, project size, land ownership, proximity to other developments, and the presence of protected or sensitive state or federal fish and wildlife species. A case-by-case approach is used to determine the focus of applicable regulations and impact minimization. Three examples of renewable energy projects in Nevada are provided, which illustrate the application of either state or federal regulations.

Project 1: Utility-scale solar PV plant that is proposed on public land managed by the BLM in the Mojave Desert within occupied desert tortoise *(Gopherus agassizii)* habitat. A potential effect on the desert tortoise due to solar development is direct mortality from vehicles or equipment that may collapse burrows or entrap tortoises within burrows.[5] The desert tortoise is listed under the US Endangered Species Act (ESA) as threatened. Section 7 of the ESA is the mechanism by which federal agencies ensure the actions they take, including those they fund or authorize, do not jeopardize the existence of any listed species. If through a biological assessment or other review process, the BLM determines its action (authorizing the PV project) is "likely to adversely affect" the desert tortoise, the BLM then submits to the USFWS a request for formal consultation. During this consultation, the USFWS will prepare a biological opinion on whether the proposed solar PV project will jeopardize the continued range-wide existence of the desert tortoise.

In this example, the USFWS finds that the PV project may adversely affect the desert tortoise but not jeopardize its continued existence. When this happens, the USFWS prepares an "incidental take statement." Under most circumstances, the ESA prohibits take, which is defined as harming (including killing) or harassing a listed species. Take that results from a federal action but is not the purpose of the action is considered an incidental take. Incidental take may be allowed when the USFWS approves it through an incidental take statement. The statement establishes the amount or extent of anticipated take, reasonable and prudent measures to minimize the take, and terms and conditions that must be observed when implementing those measures.[6]

Project 2: Utility-scale wind farm proposed on public land managed by the BLM in the Great Basin within close proximity to golden eagle *(Aquila chrysaetos)* nesting territories. The primary threat to eagles at wind facilities is collisions with the towers and turbine blades.[7] The Migratory Bird Treaty Act (MBTA)[8] and Bald and Golden Eagle Protection Act[8] prohibit take of eagles except pursuant to federal regulations. In 2009, the USFWS promulgated new permit rules for eagles that address the issue of wind farms.[9] Under these new rules the USFWS can issue permits that authorize individual instances of take of bald *(Haliaeetus leucocephalus)* and golden eagles or "programmatic" take, which means that instances of "take" may not be isolated but may recur.[9] These new rules acknowledge that even wind facilities that are designed and operated with utmost care to conserve wildlife may, under some circumstances, result in the "unavoidable" take of eagles. Wind project operators must implement eagle conservation practices that are required for the issuance of Eagle Incidental Take Permits.[9]

Project 3: Utility-scale concentrating solar power project located on public land managed by BLM. Concentrated or thermal solar power plants use tracking mirrors to reflect and concentrate sunlight onto a heat exchanger or receiver tubes. The tubes contain fluids that absorb the concentrated sunlight in the form of heat energy. The heated fluids flow through the tubes and are used to produce steam that drives a conventional turbine, which is connected to a generator producing electricity. Similar to coal-fired or natural gas power plants, water is necessary in the cooling processes and is discharged to industrial wastewater evaporation ponds. In the deserts of the southwest, these ponds are an attractant to migratory and nonmigratory birds, and potentially bats, and may represent a secondary hazard if the wastewater contains toxicants or the increase in the density of birds increases collision mortalities with mirrors and the tower.

State agencies, such as the Nevada Department of Wildlife (NDOW), may regulate these artificial or artificially created bodies of water that contain chemicals or substances that cause or will cause the death of wildlife. NDOW's Industrial Artificial Pond permit is a means to facilitate impact avoidance, minimization, and mitigation measures regarding industrial solutions that pose a risk to wildlife and are usually in an open environment like a pond. Site-specific recommendations for reducing impacts to wildlife are made on a case-by-case basis. NDOW works closely with their federal partners, including the USFWS, when considering measures proposed for species protected not only under the MBTA, but also under the State of Nevada.[10]

Wildlife Impacts From Specific Energy Sources

Wind Energy

Wind energy facilities (WEF) are either on- or offshore. Onshore WEF are one of the cheapest and most developed renewable energy sources and currently account for 0.5% of global energy production.[2] The Intergovernmental Panel on Climate Change predicts that this will increase by 5%–29% by 2030. Wind energy has probably generated the most studies on impacts to wildlife, with the majority conducted in the areas where the facilities are located, in North America and Europe.

The development of offshore installations is increasing with a trend for turbines farther from shore in deeper waters.[11] The primary environmental concerns identified include: (1) the effects of increased noise levels on marine mammals, turtles, and fish; (2) collision risks for seabirds with the turbines and tower structure; and marine mammals and sea turtles with increased boat traffic; (3) impacts to

benthic and pelagic habitats including food webs; (4) pollution from the release of seabed contaminants and construction vessels; and (5) disruption for all species to migration routes and feeding grounds.[11,12] The construction phase of the turbines potentially leads to the greatest impacts on wildlife from noise and increased vessel traffic. Driving piles into the seafloor is loud with sounds audible under water for tens of kilometers. There is concern that this generated noise could cause damage to hearing in addition to disrupting communication and leading to spatial displacement in marine mammals and perhaps sea turtles.[11] Studies have shown a number of marine fish species to be affected by noise levels equivalent to pile driving, documenting barotrauma injury and hearing loss as well as behavior changes, which could, potentially, disrupt movements for breeding, spawning and foraging.[13] Once operational, undersea noise levels from the turbines are considered low enough as to not cause severe noise-related issues in marine mammals. However, it is not known whether turtles, fish, or other benthic species may be impacted by lower frequency noise levels or by electromagnetic fields generated from cables that transfer the electricity to shore.[11,13]

The effects on avian species from offshore WEF have been assessed as (1) risk of collision, (2) disturbance, or (3) loss of foraging habitat, and are highly variable by species.[12] The risk of collision correlates with species who do not avoid wind farms and whose flight pattern is within the sweep area of the blades, with species that fly lower to the water (below the sweep) having a decreased risk of collisions.[12] Disturbance results when birds spend extra time and energy avoiding WEF, particularly during times of increased human activity (construction and maintenance), which may lead to displacement, potentially depriving them of important foraging areas.[12] Decreased use by some species of areas within 100–600 m from WEF has been documented in a number of studies.[14]

Bat fatalities at wind turbines were first documented from Australia[15] in the 1970s, but received little attention until nearly three decades later when estimates of thousands of bat fatalities from individual WEF were reported in the eastern United States.[16] At that time, the installed wind energy capacity was approximately 6 gigawatts (GW); now, through the first quarter of 2017, it exceeds 84 GW.[17] Cumulative estimates for the United States and Canada suggest that hundreds of thousands of bats are killed by wind turbines each year.[18]

Direct collision with moving turbine blades as well as barotrauma due to sudden pressure changes near the moving blades are both thought to contribute to mortality in bats. Gross necropsy, histopathology, and radiology have been used to examine carcasses recovered from around turbines.[19] Lesions consistent with blunt force trauma (fractures, abdominal wall herniation, hemothorax, and moderate to severe middle ear hemorrhage) as well as barotrauma (hemothorax and moderate to severe middle ear hemorrhage) have been documented,[19] although more information on the pressure changes required to cause barotrauma in bats can help to determine if this is a significant source of fatality. In addition, studies have suggested that bat fatalities do not seem to be random events, and available data suggest that bats—at least some species—might be attracted to wind turbines.[20] In both the United States and Canada, at least 22 species of bats have been reported as fatalities at WEF.[15] However, certain species appear to be more susceptible than others. For example, three species of migratory tree-roosting bats, hoary bats *(Lasiurus cinereus)*, eastern red bats *(Lasiurus borealis)*, and silver-haired bats *(Lasionycteris noctivagans)*, make up nearly 80% of the reported fatalities at sites in the United States and Canada.[18] The composition of fatalities may vary geographically, with high proportions of Brazilian free-tailed bat *(Tadarida brasiliensis)* fatalities reported in the southwestern United States.[21] The impact of wind turbines on other species, including the tri-colored bat *(Perimyotis subflavus)*, Indiana bat *(Myotis sodalis)*, northern long-eared bat *(Myotis septentrionalis)*, and little brown bat *(Myotis lucifugus)*, although found in relatively low numbers in the United States and Canada, is a concern because populations are already decimated by white-nose syndrome, a disease caused by the fungus *Pseudogymnoascus destructans* (see Chapter 72).[22] Because bats have low reproductive rates, large-scale fatalities may result in a population-level impact.[23] Recent research suggests that the hoary bat may suffer 90% population decline in the next 50 years if fatalities continue at the current rate,[24] although this does not consider large-scale implementation of impact reduction strategies, such as operational minimization (see discussion section for more details).

Little is known about the impact of offshore WEF on bats; however, migrating bats have been documented offshore and further investigation is warranted to understand how offshore developments may affect bats.[11]

Discussion

The majority of studies on renewable energy's impact on wildlife results from studies on avian mortalities at onshore WEF. Similar to offshore WEF, there are concerns for impacts during both the construction and the operation phases of these facilities, with multispecies studies documenting highly variable responses of species to both phases. When compared to reference sites, in a European-based study, there was a documented decline in the abundance of 10 diverse bird species during construction.[25] Some species returned to preconstruction density during the operation; however, others remained suppressed or absent at sites.[25] What is not clear from many of these studies is if displaced birds are moving to other available habitats for breeding, or are being lost from local breeding populations.[25] There is also some evidence that the impact on local avian population densities around WEF may decrease with time, thus short-term monitoring studies (<5 years) and may not be capturing the true impact on populations.[26] There is currently no published information on avoidance or disturbance effects of WEF on large mammals.[27] Approximately 250 species of birds, primarily small passerines, make up the majority

of the direct (collision-related) mortalities from terrestrial WEF; however, large raptors have received the greatest public attention. The Altamont Pass Wind Resource Area (APWRA) in Alameda County, California, is the oldest of the WEF in the United States. Brought on line in the 1980s, it is still the largest in the world with approximately 6000 turbines spread over 50,000 acres.[28] Reports of annual golden eagle mortalities (estimated at 75–110 per year) brought into public conscience the deadly impact of this new form of "green power" on avian species.[28] In addition to golden eagles, and other raptors and passerines, the mortality rates for all avian species at APWRA are estimated at 4700 per year.[28] Direct collisions have been documented at all WEF—the results of which are public—and reports indicate a range of 0 to >30 collisions/turbine/year.[14] Although hundreds of thousands of passerines are killed at these facilities annually, there is no consensus among researchers that this level of mortality is leading to population-level impact in passerine species, and this represents only a small portion of the annual human-associated cause of avian mortality.[27] Domestic cats (see Chapter 18), powerlines, communication towers, buildings, and windows cause a much greater level of mortality.[27] Due to their slow maturity and long reproductive life, there are concerns for local and potential species-level decline in raptors where facilities have been placed along migration routes—for example, in southern Spain, where raptors congregate before migrating across the strait of Gibraltar to Africa.[27]

A number of mitigation measures have been proposed and implemented to decrease the direct and indirect impact of WEF on wildlife. For proposed projects, siting to avoid areas of high conservation importance (primary migration corridors and flight paths), as well as avoiding a critical habitat for species of high conservation concern, must be considered. Increasing turbine height and spacing appear to be the critical factors at APWRA in reducing collision-related raptor mortalities.[7] The replacement of smaller low-capacity turbines at APWRA with larger, higher capacity and more efficient models is predicted to decrease collision mortalities by 50%.[27] The new, larger turbines have fewer blade rotations per minute and the towers are smooth, as opposed to the older lattice structures, which likely provided perches ideal for raptors scouting prey.[27] Turbine shutdown or removal at critical migration routes has also been implemented. In an attempt to increase visibility and prevent collisions, ultraviolet paint has been used on towers and blades. To date, limited studies have found little effectiveness in this method.[27] European researchers have developed assessment tools to apply to individual projects and individual species, to rank the risk of a facility to priority populations and to assist companies in siting offshore WEF to minimize the impact on these species.[11]

Current data suggest that, in general, bats are at highest risk of fatality under relatively low-wind-speed conditions.[29] Moreover, unlike birds, bats tend not to fly into stationary structures, and the majority of fatalities at wind turbines occur only when the turbine blades are spinning.[30] This culminated in the hypothesis that bat fatality may be reduced by preventing blades from spinning during high-risk conditions. This is accomplished by feathering the blades (i.e., pitching the blades parallel to the wind) until wind speeds are high enough to reduce the risk to bats. Given the relatively narrow period of time when bats are at risk (i.e., at night, during low-wind-speed conditions, during late-summer and autumn at least at latitudes ≥35°N), changes made to turbine operations are minimized. The first US-based study to test this strategy demonstrated a 44%–93% reduction in bat fatality at turbines feathered up to wind speeds of 5 and 6.5 meters/second (m/s) compared to normal turbine operating conditions of 3.0 m/s.[29] In addition, this study reported the annual power loss associated with this strategy was ≤1%, which means it allowed 99% of the annual power to be produced while reducing bat fatality up to 93%. Subsequent studies have reported similar reduction levels using this strategy, although effectiveness likely varies by species, wind-speed condition, and turbine model.[31] Other promising research involves the use of ultrasonic acoustic deterrents to reduce bat activity near WEF, but more studies are needed before a commercially ready device is available.[31] Given the estimated high annual fatality of bats at WEF, the potential population-level impact, and the relatively rapid growth of WEF, the timely large-scale adoption of impact-reduction strategies is necessary to reduce risk and minimize fatalities.

Solar Photovoltaic and Concentrating Solar Thermal Power

Solar facilities (SF) may consist of hundreds to millions of solar collectors and span thousands of acres. In the United States, the largest SF are primarily located in the Desert Southwest and often sited on public land. The effects of SF on wildlife are not well documented in peer-reviewed literature with the majority of reports in unpublished or non-peer-reviewed literature, such as environmental impact documents.[5] Similar to other forms of renewable energy, the greatest threat to wildlife from SF may be habitat fragmentation and loss, as well as disruption during construction with the impact lasting into site operation. Site preparation (grading and leveling) dust, noise, and soil compaction, as well as altered water flows, alterations in microclimates, and electromagnetic field generation have all been identified as causing a potential impact on wildlife.[5] Studies on the impact of noise during construction and potentially into operation have documented noise levels produced by some vehicles as reaching 110 decibels.[5] At this level, Mojave Desert wildlife species, such as kangaroo rats (*Dipodomyss* spp.), desert iguanas (*Dipsosaurus dorsalis*), and fringe-toed lizards (*Uma* spp.), have been documented to suffer hearing loss, and abnormal emergence patterns in aestivating spadefoot toads (*Scaphioous* spp.) have been triggered.[5]

In addition to wildlife, of particular concern in this fragile and arid ecosystem is the impact on vital plant communities. Dust from site disturbance may disrupt the

physiologic processes of desert plants, including photosynthesis, gas exchange, and water usage, whereas commonly used dust suppressants (chloride salts) applied to roads and graded areas may damage plant growth at the point of application as well as in adjacent habitats from runoff.[5] The alteration of water flow and soil compaction also may have a lasting impact on plant communities by potentially degrading significant amounts of habitat around SF sites.[5]

Many species may experience direct mortality from the construction and operation of SF; however, the desert tortoise, due to its threatened status listing with the USFWS, has received the most attention. If incidental take is not permitted, then mitigation involves translocation of the tortoises to an alternative habitat with subsequent monitoring. However, the science of translocation in this species is young, and long-term monitoring of moved tortoises greater than 2–3 years is currently lacking. Translocation of *G. agassizii* depends on the individual animals being located and removed from the site. It is likely that there are a certain number (especially juveniles) that are missed prior to site preparation. Additionally, soil compaction from traffic—even from fairly lightweight vehicles—has been shown to extend to the depth of shallow burrows (<33 cm). This may cause crushing or entrapment of species that use shallow subterranean habitats for hibernation, including locally or federally protected species, such as the flat-tailed horned lizards *(Phrynosoma mcallii)* and the Coachella Valley fringe-toed lizard *(Uma inornata)*.[5]

Bird collisions with structures at SF, boiler towers, and tracking mirrors have been reported at concentrating solar power facilities.[32] In addition, the concentration of a number of mirrors at stand-by points on the boiler tower may create enough heat to burn the skin and feathers of birds flying through the thermal column.[32]

Conclusion

Renewable energy facilities are coming online at unprecedented rates worldwide. According to the Renewables Global Status Report 2016, "2015 was an extraordinary year for renewable energy. Renewables are now cost competitive with fossil fuels in many markets and are established around the world as mainstream sources of energy. Globally, renewable electricity production in 2015 continued to be dominated by large (e.g., megawatt-scale and up) generators that are owned by utilities or large investors."[2] The negative impacts of climate change are one of the key drivers in the current global commitment to bring these utility-scale renewable energy facilities online. However, major impacts to wildlife, including loss of biodiversity, may potentially occur in both scenarios unless there is a multistakeholder approach or a policy supporting research to understand and quantify impacts, and to improve existing or develop new strategies to minimize the negative effects of utility-scale renewable energy on wildlife.

There are many opportunities for veterinarians to contribute to the renewable energy issue. With few published peer-reviewed literature on the impacts of utility-scale renewable energy on wildlife, there is a desperate need for research. No matter where you live, there is likely a renewable energy plant in operation or in the permitting process nearby. Veterinary expertise is likely needed to help alleviate the consequences of localized impacts to wildlife caused by a wind farm or solar plants, transmission lines, or the toxic/hyper-saline solutions of industrial ponds.

Perhaps the most important action veterinarians may take is to embrace and teach that energy conservation is the most effective method to diminish the impact of all energy-generating facilities on wildlife.

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SECTION 4

Reproduction

21

Female Infertility in Zoo Animals

BRUCE CHRISTENSEN

When investigating potential causes for lack of success in a particular breeding program, first determine what is known about that species with regard to reproductive physiology and reported causes of subfertility, as well as what is known about the individual animal's history. A general list of questions for a clinical history is included (Box 21.1). This chapter will discuss potential causes for female infertility, and group them into stages of the reproduction process.

Female Infertility

When considering female reproductive health, it is useful to categorize the stage of the reproductive process where the problem is likely occurring. Ask the following questions:

1. Does this female appear to cycle normally?
2. Has early pregnancy been diagnosed and subsequently lost?
3. Has this female experienced abortion, dystocia, stillbirth, or neonatal losses? (Late-term pregnancy losses are beyond the scope of this chapter.)

Causes of Subfertility Associated With Regular Cyclicity

Male Subfertility

When a pair of animals desired for reproduction fail to produce offspring, one of the most basic questions to answer is whether the fault lies with the male, the female, both, or neither. If this male has recently produced offspring with a different female, then the focus will be more on the female's reproductive ability, compatibility between the current pair, and methods being used currently compared to what has worked in the past. If the male's current fertility is unknown, he also must be the source of fertility investigation. Focus on male subfertility is beyond the scope of this chapter but would minimally include a thorough reproductive history, physical examination (with ultrasound), and semen analysis.

Abnormal Genitalia

Abnormalities in genital anatomy, whether congenital or acquired, may affect the ability of a female to mate, maintain a pregnancy, or successfully undergo parturition. Vaginal strictures and septa have been diagnosed in some species that make copulation painful for the female. Females with these disorders typically have a history of regular cycles, being attractive to and attracted by the male during estrus, allowing initial mating, but then objecting and not allowing the male to complete intromission, and may be reluctant to allow the male to mount in subsequent attempts. Physical examination of the vaginal tract is necessary to diagnose these conditions. The vaginal tract may be evaluated manually, digitally, or through the use of specula or endoscopic equipment. Visual evaluations must be done carefully to avoid inadvertently passing a narrow instrument beyond strictures. Strictures or septa might be corrected via manual or surgical reduction. Post-procedural fibrosis with subsequent repeat stricture may be managed with physical emollients during the healing period, but this requires daily application for at least a week. If reduction is not possible, or postoperative stricture occurs, and reproduction is still desired, then artificial insemination with planned cesarean section should be discussed as the vaginal anomalies would increase the risk of dystocia. The genetic component to vaginal abnormalities will most likely be unknown in zoo species, so this will remain an uncertain risk of breeding these animals.

Parturition-Related Injury

Dystocia related injuries may result in fibrous tissue forming inside the lumen of the reproductive tract, sometimes completely occluding the lumen and trapping fluid in the proximal portions of the tract, resulting in hydrometra, mucometra, or pyometra. Even if the fluid is resolved, subsequent damage to the uterus may be severe enough to render the female infertile. Depending on the species, and the nature of the fluid trapped, the female may or may not show systemic signs of disease. Bluntly dissecting the fibrous tissue and allowing the fluid to drain passively, or flushing the uterus with isotonic solutions, may achieve temporary relief. Recurrence is likely as the scar tissue is prone to form again. Complete resolution of the problem may be achieved only through hysterectomy.

Dystocia may also result in damage to the cervix, vestibulovaginal fold, or vulva, preventing these structures from acting as a physical barrier to ascending pathogens.

• BOX 21.1 Clinical History for Female Infertility

1. What is the normal age of puberty for this species?
2. What is the normal age for sexual maturity for this species?
3. What is the age of senescence in this species?
4. What conspecifics are housed with the individual?
   a. Where is this individual in the hierarchy?
   b. What is the past social history?
5. Have new individuals been introduced recently?
6. What is the quarantine protocol for new animals?
   a. Did this animal come from another country (or a facility not associated with the Association of Zoos & Aquariums (AZA))?
   b. Has any infectious disease screening been done (especially for known potential reproductive pathogens)?
7. Describe the vaccination and deworming history.
8. What is the general health of the individual?
   a. Weight?
   b. Body condition score?
9. What are the results of past reproductive examinations?
10. What is the reproductive history of the individual?
    a. Does this female have any history of any reproductive diseases?
    b. Is this a seasonal species?
    c. What are the results of hormone monitoring?
       i. Do we know the normal cycle for this species?
    d. What type of mate access has been allowed?
    e. Breeding dates
       i. Describe each event
       ii. Past history of aggression against potential mates
    f. Types of breeding (natural vs. artificial)
11. Has this animal received any hormone treatments for clinical conditions?
12. Describe any previous contraceptive treatments including type of contraception, dates administered, length of time on the contraceptive, and any signs of reversal.
13. What are the signs of proestrus in this species?
    a. List dates of onset and describe proestral signs.
14. What are the signs of receptivity in this species?
    a. List dates of onset and description of receptivity.
15. Are any ovulation dates known? How were they determined?
16. What are observed dates of first refusal of mating?
17. What is the length of the interestrous interval?
    a. What is normal for this species?
18. Has pregnancy ever been diagnosed? (List dates and methods.)
    a. Have methods been established for this species?
19. Has this female ever aborted a pregnancy?
    a. If so, please include necropsy data.
20. Previous parturition history
    a. What methods were used to predict the window of time for possible parturition?
    b. What methods were used to monitor the onset of parturition?
    c. What were her previous litter sizes?
    d. Has this female experienced a dystocia? If so, describe the details.
    e. Has this female experienced any stillbirths?
    f. Have any of this female's offspring had congenital malformations?
    g. Have any of this female's offspring experienced neonatal death?
    h. What is the necropsy data from fetal or neonatal deaths? Include placental lesions.
21. Describe the history of maternal care.
    a. Has this female ever committed infanticide?
22. Describe the female's lactational history.
23. Has this female ever experienced any false pregnancies? How were they diagnosed?
24. Have the males bred to this female sired any offspring with other females? If so, when? How were those breedings accomplished?

Surgical corrections are possible, but postoperative failure is a common complication.

Fibrous adhesions may form within the abdomen following cesarean section, preventing the uterus from having normal contractions and making it prone to fluid accumulation and an inability to maintain a pregnancy. Diagnosis is made by ultrasound or by exploratory surgery. Adhesions are likely to form again and hysterectomy may be the only treatment.

Age-Related Changes

Age-related pathologic changes to the female reproductive tract will happen with most females if they live long enough, but they are more pronounced in older, nulliparous females, or those with long inter-birth intervals. Pregnancy does have a protective, regenerative effect on the female reproductive tract[1–6]; females who experience the hormonal changes of multiple, nonpregnant cycles develop a variety of species-specific pathologic changes. Age-related changes reported in different species include cystic endometrial hyperplasia (CEH), pyometra, hydromucometra, adenomas, periglandular fibrosis, periovarian cysts, and leiomyomas.[1,3,5] Leiomyomas in elephants reduce in size after treatment with gonadotropin-releasing hormone vaccines.[7]

It is difficult to overstate the importance of the "use it or lose it" hypothesis when considering what is known from investigations of aging and reproduction in domestic and zoo species, and the current breeding management practices that have resulted in a skewed population in zoos toward aged, nulliparous individuals that now are having difficulties reproducing.[5] This has led to a more comprehensive, life-long view of reproductive health in captive species' breeding management. The Association of Zoos and Aquariums' Reproductive Management Center now promotes lifetime reproductive planning for genetically valuable females; this new direction is discussed in greater detail in Chapter 22. While recommendations will surely need to be adjusted for each species as we learn more, it seems wise to plan on breeding valuable females early in their reproductive life at least once, and then spreading out subsequent pregnancies throughout the lifetime of the female. Concerns regarding surplus animals[8] will need to be addressed and are discussed in Chapter 23 in this text.

Uterine Disease

Uterine disease, including endometritis, pyometra, endometriosis, hydrometra, mucometra, CEH, or neoplasia, will prevent early embryonic development or interfere with placentation. Diagnosis of uterine disease usually involves imaging of the uterus (usually with ultrasound) and observing abnormal tissue or fluid, and then sampling the abnormalities to determine their nature. Manually or endoscopically guided techniques for transcervical cytology, culture, and biopsy of the uterus have been described for large and small species.[9–11] Endometrial culture, cytology, and biopsy samples have been obtained transcervically from a variety of zoo species, ranging from small carnivores to large ungulates. Most techniques require some degree of sedation or anesthesia, but the collection techniques are minimally invasive and do not require a surgical approach. Once information is obtained as to the pathologic state of the uterus, targeted decisions may be made regarding potential therapy including uterine lavage and antimicrobial therapy.[12]

Endometriosis is reported in great apes and Old World monkeys and may be treated both medically and surgically.[13] Endometritis, a more superficial inflammation in the uterus, is known to occur in other species as a result of bacterial and fungal pathogens, as well as contact with sperm. Most females are able to clear the effects of acute endometritis through normal immune responses. Endometritis should be suspected if fluid is detected in the uterus prior to breeding, or beyond 36 hours after breeding.[14] Fluid may be detected by the observation of a mucopurulent vulvar discharge or through ultrasound evaluation. Definitive diagnosis of etiologic agents may be determined through uterine culture or biopsy. Intrauterine administration of antimicrobials through the transcervical route over the course of 4–7 days may be more effective than systemic administration, though in zoo species this may not be possible.

The etiology of CEH differs, depending on the species, but it is more commonly observed in aged, nulliparous females. As with other reproductive diseases, pregnancy is protective and allowing females to reproduce early may reduce the incidence of CEH. The presence of CEH reduces fertility by impairing the ability of endometrial glands to function. In some species, CEH predisposes the uterus to infection and subsequent pyometra.[15] If a female with CEH is able to successfully become pregnant and maintain the pregnancy, subsequent CEH lesions are decreased. CEH is significantly more pronounced in older, nulliparous female red wolves and African painted dogs, especially among those that were contracepted with deslorelin acetate implants (Suprelorin), melengestrol acetate (MGA), or simply isolated from males for prolonged periods.[1,15] The development of CEH was decreased if megestrol acetate (Ovaban) was given prior to deslorelin administration in order to suppress the initial stimulation phase of the reproductive tract.

Persistent accumulation of fluid in the uterus may result in irreparable damage to the endometrium. In the case of pyometra, inflammatory products may have systemic effects, as well. Some species, including horses, may have a dramatic pyometra without systemic effects, whereas in others, like African painted dogs or red wolves, pyometra is life threatening because of the systemic release of endotoxins from gram-negative infections. Culture of the fluid should be performed to determine if an infectious agent is present, and treatment should be appropriately chosen. In addition, efforts to drain the fluid through lavage and ecbolic agents are necessary if the uterus is to be spared. In many cases, hysterectomy will be the efficient treatment of choice. Even if the animal is not systemically ill, the prognosis for future fertility is guarded to low, and the risk for recurrence is high.

The most common neoplasia of the female tubular tract is leiomyoma. Depending on the extent and location of the lesion, leiomyoma is likely to render the female infertile. In some cases, leiomyoma has been definitively associated with prolonged nonpregnant periods, providing yet another reason for breeding valuable females early in their reproductive lives.[2–4,16,17]

Breeding Management Errors

Females in breeding management programs are sometimes fertile but are not able to reproduce because of improper breeding management decisions. These errors may include improper husbandry, poor timing of the estrous cycle, or inadequate handling of semen and the insemination process.[6] Anecdotal accounts abound in captive breeding programs documenting the importance of adequate space, certain dietary components, sociosexual structure, or even cleaning protocols. Individual mate choice is a critical behavioral component of a successful pairing.[18] Understanding the natural history of the species in question is paramount to providing the right environment for reproduction in captivity. Understanding the basic endocrinologic profile for that species is crucial. Endocrine monitoring may be achieved from blood, urine, or fecal samples. Endocrine monitoring of a male and female walrus, for example, documented that their active reproductive seasons were asynchronous. Stimulating the male to come into rut during the female's estrous period resulted in successful breeding and pregnancy.[19]

Finally, it should be accepted that even if everything is done correctly, successful reproduction does not happen all of the time, even with perfectly fertile, cycling females and fertile males. Biological variation is broad enough to ensure that many unpredictable variables may affect the success of a reproductive program.

Causes of Subfertility Associated With Irregular Cyclicity

Intersex Conditions

If a female has never been known to cycle, or has always cycled irregularly, a disorder of sexual development should

be suspected.[20] In mammals, sexual development involves the establishment of a chromosomal sex in the embryo (XX for females, XY for males), subsequent, respective differentiation of the bipotential gonad into either ovarian or testicular tissue, and finally, under the influence of gonadal hormones, differentiation of the tubular reproductive tract, accessory sex glands, and external genitalia. These processes are complex, and errors at any stage will likely result in an individual that is functionally subfertile or infertile. Often it may be impossible to define whether the individual is strictly male or female. Diagnosis of these conditions starts with determining the karyotype of the individual. Anatomic and histopathologic evaluations of the reproductive tract are also necessary to determine the nature of the specific condition. No fertility treatment exists for these conditions. Very little is known of these types of disorders in nonmammalian species. Sexual differentiation in birds is opposite to that of mammals, with the female being heterogametic (ZW) and the male homogametic (ZZ). For reptiles, amphibians, and fish, it becomes even more complicated as temperature, other environmental factors, or social status determine the current sex of the individual.

Poor Nutrition

The role of nutrition in a reproductive program is largely unknown. It is known that individuals at either extreme in body condition tend to show decreased fertility. Obesity in zoo animals is sometimes a problem and may very well contribute to subfertility. It is likely that some species require certain nutrients for successful or optimal reproduction, but data on specific examples are lacking.[21] Evidence has been presented that phytoestrogens may result in species-specific reproductive losses—for example, causing subfertility in captive-born female white rhinoceros, but not female Indian rhinoceros.[22] Phytoestrogens have been shown to negatively affect other species by causing cycle irregularity or predisposing them to reproductive diseases and lesions like pyometra, CEH, and leiomyoma.[23,24]

Endocrine Disorders

Endocrine disorders of any type should raise suspicion that reproductive hormones may also be affected through intricately balanced webs of positive and negative feedback loops. Hyperadrenocorticism, hypothyroidism, hyperthyroidism, and hyperprolactinemia all prevent normal ovarian function in females.[25,26] Treatment with cabergoline, while lowering prolactin levels, did not result in resumption of cyclicity in elephants.[27]

Neoplastic ovarian tumors will cause ovarian dysfunction either by causing cycle irregularities or complete shutdown of the estrous cycle, depending on whether or not hormones secreted by the tumor affect the contralateral ovary. The most common ovarian tumor in most species is the granulosa or granulosa-theca cell tumor (GCT). In some species, this tumor secretes inhibin, which will inhibit follicle-stimulating hormone (FSH) secretion and therefore prevent follicular growth in the contralateral ovary. Granulosa cells secrete anti-mullerian hormone (AMH), and assays measuring AMH have proven very sensitive and specific for the diagnosis of GCTs in horses.[28] Suspicion of GCT is high based on the finding of a large ovary with irregular tissue echogenicity on ultrasound. A positive AMH assay would be helpful, if normal ranges for the species in question were also established. Exogenous anabolic steroids may negatively feedback on endogenous hormones and prevent normal cycles. In zoo settings, if another animal in the enclosure is receiving hormone treatments, it should be investigated if any of the hormones are accessible to nontarget animals and therefore affecting their reproductive cycles.

Age-Related Changes

Advancing age may affect the regularity of cycles, as well as the subsequent fertility of those cycles. Typically these age-related changes are seen in, or at least exacerbated in, nulliparous females. Older, nulliparous white rhinoceros females (15–38 years old), for example, show prolonged periods where luteal activity is absent or erratic.[3,17] Simply having one calf during their early years prevents this occurrence of "flat-lining." This evidence gives further support to the already introduced idea of lifetime reproductive planning and allowing females to reproduce early in their lives in order to decrease the potential of subfertility later on.

Anovulatory Follicles and Persistent Corpora Lutea

While the etiology of anovulatory follicles is largely unknown, their occurrence in many domestic species is well documented. Typically these follicles grow larger than the typical size for dominant follicles but fail to ovulate in response to either endogenous or exogenous hormonal stimulation. They may become cystic, or they may eventually luteinize. Anovulatory follicles will often prevent a subsequent follicular wave from progressing until the anovulatory follicle itself regresses. If the anovulatory follicle does luteinize, prostaglandin administration may be used to lyse the luteal tissue and stimulate an earlier return to cyclicity by allowing the next follicular wave to progress. The presence and progression of these follicles is best documented by ultrasound.

In polyestrous species, prolonged interestrous intervals may be caused by persistent corpora lutea (CL) that do not regress after maternal recognition of pregnancy (MRP) fails to occur. This may happen because of disruption to the MRP signal or to the receptors responsible for receiving the signal. Mechanisms for MRP are species specific, so failure of MRP would vary in each species. Early loss of undetected pregnancy after MRP appropriately occurred would also result in persistent CL and prolonged interestrous interval. For unknown reasons, persistent CL are also known to occur in nonmated cycles and will result in a prolonged interestrous interval.

Causes of Subfertility Associated With Early Embryonic Loss

Old age is also associated with a higher incidence of early embryonic death (EED). It is thought that this increased incidence of EED is attributable to decreased follicular or oocyte quality as well as age-related uterine disease.[29,30] Very poor nutrition or deficits in critical nutritional requirements could also result in early loss of pregnancy, though documented cases are lacking. Access to exogenous steroids through hormonal medications or, in theory, phytoestrogens in feed, might also contribute to EED, although documented cases are lacking. Chromosomal abnormalities may allow embryos to mature through early cell divisions but fail to mature beyond the early embryonic stages, resulting in early pregnancy diagnosis with subsequent losses.[31] Diagnosis of these chromosomal abnormalities would require advanced molecular techniques in each species. Breeding pairs with high inbreeding coefficients have been shown to experience decreased fertility, lower litter sizes, and increased neonatal mortality.[32,33] Finally, luteal insufficiency is commonly suspected, though less commonly documented, in cases of EED. Progesterone assays from serum or fecal material may be evaluated in order to definitively diagnose luteal insufficiency, but normal ranges need to be established, and at least three samples should be run to be certain of the diagnosis. Secondary causes, primarily inflammatory or endocrine conditions, should be investigated as primary luteal insufficiency is considered rare. The secondary causes should be treated appropriately. Direct treatment of luteal insufficiency involves progestin supplementation. Sources of progestin will vary by species as not all progestagens are active in all species, and sources/types of endogenous progestagens may change within a species depending on the stage of gestation. Various synthetic and natural sources of exogenous progestagens are available and include injectable as well as ingestible forms.

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22

Changes in Reproductive Management

CHERYL ASA

The Sustainability Crisis

Sustainable animal populations are the ultimate measure of successful zoo and aquarium breeding programs. However, the programs for most species in regional zoo associations around the world are not reaching their demographic and genetic goals for long-term viability. Although percentages vary by region and by criteria used to define sustainability, results show that a large proportion of species are not reproducing at replacement levels.[1–3] This "sustainability crisis" demands a review of current practices affecting the reproductive management of species in these programs.

Sustainability depends on the successful reproduction of individual animals that contribute to the gene diversity of the population. Yet reproductive rates, calculated recently by Lincoln Park's Alexander Center for Applied Population Biology, are averaging less than 25% for pairs recommended by the Association of Zoos and Aquariums (AZA) (L. Faust, personal communication). As part of its Sustainability Initiative, the AZA has compiled survey results from animal program managers to produce its Sustainability Database, available through its website.

The AZA also reevaluated the activities of its various committees and advisory groups as well as its centers: the Population Management Center (PMC) at the Lincoln Park Zoo in Chicago, Illinois, and the Wildlife Contraception Center at the Saint Louis Zoo in St. Louis, Missouri. Recognizing the crucial role of improved breeding success to program viability, one of the major changes was to expand the scope of the Contraception Center to encompass reproduction in general, renaming it the AZA Reproductive Management Center (RMC). In this expanded role, the RMC now serves as a coordinating body for the AZA committees and advisory groups, whose expertise may contribute to improving the sustainability of animal programs (Box 22.1). The overall goal of this collaboration is to identify reasons for reproductive failure so that corrective measures may be developed and applied. To help achieve this goal, the RMC also works closely with the Reproductive Health Surveillance Program, based at Michigan State University, which evaluates the reproductive pathology of exotic species as it might relate to fertility or to contraceptive side effects.

Reproductive Management

Captive animal populations are carefully managed to promote their sustainability and preserve genetic variation, which increases their long-term viability.[4] To achieve these objectives, populations must maintain genetic diversity and a healthy age and sex structure, avoid inbreeding, and minimize adaptation to captivity.[5] Individual animals in AZA programs receive breeding recommendations generated by the PMC using PMx software to analyze the genetic composition of the current US populations. The role of the RMC is to assist programs in implementation of those recommendations, which includes enhancing reproduction in those receiving a breeding recommendation at a given time and preventing reproduction in the remainder.

The enhancement of reproduction may rely on hormone monitoring or assisted reproductive techniques, such as artificial insemination (e.g., black-footed ferret, elephant, rhino, Iberian lynx). More advanced in vitro techniques have generally proven less useful. In a review of the AZA Sustainability Database, the RMC found that husbandry was considered a major reason for reproductive failure in many species, particularly birds. Husbandry can include many factors—for example, habitat size and design, diet, cleaning routines, and exposure to other species. Each species must be evaluated separately, considering all potential impacts on reproductive processes, to develop the most effective approach.

Although the reasons for reproductive failure may vary among regional zoo associations, reproductive success in general requires healthy individuals that display species-typical behavior (see Chapter 14). Across species there are basic components of the reproductive process that must be in place or succeed for the production of offspring (Box 22.2).

The logistics of introducing potential mates may be challenging, especially if it requires transfer to another facility, which may be limited by temperature constraints. Introductions must often be carefully orchestrated to optimize social context and prevent undue aggression, although allowing some species-typical aggression may actually contribute to mate acceptance. That is, some level of aggression may be

• BOX 22.1 Association of Zoos and Aquariums Committees and Advisory Groups Under the Umbrella of the Reproductive Management Center

Committees	Scientific Advisory Groups
Wildlife Conservation Management	Reproduction and Endocrine
Animal Health	Nutrition
Animal Welfare	Behavior
	Small Population Management
	Biomaterials Banking

• BOX 22.2 Basic Components in the Reproductive Process Required for Successful Production of Young

Component
Introduction of male and female
Compatibility for mating
Fertility of both male and female
Full gestation or incubation
Appropriate parental care

a normal and perhaps critical component of the courtship sequence in some species. A basic familiarity with natural behavior may provide guidance.

Mate acceptance may also be enhanced by allowing some measure of choice.[6] Male–male competition and female choice is part of the reproductive process in many species, yet modern breeding programs seldom provide choice; instead, they present only the recommended partner. Recent studies show that breeding outcomes may be improved by offering more than one potential mate.[7] Logistics is one of the main challenges to presenting choice, so there is a critical need for devising and testing such models for a variety of taxa and situations. Offering choice need not compromise genetic management, because options might be limited to potential partners who also satisfy population goals.

Even if a pair is compatible and successfully mates, conception depends on both partners being fertile. Assessing fertility has become practical for many taxa with the availability of endocrine monitoring, ultrasound, and semen collection techniques. In fact, an increasing number of animal programs recommend routine endocrine monitoring, which may often use fecal samples that are relatively simple to collect for most species, to determine onset of puberty, breeding season changes, and even time of ovulation. A workshop conducted by the RMC in 2015 developed tools for assessing fertility in a representative ungulate and carnivore, something that could be accomplished for other taxa as well, by bringing together participants with the needed expertise. Approaches to addressing female infertility are presented in Chapter 21.

For most species in animal breeding programs, full-term gestation or incubation are necessary for the birth or hatching of healthy offspring that are able to survive. In mammals, a component of fertility is a healthy uterus where a conceptus may implant and be nourished until it is capable of surviving on its own. The situation may be similar for viviparous reptile species. The analogous period for birds and some other oviparous species is of course incubation, which requires appropriate behavior by one or both parents. The behavior sequence may be influenced both by learning (experience) and by the hormonal milieu, which itself may be affected by the interactions between mates (e.g., ring doves[8,9]).

Although not all species provide parental care for their young, it is a critical phase for most of those in managed breeding programs. Failure to display appropriate care may be simply a function of experience; that is, first-time parents are often less successful than those who have already gained experience. Although the hand-raising of young when the parents fail to provide adequate care may be deemed necessary for their survival, as adults they too may lack parenting skills, thus perpetuating the cycle of failure. Parenting may also be affected by the habitat provided or by social grouping, aspects usually considered of husbandry. Again, knowledge of natural history of the species may inform care.

Although mate choice, as discussed earlier, is familiar to many animal managers, *cryptic female choice* is less well known but may affect all stages of the reproductive process following mating.[10,11] The term refers to effects on conception, gestation/incubation, and parental care that may be compromised if a female is forced to mate with a nonpreferred male. The female may not actually be conscious of influencing these processes, but—perhaps through mechanisms associated with the stress response—sperm transport may be altered, resources may be shunted from eggs or embryos, and care may be withheld after birth or hatching. These phenomena have been best described for birds but are believed to affect other taxa as well. This is therefore another reason to consider offering mate choice.

Limiting Reproduction

Fulfilling PMC breeding recommendations entails not only producing offspring from chosen pairs but also preventing reproduction among those that have not received a breeding recommendation. Thus comprehensive reproductive management includes not only enhancing reproduction for some but assessing options for preventing the production of offspring in others. Options include separation of males from females, reversible contraception, or even, in some cases, permanent sterilization. Contraception recommendations in the United States are provided by the RMC (formerly the AZA Wildlife Contraception Center) through its website www.stlzoo.org/contraception. In Europe, where the availability of contraceptive products varies, a similar service is provided by the European Group for Zoo Animal Contraception through the website www.egzac.org.

Contraception has been used primarily in mammals, although it is being considered an option in an increasing number of other taxa. Even among mammals, a contraceptive product that is safe and effective in one taxonomic group may present risks in another. The best example is progestin-based methods, such as the melengestrol acetate (MGA, Wildlife Pharmaceuticals) implant and Depo-Provera injections (medroxyprogesterone acetate: Schering), that have been effective and safe in most species for decades. Efficacy is virtually 100% in females of all species treated, but few studies of reversibility have yet been conducted (golden lion tamarin,[12] tiger[13]) due to the complexity of judging reversal (e.g., mate access and need to compare to individuals matched by age and reproductive history).[14] RMC staff are conducting reversibility studies of two representative Old World monkey species, colobus and hamadryas baboon, to determine duration of implant efficacy more specifically so as to facilitate subsequent breeding recommendations.

Surveys submitted to the Contraception Database and subsequent investigation by RMC and RHSP staff have failed to identify contraceptive failures or significant health effects in the wide range of taxa that have been treated with the exception of carnivores. Studies have shown that these same progestin-based products carry a risk of endometrial and mammary pathology in felids[15] and canids[16]; and the response is likely similar in other carnivore species. Currently the best option for carnivores is a GnRH agonist (deslorelin: Suprelorin, Virbac Animal Health) paired with a short-term progestin to prevent the initial stimulation phase,[17] although time to reversal may vary greatly among individuals and species (M. Agnew, unpublished).

The GnRH agonist implant Suprelorin has also been used successfully in taxa other than carnivores.[18] Efficacy in females of most mammalian species is high once the correct dose has been determined. Although GnRH agonists should also be effective in males, higher dosages than those used in females of the same species are typically needed. However, males of most ungulate species have not been successfully suppressed. Use in some species of birds and reptiles has increased, but efficacy has been mixed. Whether the outcomes are due to species differences or inadequate doses is yet unknown.

Although not specifically reproductive management, euthanasia of individual animals judged genetically surplus to the population is an option in some countries and is being increasingly discussed in the United States.[19,20] See also Chapter 23.

Genetic Management Versus Reproductive Management

In current practice, breeding recommendations are generated for most species once annually or at least every few years based on the genetic composition at the time of analysis. That paradigm results in some females not receiving a recommendation to reproduce for many years. The paradigm also delays breeding for individual females for as long as possible, to lengthen generation time and thus lessen the loss of gene diversity over time. These strategies are designed to meet the goals of genetic population management, but they may compromise the fertility of females in the population.

However, this paradigm may be at odds with reproductive biology. In a study of canid species managed in AZA institutions, the prevalence of endometrial hyperplasia and pyometra was higher in females that had reproduced less frequently.[21] A review of the literature[22] found "use it or lose it" to be a theme common to a number of species (e.g., cheetahs,[23,24] elephants,[25] white rhinoceroses,[26] Seba's bats,[27]) as hypothesized by Lindburg and Durrant 20 years ago.[28] There are likely taxonomic differences in susceptibility to endometrial effects, and other components of the reproductive system may be affected differentially across species. Infertility resulting from long barren periods is avoided in some countries by management euthanasia (see Chapter 23).

A related phenomenon is the importance in many species of establishing fertility in young females by allowing early reproduction. In general, allowing a female to reproduce soon after puberty seems to affect the reproductive process in ways that increase fertility in later years, although the mechanisms may vary by species. However, the concern of population geneticists is that this decreases generation time and risks losing gene diversity. However, reproductive failure itself results in loss of gene diversity,

Lifetime Reproductive Planning

The success of genetic population management rests on recommended breedings resulting in the production of young, but as outlined earlier, reproductive rates are so low that most programs are not meeting their goals. Thus strategies for establishing and maintaining fertility are crucial. In the United States, the RMC is developing and testing models for lifetime reproductive planning for individual females. Breeding recommendations would not be based only on current factors related to population genetics. Instead, the potential genetic contribution of each female to the population could be developed (i.e., planned soon after her birth and before puberty). The ideal pattern for most species would allow each female to reproduce soon after reaching puberty, to establish fertility, and then at regular intervals to maintain fertility: "Breed early and often."

Various scenarios are being modeled, an analysis that must be repeated for each species owing to differences in parameters such as mating system, ages of first and last reproduction for the population, interbirth or interclutch intervals, litter or clutch sizes, and so on. For some females, the Lifetime Reproductive Planning might be to produce their entire lifetime contribution early in life without interruption. For others, breeding recommendations would be spread across a lifetime or concentrated during prime breeding age, usually middle age. During nonbreeding intervals,

these females would be either separated from males or treated with the contraceptive that is most appropriate for the species in terms of efficacy, safety, and reversibility. Once a female's genetic contribution to the population is secure, she could be permanently sterilized or given a long-term contraceptive, which could retain the possibility of later reproduction if changes in the population warranted.

Lifetime reproductive planning is proposed as a step in addressing reproductive failure in mammals, caused in particular by endometrial disease resulting from long barren periods. This appears to be a common problem in some mammalian species, but other causes of reproductive failure must be considered as well. Especially in nonmammalian taxa, adjusting husbandry protocols may be pivotal, and, for all species, behavioral sequences specific to courtship and social interactions should be considered. Similarly, overall health is central to fertility and the production of young that survive. For example, veterinarians may play a more central role in enhancing reproductive success by incorporating breeding soundness exams as a routine protocol—something that is also recommended by the AZA Animal Health Committee.

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23

Issues Surrounding Surplus Animals in Zoos

MADS FROST BERTELSEN

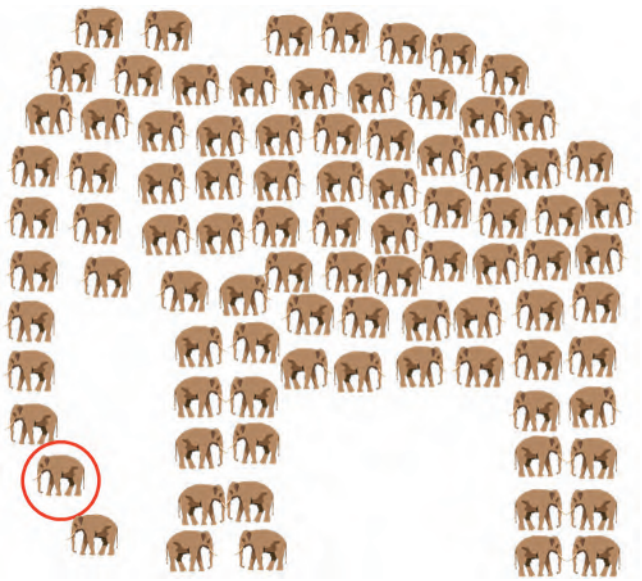
"Surplus animal" has a negative connotation, and it seems appropriate to start a discussion around this topic with a definition. Surplus animals are surplus to the needs of the population and in excess of the needs of the individual institution. In other words, a surplus is more likely to occur the better a species is doing in zoos. The more offspring that are born and the better the individuals are doing in terms of coping with disease, stress, and other problems, the more likely it is that the supply will exceed the demand. Fundamentally surplus animals are a sign of success. The day when zoos breed a surplus of all endangered animals would be a day to celebrate. However, surplus animals eat, take up space (which is ultimately always limited), and evoke the emotions of staff and visitors, so their management is a complicated issue.

Sustainable Populations

It is a declared goal of zoos to be self-sufficient with regard to animals, and indeed the ambition is to maintain genetically, demographically, and physically healthy populations over the long term to promote visitor education and to act as an assurance population for potential future reintroduction to the wild.[1,2] This can happen only through careful genetic management of the animals in the zoo's care and through continued breeding to provide a constant turnover of the population.[2–5] For many veterinarians trained to cater to the survival of the individual animal and used to contributing to species conservation one case at a time, it sometimes takes an effort to step back and see the bigger picture, where it is the long-term health and survival of the *population* that counts (Fig. 23.1). The population has become the patient, and that patient is not doing very well. Despite efforts to maintain sustainable captive populations, recent scrutiny has demonstrated that zoos are far from that goal—far enough to warrant the use of the term "sustainability crisis" (see Chapter 22).[6] To increase sustainability, a change in the culture surrounding zoo animal breeding is needed. Successful breeding of a species must become more important to an institution than maintaining specific individuals. Relocation of individuals must happen more often to allow mate choice, increase genetic diversity, and address infertility (see Chapter 21). That is, a reproductive management plan must be implemented for each individual to optimize the possibility of retaining genetic diversity through breeding while maintaining manageable yet sustainable populations.

Meeting the Demand

Contraception or even just separation of the sexes are powerful tools to reduce the number of offspring. However, the safety and reversibility in terms of future breeding are often (depending on the species) less than optimal.[6,7] More importantly though, sustainability is not just about numbers but about breeding the right animals. Although sometimes skewed,[8] the average sex ratio at birth is close to 1:1, producing an unavoidable surplus of males in species where one male breeds with several females (e.g., a "harem"



• **Figure 23.1** For veterinarians trained to cater to the survival of the individual animal and used to contributing to species conservation one case at a time, it sometimes takes an effort to take a step back and see the bigger picture, where it is the long-term health and survival of the *population* that counts.

system of breeding). This applies to most hoofstock and megavertebrates as well as a number of carnivores. Contraception cannot solve this problem, and surplus males are an unavoidable byproduct of breeding enough females.

Even if the exact production of offspring could be controlled, which of course it cannot, the demand is impossible to predict. Disease, senescence, and infertility may change the influx required to sustain a population. Therefore a certain surplus is necessary, as it provides an essential buffer for unexpected events. However, such surplus animals cannot be sustained forever. Although some bachelor herds are necessary for backup and for providing a "genetic pool" from which to draw new breeding males, permanently housing animals surplus to the breeding programs ultimately will obstruct the system by taking up space and resources that could otherwise be used for more genetically valuable breeding individuals. There are only so many seats on the bus, so to speak.

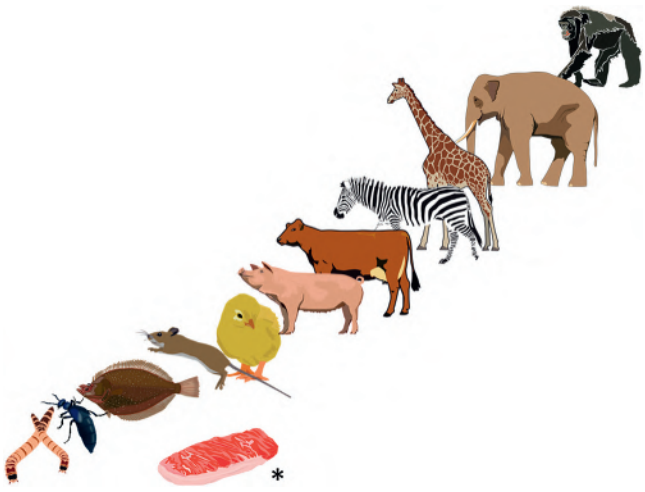
Breeding Is "Natural"

In general, zoos strive to provide "natural" conditions for their animals, although in practice numerous compromises are made; "natural" space is not available to most animals, "natural" diets are often substituted, and "natural" habitats and climates are mostly lacking. On the upside, "natural" parasites, "natural" predator stress, and "natural" competition for food are usually absent. Most would agree that "natural" behavior should be strived for, and with food provided and no predators to avoid, breeding becomes a paramount tool in providing "natural" behavior and "enrichment" in the shape of courtship, pair bonding, mating, pregnancy, nursing, feeding, mother–infant bonding, playing, sparring, and so on.[9–11] All these effects are essential parts of animal welfare, but in excess of population needs, surplus animals are the unavoidable secondary outcome.

How to Deal With Surplus Animals

So for the reasons previously mentioned, a certain surplus of animals is not only a sign of healthy populations but also an unavoidable "by-product" of sustainable breeding. As previously mentioned, simply housing surplus animals indefinitely is counterproductive to achieving sustainable populations, as these animals take up space that could be used for individuals more genetically valuable to the population. Sending such animals to private holders or institutions outside of the breeding programs raises a multitude of ethical issues and ultimately is not a long-term solution. Reintroduction into the wild unfortunately is rarely a realistic solution. Thus the only option available is to kill (or cull) those animals definitely in surplus.

It can be (and has been) argued that killing any animal is ethically wrong; however, the vast majority of human beings and every zoo known to the author have made the fundamental choice that it is acceptable to kill animals. For example, approximately 95% of the US population consume



• **Figure 23.2** What animal species may be culled to feed others? Most people have an irrational cutoff on the "cuteness index" shown here. Where is yours? Note that generic meat (*) falls very low on the scale.

meat,[12] and every zoo utilizes invertebrates, rodents, chickens, and ungulates as feed for its carnivorous inhabitants. In addition, most zoos kill invertebrates, rodents, and various other animals categorized as pest species. An old anecdote accounts for a conversation between a gentleman and a distinguished lady at a fundraising dinner. The gentleman offers the lady $100,000 if she will agree to sleep with him, an offer to which she assents. He then asks if she would do it for $10. The lady gets upset and says: "What kind of woman do you think that I am?" to which he replies: "We have already established that. Now we are just haggling over the price." The situation is very analogous to our relationship to killing animals; consciously or not, we all apply a more or less arbitrary cutoff on a scale from cockroach to great ape (Fig. 23.2), and our position on the scale is highly dependent on our nationality and cultural background.[10,13]

When the rational decision to cull has been made, the next question is when to do so. Some institutions have instituted a practice of culling infants deemed surplus shortly after birth; however, this precludes them from harvesting several of the benefits of producing surplus animals: the enormous behavioral enrichment to the parents of raising the offspring and the idea of having a buffer. A compromise, based on the three peaks of mortality observed in the wild, appears rational and "natural": In "nature" the mortality is highest in infants, animals around dispersal age, and in animals past their prime; geriatrics are not common in the wild. Zoos can mimic this by reducing litter sizes perinatally, primarily culling around dispersal age, and by minimizing the amount of postreproductive animals to a minimum deemed necessary for balanced group composition. Maintaining postreproductive individuals of solitary or monogamous species is counterproductive for population sustainability.

How the animals are used following culling has a great impact on the acceptance of the practice by zoo employees and the public alike. Also here, there are vast cultural differences around the globe, yet it appears that a utilitarian

philosophy is dominant enough that acceptance increases as people understand *why* the animal was culled and that it had a *purpose* thereafter. Carcasses may be used for feeding, for educational purposes (e.g., dissections, demonstrations, or museum exhibit), and for research.

The Public Perception

Although it is inherently logical to cull surplus animals, it certainly is not without problems. The opinion of the public, including potential zoo guests, may be unpredictable and volatile[13]; even among zoo professionals there are very differing opinions on which if any animals may be culled.[14] In certain countries (e.g., Germany) it is prohibited by law to kill animals without a purpose, and while consumption by humans and other animals, as well as research, constitute such a purpose, it has yet to be established whether welfare for the parents or population sustainability does.[11]

Despite these challenges, in this author's opinion the greatest threat to zoos currently is hypocrisy and double standards. In the long run, zoos cannot pretend to champion species conservation while putting the life of the individual in front of survival of the population. The zoo community will have to realize that there is a certain percentage of the public who will be against zoos no matter what they do, and that in compromising on biological fundamentals (e.g., all animals die, carnivores eat meat) to avoid controversy, they are missing the opportunity to influence and educate the vast majority of the people—a majority who are likely to understand and respect transparent rational zoos linking conservation efforts in situ with sustainable breeding ex situ. One place to start would be to acknowledge and overcome the "sustainability crisis" by realizing and communicating that the only alternative to a "surplus" is a "deficit," which for nonendangered animals would translate into a need to continually bring animals in from the wild. For threatened species, it could mean extinction.

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SECTION 5

Therapeutics

24

Stem Cell Therapy in Zoo Medicine

MATTHEW E. KINNEY AND ROBERT HARMAN

What Are Stem Cells?

Stem cells are defined as having both the capacity for self-renewal and the ability to give rise to differentiated cells.[1–4] Stem cells are classified based on their differentiation potential and cell origin. Cells have diverse abilities to differentiate and can be characterized based on a continuum from unipotent cells that are capable of differentiating into a single mature cell type to totipotent cells that are capable of differentiating into any cell or tissue of an organism. Classification based on cell origin is used to distinguish embryonic stem cells (ESCs) from adult tissue–derived stem cells. ESCs arise from embryos during the early stages of development. Cells of the inner cell mass are able to generate into any cell type of the body and maintain unlimited proliferation and replication under appropriate culture conditions.[5] In 1998 the establishment of human ESC lines was first reported, and although the research potential of a cell that can proliferate indefinitely into any cell type of the body is enormous, the clinical use of ESCs in veterinary medicine as a therapeutic modality has minimal direct utilizations at this time due to the high risk of tumors.[6,7] As such, the focus of this chapter is on adult tissue–derived stem cells, which are currently being utilized in therapeutic capacities in both human and veterinary medicine. Induced pluripotent stem cells (iPSCs) are adult cells that are reprogrammed to generate cell lines that have the capacity for self-renewal and pluripotency. The use of iPSCs for conserving and rescuing endangered species is an exciting and promising use of iPSCs in veterinary medicine, along with many other research applications; but iPSCs have many of the drawbacks of ESCs, including immunogenicity and tumorogenicity.[7–11] Mesenchymal stem cells (MSCs) are another type of adult-derived stem cells and are commonly used as a therapeutic modality in veterinary medicine. The reader should be aware that MSC, an abbreviation present throughout the stem cell literature, may be a reduction for any one of the following terms; *mesenchymal stem cell, mesenchymal stromal cell, multipotent stromal cell, marrow stromal cell, colony forming fibroblasts*, or *medicinal signaling cell*. The naming convention for MSCs was first based on their ability to differentiate into cells of mesodermal origin.[12]

A 2006 position statement from the international society for cellular therapy sought to clarify the definition in an attempt to better characterize MSCs and allow for improved standardization in the rapidly evolving field of stem cell therapy and regenerative medicine.[13] The minimum criteria for defining a human MSC were based on the ability to adhere to plastic, specific surface antigen expression, and multipotent differentiation potential. Plastic adherence is a common nonspecific but well-described characteristic of MSCs under standard culture conditions. Immunophenotyping using expression (CD105, CD72, CD90) and lack of expression (CD45, CD34, CD14 or CD11b, CD79 alpha, or CD19, HLA-DR) of specific surface antigens is used as a proxy to identify MSCs and make certain that contamination from other cells, such as hematopoietic cells, has not occurred. In human medicine using flow cytometry, 95% or more of MSCs should be positive for the specific antigens and less than 2% of MSCs should lack expression of the specific antigens listed previously. The use of these specific surface antigens to discriminate MSCs from other cells types cannot be universally translated from human to veterinary medicine, as there are clear interspecies differences in the expression of surface antigens.[14] The third criterion is based on cell potency and states that using standard in vitro culture conditions, MSCs should demonstrate the ability to differentiate into all three mesenchymal lineages, including osteoblasts, adipocytes, and chondroblasts. Differentiation into cells of the mesoderm is merely a minimal criterion for definition, as some authors contend that MSCs have the capacity to differentiate into cell lineages other than those of mesodermal origin.[15] In 2013 a similar position statement was proposed under the authority of the International Federation for Adipose Therapeutics and Science and the International Society for Cellular Therapy to provide guidance and establish standards for researchers investigating adipose-derived cells for use in regenerative medicine.[16]

MSCs are an attractive therapeutic modality in veterinary medicine because these cells are multipotent, present in a number of accessible tissues for cell collection, have the capacity for self-renewal, can expand using established in vitro culture methods, are genetically stable, have powerful

trophic effects, and have few ethical concerns.[17,18] For these reasons MSCs are the most commonly utilized stem cell type for therapeutic purposes in veterinary medicine and a number of both university and commercial research groups continue to facilitate the progress of this modality in research and clinical veterinary medicine.

How Do Stem Cells Work?

The mechanism of MSC function is an active and rapidly evolving area of research, and many questions remain to be elucidated in determining how MSCs behave both in vitro and in vivo. The original hypothesis that MSCs exhibit their therapeutic effect by direct tissue regeneration was logical and intuitive based on their ability for self-renewal and multipotent differentiation in vitro.[19] The capacity of MSCs to migrate to the site of injury, engraft, and differentiate into functional cells was initially postulated to be the underlying mechanism of clinical improvement observed following MSC administration; perhaps because of the relative simplicity, this viewpoint remains a popular understanding. However, the paradigm of direct tissue regeneration secondary to MSC migration and incorporation has been questioned based on clinical improvement observed despite the lack of long-term stem cell engraftment in target tissues and numerous studies proposing additional mechanisms of action. The mechanism of action of MSCs is likely a combination of differentiation into functional cells, bioactive factor secretion, cell-to-cell interactions, and the release of extracellular vesicles that are dependent on MSC origin, culture condition, administration protocols, local microenvironments, and desired therapeutic response.[20–22]

Bioactive factor secretion is intended to be a phrase that encompasses a variety of terms in the stem cell literature documenting the ability of MSCs to affect other cells. This includes autocrine, paracrine, and stem cell–related molecules as well as trophic factors. Considerable research, particularly in the area of myocardial and central nervous system ischemic insult, has focused on the investigation of cytokines, growth factors, chemokines, and immunomodulatory proteins and how they contribute to the therapeutic improvements observed following stem cell administration.[23] This area of stem cell research in humans and laboratory animals has focused on characterizing the transcriptome and proteome in an effort to explain the role played by MSCs in immunomodulation, decreasing apoptosis, promoting cell survival, accelerating progenitor cell self-renewal, stimulating angiogenesis, blocking pain, and reducing inflammation.[24–29] Numerous complete reviews are available detailing the proposed underlying mechanisms, but the crucial take away for the veterinary clinician is to recognize that the mechanism of action of MSCs is not to merely engraft into diseased tissues for replacement but rather influence the microenvironment to promote functional recovery of an organ or body system.[19–27] In fact, it is feasible that under appropriate harvest, culture, and expansion conditions, the administration of bioactive factors produced by stem cells may have therapeutic potential without administration of actual cells to the patient.

Stem Cell Collection

Autologous is a term used to describe MSCs harvested from and administered to the same individual. An *allogeneic relationship* between MSC donor and recipient indicates that both are from the same species but different individuals. A *xenogeneic relationship* indicates the MSC donor and recipient are from different species. Currently the most common donor-recipient relationship in veterinary medicine is autologous, although allogeneic MSC administration has been performed in a number of species. The use of allogeneic MSCs in veterinary medicine can potentially provide consistency and standardization of MSCs administered to the recipient and allow for immediate administration to a recipient without the need to harvest tissue prior to MSC administration. Studies evaluating allogeneic MSC administration by various routes have reported few adverse clinical concerns in canine or equine patients, and the commercialization of allogeneic MSC is a promising research direction that holds great potential for zoo medicine.[30–32]

MSCs are currently thought to reside in large part in a perivascular niche and have been termed *pericytes*.[33,34] Although MSCs reside in nearly all organs, the feasibility of cell collection is an essential clinical consideration in determining a suitable harvest location. In human and veterinary medicine, bone marrow, adipose tissue, and umbilical tissue are the most common locations for MSC harvesting. The optimal tissue source of exogenous MSCs has not been determined.[17] Bone marrow MSCs (BM-MSCs) were the original MSCs isolated; collection protocols from the fifth sternebra have been thoroughly described in sedated horses using local anesthetic and a 12-gauge Jamshidi needle.[35,36] The fifth sternebra is the preferred collection site because it is cranial to the apex of the heart, and the marrow space is of adequate size to permit the harvesting of 10–15 mL of bone marrow. Adipose tissue is an attractive harvest location due to its abundance and relative ease of collection. Adipose MSCs (AT-MSCs) have been harvested from both peripheral and visceral adipose tissue, with the most common location in the horse being the subcutaneous tissue near the tail head or over the dorsal gluteal muscles. Similarly, a lipectomy performed at the base of the tail in a domestic cow and water buffalo *(Bubalus bubalis)* has been used to collect and culture AT-MSCs, and this collection location is suitable for many zoo species.[37] In the bottlenose dolphin *(Tursiops truncatus)*, adipose-derived MSCs were harvested and subsequently cultured using ultrasound-guided liposuction from the subcutaneous fat on the dorsal midline, 15 cm caudal to the blowhole, following local anesthesia and use of a 2.4-mm diameter infusion cannula.[38] As compared with subcutaneous fat, blubber contained fewer nucleated cells per gram and was difficult to digest efficiently. The umbilical cord is another attractive harvest location because such harvesting is entirely noninvasive

and cells are collected from a young tissue source. MSCs can be obtained from umbilical cord blood or Wharton jelly, a collagen-rich matrix within the umbilical cord. The umbilical cord should be considered as a potential harvest location for MSCs in zoological medicine. MSC cultures from the umbilical cord of a greater one-horned rhinoceros *(Rhinoceros unicornis)*, giraffe (*Giraffa* sp.), and lion *(Panthera leo)*, among other species, have been established. In many species, especially megavertebrates, placental and umbilical cord tissue collection can be incorporated into the birth plan of individual animals to streamline the collection process during the periparturient time. The isolation and culture of MSCs from peripheral blood have been identified as an alternative and attractive source of MSCs owing to the ease of collection; this is an attractive option for zoo species. The demonstration of chondrogenic differentiation of human peripheral blood–derived MSCs suggests that cells derived from peripheral blood may have similar potential as MSCs derived from adipose tissue, bone marrow, or umbilical cord.[39] Similarly, immunophenotyping and histochemical staining of MSCs isolated from equine peripheral blood supports the potential for trilineage differentiation.[40] MSCs have been isolated and cultured from the peripheral blood of a giraffe (*Giraffa* spp.), further supporting the potential use of this collection site in zoo species (Valerie Johnson, personal communication, March 2, 2017).

Intravenous, intraarterial, intraperitoneal, and local intratissue routes have been used to deliver MSCs to human and veterinary patients. The size of some zoo species must be considered in determining an administration route, as intratissue administration may be precluded by organ accessibility or the need for restraint. *Homing* is a term used to describe the arrest of MSCs within the vasculature, followed by transmigration across the endothelium; it is postulated that injured or inflamed tissues release chemokines, cytokines, and growth factors that serve as a cue to direct MSCs to tissues in need of repair.[41] In general, MSC homing to target tissues occurs when there is the right combination of signaling molecules from the injured tissue and the corresponding receptors on MSCs.[42] Rats with myocardial infarctions administered intravenous BM-MSCs in the ascending aorta demonstrated MSC homing to the infarcted regions of the heart and improved fractional shortening.[43] Increased MSC engraftment in the gastrointestinal tract, liver, and spleen was documented in mice that received local radiation to the abdomen when compared to nonirradiated mice, suggesting that MSC homing occurs in radioinduced lesions.[44] The concept of MSC homing is promising in zoological medicine, as this characteristic of MSCs may be harnessed to facilitate the targeting of damaged tissue via systemic intravascular administration. In instances where the damaged tissue is extensive and diffuse or the patient is too large to facilitate local MSC administration, systemic administration may be more practical and efficacious compared with intralesional injection. MSC therapy of the lung can be easily applied via intravenous injection, as the cells will traverse to the lung capillary beds. Recently, it was reported that MSC administration to horses with exercise-induced pulmonary hemorrhage was efficacious in preventing bleeding during racing and could be considered for other respiratory diseases.[45] Knowledge gaps in the area of MSC homing, an active area of research, include the underlying mechanism of homing, the activity of MSCs once target tissue has been reached, and the ability to improve homing by promoting receptor conservation in MSC cultures. As these knowledge gaps are filled, systemic MSC administration will likely be modified to target damaged tissues and diseases in individual patients, with fewer MSCs required to attain the desired therapeutic outcome.[46]

Stem Cells in Clinical Medicine

The therapeutic use of MSCs in veterinary medicine has been best studied in lameness conditions in canine and equine patients because of the original postulated mechanism of tissue regeneration and the interest in exploring this emerging technology as an adjunctive treatment modality complementary to or in place of more traditional treatments for musculoskeletal injuries. The efficacy of MSC treatment in domestic dogs has progressed from anecdotal reports of clinical improvement to prospective, randomized, placebo-controlled clinical studies with a relatively large number of study participants.[32] Outcome metrics have evolved from owner-reported improvement to the utilization of force platform gait analysis, radiographs, synovial fluid analysis, client-specific outcome measurements using a standardized scoring system, and a scoring system gauging veterinary pain on manipulation.[32,47–50] The refinements in study design and outcome metrics provide valuable safety and efficacy data supporting the potential applications of MSCs for musculoskeletal conditions, and further studies should continue to utilize blinded, randomized, placebo controlled studies to allow clinicians to practice evidence-based medicine when considering MSCs as a potential treatment modality.

Increased understanding of the mechanisms underlying MSC activity has shifted the fundamental perception of their usefulness in veterinary medicine from a therapy based on tissue-regenerative properties to one that may be utilized to treat a variety of diseases. MSC-based therapies for the treatment of equine joint disease, tendon and ligament injuries, and bone repair were an early use and continue today.[51–55] In the domestic horse, equine recurrent uveitis, laminitis, perinatal asphyxia syndrome, chronic corneal ulceration, equine myeloencephalopathy, and laryngeal hemiplegia have been specifically identified as conditions that may benefit from MSC treatment.[56] Veterinary dermatology has been identified as a specialty where MSCs may expand the treatment options for chronic nonhealing wounds, immune-mediate skin diseases, alopecia, and scar tissue remodeling based on the antiinflammatory, immunomodulatory, revascularization, and antiapoptosis effects as

well as the differentiating and homing capacities of MSCs.[57] Domestic cats refractory to conventional treatment for feline chronic gingivostomatitis were administered two systemic intravenous injections of adipose-derived MSCs 1 month apart with oral biopsies, blood immune cell subsets, serum protein, and cytokine levels used as outcome metrics. Of the 7 cats that completed treatment, 3 demonstrated complete clinical remission, and 2 showed substantial clinical improvement. Systemic immunomodulation was documented in cats that responded to treatment with decreased circulating CD8+ T cells, decreased neutrophil counts, and normalization of CD4/CD8 ratios, among other changes in cytokine concentrations.[58] In domestic dogs and cats, MSC treatment has also been attempted for a variety of disease conditions, including intervertebral disk disease, atopic dermatitis, perineal fistulas, inflammatory bowel diseases, dilated cardiomyopathy, keratoconjunctivits sicca, granulomatous meningomyeloencephalitis, and feline chronic kidney disease.[59] In many of these studies, the safety of MSC was established; however, efficacy was difficult to evaluate due to study design; it was difficult to assess outcome metrics and follow-up of study patients. In many instances the shortcomings of these studies are intrinsic to the challenges associated with evaluating the efficacy of a treatment modality on client-owned animals that have attempted other treatments, may have additional comorbidities, and may elect to no longer enroll in the study or to euthanize their pets. Nonetheless, these studies provide a foundation for further investigations and demonstrate the potential of MSCs as a treatment modality beyond the regenerative model in domestic animal medicine.

The use of MSCs in human and research animal medicine provides an additional resource for veterinarians to consider when evaluating MSC safety and efficacy. Similar to domestic animal medicine, the preliminary view of the regenerative capacity of MSCs resulted in investigation of MSCs for treatment of diseases that affected body systems with suspected minimal regenerative capacity. Numerous human studies of MSC administration following myocardial infarction have been performed with different cell types, modes of delivery, and clinical outcomes that contribute to continued debate regarding the benefits of MSCs.[60] Briefly, AT-MSCs and BM-MSCs are the most commonly administered types with modes of delivery including peripheral intravenous and direct intramyocardial injection during coronary artery bypass graft surgery, transendocardial injection, and intracoronary infusion into the infarcted artery with or without direct adventitial delivery. Several studies have documented improved ejection fraction, wall motion, and infarct size in patients with acute myocardial infarction and decreased mortality in patients in heart failure, while others have documented no significant difference in outcome measurements when compared with conventional treatment. The mechanisms underlying these improvements continue to be discussed, and mechanisms other than the direct differentiation of MSCs into functioning cardiomyocytes replacing necrotic cells do not appear to be the sole explanation for clinical improvement. The paracrine effects of MSCs are suspected to contribute to the cardiac repair by neovascularization, angiogenesis, decreasing inflammation and infarct size, enhancing cardiomyocyte survival, mobilizing other stem cells to the infarcted area, and decreasing fibrosis. Similar to myocardial infarction, ischemic stroke has been extensively researched as a condition that may benefit from MSC treatment, with similar variability in cell type, mode of delivery, and outcome metrics. Initial studies have reported improved functional recovery, reduced infarct volume, and diminished neurobehavioral deficits in MSC-treated patients.[61] For both cardiac and cerebral ischemic conditions, rigorous investigation of the efficacy of MSCs will continue due to the lack of current treatments that offer cures instead of merely clinical compensation. MSCs have been identified as an innovative treatment option in a number of other disease processes that affect humans; current studies are evaluating MSC treatment for acute kidney injury, chronic kidney disease, diabetic nephropathy, glomerulosclerosis, kidney transplantation, retinal degeneration, glaucoma, diabetes mellitus, and liver fibrosis, among many other disease processes, thus highlighting the diversity of MSCs treatment potential.[62–65]

Mesenchymal Stem Cells in Zoo Medicine

Despite the paucity of refereed publications on MSCs in zoological medicine, MSCs have been used in multiple zoos. Some examples include the use of intravenous or intralesional autologous adipose MSC administration for the treatment of lameness in a variety of large cats, the northern white rhino *(Ceratotherium simum cottoni)*, giraffes (*Giraffa* spp.), and Grevy's zebra *(Equus grevyi)*. Allogeneic adipose MSCs administered intravenously and topically have also been used to treat pododermatitis and osteoarthritis in macaroni *(Eudyptes chrysolophus)*, emperor *(Aptenodytes forsteri)*, Adelie *(Pygoscelis adeliae)*, gentoo *(Pygoscelis papua)*, and magellanic *(Spheniscus magellanicus)* penguins. Numerous additional treatments have likely occurred in zoos around the world that have not been widely reported. The novelty of MSC treatment often attracts media coverage, but unfortunately these cases rarely transition into case reports or case series that are presented at zoological medicine conferences or peer-reviewed publications. Therefore MSC harvest sites, culture conditions, administration routes, adverse effects, outcome metrics, and long-term efficacy are largely unknown at this time. Documentation of MSC use in zoo species will serve not only to introduce basic collection and administration methods but also allow readers to critically evaluate MSCs as a potential treatment modality. As the available literature increases, harvest locations, administration routes and doses, outcome expectations, and safety concerns will become more apparent, allowing clinicians to better evaluate whether MSC treatment should be pursued as a treatment option in zoological medicine.

When compared with domestic animal veterinary medicine, MSC use in zoological species presents a number of unique challenges. Some of these challenges are simply technical and may be overcome with experience, communication, and creativity. Determination of the MSC stem cell harvest location is an initial challenge. A combination of necropsy photo review, preanesthesia ultrasonography, and review of the equine literature provided adequate background knowledge to guide adipose tissue harvest from a northern white rhino. In many instances two immobilizations are required to harvest fat or bone marrow and to administer autologous MSCs to the patient. The legitimacy of this concern may be institution-dependent, and repeat immobilizations are not always required. Currently there are commercial companies that will perform patient-side harvesting and administration under the same anesthetic event in domestic animals and the selected administration route, such as intravenous, may preclude the need for a second anesthesia in a well-trained patient. Additionally, the culturing and expansion of MSCs from peripheral blood in the horse provides a foundation to establish techniques that would allow for MSCs to be expanded in adequate numbers from a biological source that could be voluntarily acquired from many zoological species including megavertebrates in modern zoos.[40] Intravenous administration of allogeneic MSCs harvested from the peripheral blood of domestic horses involved 291 domestic horses, and no adverse clinical effects were noted in the 1 year post administration monitoring period.[66] Similarly, intraarticular administration of allogeneic MSCs harvested from the adipose tissue of donor domestic dogs were demonstrated to be both safe and effective compared with saline treatment.[32] The result of these studies holds great potential application to zoological medicine. If the safety of allogeneic MSC administration is translated for zoo species, there is potential that peripheral blood or adipose tissue could be used as a MSC source for other individuals of the same species, thus greatly reducing the investment in MSC acquisition from individual animals that may not be conditioned for voluntary blood collection or be amenable to immobilization to harvest adipose or bone marrow. The use of allogeneic or xenogeneic MSCs is a fascinating and promising prospective direction that zoos may consider to separate the source of MSCs from the animal that is in need of treatment. The use of allogeneic MSCs could better capitalize on the potential of MSCs by making this treatment modality more similar to other therapeutics that are immediately initiated once they are indicated for treatment by an attending veterinarian. The utilization of allogeneic or xenogeneic MSCs would allow for rapid turnaround time between a veterinary clinician deciding that MSCs are indicated as a treatment option and administration to the patient. Currently the San Diego Zoo Safari Park maintains MSC cultures that were established from adipose or umbilical tissue from northern white rhinoceros *(C. s. cottoni)*, greater one-horned rhinoceros *(R. unicornis)*, Ugandan giraffe (*Giraffa camelopardalis* spp.), and African lion *(P. leo)*, with plans to increase the number of species through the collection of umbilical tissue following parturition or adipose tissue collection during immobilization procedures. Although the initial investment in establishing and maintaining MSCs is significant, the potential for rapid turnaround time between identifying a potential case where MSCs may be indicated and treatment initiation will allow for the utilization of this modality more willingly and rapidly. The potential for the allogeneic or xenogeneic use of MSC is appealing, but it must be noted that aside from select species, the safety of allogeneic administration has not been elucidated, and there are also logistic considerations such as time, labor, financial investment, the selection of donor animals, ownership of biological material, and accountability for adverse effects, especially if multiple institutions are collaborating on the establishment of a central repository.

As the underlying mechanisms of MSCs continue to be better understood, the potential indications for MSCs have evolved from being a last ditch therapeutic modality for musculoskeletal conditions to a treatment option to address a range of diseases in a number of body systems. As our understanding of the mechanisms of MSCs continues to advance, the indications for their potential use expand, and many of the basic principles of MSC therapy may provide a potential treatment for diseases that affect zoo animals. In particular, as the mechanisms of homing are more thoroughly investigated and the efficacy of intravenous treatments is reported, intravascular administration of MSCs is becoming a more attractive treatment option for zoo patients due to their large size or conditions that involve entire organ systems. The antifibrotic and proangiogenic properties may have utility as a treatment option for great ape fibrotic cardiomyopathy or cheetah veno-occlusive disease. The regenerative potential of MSCs may present an alternative option for nonhealing cutaneous wounds in columbiformes, pododermatitis in penguins, or postintubation tracheal stenosis in long-necked birds. The immunomodulary effects may be useful as a potential therapy for amyloidosis in bongo *(Tragelaphus eurycerus)* or alopecia in Andean (spectacled) bears *(Tremarctos ornatus)*. Veterinary clinicians are encouraged to consider the potential of MSCs beyond simply their cell regenerative capacity. Clearly MSC use as a therapeutic modality in zoological medicine is in its infancy, and fundamental questions regarding safety and logistics remain unanswered. Basic science research, domestic animal and human clinical trials, and veterinary colleagues at other zoos may be consulted to expand our understanding of the underlying mechanisms of MSCs, provide a foundation for translation into zoological medicine, and broaden the potential applications of MSC use as a therapeutic modality for various disease processes to improve the health of animals in our care.

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Compounding Pharmacies

KATHRYN C. GAMBLE

Commercial drug manufacturing commences with an active pharmaceutical ingredient (API), also known as a bulk drug. In this terminology, it does not mean a measure of volume but that it is the base ingredient.[1] Inert additives (such as fillers, excipients, or colorants) or active agents that affect drug behavior may be added during production of a marketed pharmaceutical.[1] In the United States, in its final formulation, a pioneer *drug* will be approved by the Food and Drug Administration (FDA), after legally required time-consuming and costly evaluations demonstrate that it complies with its intended safety and efficacy parameters.[1,2]

Inherent in these factors are potency (actual concentration of active ingredient per unit of drug formulation),[2–7] accuracy (difference between actual and intended concentration, and specifically ±10% of labeled amount),[3] precision (variation around the mean of concentrations),[3] quality (absence of harmful contaminants or ingredients other than labeled),[2] purity (inclusion of only intended product),[5] integrity (retention of potency until beyond use date),[2] and lack of contamination (inclusion of actual infectious agents).[4,5] In this process, both medical and veterinary pharmaceuticals are labeled with specifically evaluated doses, routes of administration, indications, and, for veterinary medicine, particular species applicable to the testing completed, making these expressly legal uses. After such a drug has reached the completion of its patented life span, generic drugs may become available that are developed by these same manufacturing processes. These products also are FDA approved and required to be bioequivalent copies of the original product.[1,2,8] Due to market interest and commercial marketing pressures, subsequently identified adverse drug events (ADEs), or product innovations, these pioneer drugs and their generic equivalents may become temporarily or permanently unavailable to the end user.[9] It is into and within all of these means and ways that compounded pharmaceuticals (CPs) can be found both integrated, yet separate.

In the subsequent discussion, CPs are drug formulations developed from either API or the finished commercial or generic formulations, by any change from the labeled procedures or directions.[1,5–7,10] Traditional compounding includes the various basic manipulations by which a new formulation of a currently FDA-approved drug can be created.[5,7,12,13] Most simply, it can be an in-clinic processing such as grinding a tablet into a powder to facilitate administration in a patient's food (change of formulation) or diluting a parenteral product with sterile saline to facilitate more accurate measurement for a smaller patient (change of concentration).[13] To be clear, any action performed that is not specifically on the label direction is considered compounding. This includes reduction in the quantity of reconstitution diluent for concentrated end use or simply mixing two drugs together in one syringe.[7,14]

Professional compounding is available for more difficult manipulations such as creating sterile parenteral anesthetic solutions and resurrection of drugs absent from the market using APIs unavailable in the nonpharmacist role; or changing a commercial oral formulation to that of a transdermal gel.[5] Although these CPs may be evaluated independent of federal oversight, they remain unapproved by FDA regulations and thus are not ensured to create their intended effect when used in the course of patient care.[3,6,7] In addition, it is important to understand that use of CPs, even in the labeled species of the original formulation, and the use of FDA-approved drugs for any indication or species, or by any route that is not specifically labeled, constitute extralabel drug use (ELDU). In the United States, as the prescriber, the veterinarian has the privilege of selecting the pharmaceutical best suited for his or her patients' needs, whether this be a medical or veterinary pharmaceutical, or FDA-approved or compounded drug. As such, it is critical that the veterinary clinician fully understand the incumbent liability as the prescriber, particularly with CPs.[2,6]

Pharmaceutical Legislative History and Current Perspectives[1,5–8,10,14]

Although the following discussion is focused on the perspective within the United States, it should be considered that the basic issues arising from drug manufacturing and compounding will be present throughout worldwide pharmaceutical regulations where they exist.[7] In 1938,

due to rising concern over contaminated pharmaceuticals and poor production standards, legislative action resulted in the Federal Food, Drug, and Cosmetic Act (FFDCA, 21 U.S.C. § 360) and created the FDA as a regulatory body for drug manufacturing. These legislations largely ignored veterinary medicine in specific mention until its amendment in 1968.[2,11] Implemented and unaltered for decades, this initial legislation did not encompass compounding either as a procedure or end product, although it was well known that these activities were occurring for patient care. However, as veterinary medicine sophistication increased, practitioners used more medical pharmaceuticals and administered veterinary drugs to species other than those labeled in the commercial manufacturing process. Therefore to address this regulatory deficiency, the Animal Medicinal Drug Use Clarification Act (AMDUCA; 21 C.F.R. § 530.13) was passed in 1994, which provided veterinarians legal support to select, administer, and dispense medications in an ELDU fashion.[12] It included acknowledgment of compounding when no FDA-approved pharmaceutical was available.[7] Although no historic reference existed, it provided that with adherence to all relevant state laws and with professional direction from veterinarian to pharmacist, compounding was permissible; however, specific exclusion was made limiting compounding from APIs. This enhanced permission and the burgeoning standards of veterinary care and client expectations continued market pressure on compounding pharmacies to provide more individualized products and formulations.

Understandably, the FDA and commercial manufacturers as represented by the Animal Health Institute (AHI) were concerned about substandard activities in this federally unregulated field, especially when quite public, traceable ADEs, or failures of treatment occurred. Admittedly, one must consider that financial competition had some role in this opinion and resultant regulatory pressure. In 2003 FDA promulgated its Compliance Policy Guide (CPG, specifically 608.400) as replacement for its 1992 guide to its employees and actions and specifically sought to assess and address potential compounding violations. In particular, compounding from APIs and question of appropriate volume of reserves of CPs based on sales rather than for specific patient need were at issue. Unfortunately, this guide was taken in some arenas as actual legislated authority. Furthermore, it had many areas of vague definition and contradiction in purpose, which actually confounded rather than clarified the compounding issue. It particularly isolated species-care groups, such as exotic animal practitioners, and prevented their voice in patient care needs.

Since 2000, repeated testimony and veterinary industry lobbying from multiple disciplines encouraged congressional conclusion that the CPG was unfounded in its current form.[14] Inconsistent enforcement was documented, especially directed at compounding sources[1]; excessive and unnecessary product labeling was a concern[15]; and where documentation through established routes, such as ADEs, could be made to accumulate scientific evidence, these opportunities were not used.[1] As such, this publication was rescinded in its entirety in May 2015.[1,14] Although a victory in many ways, the compounding process now remains without federal regulation, guidance, or acceptance, leaving front-line clinicians in a gray zone of prescription liability. As of now, only a Guidance for Industry (GFI #230) document has been drafted and remains unfinalized.[7,11]

Compounding—What It Is and Is Not

Compounding is increasing, at quite startling rates, within all medical fields. Essentially any pharmacy can perform compounding, although professional compounders focus their procedures in these endeavors. Veterinary compounding can occur at either medical or veterinary pharmacies, although veterinary sources are considered to have deeper understanding of the variable patient species base.[6,7]

Ideally, the FDA intended that drug compounding should not even resemble manufacturing. It is expected to occur for an individual patient from a specific prescription generated by a veterinarian within a valid professional relationship with both a client and pharmacist.[5] Although formulating new drugs from FDA-approved manufactured products, compounding is not to be confused with the creation of new drugs. As such, the safest means to avoid legal contention is use of an FDA-approved commercial or generic product for compounding.[2] However, this approach is not always appropriate, as these formulations may not lend themselves to change dramatically between routes of administration in a manner safe for the patient.[6,7,11] Additives in the finalized products may be undesired, such as beef-based flavorings for food-intolerant individuals or veterinary-specific additives of concern (e.g., xylitol) that cannot be separated through a reliable procedure from the commercial preparations.[11,16] Furthermore, in situations of discontinued commercial availability, the finished product actually no longer exists.[9] In these situations, compounders obtain APIs as their base to begin the process. Although not expressly forbidden, this practice is officially within an FDA-unapproved area.[10]

Immediately, this approach calls attention to itself because it can be considered manufacturing to take a bulk product and formulate a marketed end product. However, in compounding, APIs can be the desired base to eliminate concerns of purity, need for extraction of additives, and resolve lack of availability. As occurs in manufacturing, these APIs should be obtained from an FDA-registered source for optimal assurance of quality and for reduced legal liability.[2]

Under these circumstances, regulatory concern should ebb because it is not a bypass route to the drug-manufacturing and FDA approval process but rather provision of a needed pharmaceutical. Furthermore, compounding from APIs has played a role in regulatory defined minor species—essentially all those in zoo and wildlife veterinary care—where market share will never justify the pursuit of commercially available finished products through FDA-approved procedures.

Although closer to manufacturing than traditional compounding, it remains a necessary consideration that should not raise regulatory hackles, although protest of market diversion has been made from commercial players. However, of particular caution, compounding API use as a blatant means to undercut commercial products by mimicry is considered piracy. Low-cost sourcing of APIs to provide a final product below cost of the commercial version is considered ethically inappropriate and may produce questionable efficacy if API quality is compromised for reduced price.[8,14] Ongoing and often contentious discussions between AHI and compounding pharmacists have occurred over consideration of larger volumes of drugs prepared from bulk sources that are held in reserve. By federal review, it was concluded that available compounded inventory could be necessary for rapid response of the compounding source to its clients, as would be expected for good commercial manufacturing practice. This approach should be tailored to routine market need rather than mass-volume sales. If only these deciding factors faced prescribing veterinarians, it would be a minimal minefield from which to proceed. But what quality standards exist where no standards are actually mandated?

Initially, the end-use concern of poor product sourcing should be eliminated by engagement of pharmacies that operate under API sourcing guidelines provided to manufacturing facilities. In addition, assurance of expected end result can be enhanced by prescribing from compounders using industry standards of the US Pharmacopeial Convention (USP),[7,17] which is a not-for-profit scientific organization that is referenced by FDA regulation.[4,5] Initiated in 1820, USP has gathered methodologies and details accepted as good practice basis and is accepted in more than 140 countries worldwide. It details not only production but also chemical properties and cautions, such that adherence to the guide should produce a better end product.[1,5] This document has been updated to include a dedicated chapter to veterinary compounding.[6] Although the USP is not uniformly required for use in all states and territories within the United States,[1,5] compounding regulation for pharmacies does exist at a state level. However, it is widely variable in application, so prescribers must be aware of their state's regulations.[7,18] These vague boundaries of veterinary compounding are complicated further when facilities ship across state lines. An additional measure of assurance is that compounding does have self-policing via various professional organizations, including the Pharmacy Compounding Accreditation Board (PCAB) established from industry sources to create compounding regulations; and the International Academy of Compounding Pharmacists and American College of Veterinary Pharmacists that provide training, continuing education, and certification.[4,5,10]

Yet, as non–FDA-approved products, CPs have no guarantee of safety or efficacy, even when produced with care.[2,10] Sterile formulations for parenteral use have been made that presented issues of fatal contamination.[19] Novel formulations have been created that are scientifically unproven and may enhance risk to the patient or administrator, particularly noteworthy in transdermal preparations.[6,7,11] Of greater concern is the expectation by the end user that CPs are evaluated consistently by some party to ensure the label information is accurate. In contrast, repeated random assessment by federal and state regulatory bodies and independent scientific publications have demonstrated that not only between but also within batch irregularity exists.[3,7,9] Overall concerns for variability in potency have been demonstrated routinely, and in rare instances, actual absence or multiple-fold presence of API has been confirmed.[1,4,5]

Furthermore, without the rigor of testing, it is unknown what the best use date (BUD) may be for a given CP, unlike the stability testing required of commercial and generic products. Difference between large-volume manufacturing to small-volume compounding steps may produce different results in the same product formulation. It has been published that CPs cannot be reliably assigned a BUD past 14 days, unless specifically evaluated.[2,4,6,9,12,16,17,20–22] In particular, USP standards recommend that nonsterilized, water-based oral formulations be stored at controlled cold temperatures (2°C–8°C) to even permit the assignment of this BUD, and consideration that pH changes or exposure to UV light unexpectedly can affect this recommendation.[3,6,7,12,17,21] In some situations, commercial additives are active and necessary for appropriate pharmacodynamic expectations; when these agents are proprietary, and therefore unavailable to the compounder, the resultant CP is not as effective as the commercial product or even the originating API. Often, it has required treatment failure in repeated situations to generate sufficient interest to perform scientific investigation to validate these discrepancies. An outstanding example of this concern is compounded itraconazole, which lacks the essential cyclodextrin moiety. The resultant diminished serum concentrations in clinical patients, and repeated failure of efficacy, have resulted in the recommendation that compounding not be used in this product.[6,22] Although these studies have been quite limited in exotic patient situations, it is within the wide variety of these species that one easily can extrapolate that similar concerns can exist in any compounding process. In each of these situations, rather than enhance patient care, CPs compromise clinical outcome and produce ADE potential for which the veterinary prescriber is liable.[6]

Appropriate Compounding Situations

Federal recognition exists that compounding is necessary in veterinary medicine. Use of CPs must ensure that harm and therapeutic failure do not occur to the patient and that no violative residues are produced in food source animals.[6] However, as considered and recommended by both American Veterinary Medical Association and American Association of Zoo Veterinarians documentation,[8] compounding is appropriate in many situations but must include a valid veterinary-client-patient relationship and have expected prevention of animal death and suffering and, wherever

possible, be patient specific by prescription.[1,10] In particular, compounding is condoned when it is:

1. Focused on those products that have been determined safe and effective in target species in their compounded form, and the risks of lack of efficacy or expectation of ADEs are outweighed by potential patient benefit;
2. An adjustment of formulation to accommodate individuals where no other available formulation will be successful; and
3. Possible to monitor the effects of CPs through indirect measures of physiologic function, or actual therapeutic drug monitoring (TDM).[9]

Specific indications from these considerations include:

1. Lack of appropriate drug concentration that requires adjustment to facilitate either more precise dose measurement (smaller patients) or administration of more appropriately sized dose formulations (larger patients);
2. Lack of appropriate formulation in what is available commercially;
3. Accessing essential treatment from drugs that are temporarily or permanently removed from market access for reasons other than ADEs in veterinary patients; and
4. Accessing essential drugs for minor species, as consistent with the definition established in 1994 by the Minor Use and Minor Species (MUMS) Animal Health Act.[1]

Drug compounding does have inappropriate applications. Veterinarians are advised to avoid this approach when:

1. Commercial or generic products in their marketed form are satisfactory and appropriate for the intended use, aside from minor in-office manipulation;
2. Food-producing animals are involved, including all cattle, swine, domesticated poultry, sheep, goats, deer, rabbits, and nonornamental fish, even when these species are pet breeds or managed in the captive setting. These species are restricted due to concerns of food source contamination from either intentional or inadvertent production streams.[1] Exceptions have been permitted in depopulation, euthanasia, and specific antidotes that as such will not enter the food stream[1];
3. Production is based solely on the ability to reduce cost of the treatment by the process of compounding[2]; and
4. Minimal relationship is available with the compounding source such that understanding of their practices is unknown.

Compounding Relevance and Considerations for the Zoo and Wildlife Specialty

It scarcely needs mention to this audience that compounding is critically essential to patient care. Available drugs minimally are labeled for the vast array of species and medical concerns of this discipline. Yet for completeness, and because only 5% of the total compounding effort in the United States was directed to zoo and wildlife species in 2013, consideration of compounding situations for this setting must be enumerated.[14]

As mentioned, accuracy of dosing for both large and small patients is facilitated by CPs. Immediate availability of antiinfective products in their appropriate form or at-hand availability of appropriate anesthetics in the face of escape or urgent need precludes the ability of this discipline to use prescriptions of individual and volume-limited CPs. However, each zoo and wildlife veterinarian is charged to exercise this part of their duties with the understanding that each of our actions has the potential to affect the entire discipline if sound judgment is abandoned in the face of convenience. Counsel in the use of CPs, and thorough documentation, should be made in the practice setting.

Veterinarians should be critical of their sources of CPs. This caution is not intended in a negative or punitive manner but rather encourages an educated and constructive communication. It should be ensured what state and industry oversight that is available will be exercised; measures of good practices are engaged by personally selected compounders; and a relationship should be maintained to identify concerns or ADEs more quickly by either prescriber or compounder. Appropriateness of the situation and the ethical impact of the CP choice is tantamount to the successful prescription. In this consideration the factor of larger volumes maintained for office dispensing should consider that BUD has not been determined for many CPs, such that any product provided a longer than 14-day BUD may need to be considered for more rapid disposal and replacement. Published literature on compounded formulations, even in domestic species, should be reviewed regularly to ensure that known CP issues are incorporated into or avoided in patient care.[3,12,16,20,22] In addition, initiation of such analyses is needed sorely, and it should span many species and indications.[1] Participation in legislative updates (https://www.avma.org/kb/resources/reference/pages/compounding.aspx) by the practitioner needing CPs is critical to inform future regulatory processes.

Conclusion

In conclusion, the prescriber must be engaged in the process of prescription—whether this be for a compounded or FDA-approved drug. The responsibility to select the optimal drug, appropriate formulation, and advocated administration route to their best ability requires that veterinarians understand the risks of any prescription administered. Furthermore, when considering compounding, it should be questioned whether compounding is appropriate—or even necessary.[11] In addition, diligence in reporting prescriptions and treatment success or failure are important within animal records, despite the unclear regulations existing on CP labeling. Regardless of the originating pharmaceutical, in the face of treatment failure or unexpected outcome, it should be considered that the prescription was the source. As such, antemortem treatment can be adjusted or postmortem evaluation can confirm the issue. Thorough ADE

reporting within 15 days is recommended to not only the pharmacy source but also the FDA (www.fda.org; Form FDA 1932a), even for compounded drugs that currently do not have this legal requirement.[1,10] By doing so, one not only provides excellence in individual practice care, but also precludes additional harm to similar animals or species in global zoo and wildlife veterinary care.

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SECTION 6

Anesthesia and Analgesia

26

Sustained-Release and Long-Acting Opioid Formulations of Interest in Zoological Medicine

JESSICA A. EMERSON AND DAVID SANCHEZ-MIGALLON GUZMAN

Introduction

Pain management is an important consideration for all species and has become the expected standard of care in zoological institutions. There are multiple types of analgesic drugs that are used in zoological settings, such as opioids, nonsteroidal antiinflammatories, and local anesthetics drugs. It is often ideal to use more than one type of analgesic drug in a "multimodal" approach to manage pain in a balanced manner. Challenges associated with administration of these medications include handling of the animal for drug administration, patient compliance, dosing frequency, and maintenance of steady-state therapeutic plasma and tissue drug concentrations. These challenges may lead to suboptimal results, including increased patient stress or break-through pain, with development of central sensitization and hyperalgesia. Sustained-release (SR) formulations help to overcome these challenges because they allow for less frequent dosing, thus minimizing handling and stress, and may have improved efficacy due to steady therapeutic levels.

The term *sustained release* is used in this chapter to refer to those formulations that have a vehicle or membrane that provides release of the drug at a controlled rate. This is in contrast to *long-acting* (LA), which refers to the duration of the effect whether or not there is a vehicle or membrane present. The properties of these formulations are due to the half-life of the drug itself or the dose administered. Theoretically, SR formulations should follow a zero-order kinetic profile (constant amount per unit time is metabolized)[1] compared with standard formulations with a first-order kinetics (constant fraction per unit time is metabolized). In the literature and clinical studies, these terms are often used interchangeably, contributing to confusion and inconsistency regarding their definition.

For the purpose of this chapter, we have focused primarily on currently available SR opioid formulations in the United States. These formulations also have disadvantages, which might include the inability to reverse the effects after dosing, long duration of adverse effects, and/or increased severity of adverse effects. The information available for these formulations is often limited to anecdotal reports or pharmacokinetic studies in a small number of individuals and/or species. Even in the cases that a study has been performed, for a variety of reasons, there is variability of the pharmacokinetics and difficulty predicting analgesic effects. Therefore it is of utmost importance to monitor pain in each individual patient and consider the potential adverse effects when developing a pain management protocol.

Additional modalities that are beyond the scope of this chapter include SR nonsteroidal antiinflammatory drugs, SR oral medications, liposome-encapsulated bupivacaine suspension, epidural catheters, and fenestrated ("soaker") catheters placed into wounds.

Opioid Drugs

Opioids are one of the most common analgesic and anesthetic medications used in veterinary medicine and the cornerstone of therapy for pain management. They provide analgesia by their actions on specific opioid receptors on cell membranes, mimicking the effects of endogenous opioids (endorphins, enkephalins, and dynorphins).[2] There are four types of opioid receptors, and the activation of each has specific effects: μ or MOP (mu opioid peptide), κ or KOP (kappa opioid peptide), δ or DOP (delta opioid peptide), and the nociceptin receptor, or NOP (nociceptin opioid peptide).[2] General side effects of opioids include sedation or agitation, ataxia, hypothermia or hyperthermia, respiratory depression, nausea and vomiting, ileus, increased intestinal peristalsis at first and then constipation, urine retention in the bladder, oliguria (μ-agonists), or increased diuresis (κ-agonists).

Fentanyl

Fentanyl is a short-acting, synthetic, lipophilic, μ-agonist that is approximately 100 times more potent than morphine.[3] Fentanyl is available as an injectable used for constant-rate infusions or with osmotic pumps, as a transdermal patch that may provide sustained analgesia, and as a transdermal topical solution. Fentanyl is an ideal drug for transdermal delivery due to its low molecular weight, high potency, and lipid solubility.[4]

Transdermal fentanyl delivery systems, or patches (TFP), are available in many different strengths, ranging from 12.5 to 100 μg/h and come in two broad categories.[1,3,5] Reservoir patches are typically composed of four layers—a protective polyester film backing, a drug reservoir of fentanyl in alcohol, a rate-controlling semipermeable membrane, and a fentanyl-saturated silicone adhesive layer.[3,5,6] This type of patch must remain intact because cutting the patch allows the contents of the drug reservoir to come into direct contact with the skin, which could result in overdosage.[3] Matrix adhesive patches do not have a rate-controlling membrane, and instead the drug is present throughout the adhesive layer, which controls drug delivery.[5] Drug delivery from this type of patch is dependent upon drug concentration in the adhesive, so a decrease in the release rate is noted with longer wear times.[5] For these reasons, it is important to know which type of patch you are using prior to placement.

Osmotic pumps are miniature, cylindrical, subcutaneous (SC) implants that range in length from 1.5 to 5.1 cm.[7] They do not require a battery or external power source because they operate on the basis of an osmotic pressure difference between the extracellular fluid and the osmotic agent in the pump but have to be surgically removed.[7] In short, as water diffuses across an outer semipermeable membrane, a "salt sleeve" compartment expands and presses on the flexible drug reservoir, thereby releasing the drug at a continuous rate. The pump rate is determined by properties of the semipermeable membrane and osmotic agent, so it is independent of the properties of the drug dispensed.[7]

Mammalian

TFPs have been evaluated in many species[6,8–18] but have been generally shown to have variable pharmacokinetic profiles between individuals and species (Table 26.1). Transdermal fentanyl absorption is also dependent on a number of other factors, including body temperature, stratum corneum disruption, and anatomic location of placement.[3,19–22] In addition, in rabbits, hair removal technique (clipping vs. depilatory cream) affected absorption, with use of depilatory cream associated with more rapid absorption, markedly higher serum concentrations, evidence of sedation, and shorter clinical activity.[9] TFPs may take up to 12–24 hours in mammals to reach target plasma concentrations and might require a loading dose for acute pain. TFPs also carry a risk of coming dislodged and being ingested, which has been associated with fatalities in cynomolgus macaques *(Macaca fascicularis)*.[23]

Transdermal fentanyl solution (TFS; Recuvyra, Elanco Animal Health, Indianapolis, IN, USA) for the treatment of perioperative pain in canine patients with a duration of action of 72 hours has become available.[24–26] This formulation contains 5% fentanyl with a skin penetration enhancer in an isopropanolol base and has been reported to reach target plasma concentrations in 1–3 hours, which is markedly faster than with the patches. In addition, the dose can be titrated more effectively to body weight, although it is not recommended for use in dogs weighing less than 2.7 kg. Although the benefit of this product is prolonged activity that limits handling, this is also one of the risks associated with its use. Treatment with naloxone can be initiated in the case of an accidental overdose.[27] Adverse effects of this medication in dogs include sedation, reduced food or water intake, weight loss, transient lens opacities, and minimal decreases in heart rate or rectal temperatures.[28]

In nonhuman primates, TFPs (25 μg/h) and TFS (2.6 mg/kg and 1.95 mg/kg) have been evaluated in cynomolgus macaques.[29] The study found marked interanimal variability in absorption and maximal concentration. Three of four animals in the high-dosage TFS group showed life-threatening adverse effects characterized by severe respiratory depression, hypothermia, bradycardia, and unresponsiveness. Patient status was markedly improved within 2 hours of administering naloxone. In rhesus macaques *(Macaca mulatta)* a single topical administration of 1.3 or 2.6 mg/kg TFS has also been evaluated, with very different results. A single TFS dose may provide efficacious analgesia to rhesus macaques and reduce stress, discomfort, and risk to animals and personnel, with no adverse effects noted.[30] These studies further highlight the individual and species variability associated with TFS.

The use of osmotic pumps for fentanyl administration have been also studied in domestic cats, with a shorter lag time, higher bioavailability, and faster elimination after removal compared with TFP.[7] Both the osmotic pump and the TFP were in place for 96 hours. Interestingly, there was individual variability noted with both methods, prompting the authors to postulate that individual variation in metabolism of fentanyl may be a more important source of plasma concentration variability than factors associated with patch placement or cutaneous anatomy. The osmotic pump requires surgical implantation and removal, and therefore two anesthetic events, but does not have the same level of risk for becoming dislodged or ingested. These may represent a viable option for zoological species, specifically nondomestic felids.

There are multiple reports of anecdotal TFP use in zoological medicine, with minimal to no side effects (Table 26.2). These were used for varying conditions, but minimal information regarding perceived efficacy was identified in the case reports. However, in white rhinoceros *(Ceratotherium simum)*, sedation with no apparent analgesia was noted.[31]

Avian

The TFP (25 µg/h) has been evaluated in chickens.[32] The TFP was in place for 72 hours over the left iliopsoas muscle after plucking the feathers. There was substantial interindividual difference in maximum plasma fentanyl concentrations that was attributed to absorption variability, but all chickens reached the human target plasma concentrations of 0.2–1.2 ng/mL within 2–4 hours and had rapid decrease following removal of the patch. TFS has been evaluated in Helmeted guineafowl *(Numida meleagris)*.[33] The solution was placed on the skin in the interscapular region at 5 mg/kg, which resulted in mean plasma levels greater than those considered analgesic for dogs (0.6 ng/mL) from 4 hours through at least 7 days, with no evidence of adverse effects or change in behavior. Pharmacodynamic studies are indicated to more accurately determine effective dosages for analgesia.

Reptilian

Fentanyl patches (12.5 µg/h) were recently evaluated in two 2.6-kg ball pythons *(Python regius)*.[34] The patches were placed on the dorsal midbody of the snakes. Therapeutic concentrations for mammals (1 ng/mL) were reached within 4 hours and were present for the 7 days evaluated with no adverse effects. However, a follow-up study showed no evidence of thermal antinociception at similar plasma levels in ball pythons.[35] In prehensile-tailed skinks *(Corucia zebrata)*, a 1-cm^2 portion of a 12.5 µg/h fentanyl patch (equal to 2.5 µg/h) was placed over the dorsal thoracolumbar region, and therapeutic concentrations for mammals (0.2–2 ng/mL) were reached within 12–24 hours.[36] No adverse effects were seen during this study either; however, two animals showed anorexia 2 weeks after completion of the elimination study. Conversely, 2 cm^2 of a 12.5 µg/h patch placed on the dorsolateral torso of two green iguanas *(Iguana iguana)* showed no detectable plasma fentanyl concentrations.[36] It was hypothesized that the finely nodular scaling of this area prevented close apposition of the patch (see also Chapter 60).

Buprenorphine

Buprenorphine is a semisynthetic opioid, approximately 20–50 times more active than morphine.[14] Once thought to be a partial µ-agonist, buprenorphine has recently been determined to be a full µ-agonist.[37] Buprenorphine is less likely to cause respiratory depression than some of the other opioids and has been reported to exhibit a "ceiling effect" where increasing the dosage does not increase the adverse effects in animals.[37] In addition, buprenorphine is considered to have a longer duration compared with similar opioids due to very high affinity binding that results in slow disassociation from the receptor.[37] There are several SR buprenorphine formulations available at different concentrations that rely on a proprietary matrix vehicle for their SR properties. Although not approved by the US Food and Drug Administration (FDA) at the time of this writing, a portion of these SR formulations were under review to gain FDA indexing status and meet the USP<797> guidelines regarding sterile pharmaceutical compounding (buprenorphine SR and buprenorphine SR-LAB; ZooPharm, Wildlife Pharmaceuticals Inc., Windsor, Colorado). In addition, there is an FDA-approved concentrated injectable formulation for domestic cats (1.8 mg/mL; Simbadol, Zoetis Inc., Kalamazoo, Michigan) that relies on a higher-dose administration than standard buprenorphine hydrochloride formulation for the LA properties of approximately 24-hour duration of action in cats. Finally, buprenorphine is available in a transdermal patch formulation ranging in concentrations from 5 to 70 µg/h, depending on the manufacturer.

Mammalian

SR buprenorphine formulations (buprenorphine SR and buprenorphine SR-LAB) have been evaluated in many companion and domestic species[4,38–50] and are summarized in Table 26.1. The most common adverse effect noted across species is varying forms and severity of skin reactions (see Table 26.1). In a 2014 study a new formulation of the product (buprenorphine SR-LAB) was evaluated in 12 mice and there were no skin lesions noted, so this adverse effect may pose less of a concern moving forward.[45] There also appears to be a trend toward marked interspecies and interindividual variation in pharmacokinetics. For example, in alpacas dosed with 0.12 mg/kg buprenorphine SR, two of six animals did not have detectable plasma concentrations at 8 hours post injection.[48] However, in minipigs treated with 0.18 mg/kg of buprenorphine SR, five of five animals had plasma levels considered therapeutic for 10 days.[49]

In zoological medicine, buprenorphine SR has been evaluated in a limited number of species. In five rhesus and five cynomolgus macaques, the pharmacokinetic profile of buprenorphine SR at 0.2 mg/kg following SC administration was evaluated.[51] The results demonstrate that a single 0.2 mg/kg SC injection of buprenorphine SR could maintain plasma concentrations greater than 0.1 ng/mL for 5 days in these species. Injection site reactions were noted in 4 of 10 macaques following injection with buprenorphine SR, but they were considered generally mild and did not require treatment. No obvious sedation, respiratory depression, or changes in appetite were noted in the 72 hours following injection.

In juvenile northern elephant seals *(Mirounga angustirostris)* the pharmacokinetic profile was evaluated following SC administration of 0.12 mg/kg of buprenorphine SR.[52] There was a high degree of individual variation in plasma concentration at all time points, and most seals had a plasma concentration less than 1 ng/mL after 24 hours. Local reactions, including cellulitis and abscess formation, occurred at the injection site in 6 of 26 (23%) of the seals. Some abscesses required local treatment (lancing, flushing with dilute betadine and saline solution), but none required treatment with antibiotics or naloxone.

Text continued on p. 160

TABLE 26.1 Sustained-Release Opioid Drugs of Interest in Zoological Medicine

Drug	Route	Dosage	Study Type and Reference	Frequency
Fentanyl	Transdermal patch	25 μg/h	PD,[8] postoperative OHE	Once, in place for 73 h, placed 11.5 h prior to surgery
Fentanyl	Osmotic pump SC and transdermal patch	25 μg/h	PK,[7] crossover design, comparison with transdermal fentanyl	Once (for each modality), in place for 96 h
Fentanyl	Transdermal patch	50 μg/h; 75 μg/h; 100 μg/h	PK,[6] crossover design	Three times, in place for 72 h
Fentanyl	Transdermal patch	25 μg/h	PK[9]	Once, in place for 72 h
Fentanyl	Transdermal patch	25 μg/h	PK[29]	Once, in place for 96 h
Fentanyl	Transdermal solution	2.6 mg/kg	PK[29]	Once
Fentanyl	Transdermal solution	1.95 mg/kg	PK[29]	Once
Fentanyl	Transdermal solution	1.3 mg/kg (25 μL/kg)	PK[30]	Once
Fentanyl	Transdermal solution	2.6 mg/kg (50 μL/kg)	PK[30]	Once
Fentanyl	Transdermal patch	2 × 100 μg/h	PK[10]	Once transdermal, in place for 48 or 72 h; multiple transdermal, replaced every 48 or 72 h over 9 days; Once IV (2 mg)
Fentanyl	Transdermal patch	100 μg/h	PK and PD,[11] all horses had painful condition that did not respond to 24–72 h of treatment with a nonsteroidal antiinflammatory	Once, in place for 48 or 72 h
Fentanyl	Transdermal patch	50 μg/h	PK and PD[12]	Twice (Crossover study design), in place for 60 h
Fentanyl	Transdermal patch	25 μg/h; 50 μg/h	PK and PD[13] (post-operative left lung allograft transplant)	Once, in place for 72 h

Species	Location of Placement (if applicable)	Comments
Domestic cat	Lateral thorax, covered with a light bandage	*N* = 12; cortisol levels significantly lower in group with patch undergoing OHE than group without, effective in alleviating perioperative pain
Domestic cat	Pump: 2 cm incision dorsal scapular region with 8–10 cm SC tunnel Patch: Lateral thorax, secured with adhesive bandage	*N* = 8; osmotic pump had shorter lag phase, higher bioavailability, and faster elimination than transdermal patch; evidence of individual variation in fentanyl metabolism for both methods
Domestic dog	Lateral thorax	*N* = 6, weight range 16.5–23.3 kg, high variability between and within individuals, skin reactions common (15/18 applications), steady-state plasma concentrations took up to 24 h to reach, plasma fentanyl concentrations declined rapidly after patch removal
Rabbits (New Zealand White)	Dorsal interscapular region	*N* = 9 hair clipped, *N* = 6 depilatory cream; rapid hair regrowth appeared to prevent absorption in some clipped animals, patients without rapid hair growth had therapeutic levels (0.5–2 ng/mL) for 72 h; depilatory treated animals had rapid absorption, higher peak plasma concentrations, sedation, and <72 h efficacy
Cynomolgus macaques *(Macaca fascicularis)*	Dorsal scapular area, jacket worn to prevent tampering	*N* = 16; significant interanimal variability, 2/12 animals had undetectable serum levels, peak concentration occurred at a mean of approximately 36 h, C_{max} ranged from 0.7 to 3 ng/mL
Cynomolgus macaques	Dorsal scapular area, jacket worn to prevent tampering	*N* = 4; 3/4 had significant adverse effects (respiratory depression, unresponsive, bradycardic) that necessitated treatment, peak concentration occurred at 56 ± 18.1 h, median C_{max} was 159.8 ± 35 ng/mL
Cynomolgus macaques	Dorsal scapular area, jacket worn to prevent tampering	*N* = 8; significant interanimal variability, no adverse reactions, peak concentration occurred at 56 ± 17.6 h, median C_{max} was 177.1 ± 160.6 ng/mL
Rhesus macaques *(Macaca mulatta)*	Clipped dorsal skin	*N* = 6; maximal plasma concentration was 1.95 ± 0.40 occurring at 21.3 ± 4.1; terminal elimination half-life was 93.7 ± 7.1 h. No adverse effects.
Rhesus macaques	Clipped dorsal skin	*N* = 6; maximal plasma concentration was 4.19 ± 0.69 ng/mL, occurring at 30.7 ± 8.7 h; terminal elimination half-life was 98.8 ± 5.4 h. No adverse effects.
Domestic horse	Medial or lateral antebrachium or gaskin, covered with nonadherent bandage material and adhesive elastic tape	*N* = 6; rapidly and completely absorbed; mean serum fentanyl concentrations >1 ng/mL reached in 1 h; less fluctuation when patches applied every 48 h; 3/6 horses exhibited brief episodes of increased body temperature; 7/54 patches lost, mild scaling of skin at patch site for several days following removal
Domestic horse	Proximal lateral antebrachium, secured with adhesive elastic tape	*N* = 9, combined treatment with NSAIDs, significantly decreased pain scores after fentanyl and NSAID administration, no significant change in lameness score, serum fentanyl concentrations >1 ng/mL by approximately 6 h but decreased to <1 ng/mL by 72 h, high individual variability, no significant adverse effects
Domestic swine (growing)	Skin behind ear, canvas sutured over patch for protection; placed after 30 min of isoflurane anesthesia or no anesthesia	*N* = 8, 20–30 kg pigs, no sedation noted, large variations in serum fentanyl concentrations, no difference in absorption associated with anesthesia
Domestic swine	Dorsal interscapular region	*N* = 3 at 25 μg/h, *N* = 2 at 50 μg/h; pigs treated with 25 μg/h had higher pain scores and lower serum concentrations (0.1–0.3 ng/mL); pigs treated with 50 μg/h had the lowest pain scores and serum concentration >0.5 ng/mL

Continued

TABLE 26.1 Sustained-Release Opioid Drugs of Interest in Zoological Medicine—cont'd

Drug	Route	Dosage	Study Type and Reference	Frequency
Fentanyl	Transdermal patch	75 µg/h; 150 µg/h; 300 µg/h	PK and PD[16]	Once
Fentanyl	Transdermal patch	2 µg/kg IV; 2 µg/kg/h transdermal (varying combinations of patch sizes used based on weight of animal)	PK and PD[17]	Once IV and once transdermally, in place for 72 h
Fentanyl	Transdermal patch	2 µg/kg/h (varying combinations of patch sizes used based on weight of animal)	PD[14]	Once; in place for 72 h
Fentanyl	Transdermal patch and IV	2.5 µg/kg IV; 2.05 µg/kg/h transdermal (varying combinations of patch sizes used based on weight of animal)	PK[15]	Once; patch in place for 72 h
Fentanyl	Transdermal patch	50 µg/h	PK[18]	Once IV (2.5 µg/kg); Once transdermally, in place for 72 h
Fentanyl	Transdermal patch	25 µg/h	PK[32]	Once, in place for 72 h
Fentanyl	Transdermal solution	5 mg/kg	PK[33]	Once
Fentanyl	Transdermal patch	12.5 µg/h	PK[34]	Once, in place for 7 days
Fentanyl	Transdermal patch	3 µg/h; 12 µg/h	PK and PD[35] (thermal nociception, incomplete crossover design)	Once, in place for 48 h
Fentanyl	Transdermal patch	2.5 µg/h (1 cm^2 of a 12.5 µg/h patch)	PK[36]	Once, in place for 72 h
Fentanyl	Transdermal patch	5 µg/h (2 cm^2 of a 12.5 µg/h patch)	PK[36]	Once, in place for 72 h
Buprenorphine: Long-acting (Simbadol)	SC injection	0.24 mg/kg	Zoetis, Clinical efficacy trial	Once daily for 3 days
Buprenorphine SR	SC injection	0.12 mg/kg	PD[38]	Once
Buprenorphine	Transdermal	35 µg/h	PK and PD[56] (preliminary, thermal threshold)	Once, in place for 72 h
Buprenorphine SR	SC injection	0.2 mg/kg	PK and PD[39]	Once
Buprenorphine	Transdermal	52.5 µg/h	PK and PD[55]	Once, in place for 72 h

Species	Location of Placement (if applicable)	Comments
Llama	Medial antebrachium, corners stapled, and circumferential adhesive bandage placed	*N* = 9; 3 llamas per dosage; 4 × 75 μg/h (total 300 μg/h) patches provided sustained serum fentanyl concentrations from 12–72 h of 0.3 ± 0.08 ng/mL; no sedation noted
Alpaca	Medial antebrachium, circumferential adhesive bandage placed	*N* = 6, no significant changes in heart rate or respiratory rate, individual variability noted, peak plasma concentrations of mean 1.2 ng/mL occurred at approximately 24 h
Domestic sheep	Lateral antebrachium	*N* = 15, unilateral tibial osteotomy performed 12 h after placement, superior analgesia to 0.01 mg/kg buprenorphine q6h IM
Domestic sheep	Lateral antebrachium	*N* = 6 IV, *N* = 15 transdermal; maximum plasma concentration reached at median of 12 h and a median level of 1.3 ng/mL, plasma concentrations >0.5 ng/mL for 40 h after patch placement
Domestic goats	Right lateral neck, covered with gauze and elastic tape around the neck to secure	*N* = 6; variable plasma concentrations, time to peak concentration ranged from 8 to 18 h, from 4 to 36 h the plasma concentration remained >2 ng/mL, but then declined so a steady state was never achieved
Domestic chickens	Left iliopsoas muscle following feather plucking	*N* = 10; marked individual variability, all chickens had levels within target range (0.2–1.2 ng/mL) through 72 h, peak plasma concentrations of 2.86 ± 2.58 ng/mL at 14.9 ± 8.2 h after placement
Helmeted guineafowl *(Numida meleagris)*	Interscapular skin	*N* = 21, no adverse effects or changes in behavior, Mean peak plasma concentration 228.8 ng/mL at 4 h, plasma concentrations >0.6 ng/mL for at least 7 days
Ball pythons *(Python regius)*	Dorsal mid-body, attached with 4 staples	*N* = 2, plasma concentrations >1 ng/mL within 4 h
Ball pythons *(Python regius)*	Epaxial musculature lateral to spine	*N* = 16, no evidence of thermal antinociception at either dose, evidence of respiratory depression, plasma concentration >1 ng/mL within 6 h
Prehensile-tailed Skink *(Corucia zebrata)*	Dorsal thoracolumbar area, secured with elastic bandage	*N* = 8, plasma concentrations 0.2–2 ng/mL within 12–24 h
Green iguana *(Iguana iguana)*	Dorsolateral torso	*N* = 2, no detectable fentanyl concentrations noted at any time point
Domestic cat (FDA approved)		*N* = 221; statistically significant increase in treatment success (defined as lack of necessary rescue analgesia) compared to placebo following soft tissue surgery and orthopedic surgery
Domestic cat		*N* = 11 cats undergoing ovariohysterectomy; comparable efficacy of twice daily oral transmucosal buprenorphine (0.02 mg/kg) with one preoperative dose of SR buprenorphine over 72 h
Domestic cat		*N* = 6 for PD and *N* = 5 for PK; no change in thermal thresholds, peak buprenorphine concentration 10 ± 0.81 ng/mL at time 34–72 h, visible hair growth noted under patch and *N* = 4 cats needed to have patches replaced during study period
Domestic dog	Dorsal cervical area	*N* = 10 undergoing ovariohysterectomy; plasma buprenorphine above hypothesized therapeutic values (0.6 ng/mL) for >5 days; 1/10 had breakthrough pain, 7/10 had small nonpainful dermal injection site reactions
Domestic dog	Left lateral thorax	*N* = 10, peak plasma concentrations of 1.54 ng/mL 60 h after application, significant increase in thermal threshold from 36 to 72 h after placement, 3/10 had no detectable concentrations

Continued

TABLE 26.1 Sustained-Release Opioid Drugs of Interest in Zoological Medicine—cont'd

Drug	Route	Dosage	Study Type and Reference	Frequency
Buprenorphine	Transdermal	70 μg/h	PK[54]	Once, not removed during 108-h study
Buprenorphine	Transdermal	70 μg/h	PD[53] (post-operative pain)	Once, in place for 86 h (applied 48 h preoperatively)
Buprenorphine SR-LAB	SC Injection	0.12 mg/kg	PD[40]	Once, immediately preoperatively
Buprenorphine SR	SC injection	1.5 mg/kg	PD[45] (Thermal withdrawal)	Once
Buprenorphine SR	SC injection	0.6 mg/kg	PK[43]	Once
Buprenorphine SR	SC injection	0.6 mg/kg	PD[44]	Once
Buprenorphine SR	SC injection	0.3 mg/kg, 1.2 mg/kg, 4.5 mg/kg	PD[42] (mechanical and thermal latency following plantar incision)	Once
Buprenorphine SR	SC injection	1.2 mg/kg	PK and PD[41]	Once, 10 min prior to incision
Buprenorphine SR-LAB	SC injection	0.3 mg/kg	PK and PD[46] (paw withdrawal pressure)	Once
Buprenorphine SR	SC injection	0.9 and 1.2 mg/kg	PK[47]	Once
Buprenorphine SR	SC injection	0.2 mg/kg	PK[51]	Once
Buprenorphine SR	SC injection	0.12 mg/kg	PK[52]	Once
Buprenorphine SR	SC injection	0.18 mg/kg	PK[49]	Once
Buprenorphine	Transdermal	30 μg/h (1 each of 20 μg/h and 10 μg/h)	PK[49]	Once, in place for 72 h
Buprenorphine SR	SC injection	0.12 mg/kg	PK and PD[48]	Once
Buprenorphine SR	SC injection	0.27 mg/kg	PK and PD[50] (thermal nociception)	Once
Buprenorphine SR-LAB	SC and IM	1.8 mg/kg	PK[57]	Once
Buprenorphine SR-LAB	IM	1.8 mg/kg	PD[61] thermal antinociceptive thresholds	Once

FDA, US Food and Drug Administration; *IM,* intramuscularly; *IV,* intravenous; *OHE,* ovariohysterectomy; *PD,* pharmacodynamics study; *PK,* pharmacokinetic study; *SC,* subcutaneous; *SR,* sustained-release.

Species	Location of Placement (if applicable)	Comments
Domestic dog	Ventral abdomen, light bandage used to keep patch in place	*N* = 4, concentrations increased for first 36 h, then remained in target range of 0.7–1.0 ng/mL through 108 h, 1 dog did not show significant absorption
Domestic dog	Left lateral thorax	*N* = 8 undergoing ovariohysterectomy, no significant difference in pain score compared to 0.02 mg/kg buprenorphine SC q6h
Rabbit (New Zealand White)		*N* = 12 receiving tibial implants, similar effect to 0.02 mg/kg regular buprenorphine q12h, 1/12 had dermal reddening that spontaneously resolved
Mice		*N* = 12, improved formulation from prior studies, antinociceptive effects for 48 h, no skin reactions noted
Mice		*N* = 21, >1 ng/mL for 24 and >0.5 ng/mL for 48 h, no injection site reactions
Mice		*N* = 8, adequate analgesia following experimental laparotomy, significant increased general activity and decreased orbital tightening for 6 h postoperatively compared with regular buprenorphine (0.1 mg/kg q12h)
Rat		*N* = 6 at each dose, plantar incisional pain model, 0.3 mg/kg effective at least 48 h; 1.2 mg/kg effective at least 72 h, 4.5 mg/kg led to weight loss and sedation
Rat		*N* = 6 plantar incisional pain model, *N* = 12 PK; attenuated mechanical and thermal sensitivity days 1–4
Guinea pig		*N* = 7, plasma concentrations >0.5 ng/mL (targeted therapeutic levels) through 24 h, significantly increased paw withdrawal pressures through 26 h, estimated dosing interval of 24–48 h
Prairie dog *(Cynomys ludovicianus)*		*N* = 4 per dosage group, plasma concentrations above 1.0 ng/mL (proposed therapeutic levels) for at least 96 h, injection site reactions including erythema and scabbing in 4/8 animals
Cynomolgus and rhesus macaque		*N* = 5 of each species, remained greater than 0.1 ng/mL (hypothesized therapeutic) for 5 days, 4/10 animals had injection site reactions (mild, no scratching noted)
Northern elephant seal *(Mirounga angustirostris)*		*N* = 26, plasma concentrations >1 ng/mL (hypothesized therapeutic threshold) for up to 24 h, high individual variability, 6/26 developed injection site cellulitis or abscessation, use with caution
Swine (Göttingen minipigs)		*N* = 5, plasma concentrations >0.1 ng/mL (hypothesized therapeutic threshold) for 10 days, high individual variability, injection site reaction (firm SC nodules) in 4/5 animals
Swine (Göttingen minipigs)	Shaved area of dorsal trunk (between 12th thoracic and 2nd lumbar vertebrae)	*N* = 5, plasma concentrations >0.1 ng/mL (hypothesized therapeutic threshold) achieved in 12–24 h and lasted through 72 h (when patch was removed), 1/5 animals did not develop plasma concentrations, 1/5 animals had a mild dermal reaction (erythema with a small number of papules)
Alpaca		*N* = 6, detectable plasma concentrations in only 2/6 at 8 h; no significant difference in thermal and mechanical withdrawal latencies
Domestic sheep		*N* = 4, reached plasma concentration of 0.1 ng/mL (considered minimal therapeutic threshold) within 12 h and maintained for at least 72 h, thermal thresholds increased significantly by 12 h and remained for at least 72 h
American kestrels *(Falco sparverius)*		*N* = 14, C_{max} reached at 15 min, mean plasma concentrations remained above target concentrations (>1 ng/mL) 48 h after both IM and SC administration
American kestrels		*N* = 12, increased thermal thresholds at 6, 12, and 24 h post drug administration, mild sedation

TABLE 26.2 Anecdotal Reports of Sustained-Release Opioid Formulations in Zoological Medicine

Drug and Type	Species and Reference	Condition
Fentanyl, transdermal patch	African lion *(Panthera leo)*[62]	Dorsal laminectomy of C_1 (atlas)
Fentanyl, transdermal patch	Snow leopard *(Uncia uncia)* cubs[63]	Surgical correction of stifle osteochondritis dissecans
Fentanyl, transdermal patch	Leopard *(Panthera pardus)*[64]	Sternectomy for removal of an ectopic thyroid carcinoma
Fentanyl, transdermal patch	Maned wolf *(Chrysocyon brachyurus)*[65]	Rostral maxillectomy
Fentanyl, transdermal patch	Red wolf *(Canis rufus)*[66]	Hypertrophic osteodystrophy
Fentanyl, transdermal patch	Binturong *(Arctictis binturong)*[67]	Spinal decompression for intervertebral disc extrusion
Fentanyl, transdermal patch	Slender-tailed meerkats *(Suricata suricatta)*[68]	Acute pancreatitis
Fentanyl, transdermal patch	Asian elephant *(Elephas maximus)*[31]	Unknown
Fentanyl, transdermal patch	African elephant *(Loxodonta africana)*[31]	Unknown
Fentanyl, transdermal patch	Sichuan takin *(Budorcas taxicolor tibetana)*[69]	Laminitis
Fentanyl, transdermal patch	Alpine ibex *(Capra ibex ibex)*[70]	Surgical correction of a lateral scapulohumeral luxation
Fentanyl, transdermal patch	White rhinoceros *(Ceratotherium simum)*[31]	Unknown; sedation, but no apparent analgesia
Fentanyl, transdermal patch	Beaded lizard *(Heloderma horridum horridum)*[71]	Surgical resection of a renal adenocarcinoma (placed 24 h prior to surgery)
Buprenorphine SR	Prehensile-tailed porcupine *(Coendou prehensilis)*[72]	Postoperative gastrotomy for gastrolith removal
Buprenorphine SR	California sea lions *(Zalophus californianus)*[73]	Corneal ulceration, used when oral tramadol and carprofen were unable to alleviate discomfort
Buprenorphine SR	Hoffmann's two-toed sloth *(Choloepus hoffmanni)*[74]	Acute respiratory distress following recent foot abscess
Buprenorphine SR	Southern three-banded armadillos *(Tolypeutes matacus)*[75]	Ovariohysterectomy

SR, Sustained-release.

Buprenorphine transdermal patches have been evaluated in dogs,[53–55] pigs,[49] and cats.[56] Overall, target plasma concentrations have been reached and resulted in pain control in dogs undergoing ovariohysterectomy,[53] as well as antinociceptive effects using thermal thresholds.[55] In cats, there was no antinociceptive effect noted using the thermal threshold model.[56] Interestingly, undetectable plasma concentrations occurred in some dogs[54,55] and one pig,[49] indicating that there may be individual absorption variability with these patches as well. More research and use of these patches is indicated in zoological medicine.

Avian

In a pharmacokinetic study in American kestrels *(Falco sparverius)*, SC and intramuscular (IM) administration of 1.8 mg/kg of buprenorphine SR-LAB were both characterized by rapid absorption and elimination kinetics.[57] Forty-eight hours after both IM and SC administration, mean plasma concentrations remained greater than target concentrations (>1 ng/mL). SC hematomas in three of the birds that received buprenorphine SR-LAB SC were attributed to traumatic administration but resolved without intervention. A follow-up study concluded that depending on the severity and type of pain, adjunctive therapy, and the individual response, buprenorphine SR-LAB administered at 1.8 mg/kg IM to American kestrels would require administration every 24 hours to manage pain.[61]

In red-tailed hawks *(Buteo jamaicensis)*, the pharmacokinetics of two dosages of a concentrated formulation of buprenorphine (Simbadol) were evaluated after SC administration.[58] Maximum buprenorphine concentration was achieved at 5 and 15 minutes for the 0.3 mg/kg and 1.8 mg/kg doses, and plasma concentrations were maintained at greater than 1 ng/mL for at least 24 and 48 hours,

respectively. Baseline sedation scores were significantly lower than all other time points for each individual, with return to near baseline by 24–48 hours, consistent with mild to moderate sedation. No adverse effects were noted in any birds.

Butorphanol

Avian

In Hispaniolan Amazon parrots *(Amazona ventralis)*, a formulation containing butorphanol in a 25% poloxamer 407 base (But-P407 25%) has been evaluated following SC administration.[59] P407 (Sigma-Aldrich, Oakville, ON, Canada) is a thermosensitive hydrogel, a compound that exhibits a property known as reverse gelation. P407 is liquid at room temperature, allowing easy mixing with therapeutic agents and routine handling for injection. Once injected into homeothermic animals, micellar packing forms at the warmer temperature of the body and the compound becomes a gel.[4] This micellar packing is responsible for the high viscosity, partial rigidity, and slow dissolution of the gel, which makes it a highly effective SR system for both hydrophilic and hydrophobic drugs. Butorphanol was well absorbed from the But-P407 25% with maximal plasma butorphanol concentration reached at 90 minutes. Plasma concentrations of butorphanol remained greater than 100 ng/mL for more than 3 hours but less than 8 hours, with no noted adverse effects. A dosage of 12.5 mg/kg SC would theoretically provide analgesia for 3–8 hours.

In common peafowl, the pharmacokinetics of butorphanol administered via an osmotic pump were assessed.[60] Two osmotic pumps containing 2 mL butorphanol at a concentration depending on body weight of the bird, and administering the drug at a rate of 247 µg/kg per hour each, were surgically implanted in 12 birds (two pumps per bird) and removed 7 days later. The osmotic pumps used in the study were 5.1 cm in length and weighed 5.1 g. Plasma butorphanol concentration was measured before implantation SC in the left inguinal region, for several hours to days after implantation, and 3 and 6 hours after the implant removal. Plasma concentration reached 60 ng/mL after 24 hours in most birds, and in most birds stayed above this threshold for 7 days and then decreased rapidly after implant removal. There was no evidence of sedation or adverse effect.

Summary

There is a growing body of evidence-based and anecdotal use of SR and LA opioid drugs in zoological medicine. These drug formulations show promise for continued improvements in the way we manage pain and serve as additional treatment options. Caution is still warranted with the use of any of these drug formulations when extrapolating to species in which they have not been evaluated, because there appears to be unpredictable interspecies and intraspecies variability that could affect efficacy and adverse effects.

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27

Use of Naltrexone and Atipamezole in Emergency Response to Human Exposure to Ultra-Potent Opioids and Alpha-2 Agonists in Zoo and Wildlife Medicine

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Introduction

Numerous advancements during the past several decades have contributed to our ability to provide safe and effective anesthesia for captive and free-ranging wildlife species. This includes the development of accurate and dependable remote delivery equipment, as well as the availability of potent and concentrated anesthetic agents and their antagonists. Ultra-potent opioids (UPOs) such as carfentanil, etorphine, and thiafentanil have been available to the zoo and wildlife veterinarian for decades, and their inherent danger has been thoroughly reviewed in the literature.[1–5]

More recently, concentrated and potent forms of the alpha-2 (α-2) agonist medetomidine have become available to supplement and provide balanced anesthesia by intramuscular (IM) injection. This is especially true in larger zoo and wildlife species including hoof stock (equids, bovids, cervids, camelids, etc.), megavertebrates (elephant, rhinoceros, hippopotamus, giraffe), primates (gorilla), and carnivores (bear, lion, tiger). The high doses and volumes of α-2 agonists required in these species present a potential danger to humans in case of an accidental exposure. There are also concentrated forms of other supplemental agents such as butorphanol, midazolam, azaperone, and ketamine, but they are not considered as dangerous as the UPOs or α-2 agonists and therefore will not be discussed in detail here.

This chapter will focus on the UPOs and α-2 agonists used in wildlife species and the potential advantages of naltrexone and atipamezole in the emergency response to an accidental exposure of a human to these dangerous anesthetic agents.

Ultra-Potent Opioids and Alpha-2 Agonists Used in Zoo and Wildlife Anesthesia

UPOs have been used by zoo and wildlife veterinarians for the past 30–40 years to anesthetize captive and free-ranging ungulates. To increase the safety and quality of anesthesia, potent α-2 agonists are commonly added to the dart for remote delivery for IM injection. Furthermore, these potent α-2 agonists are used in combination with other non-UPO anesthetic agents in captive and free-ranging ungulates, primates, and carnivores. Therefore both drug classes are a potential danger to humans. Emergency treatment for a human exposure to these drugs will be discussed later in this chapter.

Specific antagonists to these drugs will also be reviewed here, because they are commonly used in veterinary medicine for rapid reversal of anesthetized animals. This is especially true in our large ungulates, primates, and carnivores, which are dangerous when awake, so having the ability to reverse and move away to safety during recovery offers a great advantage. These potent anesthetic agents may cause significant cardiovascular changes and respiratory depression, so rapid reversal is essential. Furthermore, in case of an anesthetic emergency, we may want to reverse quickly for added patient safety.

Ultra-Potent Opioids and Antagonists

Etorphine

Etorphine HCl (M99, 10 mg/mL, Wildlife Pharmaceuticals, Inc., Windsor, Colorado) is considered the most

TABLE 27.1 Equipotency Data of Clinically Significant Doses of Morphine and Ultra-Potent Opioids Used in Zoo and Wildlife Anesthesia

Ultra-Potent Opioid and Injectable Solution Concentration (mg/mL)	Equianalgesia Potency Compared to Morphine	Equipotent Dose for Respiratory Depression, 20 mg Morphine IV* (mg)	Volume† of Ultra-Potent Opioid for Equipotent Dose of 20 mg Morphine IV* (mL)
Carfentanil (3)	10,000‡	0.002§	0.00067
Etorphine (10)	6,000**	0.0033††	0.00033
Thiafentanil (10)	6,000‡‡	0.0033§§	0.00033

Note: The morphine dose provided is known to cause respiratory depression in humans. The volume of UPO necessary to provide this hypothetical accidental exposure is also listed.
*Morphine IV dose causing respiratory depression in 65 kg opioid naïve human (see Refs. 16 and 42).
†Volume (mL) equals equipotent dose (mg) times the UPO concentration (mg/mL).
‡Carfentanil equipotency to morphine, 10,000:1 (see Refs. 11 and 12).
§20 mg morphine divided by 10,000.
**Etorphine equipotency to morphine, 6000:1 (see Ref. 2).
††20 mg morphine divided by 6000.
‡‡Thiafentanil equipotency to morphine, 6000:1 (see Ref. 10).
§§20 mg morphine divided by 6000.
IV, Intravenous; *UPO*, ultra-potent opioids.

widely used UPO in zoo and wildlife anesthesia,[5] and is the induction agent of choice for elephant, rhinoceros, nondomestic equids, and other hoofstock. It is often combined with azaperone, medetomidine, midazolam, or azaperone to produce muscle relaxation.[6] The availability of etorphine since its first use and description in the late 1960s revolutionized the ability of veterinarians to safely capture and restrain many species that previously could not be handled.[2]

Thiafentanil

Thiafentanil (Thianil, 10 mg/mL, Wildlife Pharmaceuticals, Inc.), previously known as A-3080, was introduced in the early 1990s and has similar characteristics to etorphine and carfentanil, but with faster inductions in certain species.[2,7] Since thiafentanil has a shorter half-life than etorphine and carfentanil, there is less chance for renarcotization, which is particularly important in free-ranging wildlife.[7] Some studies have shown little advantage of thiafentanil as an immobilizing agent over other UPOs in most species of ungulates.[8,9] Its use in zoo and wildlife will likely increase due to the recent removal of carfentanil from production. It is often combined with supplemental drugs to produce balanced anesthesia. A comprehensive review of this drug with dose recommendations is available in zoo and wildlife species.[7]

Carfentanil

Carfentanil was the most potent of the UPOs and, in general, had similar immobilizing properties as etorphine and thiafentanil in ungulates.[1,2,5] It should be noted that as of 2016, carfentanil was no longer available from the manufacturer (Wildlife Pharmaceuticals, Inc.). It was a commonly used UPO in the United States in a variety of zoo and wildlife ungulate species for nearly 30 years.

Etorphine, Thiafentanil, and Carfentanil Potency Comparisons

Carfentanil was considered the most potent of the UPOs in most zoo and wildlife species, followed by thiafentanil and then etorphine. On a mg:mg basis, a rough estimate of clinical equipotency for most captive ungulate species is 1 mg carfentanil to 1.75 mg thiafentanil to 2.0–2.5 mg etorphine.[4] Although somewhat variable in the veterinary literature, etorphine and thiafentanil are estimated to be 6000 times more potent than morphine,[2,10] and carfentanil 10,000 times more potent.[11,12] See Table 27.1 for a review of equipotency data.

Butorphanol

Butorphanol is a mixed opioid agonist-antagonist commonly used in domestic and nondomestic veterinary species for analgesia, sedation, or improved quality of anesthesia.[6,13,14] It is estimated to be four to seven times more potent than morphine but has not been considered to be a UPO. However, it is mentioned here because it is now available in a concentrated solution (50 mg/mL, Wildlife Pharmaceuticals, Inc.) and as a constituent of BAM (Wildlife Pharmaceuticals, Inc.), a commercially available combination of butorphanol, azaperone, and medetomidine, which is reviewed below. A thorough review of the utility of butorphanol in zoo and wildlife species is found in the literature.[13]

Opioid Antagonists

Naloxone is a short-acting opioid antagonist and the drug of choice for reversal of acute opioid intoxication in humans.[14–16] It is commonly found in zoo and wildlife veterinarians' emergency response kit in case of accidental human exposure. Due to its short half-life (30–60

minutes),[16] it is not used to reverse the effects of UPOs in zoo and wildlife, which have considerably longer durations of action. Naltrexone is a long-acting pure opioid antagonist and a relative of naloxone.[6,14] It is the reversal drug of choice for the long-acting UPOs.[7] Nalmefene is another long-acting opioid antagonist that has been studied in captive ungulates but is not commercially available and its half-life is shorter than naltrexone.[6,16,17]

Ultra-Potent Alpha-2 Agonists and Antagonists

α-2 agonists are commonly used in domestic and nondomestic large and small animals to produce sedation, muscle relaxation, and analgesia.[18,19] Medetomidine, dexmedetomidine, detomidine, and xylazine are α-2 agonists used in zoo and wildlife and are often combined with other agents for complete anesthesia. It is important to note that higher doses of α-2 agonists are used in veterinary anesthesia than in human anesthesia due to apparent increased sensitivity in human patients.[20,21] Important α-2 antagonists will be reviewed with emphasis on atipamezole because it has, in general, replaced the others in veterinary anesthesia.

Medetomidine

Medetomidine is considered the most selective of all the α-2 agonists and is currently prepared only in formulations intended for zoo and wildlife species.[6,19] It is available in highly concentrated injectable solutions (20 and 40 mg/mL, Wildlife Pharmaceuticals, Inc.); therefore it may be combined in darts with UPOs and other drugs intended for nondomestic ungulate and megavertebrate species. It has been replaced by dexmedetomidine in domestic dog and cat anesthesia, because medetomidine is currently no longer available in a small animal formulation. Medetomidine is a commonly used supplemental drug combined with ketamine and other injectable anesthetic agents for use in great apes,[22–24] nonhuman primates, and carnivores. It is an important constituent of BAM (Wildlife Pharmaceuticals, Inc.), which is reviewed below. Due to the large doses used in zoo and wildlife species, this drug must be considered dangerous in case of a significant accidental human exposure. Atipamezole is the recommended antagonist for medetomidine in veterinary species.[19]

Dexmedetomidine

Dexmedetomidine is the dextrorotary isomer of medetomidine and is the newest and most commonly used α-2 agonist in small animal anesthesia.[6,18,19] It is also a popular α-2 agonist in human sedation and anesthesia.[25,26] Dexmedetomidine is considered to be twice as potent as medetomidine and is used in combination with other anesthetic agents in smaller zoo and wildlife species. It has limited use in larger animals because it is only available in a low concentration injectable solution. Atipamezole is the recommended antagonist for dexmedetomidine and is routinely used in veterinary species.[6,18,19]

Other Alpha-2 Agonists

Detomidine is an injectable α-2 agonist available and widely used, especially in domestic horses.[19] It does not come in a highly concentrated form like medetomidine, but it is still a very useful drug in zoo and wildlife anesthesia. Xylazine is now available in a highly concentrated injectable solution (300 mg/mL, Wildlife Pharmaceuticals, Inc.), which allows it to be used in darts combined with other drugs for remote delivery. This also adds to the danger to humans at high doses.[27]

BAM

BAM is a combination of butorphanol, azaperone, and medetomidine and is mentioned here due to its high concentration of the α-2 agonist medetomidine. The manufacturers' recommended doses (BAM, package insert, Wildlife Pharmaceuticals, Inc., 2017) and those used in wildlife species pose a danger to humans if accidentally exposed.[28] It is an attractive alternative drug combination providing reversible anesthesia in a variety of species while avoiding the use of potentially dangerous UPOs and easier compliance with drug regulating agencies. According to the manufacturer, BAM contains a combination of 27.3 mg/mL butorphanol, 9.1 mg/mL azaperone, and 10.9 mg/mL medetomidine. The recommended antagonists for BAM in veterinary species are naltrexone for butorphanol and atipamezole for medetomidine.[28] As an upper limit example, the recommended dose for a 350 kg zebra is 6 mL of BAM, which would contain 164 mg butorphanol, 55 mg azaperone, and 65 mg of medetomidine. The resulting syringe or dart has high amounts of potentially dangerous drugs.

Alpha-2 Antagonists

Reversibility of the α-2 agonists offers a great advantage in zoo and wildlife anesthesia by providing quick and smooth recovery, especially in our patients that receive high doses. Atipamezole has the highest selectivity as an α-2 antagonist and is the recommended antagonist for medetomidine and dexmedetomidine.[6,19] It is also capable of reversing detomidine and xylazine. Yohimbine and tolazoline are relatively nonselective antagonists compared with atipamezole. They are both reasonably effective in reversing xylazine in most species but not the newer α-2 agonists such as medetomidine.[6]

Routes and Significance of Accidental Exposure

Zoo and wildlife veterinarians have a risk for occupational exposure to dangerous drugs. Human error or accidents may occur anywhere in the continuum of planning and execution of tasks before, during, and after an anesthetic event involving potent drugs. Mistakes are most likely to occur during preparation, delivery, or retrieval of the dart

TABLE 27.2 Hypothetical Exposures to Dangerous Drugs Used in Zoo and Wildlife Anesthesia

Type of Exposure	Needle Volume* (mL)	Spray Droplet Volume† (mL)	Dose of Etorphine (10 mg/mL) in Volume (mg)	Dose of Thiafentanil (10 mg/mL) in Volume (mg)	Dose of Carfentanil (3 mg/mL) in Volume (mg)	Dose of Medetomidine (40 mg/mL) in Volume (mg)	Is This a Significant Volume of Exposure? Significance Is Expressed as Multiple of Known Harmful Dose of Morphine‡
27 ga × ½ in. needle stick	0.00044§		0.0044 [0.0033]**	0.0044 [0.0033]††	0.00132 [0.002]‡‡	0.018 [0.13]§§	Etorphine, 1.3 times Thiafentanil, 1.3 times Carfentanil, 0.66 times Medetomidine, 0.14 times
22 ga × 1 in. needle stick	0.00343***		0.0343 [0.0033]**	0.0343 [0.0033]††	0.0103 [0.002]‡‡	1.37 [0.13]§§	Etorphine, 10.4 times Thiafentanil, 10.4 times Carfentanil, 5.2 times Medetomidine, 10.5 times
Spray droplet		0.05	0.5 [0.0033]**	0.5 [0.0033]††	0.15 [0.002]‡‡	2.0 [0.13]§§	Etorphine, 152 times Thiafentanil, 152 times Carfentanil, 75 times Medetomidine, 15.4 times

Note: Estimated volumes and mg dose of ultra-potent opioids or medetomidine from a hypothesized needle stick or spray droplet exposure. Clinical significance of the dose is estimated from comparative data in the literature for morphine, ultra-potent opioids, medetomidine, and dexmedetomidine.

*Volume of a cylinder = $\pi r^2 h$

- Refer http://www.math.com/tables/geometry/volumes.htm
- $\pi = 3.14$, r is the radius of needle lumen, h is the length of needle lumen

†Volume of a droplet = 0.05 mL, http://www.endmemo.com/sconvert/milliliterdrop.php.

‡Estimated dose of morphine that causes respiratory depression is 20 mg IV (see Refs. 16 and 42).

§Volume of a 27 ga × ½ in. needle = $\pi r^2 h$

- Refer http://www.sigmaaldrich.com/chemistry/stockroom-reagents/learning-center/technical-library/needle-gauge-chart.html
- $\pi = 3.14$, r is the radius = 0.105 mm, h is length = 12.7 mm
- Therefore: (3.14) (0.105 mm)^2 (12.7 mm) = 0.44 mm^3 or 0.44 µL or 0.00044 mL

**Estimated dose of Etorphine that would cause respiratory depression using morphine equipotency in human (see Table 27.1).

††Estimated dose of Thiafentanil that would cause respiratory depression using morphine equipotency in human (see Table 27.1).

‡‡Estimated dose of Carfentanil that would cause respiratory depression using morphine equipotency in human (see Table 27.1).

§§Estimated dose of Medetomidine, (2 µg/kg, 65 kg human, 0.13 mg dose) that would cause cardiovascular symptoms using dexmedetomidine equipotency in humans (see Refs. 49 and 59).

***Volume of a 22 ga × 1 in. needle = $\pi r^2 h$,

- Refer http://www.sigmaaldrich.com/chemistry/stockroom-reagents/learning-center/technical-library/needle-gauge-chart.html
- $\pi = 3.14$, r is the radius = 0.2065 mm, h is length = 25.4 mm
- Therefore: (3.14) (0.2065)^2 (25.4 mm) = 3.43 mm^3 or 3.43 µL or 0.00343 mL.

due to distractions, carelessness, inexperience, procedural pressures, or challenging environmental conditions.

Routes of Exposure

Accidental injection is the most obvious exposure and must be dealt with as life-threatening. In one survey, needle stick exposures in zoo veterinarians were reported as high as 87%.[29] Significantly, 17.2% of those exposures were while working with immobilizing agents. The most catastrophic type of accidental exposure would be a deep IM injection due to a dart hitting a human. See Tables 27.2 and 27.3 for hypothetical exposure of this type of accidental injection.

Aerosolization of potent anesthetic agents may occur due to spray exposure resulting in direct contact with skin or mucous membranes. Transdermal and transmucosal absorption of certain drugs, such as fentanyl, is well documented and capitalized upon in the development of human and veterinary drug delivery routes. This type of absorption depends on the concentration and amount of drug; duration, location, and surface area of exposure; temperature and integrity of the skin or mucosa; and lipophilicity, molecular weight, and solubility.[30,31] Fentanyl and most of its derivatives are highly lipid soluble and are known to be absorbed across the skin.[2,32,33] Presumably, all of our UPOs would have similar disposition. In human medicine, transdermal and transmucosal fentanyl are used for analgesia

TABLE 27.3 Hypothetical Scenarios With Known Accidental Human Exposure to Potent Anesthetic Agents and Suggested Response With Available Antagonists

Species Information	Anesthetic Induction Protocol	Spray or Needle Stick, Minor Exposure: Antagonists to Consider, Only If Justified	Dart Injection, Significant Exposure: Antagonists to Consider, Only If Justified	Comments
Southern white rhinoceros *(Ceratotherium simum)*, adult, male, 2050 kg, in zoo	ETOR 3.6 mg MED 40 mg BUT 40 mg MIDAZ 30 mg	• NAL 1 mg IN or IM • TREX 50 mg IM • AT 25 mg IM	• NAL 5 mg IM or IN then IV, repeat as needed • TREX 50–100 mg IM • AT 50–100 mg IM, then 0.3 mg/kg IV boluses as needed • FLU 0.5 mg IM	Darts for rhinos, and other large ungulates, are extremely dangerous due to drug combinations (UPO, MED) and amounts used for immobilization
Western lowland gorilla *(Gorilla gorilla)*, adult, male, 200 kg, in zoo	KET 500 mg MED 7 mg MIDAZ 10 mg	• AT 25 mg IM	• AT 50–100 mg IM, then 0.3 mg/kg IV boluses as needed • FLU 0.5 mg IM	The amount KET and MED in a dart exposure would be significant in a human
African elephant *(Loxodonta africana)*, adult, male, 5000 kg, in zoo	ETOR 15 mg MED 15 mg	• NAL 1 mg IN or IM • TREX 50 mg IM or PO • AT 25 mg IM	• NAL 5 mg IM or IN then IV, repeat as needed • TREX 50–100 mg IM • AT 50–100 mg IM, then 0.3 mg/kg IV boluses as needed	Darts for elephants, and other large ungulates, are extremely dangerous due to drug combinations (UPO, MED) and amounts used for immobilization
White-tail deer *(Odocoileus virginianus)*, adult, female, 80 kg, in field location	BAM 2 mL (BUT 54.6 mg, AZAP 18.2 mg, MED 21.8 mg)	• NAL 1 mg IN or IM • TREX 50 mg IM • AT 25 mg IM	• NAL 5 mg IM or IN then IV, repeat as needed • TREX 50–100 mg IM • AT 50–100 mg IM, then 0.3 mg/kg IV boluses as needed	Remote location may magnify incident. There is no antagonist for AZAP so provide supportive care.

AT, Atipamezole; *AZAP*, azaperone; *BUT*, butorphanol; *ETOR*, etorphine; *FLU*, flumazenil; *IM*, intramuscular; *IN*, intranasal; *IV*, intravenous; *KET*, ketamine; *MED*, medetomidine; *MIDAZ*, midazolam; *NAL*, naloxone; *TREX*, naltrexone; *UPO*, ultra-potent opioid.
Note: In these cases, the veterinarian justifiably provides immediate action due to dire, life-threatening circumstances and lack of options. Patient may be exhibiting symptoms or they are expected. Please refer to Figs. 27.1–27.3 for emergency response algorithms. The reader must understand this table is hypothetical, and authors cannot recommend the use of antagonists but provide this information based on evidence to support its consideration in a known emergency.

by skin patch, lozenge, buccal patch, and lollipops.[32,33] In zoo and wildlife species, transmucosal delivery of UPOs for anesthesia and sedation has been reported in the black bear[34] and brown bear[35,36] and UPOs and α-2 agonists in a tapir.[37] Therefore the literature supports transdermal and transmucosal absorption as a predictable method of controlled, and likely accidental, administration of potent opioids and α-2 agonists. See Tables 27.2 and 27.3 for hypothetical exposure of this type of accidental injection.

Significance of an Exposure

Determining what constitutes a clinically significant exposure is difficult, but for safety purposes, we must assume that any accidental exposure should elicit an emergency response. Fortunately, significant exposures appear to be rare and are attributed to redundancy in safety measures, training, and careful attention to detail by the attending veterinarian.[1,29] Minor exposures are more probable but are likely not to be reported in the literature.[2,38]

The clinically significant or lethal dose of the UPOs in humans is unknown, but if we use the estimated analgesic potency of these drugs referenced in the literature,[1,2,38] we can create hypothetical examples. Morphine is considered the gold standard for comparing analgesic potency of opioids and other analgesics.[39–41] The clinically significant dose of intravenous (IV) morphine causing respiratory depression in the opioid naïve 65 kg human is approximately 20 mg.[41,42] Doses higher than this are expected to become more severe and life-threatening. Therefore a clinically equipotent morphine dose for etorphine and thiafentanil (6000 × morphine) would be approximately 3.3 µg, whereas the carfentanil (10,000 × morphine) equipotent dose would be only 2.0 µg. See Table 27.2 for hypothetical examples demonstrating the extremely small volumes of each UPO that may cause respiratory depression in an accidental exposure. It must be restated that the potency of the UPOs presented here originate from referenced extrapolated analgesia data but then provides the only data available to predict the dangers of the UPOs.

Mathematics of Exposures

To better understand the clinical significance of an exposure to a UPO or concentrated medetomidine, a hypothetical example with quantification of the exposure is necessary. Because minor exposures to these drugs are more likely, a situation is presented where a person is accidentally exposed by a needle stick or a minor spray from a mishandled dart or syringe. A more dangerous situation is possible with exposure to larger amounts or a combination of these potent drugs. The consequence of a human exposure to medetomidine will be compared with clinically significant doses of dexmedetomidine used in human anesthesia. This information is reviewed in Tables 27.1–27.3.

Needle Stick Exposure

Needle stick exposures by zoo and wildlife veterinarians are a potential source of accidental injection of dangerous drugs.[29,38,43] To demonstrate, we pose a situation in which a person accidentally sticks himself or herself with a needle with an unpressurized syringe. Only the volume of the drug found in the lumen of the needle is hypothesized to be injected. The lumen volume of a needle is estimated to be the volume of a cylinder, as per the mathematical equation $\pi r^2 h$, where $\pi = 3.14$, r is the radius of the needle's lumen, and h is the needle length. A needle gauge chart is then used to determine the radius and length (http://www.sigmaaldrich.com/chemistry/stockroom-reagents/learning-center/technical-library/needle-gauge-chart.html). Veterinarians commonly use 27-ga × ½ in. (from a TB syringe) and 22-ga × 1 in. needles to withdraw small volumes of concentrated drugs, and these will be used as examples in this hypothetical exposure. Results are found in Tables 27.2 and 27.3 and represent only a minor exposure of a needle stick with the volume found within the lumen.

Spray Exposure

Aerosolization of these drugs with exposure to the skin or mucosa may occur by mishandling a syringe or dart or in the event of an accident. In this example, we pose a hypothetical situation where a person is exposed to a single droplet of a potent anesthetic solution. This may easily be amplified, of course, if exposed to numerous droplets. The volume of a fluid droplet is estimated to be 50 µl, or 0.05 mL (http://www.endmemo.com/sconvert/milliliterdrop.php). Results from this type of exposure by various drugs and proposed clinical significance can be found in Tables 27.2 and 27.3.

Medical Management for Accidental Veterinary Anesthetic Exposure

Agent-Specific Resuscitation Protocols

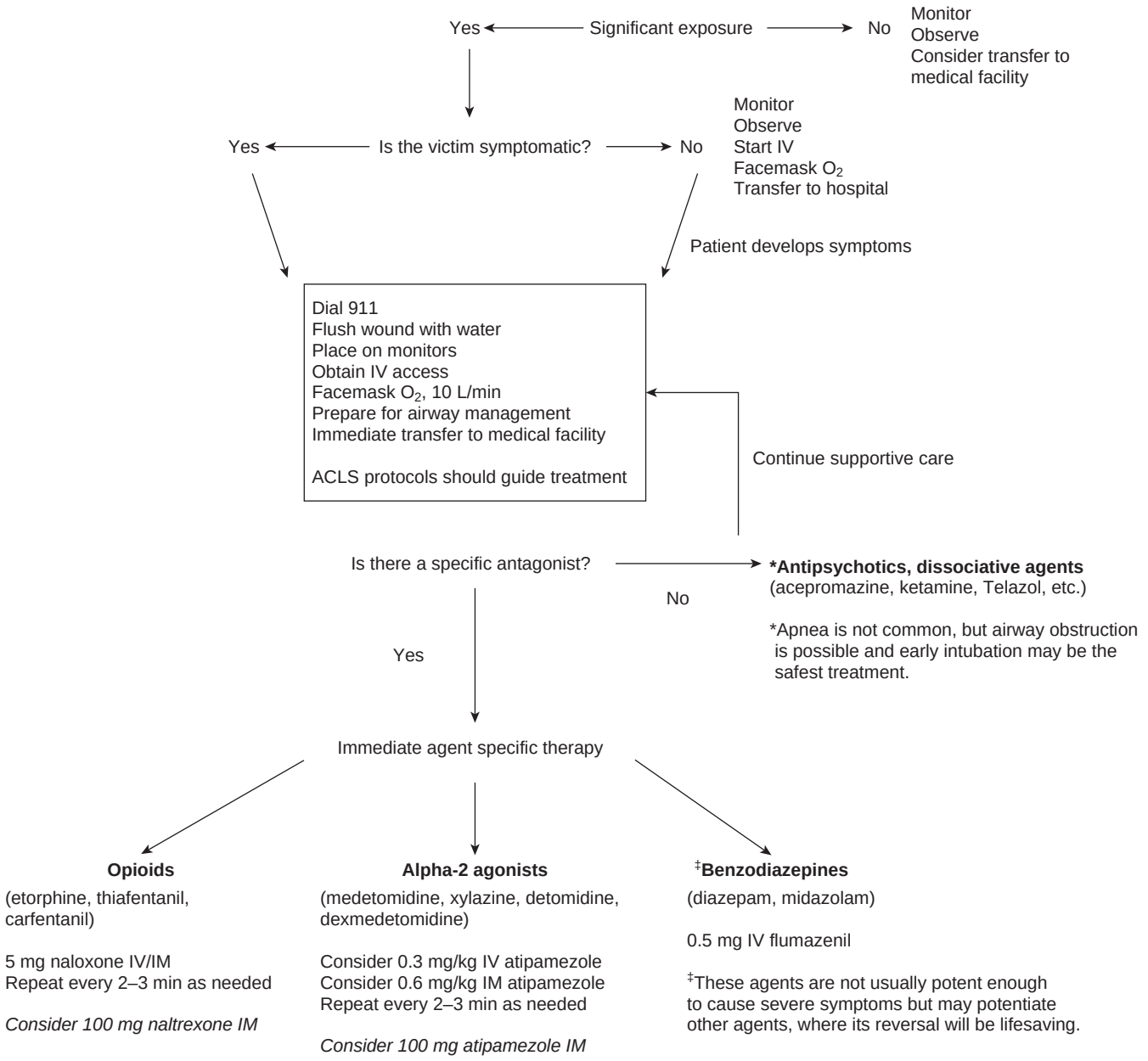
After initial evaluation of the victim, the next steps of the resuscitation will depend on which agents are involved (Fig. 27.1). If the exposure agent has no antagonist (tranquilizers, dissociative agents), then care of the victim will be supportive using the principles of Basic Life Support (BLS)[54] until medical transport arrives. If the anesthetic agent has a pharmacologic antagonist and one is available, the next immediate steps will be to consider administering the antagonist (see Fig. 27.1) and securing IV access. For victims exposed to a dangerous drug in a remote area and far from medical care, a rapid response will be needed to prevent a fatality. For benzodiazepines, flumazenil will be helpful in most cases. In the next few sections, specific medical response to individual agents will be addressed.

Ultra-Potent Opioids: Etorphine, Carfentanil, and Thiafentanil

Due to extremely high potency, needle stick accidents containing etorphine, and presumably the other UPOs, have resulted in respiratory arrest.[38] As a safety measure, strict protocols are in place for veterinarians who handle these dangerous drugs.[1,3,4,55] However, despite careful handling practices, accidents do occur, and thus an UPO exposure treatment protocol is necessary (see Fig. 27.2). Having an opioid reversal kit that includes airway management equipment, IV placement kit, and enough naloxone for a 100-kg patient may be lifesaving.[3]

When exposure of a human to a UPO occurs, the first step is to notify other personnel and activate the EMS by dialing "911" or the appropriate local emergency alert system. Of all the dangerous anesthetic agents, UPO exposure has the potential to cause a fatality the fastest and with only a seemingly minor exposure dose. An opioid antagonist is the antidote to reverse the central nervous system and respiratory depression effects. If time allows, attach monitors and obtain IV access. The patient should not be left unattended while waiting for EMS to arrive. If the patient is already unconscious, consider immediate administration of an opioid antagonist. If the victim is not breathing, open the airway and begin rescue breathing with a bag and mask or using mouth-to-mouth ventilation.

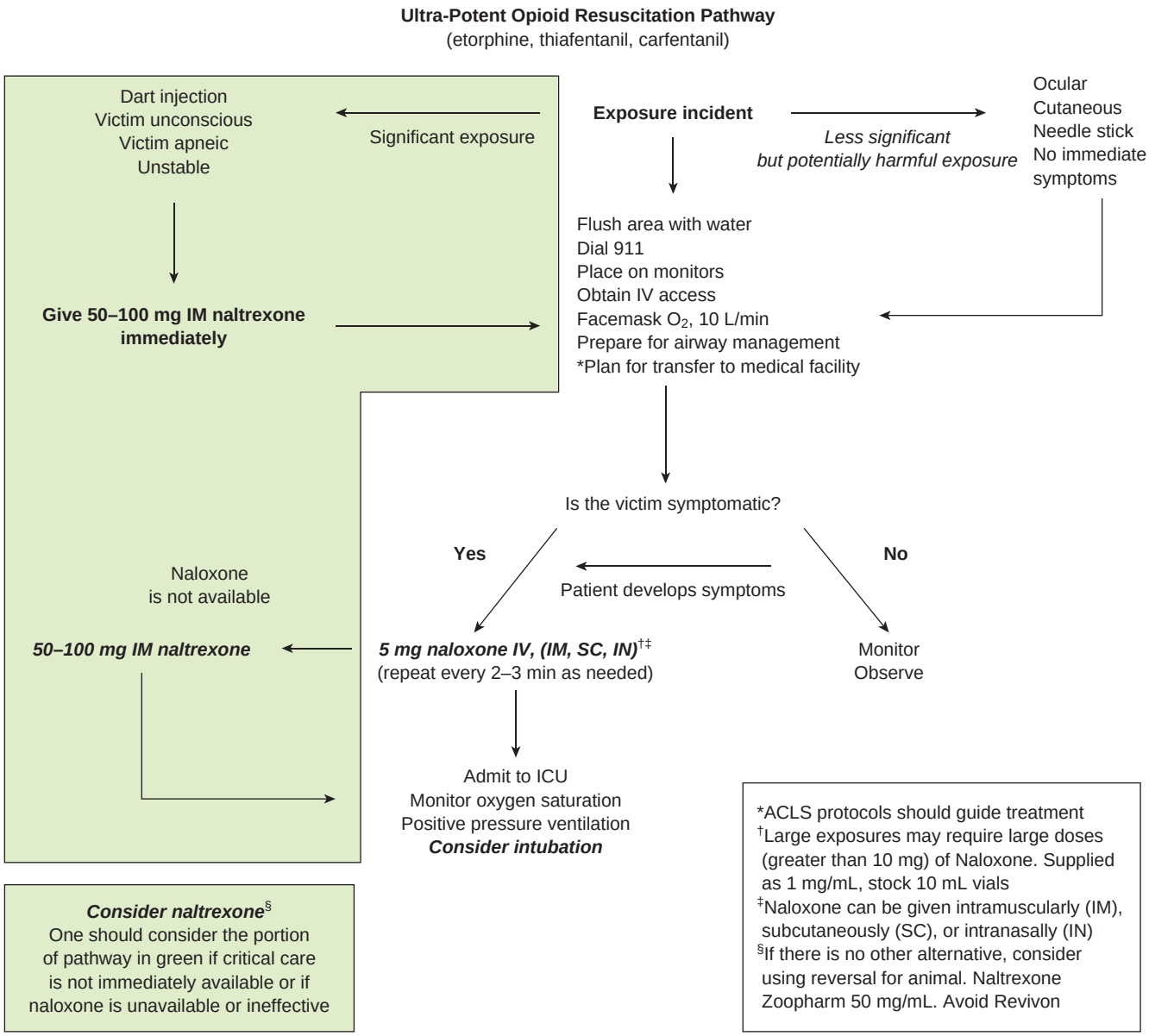
With UPO exposure, respiratory depression leading to apnea will be the principal clinical symptom, with the effects resulting in hypoxia and death. The primary resuscitative measure for UPO overdose is administration of an opioid antagonist. Naloxone is a competitive mu opioid–receptor antagonist that reverses all signs of opioid intoxication. It is typically supplied as a 0.4 mg/mL or a 10 mL multidose vial of 1 mg/mL formulation and can be given IM, IV, subcutaneously (SC), intranasally (IN), or intratracheally (IT).[32,41] The initial dose of naloxone for a victim who is symptomatic should be a 5 mg naloxone IV push.[56] If there is no IV in place, then naloxone should be given IM. For a significant UPO exposure, the effective dose may need to be much higher and repeated every 2–3 minutes, depending on the clinical picture.[38] The onset of action of naloxone is less than 2 minutes when administered intravenously.[44] The effective duration of action is only 20–30 minutes, which may be much shorter than that of a UPO. Thus, after



• **Figure 27.1** Algorithm for management of accidental exposure to anesthetic agents. *ACLS*, Advanced Cardiac Life Support; *CNS*, central nervous system; *IM*, intramuscular; *IV*, intravenous.

stabilization, intensive patient monitoring will be needed for all significant UPO exposures watching for re-narcotization. An algorithm for UPO accidental exposure and overdose is presented in Fig. 27.2. In critical cases, a significant concern will be the availability of sufficient opioid antagonist.

Naltrexone is a long-acting opioid antagonist and another potential option for UPO exposures. It is routinely used in zoo and wildlife anesthesia to reverse UPO during immobilization of megavertebrates and other ungulate species. Naltrexone is used in human medicine primarily as an oral agent to treat opioid addiction or for IM use in depot form with monthly injections.[46] It is not currently available for emergency parenteral use in humans. It has been studied in humans as part of EMBEDA (morphine sulfate and naltrexone hydrochloride, extended-release capsules, Pfizer, Inc., New York), as a failsafe to prevent opioid abuse.[57] Naltrexone has opioid antagonist effects when given orally and has been used IM in humans in emergency situations.[38] If an accident occurs in a remote area, it is unlikely naloxone will be available in sufficient quantities to antagonize a long acting UPO. In a reported case of carfentanil overdose, up to 50 mg of naltrexone was necessary to keep the patient breathing.[38] Therefore naltrexone 50–100 mg IM, which should be available for reversal of the anesthesia for the animal, would be expected to reverse significant opioid toxicity. **Due to its proven safety in humans, 50–100 mg IM naltrexone should be strongly considered in emergency situations where other therapy**

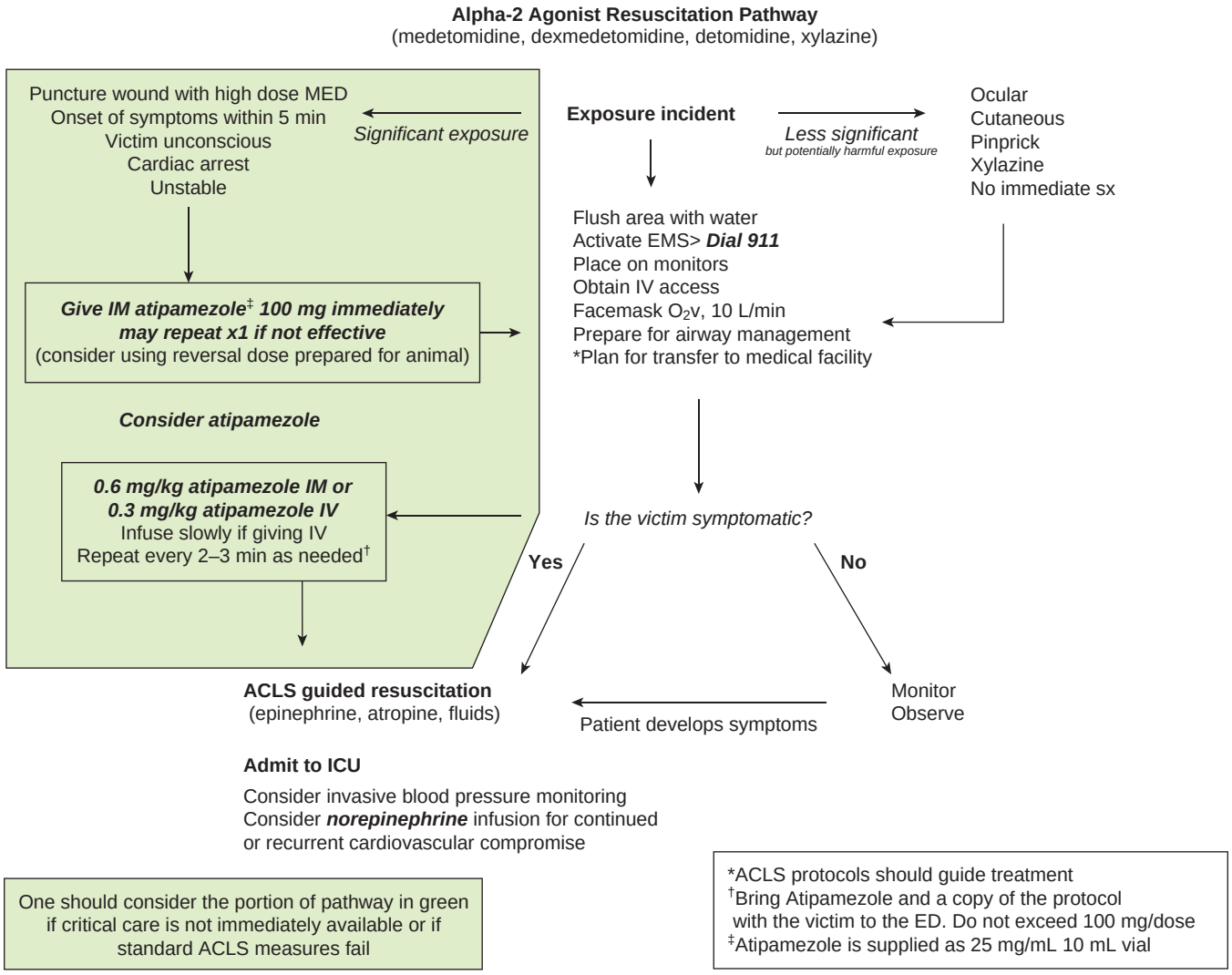


• **Figure 27.2** Opioid resuscitation pathway. *ACLS*, Advanced Cardiac Life Support; *ICU*, intensive care unit; *IM*, intramuscular; *IN*, intranasal; *IV*, intravenous; *SC*, subcutaneous.

is ineffective or not available. Due to its long duration of action, re-narcotization following naltrexone use in animals is uncommon, making naltrexone a potentially attractive option in a UPO emergency. Naltrexone, like naloxone, will cause opioid withdrawal in opioid-dependent patients.[46,58] It should be noted that diprenorphine ([Revivon, VetaPharma Ltd., Leeds, UK], a reversal agent that comes packaged with some formulations of etorphine; Immobilon, VetaPharma, Ltd.) should not be given to a human as an antagonist due to further depression of the patient.[38] Diprenorphine has agonist-antagonist properties, which may be responsible for its lack of efficacy in UPO overdose. Thus, when working with any UPO, we recommend having an adequate supply of naloxone or parenteral naltrexone available for emergency resuscitation.

Alpha-2 Agonists: Medetomidine and Dexmedetomidine

Medetomidine, a highly selective α-2 agonist, is currently available in a highly concentrated form (40 mg/mL, Wildlife Pharmaceuticals, Inc.) for use in zoo and wildlife species. In comparison, dexmedetomidine for human use (Precedex, Pfizer, Inc.) is available in a less potent formulation of 100 mcg/mL. Medetomidine is approximately 50% as potent as dexmedetomidine.[6,18,19] Therefore, in comparison with human dexmedetomidine, this veterinary formulation of medetomidine is approximately 20,000 times more concentrated on a per mL basis. In one report, dexmedetomidine was found to cause cardiac arrest at doses of 1–2 mcg/kg in humans.[59] IM dexmedetomidine at 2.5 mcg/kg



• **Figure 27.3** α-2 agonist resuscitation pathway. *ACLS*, Advanced Cardiac Life Support; *ICU*, intensive care unit; *IM*, intramuscular; *IV*, intravenous; *MED*, medetomidine.

provided sedation in human trial and was reversed with atipamezole.[49] Medetomidine would be expected to have sedative hypnotic effects in humans in quantities as small as 100 mcg, which is only 20 µL.[49] In contrast to exposure with an UPO that primarily causes respiratory depression and apnea, α-2 agonist overdose is likely to result in severe bradycardia.[60,61] In these cases, at least initially, respiratory drive may still be preserved; thus providing airway support alone will not likely be sufficient to prevent death. Cases of accidental and nonaccidental exposure have been reported, some with serious consequences.[2] For practitioners of zoo and wildlife medicine, accidental medetomidine overdose is a major concern.[3,38]

Treatment for accidental α-2 agonist overdose (clonidine, dexmedetomidine) in humans is generally supportive. Treatment with naloxone and atropine has been tried, with inconsistent results.[62] Currently therapy consists of supporting the victim with adrenergic agonists, activated charcoal, fluids, and treating respiratory failure with endotracheal intubation and mechanical ventilation.[63] Yohimbine and tolazoline have also been used for α-2 agonist overdose, but tolazoline is a nonspecific α-inhibitor, and there is no parenteral preparation of yohimbine available for human use.[64,65]

There is no FDA-approved α-2 specific antagonist available for parenteral use in humans. However, atipamezole, a highly specific α-2 antagonist, is readily available for veterinary use and is common in wildlife anesthesia. During an immobilization with medetomidine, or other α-2 agonist, atipamezole usually would be available and prepared for use in the veterinary patient. Atipamezole has been tested in dexmedetomidine sedated humans and was found to be both safe and effective.[49,66,67] Atipamezole, 100 mg IV, has been administered to anesthetized humans with minimal side effects.[66,67] It readily reverses dexmedetomidine sedation in humans when administered intravenously in greater than a 40:1 ratio.[49] It also increases norepinephrine levels, in both anesthetized and dexmedetomidine-sedated humans. Using published data, it appears that humans require approximately 10 times less α-2 agonist such as

dexmedetomidine for deep sedation, but require 10 times more atipamezole for adequate reversal when compared with veterinary species.[20] Although atipamezole is not FDA approved for use in humans, dose recommendations for emergency atipamezole use in humans do exist.[20,21] (See Fig. 27.3 for a resuscitation algorithm in the event of a significant α-2 agonist human exposure.)

Using the data from human trials and anesthetic experience in primates and other animals, the α-2 agonist resuscitation pathway in Fig. 27.3 was developed. If the victim is stable, no immediate antagonist would be given. The rescue team should monitor the victim for signs of shock and prepare for transport. However, after a significant medetomidine exposure, especially in a remote area without medical support, the rescuers should consider the use of atipamezole as shown in Fig. 27.3. **If the victim is unstable, 100 mg atipamezole IM would be reasonable. If that is not effective, it should be repeated. If the patient was initially stable and then begins to develop symptoms, the rescuers should consider giving atipamezole 0.6 mg/kg IM or 0.3 mg/kg slow IV push**. Because the dose of the α-2 agonist in an accidental exposure will not be precisely known, it seems more logical to administer atipamezole using a mg/kg weight-based dose instead of a ratio of atipamezole: α-2 agonist (see Fig. 27.3). As with UPO, it is likely the reversal agent for the animal being immobilized will be ready for use before the start of the procedure. If there is a catastrophic medetomidine exposure and no other option is available, it is reasonable to consider using the animals' reversal dose in the human victim.

If the victim develops cardiac arrest, or the cardiac rhythm becomes nonperfusing, the rescuers should immediately start chest compressions. Adequate depth of compressions during cardiopulmonary resuscitation (CPR) will be essential to maintain perfusion to vital organs.[8] If available, atipamezole should be administered.[20,21] Prompt administration of the reversal agent in this situation may reverse the cardiac arrest. Once at the hospital, ongoing care will be dictated by Advanced Cardiac Life Support (ACLS) protocols.[68] The resuscitating physicians should consider implementing a norepinephrine infusion for severe shock, as α-2 agonists lower norepinephrine levels.[20,66,67] It will be important for a member of the veterinary team to accompany the patient with sufficient atipamezole and to bring the protocol in Fig. 27.3 along with the victim to the hospital for the benefit of the emergency room physicians. Human hospitals will **not** have atipamezole, and most emergency medical staff will have no knowledge of the use of α-2 specific antagonists and are not experienced in the treatment of severe medetomidine overdose.

Exposure to a Combination of Potent Anesthetic Drugs

Accidental human exposure to a combination of drugs may significantly impact the victim. Combining a UPO with midazolam and/or medetomidine may have a multiplicative effect on depression of cardiorespiratory function. From a practical perspective, if there is an antagonist for any of the agents, it is most important to administer it quickly. An example would be if a human is exposed to high doses of concentrated midazolam, the rescue team should consider administering flumazenil up to 500 mcg (0.5 mg) IV or IM as part of the resuscitation.[68] Similarly, BAM is a combination anesthetic that also could be very dangerous in an accidental human exposure due to high doses of butorphanol and medetomidine. Atipamezole and naltrexone should be prepared as an antidote and immediately injected IM into the victim. If administering the antagonist for one of the agents doesn't result in complete resolution of symptoms, medical support of the victim may be required.

Controversies in the Use of Antagonists in Human Exposure to Dangerous Drugs

A professional dilemma exists when presented with a medical emergency involving a person with a known exposure to UPO or α-2 agonists. Veterinarians are concerned about treating a human without a license. Emergency department physicians in the United States do not have approval from federal regulating agencies, such as the US Food and Drug Administration (FDA), to provide a patient with possibly the most pharmacologically appropriate antidote. During such an emergency, the gray, unclear boundaries of malpractice, ethics, and law must be considered. Legal concerns are reviewed in the next section. The authors are careful not to go beyond the scope of our training by making overconfident or unsubstantiated statements. Rather, we provide a review of the most current literature on the use of naltrexone and atipamezole in humans as an emergency treatment option based on scientific evidence, logic, and experience. Inevitably, it will be the decision of the attending healthcare professional on how to proceed, given the circumstance. It should be noted that the use of any antagonist is not a substitute for seeking emergency medical care for a person exposed to a dangerous anesthetic agent.

Naltrexone Use in Humans

Naltrexone and naloxone are pure opioid antagonists safe for use in humans. Naloxone has a short half-life of approximately 60 minutes and is available in many forms of administration (IV, IM, and nasal spray).[16,44,45] It is often referenced in zoo and wildlife literature as the emergency antagonist of choice for UPO exposure.[1–4] Because the short-acting naloxone does not pharmacologically match the long duration of action of UPOs, multiple doses may be required in clinically significant exposures. Naltrexone is a close relative of naloxone but has a significantly longer duration of action.[2,15,16,44,46] It is available for humans in tablet and extended-release injectable suspension forms and is used primarily to manage chronic conditions such as alcohol and opioid dependence. The veterinary formulation is an injectable solution (50 mg/mL, Naltrexone HCl, Trexonil,

Wildlife Pharmaceuticals, Inc.) and is the reversal agent of choice for UPO anesthetized species, because its pharmacology better matches the long duration of the UPO.[2–4]

As mentioned, naloxone is often referenced for use in case of an emergency UPO exposure. Due to the current human opioid overdose dilemma, some state health officials are even allowing nonmedical professionals in prehospital locations to administer naloxone because it has been used to safely reverse over 10,000 opioid-related overdoses in the United States.[45,47] Recommendations for the use of naltrexone in a UPO exposure have been made in the zoo and wildlife literature.[3,4] These authors suggest providing 25–50 mg IM followed by 25–50 mg IV, with the likelihood of naltrexone side effects being minimal. Because there are currently no injectable solutions available for humans, the veterinary formulation would need to be used. It should be noted that the injectable veterinary formulation of naltrexone follows the same US Pharmacopeia (USP) reference standards enforced by the FDA to ensure the identity, strength, sterility, quality, and purity of human medicines (Bill Lance, Wildlife Pharmaceuticals, Inc., personal communication). There is no reference in the literature of the use of oral naltrexone to treat a human exposed to an UPO. This may be considered only in a coherent, conscious patient with the ability to swallow a tablet. The slower onset of action of the oral antagonist would need to be considered. A possible scenario might include a minor or questionable exposure where the injectable product was not available, refused by the patient, or considered unnecessary given the circumstance. The authors agree that both naltrexone and naloxone should be considered in the case of an emergency UPO exposure. See Fig. 27.2 for the resuscitation algorithm for human exposure to a UPO.

Atipamezole Use in Humans

The use of atipamezole in the emergency response to an accidental exposure to the ultra-potent concentrated form of medetomidine used in zoo and wildlife has not been thoroughly reviewed until recently. The safety and efficacy of atipamezole in humans has been investigated[20,21,48,49] and is discussed further later in this chapter. It should be noted that atipamezole is currently not available for use in humans, internationally. In comparison with veterinary species, the human is more sensitive on a mg/kg basis to the α-2 agonist effects of dexmedetomidine and presumably to its close relative, medetomidine.[20,21] Furthermore, humans appear to be less sensitive to the effects of atipamezole when used as an α-2 antagonist for dexmedetomidine, and this would require higher doses than in veterinary species. In domestic and nondomestic animals, atipamezole is recommended at a 5:1 ratio to the mg dose of medetomidine and 10:1 ratio to dexmedetomidine.[6,18,19] It is significant to note that atipamezole is routinely and safely used to reverse medetomidine and dexmedetomidine for anesthetic procedures in the gorilla, chimpanzee, and orangutan—our closest human relatives.[22,24,50]

Atipamezole has recently been recommended in case of an accidental exposure to these potent α-2 agonists in humans[20,21] and is further reviewed later in this chapter. See Fig. 27.3 for the recommended emergency response to an exposure of potent α-2 agonists.

Legal Concerns

It is beyond the scope of this chapter to review and interpret the laws pertaining to the actions of veterinarians or emergency care personnel responding to a catastrophic human exposure to a dangerous anesthetic agent used in zoo and wildlife. The dilemma for both professions is the legal ramifications if we choose to intervene responsibly. Fortunately, this type of incident is rare, but it behooves us to understand the moral, ethical, and legal principles of the law which are in place to protect us in case we are presented with this type of unfortunate incident. Two important terms found in the legal literature are germane when considering the risks involved in intervening in a human medical emergency: Good Samaritan and Duty to Rescue.

Good Samaritan Law

This law states that citizens should not be discouraged from helping others at a fundamental level commensurate with their expertise by fear of liability.[51–53] All 50 states and the District of Columbia in the United States have some type of Good Samaritan law, but they vary by jurisdiction who is protected from liability and under what circumstances. This is especially true if your action is reasonable, without deception and commensurate with your training, knowledge, and ability. Prior to engaging in a compassionate act, the cautious veterinarian would assess the situation, determine if he or she would competently be able to provide some assistance without doing further damage, and then do what he or she can to help. If the patient is unconscious, delusional, intoxicated, or in imminent peril, there is implied consent if the assistance is reasonable and not negligent. It appears doubtful that a court would consider a compassionate veterinarian illegally practicing human medicine for reasonably tending to a catastrophic exposure of a drug we know is extremely dangerous to humans.

Duty to Rescue

In general, there is no law in the United States that obligates you to aid someone who is in danger, but there are special circumstances that may impose a moral or ethical duty upon you to rescue.[51,52] An example may include a situation in which you have a unique relationship and perceived obligation with the person in danger or if your negligence or action caused the need for rescuing. Legal definitions and responsibilities for duty to rescue may vary by state and municipality. If an accident occurs, you may have a duty to rescue at some level due to your leadership role as veterinarian, knowledge of the drugs and their effects, and ability to provide competent assistance due to medical training.

Summary

The authors endorse the use of naltrexone and/or atipamezole at suggested doses as an immediate response to significant exposure to a UPO or concentrated medetomidine, respectively, in a situation where critical care is not immediately available. Extensive review of the medical literature and the human medical experience of one of the authors (MG) validate the emergency response algorithms found in this chapter. Veterinarians and anesthesia team participants handling these dangerous drugs, along with human emergency response team members, should be aware of these potentially lifesaving recommendations.

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28

Vaporizers and Field Anesthesia Equipment for Free-Ranging Wildlife

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Introduction

Performing general anesthesia in the field may be technically challenging when equipment designed for a single location (e.g., hospital use) must be modified for use in remote locations with limited access to electrical power and oxygen. There is the additional challenge of having sufficient supplies transported to and stored at a field site.[1] Capture- and anesthesia-related morbidity and mortality may occur with field immobilizations, regardless of anesthetic protocols.[2,3] In general, the two broad categories of anesthetic agents are inhaled or injectable agents. Because field anesthesia needs to be simple, safe, and easily mobile, injectable anesthesia is often used instead of inhalant anesthesia for logistical reasons.[1] Use of volatile inhalant anesthesia in the field is a tradeoff between the rapid induction and recovery associated with inhalants and the logistical difficulty of moving compressed gas cylinders and vaporizers into the field. Injectable only protocols lack some of the ability for "fine-tuning" by means of incremental adjustments in depth. In addition, there is the possibility of prolonged drug effect and renarcotization. This chapter focuses on the use of inhalant anesthesia, with descriptions of the types of vaporizers and novel delivery systems and ventilators that may be adapted to the challenges of the field setting.

Inhalant Anesthesia

Inhalant anesthetics are widely used in a clinical setting and possess unique advantages and disadvantages for use in a field setting. With careful attention to the principles of inhalant anesthetic pharmacology and appropriate anesthetic equipment use, wildlife veterinarians have successfully used inhalants in a wide variety of species in a multitude of natural settings. Use of inhalants for avian, marine mammal, and small rodent anesthesia is well described in the wildlife medicine literature.[4–9] Unlike injectable anesthetics, inhalants are administered and eliminated via the respiratory system. This allows for a rapid and precise adjustment of the anesthetic depth of the patient.[10] Because many of these drugs are readily expelled from the body during the course of recovery, there is minimal potential for prolonged drug residues.[11] Disadvantages include the need to transport volatile fluids, expense and bulk of the vaporizers, and logistical concerns of transporting compressed gases.[12,13] In some cases, inhalant anesthesia may be chosen to avoid having to use tightly regulated controlled substances; this is especially true crossing international boundaries.

Basic Inhalant Pharmacology

A set of basic definitions necessary for understanding inhalant anesthetic and vaporizer use is provided in Box 28.1. With the exception of nitrous oxide, all commonly used inhalant anesthetics are a vapor at room temperature and not truly a gas. A vapor is the gaseous state of a substance that is a liquid at ambient temperature and pressure. A gas may be delivered at a concentration between 0% and 100%, whereas a vapor has a maximum concentration that is determined by its vapor pressure.[10] The amount of an inhalant anesthetic in a mixture may be expressed as a volume percent or a partial pressure. When administering a vaporized inhalant anesthetic, the end goal is to achieve a partial pressure of anesthetic in the brain and spinal cord that results in anesthesia.[14] A series of concentration gradients needs to be established to allow movement of anesthetic from the vaporizer, through the circuit to the lung, and then from the alveoli to the blood and then the central nervous system. Multiple factors may affect the rate of equilibration between the vaporizer and the brain, including vaporizer setting, fresh gas flow rate, inspired anesthetic concentration, circuit volume, alveolar ventilation, cardiac output, and solubility of the inhalant.[10,14]

The commercially available inhalant anesthetics differ in terms of potency, solubility, and vapor pressure. Isoflurane and sevoflurane are the two most commonly used inhalant agents in field veterinary medicine. Isoflurane has a higher blood solubility compared with sevoflurane, owing to a higher blood to gas partition coefficient. This higher solubility translates to a slower induction and recovery in

most species. Isoflurane is also more potent than sevoflurane, meaning that a lower partial pressure of isoflurane in the brain is necessary for anesthesia. Potency is typically described in terms of minimum alveolar concentration (MAC; see Box 28.1). The commonly used inhalation anesthetics in veterinary medicine are described in Table 28.1. A volatile anesthetic allowed to vaporize in a closed container (i.e., the vaporizer) will reach a concentration proportional to its saturated vapor pressure. For example, isoflurane has a saturated vapor pressure of 240 mm Hg. So, if liquid isoflurane is allowed to vaporize at atmospheric pressure (760 mm Hg) in a closed container, the saturated vapor concentration of isoflurane in the container will be 240/760 × 100, or 32%. In contrast, sevoflurane has a saturated vapor pressure of 160 mm Hg and thus reaches a maximum concentration of 21% (160/760 × 100 = 21). This is important to understand for open drop anesthesia and if a vaporizer is tipped, as described later.

Most anesthetics have a saturated vapor concentration well above therapeutic levels and, if they were allowed to vaporize freely and be breathed by the patient, could be lethal. For this reason the preferred way of delivering inhalant gas anesthetic is by using a precision, agent-specific vaporizer and compressed oxygen.

• BOX 28.1 Terminology, Abbreviations, and Units of Measure Used to Describe Vaporizers and Inhalant Anesthetics

Vapor pressure: Partial pressure of a vapor over the liquid. Vapor pressure is dependent on temperature; at higher temperatures, a liquid will have a higher vapor pressure.

Saturated vapor pressure: The maximum vapor pressure in a closed container at equilibrium. At this point, for every molecule that enters the gaseous phase from the liquid, one molecule enters the liquid phase. Like vapor pressure, saturated vapor pressure is temperature dependent.

Delivered anesthetic concentration: The concentration of the anesthetic vapor in a mixture of gases. Concentration is expressed as a volume percentage (%). The concentration is dependent on the partial pressure of the vapor or gas being described and the other gases in the mixture.

Blood to gas partition coefficient: A partition coefficient is the ratio of the concentration of a gas in two separate media at equilibrium and is a measure of solubility in dissimilar fluids. The blood to gas partition coefficient for an inhalant is the ratio of the concentration of the inhalant in the blood versus in the gas phase. Drugs with a higher blood to gas coefficient are more soluble in blood and thus have slower induction and recovery times.

Potency: Potency of inhalant anesthetics is typically defined in terms of MAC. The term "minimum anesthetic concentration" is used for those species without alveoli. MAC may be used to compare anesthetics with one another.

Latent heat of vaporization: Calories needed to change 1 g of liquid to vapor. As a liquid vaporizes, the remaining liquid will cool due to energy lost in the vaporization process. This is the reason that an anesthetic liquid will cool during vaporization.

Specific heat: Heat needed to raise 1 g of material 1°C. Copper in a vaporizer has a high specific heat, so the temperature change is minimal during vaporization.

Units of measure for pressure and their conversion: 100 kPa = 1000 mbar = 1 bar = 760 mm Hg = 1030 cm H_2O = 14.7 psi = 1 atm.

MAC, Minimum alveolar concentration.

Vaporizers

Most commonly used inhalant anesthetics are delivered by a vaporizer with some fresh gas source. The only common exception is nitrous oxide, which is regulated by flowmeter alone. Most modern vaporizers are variable bypass, concentration calibrated, and temperature and flow compensated. These features are designed to reduce the chance of user error. Nonprecision (uncalibrated) vaporizers are rarely in use in veterinary medicine but are, on occasion, used in field settings.[15] As mentioned previously, delivered amounts of inhalant anesthetics may be quantified either by partial pressure or concentration in volume percentage. Most clinicians are more familiar with delivered concentrations as determined by the percentage setting on the vaporizer. For example, a precision isoflurane vaporizer set to 3% should deliver 3% isoflurane in 97% carrier gas (typically oxygen).

To understand the function and use of vaporizers in the field, it is essential to understand a certain set of terminology (see Box 28.1). Because the saturated vapor concentration of an anesthetic is always well above the clinically useful volume percentage used, it is necessary to dilute the inhalant vapor with fresh gas (oxygen, a combination of oxygen and nitrous oxide, or room air). For example, the saturated vapor concentration of isoflurane at 20°C and 1 atm is

TABLE 28.1 Selected Properties of Commonly Used Inhalant Anesthetics

Anesthetic	Vapor Pressure (mm Hg) @ 20°C	Vapor Pressure (mm Hg) @ 24°C	Blood to Gas Partition Coefficient	MAC (in Dogs, %)
Isoflurane	240	286	1.4	1.3
Sevoflurane	160	183	0.68	2.36
Desflurane	700	804	0.42	7.2–9
Methoxyflurane	23	28	12	0.29

32%, which is likely lethal to all vertebrate species. The vaporizer functions to dilute the 32% isoflurane vapor with oxygen to achieve a clinically useful percentage (0%–5%).

Most vaporizers in clinical use for both veterinary and human medicine are precision, out-of-circuit vaporizers. These vaporizers are agent-specific, concentration-calibrated machines that provide a regulated volume percentage of inhalant anesthetic.[16] Typically, they function by a variable bypass mechanism that allows a certain portion of the carrier gas (typically oxygen) to flow over a pool of liquid anesthetic until it vaporizes to saturation. The remainder of the carrier gas passes through a bypass chamber, and the two paths are mixed to achieve the desired concentration set on the vaporizer dial. If a vaporizer is tipped over (typically greater than 45 degrees), liquid anesthetic will flood the bypass chamber and the vaporizer will release an incredibly high concentration of anesthetic, at or near its saturated vapor concentration (e.g., 32% for isoflurane). Most modern vaporizers are temperature, flow, and back pressure compensated, although these factors are rarely considered in controlled hospital use. These compensation mechanisms have limits, and it is important to remember that the vaporizers are only temperature compensated for a range of 15°C–35°C such that the amount of vaporized anesthetic will be decreased at colder temperatures and increased at higher temperatures.[16] Similarly, regarding flow compensation, most modern vaporizers are accurate between 0.25 and 15 L/min of fresh gas flow.[12]

One notable exception to the typical vaporizer design is the desflurane vaporizer. Desflurane is highly volatile and boils at room temperature. Due to its high latent heat of vaporization, desflurane rapidly cools during vaporization, and this would overwhelm the insulating capacity of a regular vaporizer, so the desflurane vaporizer needs to be thermostatically controlled to keep it at 39°C. In addition, because desflurane vaporizes so extensively, it would need an infeasibly high fresh gas flow rate in a traditional vaporizer. Instead, no fresh gas goes into the desflurane sump, and it releases pure desflurane vapor into the mixture, which is then diluted with oxygen.[12] The need for electrical power limits the use of desflurane in the field.

Nonprecision, in-circuit vaporizers are no longer in common use in veterinary medicine, but they do still occasionally find a place in field anesthesia. The units are small and light and have low resistance to breathing.[15] These devices may be used with low-potency anesthetics with a low vapor pressure. It should be noted that the delivered percentage cannot be precisely controlled with a dial, so use with currently available inhalant anesthetics may lead to a fatal overdose. The delivered concentration of anesthetic gas may vary with patient ventilation and fresh gas flow rate; thus the potential for user error is high. The only way to measure delivered inhalant concentrations is by using a gas analyzer, which is rarely feasible in a field setting. Another, less traditional, mechanical vaporizer uses a syringe of liquid anesthetic delivered in a precise amount to achieve a desired concentration when mixed with pumped ambient air. It is available for laboratory animal use and has been modified for field use.[17]

Inhalant anesthetics delivered by an "open drop" method, in which the anesthetic is applied to a cotton ball or gauze and allowed to spontaneously vaporize in a closed container, have been used extensively in rodent anesthesia.[18] As mentioned previously, anesthetics allowed to vaporize in this fashion will reach a concentration dictated by their vapor pressure. Isoflurane will reach a maximum concentration of 32%, whereas sevoflurane will reach a maximum concentration of 21%. Open drop delivery results in extremely high concentrations of volatile anesthetic, in many cases far exceeding lethal doses. This method should be used only by experienced personnel, with a previously measured container volume and calculated amounts of anesthetic. In addition, the open drop method should be used only for brief induction, not continued maintenance, of anesthesia and reserved for situations when transport of the compressed oxygen and a vaporizer are impossible.[1]

Gas Anesthesia at Altitude

Changes in barometric pressure may affect the output of a precision vaporizer. This issue is rarely encountered in the hospital setting but may be common in field settings. This is due to the changing ratio of ambient pressure and anesthetic vapor pressure that depends on altitude. Vaporizers are calibrated at 20°C at sea level (1 atm or 760 mm Hg barometric pressure). At a higher altitude, ambient pressure is less than 1 atm, but the vapor pressure (in mm Hg) of the anesthetic does not change; thus delivered concentration for a given vaporizer setting will increase.[10] For example, at 760 mm Hg, the saturated vapor concentration of sevoflurane is 21% (equation earlier). If the same drug vaporizes at an ambient pressure of 632 mm Hg (5000 ft elevation), the saturated vapor concentration is 25% ($160/632 \times 100 = 25$). Thus a sevoflurane vaporizer set at 5% will deliver a slightly higher concentration when used at 632 mm Hg than at 760 mm Hg.

This is made more confusing by the fact that the effect of the anesthetic is still determined by potency, as expressed in terms of MAC. MAC as a partial pressure does not change with altitude, but MAC expressed as a volume percentage does change. At higher altitude, the vaporizer will put out a higher volume percentage but the same partial pressure. So, although the delivered percentage might be higher, the animal's MAC expressed as a volume percentage increases proportionally and the effect of a given vaporizer setting will be similar at different elevations.[10] Changes in ambient pressure may also affect the accuracy of a flowmeter that is calibrated at sea level.

Anesthesia Machines

The vaporizer is just one component needed for volatile anesthetic administration. The anesthetic circuit requires oxygen or another compressed carrier gas, a pressure regulator with

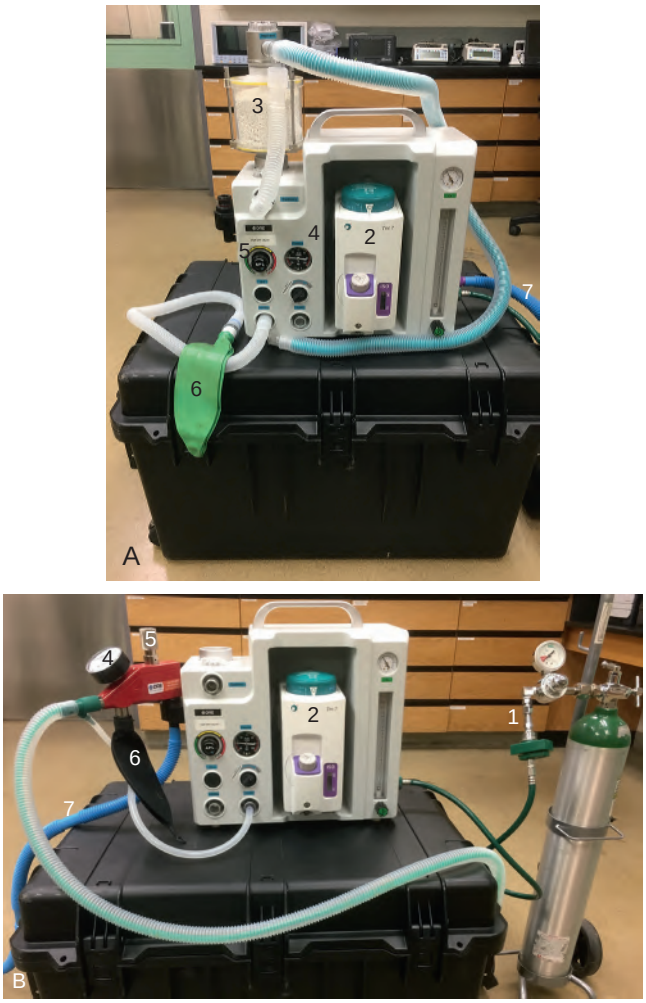
an integrated or separate flowmeter, a vaporizer, breathing circuit, and an endotracheal tube or face mask. Rebreathing systems also require some type of carbon dioxide adsorbent and a reservoir bag. Although most veterinarians are used to these components as parts of a stand-alone, preassembled anesthesia machine, understanding the role and relative importance of the various constituent pieces will allow a field veterinarian to customize or purpose-build a small and simple machine for delivering inhalants in the field. Fig. 28.1 shows a custom-built, self-contained anesthesia machine made from commercially available components that may be used as a circle (rebreathing) and nonrebreathing system and run off of a small oxygen cylinder in the field. Fig. 28.2 shows a commercially available machine that serves the same purpose, although it is slightly bulkier.

The anesthesia machine is typically divided into high-, medium-, and low-pressure systems. The high-pressure system is maintained at 1900–2200 psi and includes the oxygen cylinder, the yoke, regulator, and pressure gauge. The medium-pressure system is maintained at 40–55 psi and includes lines from the pressure regulator to a flowmeter. As mentioned later, some regulators have an integrated flowmeter and thus do not have a separate medium pressure system. The low-pressure system includes the flowmeter, vaporizer, and anesthesia circuit. Any pressure in the low-pressure system is transmitted directly to the patient and should not exceed 30 cm H_2O (0.42 psi).[16]

Field anesthetic circuits may be designed as either a circle (rebreathing) system or a nonrebreathing system. The two types of systems differ in the mechanism by which they prevent the patient from rebreathing its own exhaled carbon dioxide. Clinicians are advised to consider their patient size when deciding which type of circuit to use. Rebreathing systems are traditionally used for animals greater than 7–10 kg and use a carbon dioxide adsorbent (soda lime) to remove the patient's exhaled carbon dioxide from the circuit, allowing that exhaled gas to be rebreathed by the patient. Use of a circle system conserves body heat, oxygen, and anesthetic gases.[16] The disadvantage is the relative complexity and bulk of the system compared with a very light, nonrebreathing system. A traditional rebreathing system contains the following pieces: CO_2 adsorbent and container, one-way valves, patient breathing circuit, reservoir bag, vaporizer, manometer, and adjustable pressure limiting valve (pop-off valve). These components are labeled in Figs. 28.1 and 28.2.

In many cases, due to logistical ease, nonrebreathing systems are used for field anesthesia machines. It should be noted that a nonrebreathing system is not physiologically appropriate for animals larger than 30 kg and ideally is not used on animals greater than 10 kg. The perceived advantage of a nonrebreathing system is the simpler design, with fewer parts and less potential for mechanical failure. Unfortunately, a nonrebreathing system also requires a much higher oxygen flow rate and expends more volatile anesthetic agent, making it much less suitable for field use. Maintaining a 10-kg animal on a nonrebreathing system would require an oxygen flow rate of 3 L/min, whereas a circle system would require 300 mL/min (Box 28.2). If that flow rate is not met, it is highly likely that the patient will inhale its own exhaled gases, including carbon dioxide. When using a nonrebreathing system on a larger animal in

• **Figure 28.1** Custom-built Portable Anesthesia Machine With the Components Labeled. The machine may be set up as a rebreather (A) and a nonrebreather (B). The components of the system include *(1)* oxygen tank and regulator, *(2)* vaporizer, *(3)* carbon dioxide adsorbent canister (rebreathing system only), *(4)* pressure manometer (nonrebreathing system only), *(5)* adjustable pressure limiting (pop-off) valve, *(6)* rebreathing bag, and *(7)* scavenge hosing.



• **Figure 28.2** Commercially Available Portable Anesthesia Machine With Labeled Schematic of the Components. This machine may be set up as a rebreather (A) and a nonrebreather (B). The components of the system include *(1)* oxygen tank and regulator (visible in B only), *(2)* vaporizer, *(3)* carbon dioxide adsorbent canister (rebreathing system only), *(4)* pressure manometer, *(5)* adjustable pressure limiting (pop-off) valve, *(6)* rebreathing bag, and *(7)* scavenge hosing.

• BOX 28.2 Suggested Oxygen or Fresh Gas Flow Rates for Different Veterinary Anesthesia Breathing Circuits

Nonrebreathing system. This amount may vary slightly depending on the style of nonrebreathing system used.

$$3 \times \text{minute ventilation}$$
$$= 3 \times \text{respiratory rate (breaths/min)} \times 10\ \text{mL/breath} \times \text{body weight (kg)}$$
$$= \text{body weight (kg)} * 300\ \text{mL/min}$$

Rebreathing (circle system)

$$3 \times \text{oxygen consumption}$$
$$= 3 \times 10\ \text{mL/kg/min} \times \text{weight (kg)}$$
$$= \text{body weight (kg)} * 30\ \text{mL/min}$$

the field, it is imperative to use capnography to assess possible rebreathing of carbon dioxide by the patient. Simply relying on respiratory rate will not allow the clinician to diagnose carbon dioxide rebreathing and may result in extreme hypercapnia. In addition to expending much more oxygen, nonrebreathing systems also use much more liquid inhalant anesthetic.

All inhalant anesthetic systems produce some degree of waste anesthetic gas. Although the negative health effects of currently used anesthetic gases are not well established, it is commonly accepted that medical professionals should be exposed to the minimum amount of waste anesthetic gas possible.[19] In addition to possible health effects, these waste gases should be considered environmental pollutants. In a field medicine setting, unscavenged gases are being released directly into the environment. In a hospital setting, these gases should ideally be scavenged to reduce exposure to personnel. Outside a hospital setting, it is common to use portable, limited duration use charcoal canisters. It is critical to understand that these canisters are limited in the amount of waste they may scavenge. Based on manufacturer specifications, if the canister gains more than 50 g since starting use, it may no longer absorb waste gas and should be discarded.[12,20] The only way to easily determine the longevity of the canister is to regularly weigh it, which may not be feasible in a field site. On average, after 12–15 hours of use, the canister is essentially useless and many may fail before this time. In addition, the canisters are not able to scavenge nitrous oxide.[12] A more practical and effective means of reducing waste gas exposure and pollution is to limit the amount of gas anesthetic used. This goal may be achieved by reducing the fresh gas flow rate and using a rebreathing system instead of a nonrebreathing system.[10] For example, an isoflurane vaporizer set at 2%, with a fresh gas flow rate of 1 L/min uses 6 mL of isoflurane per hour, whereas the same vaporizer set at 2% with a fresh gas flow rate of 2 L/min uses 12 mL of isoflurane per hour.

Field Oxygen Support

Oxygen is most commonly supplied in compressed gas cylinders. Oxygen regulators, or pressure reducing valves, reduce the high pressure in the tank (1900–2200 psi) to a safe working pressure of 40–60 psi.[12] The pressure regulator will ideally also have a pressure gauge and may have an integrated flowmeter to allow controlled delivery of oxygen at a clinically appropriate flow rate (1–10 L/min) to a vaporizer or nasal insufflation line. Fig. 28.3 shows a typical small oxygen regulator for field use. This type of regulator may be connected to an insufflation line, vaporizer, or demand valve. The most common types of compressed oxygen cylinders used in field settings are small E tanks and large H tanks. The filling capacity and pressure for E tanks in the United States is 660 L and 1900 psi at 21°C. H tanks contain 6900 L at 2200 psi. Because there is no convenient way to measure the exact volume in the tank, knowing the relationship of the filling pressure and volume

• **Figure 28.3** Oxygen pressure regulator with a pressure gauge and an integrated flowmeter that may be used to supply a portable anesthesia machine through the attached hose or may be connected to an oxygen demand valve.

TABLE 28.2 Oxygen Cylinder Size, Volume, and Pressure When Filled at 21°C

Cylinder Size	Volume When Full	Pressure When Full
B	200	1900
D	400	1900
E	660	1900
M	3450	2200
H	6900	2200

is critical in a field setting where replacement oxygen tanks and filling sources may be days away. Tank pressure is read out on a gauge on the pressure regulator. Because oxygen is a gas, the volume in the tank is directly proportional to the pressure in the tank, assuming that the tank is kept at a constant temperature. So a tank that was filled to 660 L at 1900 psi will contain 330 L when the tank pressure reads 950 psi and 165 L when the pressure is 475 psi.[12,16] Table 28.2 lists common cylinder volumes and pressures. Oxygen cylinders may be made of steel alloy, which is stronger, or aluminum, which is lighter.

In the planning stages of a field project, it is critical to understand how much working time is possible with a given number of tanks. Similarly, efficient use of oxygen in the field is critical to avoid running out. For example, the following equations may be used to determine how much oxygen is needed if the plan is to anesthetize three 50-kg animals for 1 hour each. On a circle system, the ideal fresh gas flow rate from Box 28.2 would be 1.5 L/min (30 mL/min × 50 kg = 1500 mL/min = 1.5 L/min).

$$3 \text{ animals} \times 1 \text{ h/animal} \times 60 \text{ min/h} \times 1.5 \text{ L/min} = 270 \text{ L of oxygen.}$$

The 270 L of oxygen is less than half the volume of a full E tank, and so this could be easily accomplished.

Oxygen Safety

Transporting oxygen into the field is not without hazard. Because the cylinders are under high pressure, it is possible for them to rupture, if not handled appropriately, and should be handled only by trained personnel. Unsafe practices include storing cylinders upright, instead of on their side, transporting them for distances by hand without a proper cart, and exposure to extreme temperatures. Unfortunately, many of these practices are commonplace and occasionally necessary in a field setting but should be minimized whenever possible. Exposure to extreme temperatures should be avoided at all costs; temperatures greater than 54°C (130°F) and less than −7°C (20°F) may damage an oxygen tank, making them dangerous to use.[12] Even more modest fluctuations in temperature may affect the pressure of the gas inside a fixed volume canister. Most tanks have a pressure relief mechanism built into the valve. This pressure relief system will allow the contents of the tank to vent rapidly before the tank itself would explode due to over pressure. Dropping a tank could result in damage to the tank or release of the pressure relief mechanism and rapid discharge of the contents, which may turn the tank or any surrounding loose material into a deadly projectile.

Oxygen is not flammable by itself but is an oxidizing agent, and, if a flammable material and a source of ignition (flame or spark) are present, a fire may occur. Fires in an enriched oxygen environment will burn hotter and faster, and, if the fire involves oxygen under pressure, an explosion may occur. Transfilling, or filling a smaller oxygen cylinder (E tank) from a larger one (H tank), may be common practice in a field setting. Rapidly filling an empty small container from a large one at high pressure will cause the smaller cylinder to heat up rapidly due to recompression of the gas, and the resulting heat could ignite nearby flammable materials.[12]

Multiple safety mechanisms are used to prevent accidental mixing of compressed gases; the diameter index safety system (DISS) uses a standard diameter and thread configuration to prevent oxygen lines from being connected to fittings for other gases and vacuum. Similarly, the pin index safety system prevents oxygen tank valves from being connected to regulators for other gases. It is imperative that all connections and fittings be tested before transporting equipment to a remote setting, because appropriate connections and fittings may not be easily available.

Portable oxygen concentrators are battery-powered, compact, portable devices that may be used in the field for supplemental oxygen. They may be more convenient and portable than oxygen cylinders. These units use room air and, by extracting the nitrogen, may dispense 90%–96% oxygen.[21]

Field Ventilatory Support

Multiple devices may provide positive pressure ventilation in remote locations. Bag-valve devices, also known as manual

• **Figure 28.4** An anesthetized South American sea lion *(Otaria flavescens)* being ventilated with a bag-valve mask and monitored with battery-powered pulse oximetry and capnography. (Photo courtesy of the Chicago Zoological Society.)

• **Figure 28.5** A specially designed megavertebrate demand ventilator used to ventilate an anesthetized African elephant *(Loxodonta africana).* (Photo by Jeffery R. Zuba.)

resuscitators, are small, self-inflating devices that may easily administer room air with or without oxygen enrichment under positive pressure. These devices are lightweight and come in a variety of sizes. Most consist of a self-expanding bag, a one-way valve, a reservoir bag, and a line for additional oxygen supplementation (Fig. 28.4).

Oxygen demand valves are high-flow devices and may supply 100% oxygen at high pressure. Traditionally they are available in equine and human models. Equine models are equipped to deliver 160 L/min of oxygen, whereas the adult human standard is 40 L/min. To meet the needs of ventilating megavertebrates, a specially designed demand valve may be used to provide high-flow, high-pressure ventilation to very large animals (Fig. 28.5).[22] Similarly, commercial battery-powered leaf blowers (Fig. 28.6) or custom adaptations of equine demand valves may be used to ventilate elephants and other megavertebrates.[23,24]

Patient Monitoring

Ideally, personnel should have the equipment needed to deal with hypoxia, hypotension, hypoventilation, and hypothermia. Accurate responses require reliable monitoring of oxygen saturation, carbon dioxide excretion, body temperature, and blood pressure, which may be a challenge

• **Figure 28.6** A modified leaf blower used to ventilate an anesthetized African elephant *(Loxodonta africana).* (Photo courtesy of the North Carolina Zoo.)

in a field setting. The most basic anesthetic monitoring consists of measuring heart rate, respiratory rate, and (for homeotherms) body temperature. Although expensive instruments are available for specialized situations, these parameters are easily measured with the eyes and ears of the anesthetist, a stethoscope, and a rectal thermometer. Monitoring of oxygenation, ventilation (carbon dioxide excretion), and blood pressure require additional monitors. Pulse oximeters, capnographs, electrocardiograms (ECGs), and oscillometeric blood pressure monitors are the commonly used "standard anesthetic monitors" in veterinary anesthesia but require some accommodation for field use. It is important to remember that even though these devices are widely used in wildlife medicine, very few have been objectively evaluated in nondomestic species.

As with any field equipment, portable monitoring devices should be readily powered by commercial available batteries. Use of field-ready, portable anesthetic monitors is shown in Fig. 28.4. Accommodations may be made to protect incredibly valuable monitoring equipment from the challenges of field use. Waterproof or resistant rugged cases (Pelican) may be modified to house an anesthesia machine and its components or monitoring equipment. Battery-powered mainstream capnographs are commercially available and provide easy, quick, and continuous assessment of ventilation. Similarly, pulse oximeters are small, portable means of assessing oxygenation in the field.

Chapter 3 in this volume covers each of these monitors in considerably more depth; their mention here is simply to point out that they are easily adaptable to field use and their routine use in field and hospital settings may reduce patient morbidity and make anesthetic events safer.

Newer technology allows continuous collection of ECGs with use of a module that pairs with a smartphone. These devices are capable of recording and electronically storying or emailing ECG information.

Blood glucose (BG), lactate, or blood gases may be evaluated with point-of-care analyzers. In many cases, this is not necessary or possible, but for procedures on marine

mammals or large ungulates, these data may prove essential in assessing the patient under anesthesia before a crisis or cardiopulmonary arrest occurs. Such information may also prove useful in assessing instances of postrelease mortality of treated animals. Lactate measurements provide invaluable information about adequacy of perfusion and oxygen delivery and may be used as an indicator of exertional myopathy.

Conclusion

Inhalant anesthetics may be safely and effectively used in field settings on a wide variety of species. A basic understanding of inhalant pharmacology and vaporizer and anesthetic circuit design and function is needed to use this equipment safely. Each type of machine has inherent benefits and limitations that determine its appropriateness for the species of interest. In most situations, wildlife veterinarians and anesthetists will need to modify commercially available equipment or purpose-build units to meet the needs of the species of interest in their natural habitat.

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29

Perianesthetic Monitoring: Equipment and Interpretation

KHURSHEED MAMA

"For every mistake that is made for not knowing, a hundred are made for not looking."

Anonymous

Introduction

Most anesthetic and adjuvant drugs compromise patient homeostasis. Further compromise may result from the animal's physical condition, disease processes, and planned medical or surgical procedure. Untoward events may occur suddenly and, in the absence of intervention, have disastrous consequences. The degree of animal monitoring should be risk-based and inform about physiologic changes. This chapter reviews available monitoring tools and discusses their usefulness and limitations. Interpretation and integration of information provided by the selected equipment in conjunction with observation of the animal facilitate appropriate intervention.

Monitoring the Cardiovascular System

Normal cardiovascular function is essential for the maintenance of adequate oxygen delivery to the tissues. Oxygen delivery = cardiac output (CO) × O_2 content. CO is determined by two intrinsic factors (heart rate and myocardial contractility) and two extrinsic factors (preload and afterload) that functionally couple the heart and vasculature. Although CO is occasionally measured in clinical veterinary patients, parameters such as heart rate and rhythm, arterial blood pressure, central venous pressure (CVP), mucous membrane color, and capillary refill time are often used to estimate this and thus the adequacy of tissue perfusion.

Monitoring Heart Rate

Auscultation Using an External or Esophageal Stethoscope

This is feasible in many species but not practical in others, either due to size considerations or inability to gain access. It may also be disruptive to the surgeon during the procedure.

Palpation of a Pulse

Although straightforward, pulse palpation is not always possible, either as a result of extreme vasoconstriction due to large doses of certain medications (e.g., alpha-2 adrenergic agonists such as medetomidine) or lack of easy access to externally palpable arteries (e.g., snake).

Use of Pulse Monitors (e.g., Doppler, Pulse Oximeter, Arterial Pressure Waveform)

Although these tools too are not uniformly applicable and may not provide accurate values for blood pressure or saturation in all species, they can inform on heart rate. For example, the Doppler may be placed over the heart in a reptile and provide an audible signal. Both the Doppler and pulse oximeter are available in compact and battery-powered units.

Electrocardiogram

The rate is derived from a tachometer and displayed digitally—the tachometer should record a beat with every QRS complex. On occasion, depending on the amplitude of the other depolarization waves, it may count them or other artifacts caused by motion as well, yielding an inaccurate value.

The first three methods allow for assessment of mechanical activity of the heart (or circulation) and, in the case of the Doppler or arterial pressure monitor, may provide some quantification of this. Ranges for expected heart rates are unlikely to be available for all species and circumstances and are further influenced by anesthesia medications. For example, the alpha-2 agonist drugs (e.g., medetomidine) are known to cause bradycardia secondary to centrally mediated sedation and hypertension resulting from vasoconstriction.[1–3] Opioid agonists are reported to have more variable effects. In canine species and nonhuman primates, they may cause bradycardia and bradyarrhythmias, whereas in equine and megavertebrate species, they may cause tachycardia.[4–6]

Monitoring Heart Rhythm

Electrocardiogram or Electrocardiograph

This maps the spread of depolarization and repolarization waves in the atria and ventricles and provides information about heart rate and rhythm that may be a result of anesthetic drugs, variations in anesthetic depth, autonomic tone, or the result of hypoxemia, acidemia, electrolyte imbalances, and so on. Although the electrocardiogram (ECG) provides valuable information, it is only an indicator of electrical activity and does not quantitate CO. Said differently, the ECG (representing electrical activity) may continue for minutes after complete cessation of circulation.

During anesthesia, ECG lead placement configuration is often varied as necessitated by procedure and access to the animal. Leads may be placed such that the heart lies between them, with the understanding that interpretation of electrical axis or chamber size should not be made. Leads are connected to the animal via atraumatic or alligator clips, needles, or ECG electrodes (patches). Electrode gel or saline will improve contact; note that alcohol, which may be used for this purpose, is flammable. Small portable monitors have a battery facilitating short-term use for field procedures; for longer procedures, an electrical source is necessary.

Bradycardia is a heart rate lower than expected. For example, a heart rate of 40 beats per minute (bpm) would be too low in a Hispaniolan Amazon parrot *(Amazona ventralis)* but within the normal range for a standing, awake 1000-kg white rhinoceros.[7,8] The individual responsible for anesthesia management should have an awareness of what to expect for the species and circumstances they will be working in.

If the heart rate is assessed as being unexpectedly low, treatment may be warranted, as a decrease in heart rate is usually associated with a decrease in CO. Anticholinergics (e.g., atropine) are a nonspecific treatment for bradycardia in small animal patients except when alpha-2 agonists are utilized.[9] Although anticholinergics may be used in equine patients, ileus is a recognized side effect.[10,11] Certain species (e.g., rabbits) have atropinase, which will rapidly break down atropine, shortening its effective duration.[12] Thickening of salivary secretions is also reported in ruminant species.[11,13] Sympathomimetic drugs (e.g., dopamine) may be used as alternatives to increase heart rate. Occasionally and most notably in the case of alpha-2 agonists, an antagonist (e.g., atipamezole) may be administered in place of an anticholinergic. This is not usually feasible during the procedure in exotic mammals as reversal will result in arousal.

When the cause of bradycardia is known, it should be addressed. Hypothermia may also contribute to bradycardia, stressing the importance of maintaining body temperature in the normal range for the animal.

Tachycardia is a heart rate higher than expected. For example, a heart rate of 164 bpm would be considered too high for a 700-kg wood bison but normal for a ferret.[14,15] Markedly elevated heart rates may decrease cardiac filling. If anesthetic depth is inadequate during periods of noxious stimulation, administration of analgesic drugs will frequently bring the heart rate back to the normal range. In species where a sympathetic response to analgesic medications (e.g., opioids in equids or felids) is expected, this will not hold true.[4] In a well-monitored patient, tachycardia (perceived to compromise the animal) that does not respond to targeted treatments may be treated with nonspecific therapies, as for example (in domestic canines), beta blockers (e.g., esmolol 50–100 μg/kg) or cholinesterase inhibitors (e.g., edrophonium 0.5 mg/kg titrated slowly). Alpha-2 agonists may be of value in the otherwise healthy animal.

Monitoring Arterial Blood Pressure

Indirect (Riva-Rocci—"Return of flow") or Noninvasive Methods

Pulse palpation is not considered a reliable method for the estimation of blood pressure but provides qualitative information of stroke volume (the difference between systolic and diastolic pressure). Pulse palpation or auscultation may be used with a cuff to estimate pressures.

Automation of cuff inflation and deflation has led to the development of oscillometric techniques to measure systolic, diastolic, and mean pressures indirectly; the pressure is measured in the cuff and not the artery, resulting in variable accuracy. Factors influencing accuracy are both internal (e.g., programmed algorithms) and external (e.g., cuff size and placement) to the monitor. Some monitors determine the mean arterial pressure (MAP) at maximum amplitude, which increases the accuracy of this measurement. Alternatively, the monitor may determine systolic pressure at the first detection of pulse oscillations, rendering this the most accurate value. The other values are then estimated by use of proprietary algorithms, and accuracy may vary.[16,17]

The Doppler ultrasound detector is useful for monitoring trends in systolic blood pressure. The crystal is lubricated and placed over a peripheral artery of the distal limb, underside of the tail, wing, etc., to provide an audible pulse signal. Where possible, a cuff is placed proximal to the crystal. Alternatively, a pencil Doppler probe may be placed over an accessible artery (e.g., carotid in a turtle or rodent) to provide an audible (pulse) flow signal without a cuff. The Doppler is nonautomated, which can be limiting.

The accuracy of indirect methods varies from patient to patient and is influenced by the species, location of the cuff (the bladder should be placed directly over the artery), size of the cuff (width should be about 40% of the circumference of the extremity; too wide a cuff will give erroneously low readings, whereas too narrow a cuff will give erroneously high readings), rapidity of deflation, etc.[18–22] The distance of monitoring site above or below the level of the right atrium or left ventricular outflow tract (pressures will increase or decrease as recording site falls below or rises above heart level due to the hydrostatic pressure gradient); for each centimeter below or above the

heart, 0.73 mm Hg should be subtracted from or added to the recorded value (1.36 cm water = 1 mm Hg).

As a general rule the Doppler may be used on a wide range of animals (to provide an audible pulse signal), whereas oscillometric technology appears to work best on patients with regular heart rhythms and a heart rate within the stated "normal" range. Techniques utilizing a cuff may be inaccurate in patients with irregularly shaped or unusually muscular extremities, in patients with poor tissue compliance (e.g., reptiles), etc. Much work has been done correlating noninvasive and invasive methods in domesticated and nondomesticated species.[20,22–26]

Complications reported with use of noninvasive blood pressure monitoring in people include pain, venous stasis, compartment syndrome, peripheral neuropathy, and petechiae/ecchymosis.

Direct Methods

An aneroid manometer or a strain gauge/transducer requires cannulation of an artery and connection of the catheter to a "detector," using tubing. To maintain accuracy, the tubing should be relatively short and noncompliant. When using a strain gauge with a physiologic monitor, a waveform summating sine waves is produced.

To ensure accuracy of the readings, it is also essential that the transducer be appropriately balanced (zeroed relative to atmospheric pressure). The zero reference level is based on an estimate of the location of the left ventricular outflow tract (or alternatively the right atrium) and should be maintained for the duration of blood pressure measurement. This is considered the point of the shoulder (or thoracic inlet) for patients in dorsal recumbency and midline for patients in lateral recumbency. With single use of modern transducers, calibration may not be necessary; but with repeated use or in the research environment, calibration against a standard (e.g., mercury or water manometer) is recommended. This is especially important when working out of the range of blood pressures of commonly anesthetized domesticated species and people (e.g., giraffe or elephant where hypertension is anticipated). Care should also be taken to adjust the volume of flush and heparin concentration so as to minimize volume overload or heparinization.

A strain gauge provides systolic, diastolic, and mean pressure values, whereas the aneroid manometer provides mean arterial blood pressure values. In field conditions, the latter provides a functional and inexpensive way to assess blood pressure directly without the need for electricity and expensive equipment. When using the aneroid manometer, it is the interface between the fluid-filled portion of the line connected to the arterial catheter and the air in the line and not the manometer itself that serves as the zero reference point.

Peripheral sites for catheter placement vary with species and include the dorsal pedal, digital, auricular, tail, medial, or lateral saphenous artery. Complications of arterial catheterization, while low, include infection, ischemia, and hemorrhage. In human patients, peripheral neuropathy, pseudoaneurysm, and fistula formation are also reported, especially following long-term catheterization. Inappropriate use may result in misinterpretation of data and air or thrombus embolization.

Anesthesia generally lowers blood pressure values, and to ensure perfusion of vital organs, maintenance of a MAP of no less than 60 mm Hg or a systolic arterial pressure (SAP) of greater than 90 mm Hg is recommended in small animals. It is recommended that the MAP during equine anesthesia be maintained at least in the range of 70–80 mm Hg and that the SAP remain at a value above 100 mm Hg to ensure adequate muscle (and organ) perfusion. Indirect and direct arterial blood pressure values are available for other domesticated and nondomesticated species; the latter are often obtained during anesthesia, where the influence of drugs may confound the interpretation of "normal."[6,8,27–29]

Hypotension is common under general anesthesia and frequently warrants intervention. An intravenous crystalloid or colloid fluid bolus and decreasing the dose of inhalation anesthetic often resolves the problem. Inotropes and vasopressors are used when the former do not lead to resolution in a timely manner and have dose-dependent and species-specific actions. For example, dobutamine is the preferred inotrope in horses during inhalation anesthesia and is typically effective at low doses (0.5–2 μg/kg/min).[30] Conversely, higher doses (5–10 μg/kg/min) are needed to improve CO in anesthetized dogs, but a change in blood pressure may not be observed. Dopamine (5–7.5 μg/kg/min) will increase blood pressure in dogs and cats; in horses, tachycardia or tachydysrhythmias may be observed.[31,32] Rabbits, which often exhibit hypotension during inhalation anesthesia, show no change even with high doses of dopamine and only minimal improvement after administration of the vasopressor phenylephrine.[33] These examples highlight the importance of careful monitoring when using these vasoactive drugs, especially in species where basic knowledge of their response is not known. An alternative that is useful especially when ionized calcium values are low is the titrated administration of calcium.

Hypertension is not commonly observed during inhalation anesthesia with the exception of adult cattle. Conversely, during injectable anesthesia, most notably when alpha-2 adrenergic agonist drugs are used, blood pressure is often elevated. Hypertension to varying degrees is observed in animals with disease (e.g., renal, adrenal) and may necessitate management in the perianesthetic period. Although the range of normal blood pressure values is not known for all exotic species, hypertension is likely to be more common with the use of injectable sedative and anesthesia drugs, because many cause vasoconstriction and sympathetic stimulation. Hence, interpretation of values (if deemed accurate) must be made in light of medications and the species prior to any intervention. A simple treatment for hypertension in animals maintained on inhalation anesthetic agents is to increase the dose and see if blood pressure decreases. If pain is considered the cause, analgesic medications should be

provided. Alternatively, vasodilating drugs or sympatholytic drugs may be used. As an example, acepromazine has been used to counter the hypertension caused by ocular administration of phenylephrine in dogs.[34] Vasodilators such as hydralazine may also be used if vasoconstriction is suspected as the cause of hypertension. For sympathetically mediated hypertension, beta blockers such as esmolol or propranolol provide a more specific alternative.

Monitoring Central Venous Pressure

CVP is the pressure measured in the thoracic vena cava and used as an indicator of adequate preload in patients with normal myocardial function. It is determined by a complex interaction of the pumping action of the right heart, blood volume, and vascular tone. It may be measured as described previously for arterial pressure using a calibrated (in the range of measurement) and zeroed strain gauge or by measuring the rise of a column of fluid connected to a catheter placed in the thoracic vena cava. CVP should be recorded at the end of exhalation and in the absence of positive end-expiratory pressure (PEEP). CVP monitoring is not routine but is useful in high-risk patients. Detailed information regarding CVP waveforms and pulmonary artery catheter monitoring is available.[35]

Monitoring the Pulmonary System

Ventilation

Ventilation is the means by which the lung removes carbon dioxide (CO_2), a product of metabolism, from the body. Its regulation is important in the maintenance of acid-base balance. For each 10 mm Hg increase in $PaCO_2$, the pH will decrease approximately 0.05 unit. Elevated $PaCO_2$ increases cerebral blood flow, and an abnormally low $PaCO_2$ (<20 mm Hg) reduces it (in mammals). The $PaCO_2$ has an impact on oxygenation in air-breathing animals or those residing at higher elevations where the barometric pressure is low.

For most domestic species, breathing room air at sea level (barometric pressure 760 mm Hg or torr), $PaCO_2$ is maintained between 35 and 45 mm Hg. However, species variations occur; domesticated cats maintain their $PaCO_2$ at or below the low end of the range, whereas horses maintain values at or above the upper end of the range.[36,37] At higher elevations, mammalian species tend to hyperventilate to varying degrees to maintain oxygen tensions. Limited data are available for other species.[38,39] When interpreting blood gas (PCO_2 and PO_2) values, especially from species where body temperature differs significantly (e.g., reptiles), the reader should consider whether values should be assessed at analyzer temperature (37°C), where normal reference ranges are best defined, or at measured body temperature.[40] Hypothermia increases solubility in the blood and therefore decreases the partial pressure; the converse is true with a higher body temperature. Anatomic differences (e.g., three- vs. four-chambered hearts) will also influence "normal" values in nonmammalian species.

The $PaCO_2$ is directly proportional to the CO_2 produced and inversely related to alveolar ventilation. During anesthesia, CO_2 production should remain relatively consistent if body temperature does not vary. Alveolar ventilation, on the other hand, may vary significantly, as it is influenced by the depressant effects of anesthetic drugs, positioning, and so on. Abdominal distention, surgical manipulation, brain or spinal cord disease, severe metabolic illness, hypothermia, airway obstruction, and other factors may further influence ventilation in both the awake and anesthetized states.

Blood Gas Analysis

The $PaCO_2$ may be measured using an anaerobic sampling technique and blood gas analyzer. Blood gas analysis provides intermittent information; but unlike the case with PaO_2 measurements (discussed later), a venous sample may be used, allowing for easier sample acquisition. Venous values are normally 3–5 mm Hg higher than arterial values.[41]

Hypercapnia or *hypoventilation* is common during anesthesia; in healthy mammals breathing a high fraction of inspired oxygen, some degree of elevation in CO_2 is acceptable and even beneficial for cardiovascular function. In healthy mammals, an end-tidal (exhaled breath) carbon dioxide ($ETCO_2$) of approximately 50–55 mm Hg or a $PaCO_2$ of 60–65 mm Hg is often considered acceptable if the pH is maintained above 7.2 units. Interestingly, cardiovascular function is not well maintained in birds with the elevations in CO_2, so ventilation should be supported.[42,43] Although ventilators are most commonly designed for animals breathing inhalation anesthetics, tools such as the megavertebrate demand ventilator and Hudson demand valve are used to support breathing and also provide oxygen. The Ambu bag will support ventilation (with room air) in smaller animals. Guidelines for ventilation are based on normal parameters and are provided in the section on ventilometry and clinical assessment further on.

Capnography

Estimates of $PaCO_2$ may be made clinically on a continuous basis using capnography (graph and value provided) or capnometry (value only). Because the source of exhaled gas is alveolar (A) gas (which arises from blood returning to the lung from tissues where metabolic processes have occurred), the $ETCO_2$ may be used to estimate alveolar and therefore arterial CO_2. The relationship between alveolar ($PaCO_2$) and end-tidal $ETCO_2$ is influenced by the breathing circuit, character and rate of breathing, and for sidestream analyzer, the inspiratory and sampling flow rates and site of sampling. In normal clinical circumstances, the $ETCO_2$ will be lower than the alveolar or arterial CO_2 for mammals; in birds, the relationship is less clearly defined, with reports of both higher and lower ET values.[44–46] In small animals when the ventilation/perfusion ratio is well maintained, the values closely approximate (within 1–3 mm Hg) each other. The

difference widens due to increased sampling from dead space during certain procedures (e.g., thoracotomy).[47] In large mammals, wide and often variable differences in the range of 10–20 mm Hg may be observed.[41]

Two types of analyzers (sidestream and mainstream) are available, each with advantages and disadvantages. Sidestream analyzers are available in portable units and as part of a multiparameter physiologic monitor; with adaptation, it may be applied to broad circumstances. Mainstream analyzers are suited for small animals and afford the advantage of being battery-powered and portable. They are placed between the endotracheal tube and breathing circuit; in addition to adding bulk, they may increase work of breathing.

In addition to providing information about CO_2 during anesthesia maintenance, this technology may be used to confirm intubation and provides valuable information regarding equipment (e.g., exhausted CO_2, absorbent or malfunctioning values) and circulation. Monitors are typically equipped with alarms to alert the observer to low or high CO_2 readings that may result from other causes (e.g., apnea, hypoventilation).

Ventilometry and Clinical Assessment

Blood gas analyzers and capnographs are available with increasing frequency in veterinary practice, but cost issues and lack of user comfort with the equipment still remains. Under conditions of normal CO_2 production, guidelines enable the use of easily observed parameters to estimate CO_2. These also guide settings of the mechanical ventilator or demand valve.

"Minute ventilation" (product of tidal volume and respiratory rate over 1 minute) approximates 150–250 mL/kg/min. For domestic mammals, tidal volume ranges from 10 to 20 mL/kg and the respiratory rate from 6 to 20 breaths per minute (smaller mammals maintain higher respiratory rates and birds usually require larger volumes due to their unique respiratory anatomy). Respiratory rate is obtained by observing the rebreathing bag or the animal's chest. Tidal volume (two-thirds to alveolar ventilation and one-third to dead space ventilation) may be estimated by excursions of the rebreathing bag or quantitated by a ventilometer or respirometer placed on the expiratory limb of the (small animal) anesthetic breathing circuit.

Variations in ventilatory management (low pressure, high rate) and addition of supportive measures such as PEEP to improve lung compliance and oxygenation may be applied when deemed necessary (e.g., pneumonia).

Oxygenation

Oxygen delivery is the product of CO and arterial oxygen content. Oxygen content is commonly calculated from other parameters, namely hemoglobin concentration, its saturation (SO_2), and the partial pressure of oxygen (PO_2) dissolved in plasma. The relationship of these parameters to oxygen content is defined by the following formula:

$$[\text{Oxygen content (mL/dL)} = (1.36 \times \text{Hemoglobin concentration} \times \%\,\text{Sat}/100) + (PO_2 \times 0.003)]$$

The value 1.36 refers to the oxygen-binding capacity of 1 g of hemoglobin when well saturated. Hemoglobin is expressed in terms of grams per 100 mL blood; PCV/3 is a clinical estimate. The percent saturation refers to the relative saturation of hemoglobin and is measured with an oximeter or estimated from the hemoglobin dissociation curve after measuring the PaO_2. For a PaO_2 greater than 150, this is regarded as 100%. The PO_2 is the partial pressure generated by dissolved oxygen (0.003 accounts for the solubility of oxygen in the blood at 37°C; i.e., 0.003 mL O_2 will be dissolved in each 100 mL of blood per mm Hg PO_2). From this formula it becomes evident that hemoglobin plays the major role in carrying oxygen in the blood. However, the driving pressure for the transport of oxygen from blood to tissues is the partial pressure.

The alveolar gas equation

$$P_A = (P_B - H_2Ovap) \times FiO_2 - PaCO_2/0.8$$

accounts for barometric pressure (P_B), water vapor pressure (H_2Ovap), inspired oxygen (FiO_2), and $PaCO_2$. Once the alveolar pressure of oxygen has been calculated, the expected PaO_2 may be derived. A normal alveolar (A) to arterial (a) gradient of 10 (room air) to 100 (100% oxygen) mm Hg may exist. Clinically a simple way to arrive at the expected PaO_2 is to multiply the FiO_2 by 5 at sea level and by 4 at a mile high (barometric pressure approximately 640 mm Hg). The P/F ratio is also advocated to estimate adequacy of lung function. These tools, while simple, do not take into account the influence of CO_2, which may play a significant role.

Measurement of PaO_2

The PO_2, like PCO_2, is measured using a blood gas analyzer. The sample may be obtained by percutaneous puncture of an artery or sampled from an arterial catheter. Sites for puncture or catheter placement differ by species. The facial, transverse facial, lateral metatarsal, dorsal pedal, radial, superficial ulnar, and auricular vessels are possible sites. Lingual venous sampling has been used as an alternative in anesthetized carnivores, as studies have shown that values closely approximate those from an arterial source.[48] A consistent sample volume (commonly 1 mL, but capillary sampling is an option in smaller patients with some analyzers) is collected anaerobically (to preserve accuracy of the reading) in a heparinized syringe (to prevent blood clots entering the machine). All air should be removed from the sample and the sample corked and placed on ice water if it is not to be run immediately; analysis should be completed as soon as possible and ideally within an hour of sampling for mammals and contemporaneously in birds. An excess of heparin or contamination with air will alter PaO_2 values (raise it for samples collected when an animal is breathing room air and lower it for animals breathing a high FiO_2).[41,49,50]

Portable analyzers offer the veterinarian working in the field a practical option for blood gas analysis. Many utilize single-use disposable self-calibrating cartridges, but these may function only within certain temperature ranges or require cartridge refrigeration.

Measurement of SaO_2

Blood gas analyzers provide intermittent information and may not be cost-effective. The measurement of oxygen saturation using a pulse oximeter provides a means of continuously monitoring the patient's oxygenation, usually at a lower cost. Additionally, the equipment is easily portable. Pulse oximeter probes are of two types, transmittance and reflectance. The transmittance probe is attached to the patient externally (tongue, lips, ear, etc.), whereas the reflectance probe may be placed in the oral or nasal cavity, rectum, vagina, inside of the eyelid, and so on. Accuracy is influenced by pigment, tissue thickness, and movement, among other factors.

Oximetry is based on the intensity of light transmitted through a blood sample. The different forms of hemoglobin (oxygenated and reduced) absorb different wavelengths of light to different degrees. The pulse oximeter further distinguishes background absorption from that during pulsatile (arterial) flow. Accuracy is greatest in the range of saturations between 80% and 95%.

Relationship of SaO_2 and PaO_2

Hemoglobin saturation is related to the PaO_2 by a sigmoid curve. Clinically, the information is analogous but the interpretation of the numbers differs. A PaO_2 of 80 mm Hg or greater is acceptable, as is a SaO_2 of 95% or greater. A PaO_2 of 60 mm Hg is approximately equivalent to a SaO_2 of 90% for commonly anesthetized small animal species and is an indicator of hypoxemia; variations in this relationship likely exist in nondomestic species but are not fully elucidated.[41,51] Due to the nature of this relationship, an SaO_2 of 100% (hemoglobin maximally saturated) could reflect a PaO_2 ranging from 100 mm Hg to upward of 500 mm Hg. Clinically, this becomes limiting if the goal is to assess pulmonary function in the face of high FiO_2.

Monitoring Body Temperature

Although simple to perform either by intermittent monitoring (using a thermometer placed in either the rectum or auricular canal) or by continually using a thermistor probe (placed in the esophagus or rectum), this is often ignored in clinical practice. Knowledge of normal ranges for the different species is critical for appropriate interpretation. For example, normal body temperature in many avian species would be considered hyperthermic for mammals. Conversely some marsupials (e.g., kangaroo, wallaby) and most reptiles have normal body temperatures that are lower than those of mammals.

Body temperature is controlled by thermoregulatory centers in the brain and reflects the balance of heat generated from metabolic processes and heat dissipated (by conduction, convection, and evaporation). Anesthesia affects thermoregulatory centers in the brain and influences the generation and dissipation of heat. Due to a decrease in metabolic rate induced by the sleep state of anesthesia, heat generation is decreased. Heat loss is increased by a number of mechanisms related to anesthesia and surgery; cool intravenous fluids and inspired gases, cold tables, surgical clips and prepped areas, open body cavities, and so on all contribute. In general, most animals regardless of body size tend to lose heat during anesthesia.

The implications of hypothermia are many. Under extreme conditions one may alter blood viscosity and coagulation and increase the likelihood of myocardial fibrillation. Smaller decreases in body temperature will affect anesthetic dose requirements (MAC is reduced 5%–8% per degree centigrade decrease in body temperature) and rate of clearance of anesthetic drugs.[52]

Although hypothermia is the more likely, the opposite extreme in body temperature may also be seen (as, e.g., in an animal with increased muscle activity) and is just as consequential to the patient. Malignant hyperthermia is the extreme situation in which a patient may react to the inhaled anesthetic and enter a hyperdynamic metabolic state that, without early detection and intervention, is often fatal.

Laboratory Parameters

In addition to the measurement of blood gases, "blood gas" analyzers provide additional useful measured and calculated laboratory values. Measured values may include pH, lactate, glucose, creatinine, hemoglobin, and electrolytes. Bicarbonate, base excess, and saturation are derived.

The range (7.35 and 7.45 units) for normal pH (at 37°C, approximating normal human body temperature) is well described for mammals. A pH below this range indicates acidemia and one above this range, alkalemia. Carbon dioxide (as previously described) and bicarbonate (20–28 mEq/L) describe the respiratory and metabolic contributions. As with carbon dioxide, bicarbonate values vary between mammalian species; carnivores tend to present with lower (17–24 for cats) values, whereas herbivores often have higher (24–32 for horses) values. Bicarbonate provides an indication of the metabolic contribution to pH (high values indicate metabolic alkalosis and low values metabolic acidosis). Bicarbonate values are, however, influenced by carbon dioxide (as described by the Henderson-Hasselbalch equation). Broadly, an increase in $PaCO_2$ of 10 mm Hg will result in an increase of 1–3 mEq/L in the bicarbonate value; note that this is not compensation but simply the result of a mass shift. Base excess provides a true measure of the acid-base balance by removing the influence of CO_2 on bicarbonate. Base excess values (mEq/L) tend to be more negative in carnivores (−7 to +3) and more positive (0 to +4) in herbivores. A negative value outside these ranges indicates metabolic

acidosis, whereas a positive value indicates metabolic alkalosis.

Lactate and blood glucose values should be interpreted based on typical normal ranges. A lactate value greater than 2 mmol/L indicates anaerobic metabolism and a blood glucose value less than 60 mg/dL points to hypoglycemia. Electrolyte values may vary slightly among species but tend to be held to fairly tight ranges; ionized values for potassium and calcium are influenced by pH (a low pH will falsely elevate both values) and should be interpreted in light of this.

In animals that are anemic or at risk for hemorrhage, measurement of the packed red cell volume and total protein provides additional useful information.

Summary

Although not an exhaustive list, this chapter provides options for monitoring animals during heavy sedation and general anesthesia. Based on working conditions and degree of compromise, the anesthetist may select from these to complement subjective observations. Appropriate use and interpretation are key to correctly applying the information to benefit the animal.

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SECTION 7

Diagnostics

30

Wildlife Necropsy Primer

DENISE MCALOOSE

Introduction

Thorough postmortem or necropsy examinations capture all of the information that is relevant to the death of an animal or group of animals. When considered narrowly, the results provide information about an individual. More broadly and depending on the context, the compilation of these data forms the basis of understanding disease and the impacts of pathogens at the individual and species, population, and ecosystem levels. These data may be locally, regionally, or internationally relevant for wildlife, zoo, agriculture, and companion animals and for human public health. A necropsy may be performed for many reasons, including (not limited to) characterization of normal and abnormal gross and morphologic anatomic features; establishing baseline health parameters and normal reference ranges; identification of the cause(s) of morbidity and mortality in individual animals, groups, or populations; contribution of data to short- and long-term health and disease surveillance and monitoring programs; establishment of the presence and significance of pathogens and disease in individuals, groups/populations, and ecosystems; determination of the effectiveness of medical or husbandry interventions or mitigation activities; collection of forensic information necessary in legal proceedings and prosecution; and teaching and training. In conservation efforts, necropsy data may be important in recovery, reintroduction, and translocation programs (e.g., to understand disease presence/absence in assurance colonies or relocation animals and endemic populations to prevent unintended disease transmission). Results may also be used in establishment of protected areas or to influence policy decisions (e.g., habitat use, resource extraction).

Protocols and procedures for laboratory- or field-based necropsies for many terrestrial and aquatic domestic and nondomestic species are available through a number of sources. These include governmental, nongovernmental, university- or zoo-based biology, veterinary medicine, and conservation organizations. Historically and to date, most were only available in textbooks or printed manuals. Much information can now also be accessed online, sometimes in the form of instructional videos. A few examples of online protocols are those available through the World, European, or Association of Zoos and Aquariums (WAZA, EAZA, AZA, respectively) Taxon Advisory Group (TAG) or Species Survival Plan Programs (SSP, EEP) and the International Union for Conservation of Nature (IUCN), although there are many others.[1,2] Some provide general instructions, whereas others are taxon, genus, or species based. In some cases, access to online protocols may need to be requested. For others, for example necropsy of nontraditional species like invertebrates, there is value in reviewing basic zoology and biology references in addition to contacting colleagues with species-specific expertise to discuss unique anatomic features and common and emerging diseases.

Necropsy Basics

Personal safety, the safety of the team (which may include experienced individuals and groups, as well as enthusiastic but inexperienced staff, colleagues, or volunteers), biosecurity, and related communication are of paramount importance during any necropsy examination, regardless of scale or scope. In addition, the complexity and coordination of activities will vary and differ between an individual animal death and disease outbreak or mass mortality event.

Basic personal protective equipment (PPE) including gloves, surgical masks (respirators), aprons, boots, and dedicated clothing (e.g., coveralls, surgical scrubs) (Box 30.1) should be worn during all necropsy procedures, clean-up and carcass disposal, and any time equipment that may aerosolize tissues or pathogens is used (e.g., high-pressure hoses, drills, saws). In addition, an understanding of the common diseases in a particular and sympatric species and in a collection or region and risk assessment inform additional PPE and prophylactic vaccinations that are needed to safely perform necropsy procedures (e.g., eye protection for venomous animals or infectious disease splash risk, properly fitted N95 respirators for aerosol transmitted zoonotic pathogens such as tuberculosis, rabies vaccination if working with carnivores, bats, or other susceptible species). In cases in which the risk to human health goes beyond management with available PPE (e.g., suspected hemorrhagic fever, Ebola, anthrax) or vaccination status, *a necropsy should not be performed.* It is also important, especially in field situations, to evaluate the local environmental conditions to

• BOX 30.1 Necropsy Examination Personal Protective Equipment (PPE), Supplies, Equipment, and Tools

Documentation

- Forms: necropsy protocol and form, morphometrics data sheets, tissue sample checklist, human interaction form, notebook/paper (regular and waterproof [e.g., Rite in the Rain®])
- Labeling: tape, laundry tags with metal clips, pencils, waterproof marking pens and pencils (to label samples that will be immersed in liquid fixatives), Tyvek®*
- Photodocumentation: digital or film camera, batteries, memory cards, labels (include in every image)

Safety and Basic PPE

- Clothing: gloves, mask (e.g., surgical, N95, masks with integrated face shield), eye protection (goggles, face shield), surgical scrubs, lab coat, coveralls, aprons, boots, gloves, caps (head cover)
- Disinfection: sponges, dish soap, scrub brushes, disinfectant, bleach, alcohol (70% ethyl alcohol)
- Other: first aid kit, communication link (e.g., satellite phone), SDS/MSDS

Tools

- Cutting: scalpel blades, scalpel handles, knives (6- or 8-in blade), knife sharpeners†, scissors (small and large), bone shears, handsaws (e.g., hacksaw, reciprocating saw), axe/hatchet, wedges, mallet/hammer, cutting boards, rongeurs, loppers (hedge clippers), chisel/wedge (e.g., T shaped)
- Tissue handling: forceps, meat hook
- Containers: rigid leakproof wide-mouth spillproof screw-top containers (various sizes), zip-top plastic bags and Whirl-pak® (various sizes), serum tubes for fluid, blood, and urine collection, aluminum foil, Teflon® bags, cryovials, sterile vials/containers/bags, sterile needles and syringes (various sizes), trochar (various sizes)
- Morphometrics (metric): ruler, calipers, tape measure, scales
- Sterilization: sterile instruments, matches or propane torch and searing blade/spatula, isopropyl alcohol for flaming instruments
- Culture: sterile in-date swabs, urine cups, and bags, culture transport media/tubes (for bacteria, virus)
- Tissue fixation: 10% neutral buffered formalin, 4% glutaraldehyde or other EM fixative in small screw-top vials, isopropyl alcohol (for ectoparasites and endoparasites, cytologic preparations, etc.)
- For genetic/molecular diagnostics (aliquot into small screw-top vials): 20% DMSO/saturated saline solution (genetics), RNA-later® or TRIzol® (molecular diagnostics)
- Lighting: head lamp, flashlight, batteries, light bulbs, generator with extra bulbs and fuel
- Cold chain: ice chest, ice packs, refrigerator, freezer (–20°C, –80°C, dry ice, liquid nitrogen), absorbent packing materials
- Laboratory equipment: microscope (for field settings: field adapted [mirror as a light source], car battery–adapted power source, generator), centrifuge
- Other: ropes/straps/chains, string/suture, parafilm, glass slides and slide boxes, water supply/source (for cleaning/clean-up), plastic tarps, plastic tape/ropes to cordon off necropsy site, garbage bags, biohazard bags, disinfectant, bleach, sponges/scrub brushes, paper towels, portable generator (for electric powered saws, refrigerators, etc.)

Additional Equipment for Small Carcasses (<5 g)

- Magnification: dissecting microscope, magnification headband, surgical loupe
- Cutting: microdissection forceps, scissors

Additional Equipment for Megavertebrate (whale, elephant, etc.)

- Cutting: flensing knives, large reciprocating saw, large hammer/mallet and chisel, appropriate sharpener(s), shovel
- Tissue handling: large meat hooks, gaff hook
- Morphometrics (metric): 20 m long (min) tape measure
- Mobility (for moving carcass, appendages, etc.): hoist/crane with large-capacity mounted scale
- Containers: large sealable containers (e.g., vials to garbage cans)
- Other: thick rope/chain (min of 20 m long), block and tackle

*When labeled with permanent marker or pencil, may be placed in liquid (e.g., formalin) for identification purposes.
†For standard knives, recommend hand held sharpeners not sharpening stones because the use of stones requires significant expertise, and inexperienced operators may dull rather than sharpen cutting instruments (e.g., knives, scissors).
This is a general guide that may be tailored to meet the specific situational needs of a particular necropsy examination(s).
MSDS, Material safety data sheets; *PPE*, personal protective equipment; *SDS*, safety data sheets.
Data from http://www.seadocsociety.org/wp-content/uploads/Orca-necropsy-protocol-FINAL-May-15-2014.pdf; Necropsy procedures for wild animals. In White L, Edwards A, editors: *Conservation research in the African rain forests: a technical handbook*, New York, 2000, Wildlife Conservation Society, pp 196–217; Woodford M, Keet D, Bengis R: A guide to post-mortem procedures and a review of pathological processes identified in the elephant. In Woodford M, editor. *Post-mortem procedures for wildlife veterinarians and field biologists*. Paris, France, 2000, Office International des Epizooties, Care for the Wild and the Veterinary Specialist Group/Species Survival Commission of the World Conservation Union (IUCN).

identify problems that pose safety risks such as cold or rain, rising tides, or suboptimal animal position; in some cases where safety cannot be ensured, a necropsy should be postponed or abandoned. Wildlife and veterinary pathologists and clinicians and human medical health professionals are typically responsible for making these decisions based on a risk assessment that relies on an understanding of specific and relative risk, knowledge about infectious diseases and circumstantial situations, institutional standard operating protocols, and best practice protocols. In addition, if at any point there is a suspicion or confirmation of a locally, regionally, or internationally reportable/notifiable disease (e.g., USDA [US Department of Agriculture], CDC [Centers for Disease Control and Prevention], OIE [World Organization for Animal Health] reportable disease) or illegal activities (e.g., poaching, poisoning) are suspected, the scene should be secured and appropriate authorities contacted before proceeding. For reportable/notifiable diseases, direct contact with regulatory agencies or online information will provide the most up-to-date information about those diseases that are listed (see http://www.oie.int/animal-health-in-the-world/oie-listed-diseases-2017/).

In advance of a necropsy, it is important to have organized plans and assign roles and responsibilities. Standard tasks include identification of a lead prosector/pathologist, and individuals or teams to collect morphometric data, perform prosection, label and manage samples (diagnostic, research, archival), perform photodocumentation, manage data/data sheets (record, upload, organize), and ensure fulfillment of all protocols (including sample collection for research requests), ship samples, perform clean-up/disinfection and carcass disposal, and manage communication within and between necropsy investigative team members and stakeholders, including the media. Having organized plans and assigned roles is particularly important in megavertebrate necropsies or mass mortality events, due to the size or number of animals, participants of varied skill levels, and numerous different agencies that may be involved.

Necropsy Examination

Before a postmortem examination begins, it is worth remembering that *you*, whatever your role, are the most valuable asset in the process. All conclusions drawn in a mortality investigation rest on the information and samples you and your team collect. Best intentions and memory are no substitute for the collection of real-time verbal, written, and photographic documentation. Each person involved in the necropsy must take personal responsibility for his or her actions. Outcomes are as good as the data you collect and the focus you bring to the task, and what you do during and after the examination will make the impossible, possible.

All mortality investigations, regardless of whether they involve a single animal or a large mortality event, contain a set of consistent elements: a systematic, iterative diagnostic plan that applies evidence against a set of case definitions and differential diagnoses to establish a cause of death (Fig. 30.1). Central in this process is the necropsy examination. Providing specific necropsy protocols for all taxa and for every situation is beyond the scope of this chapter. However, all necropsy procedures follow a similar, basic format (described below).

Collection and Documentation of Relevant Historical Information

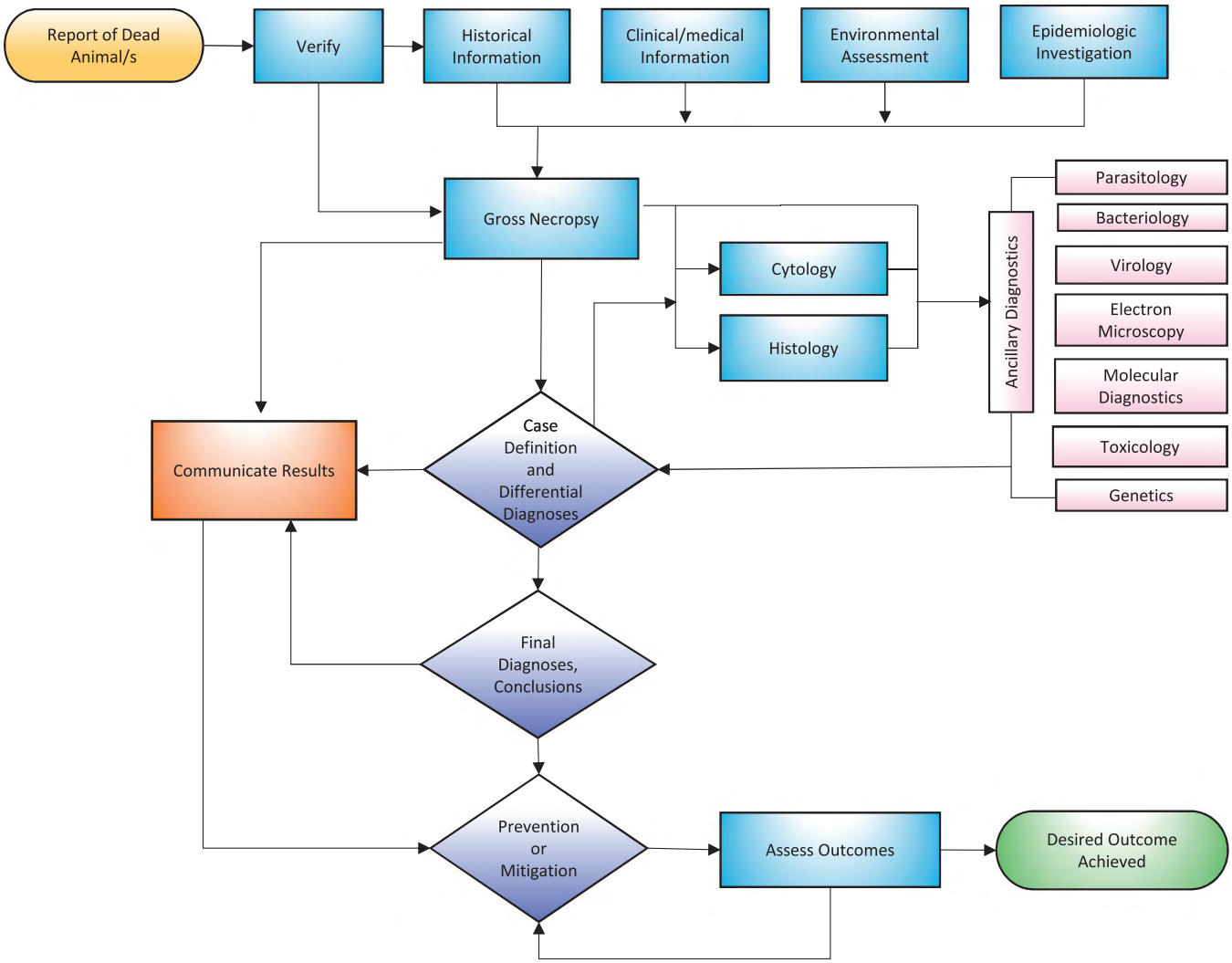
In addition to information that will be collected from the carcass, it is quite important to collect and record relevant death-related information. This includes basic animal information (e.g., species, breed, gender, age/age group, birth and death dates) and clinical medical information about the subject animal, contact animals, and other sympatric species (including other wildlife/vermin, humans, domestic animals, livestock, etc.), environmental, and epidemiologic information. It is also important to record information about the location in which an animal was found dead, environmental conditions/enclosure information, husbandry changes, and to note other findings (e.g., evidence of a struggle) that may be relevant to the circumstances of the animal's death.

External and Internal Examination

A good rule of thumb before performing a necropsy procedure is to familiarize yourself with the normal anatomy and natural history of the species on which you will be working (e.g., normal diet, fecal consistency, color patterns, sexual dimorphism, common and zoonotic diseases). This is particularly important when performing a necropsy on a species with which you are not familiar (e.g., what is the best approach to opening the body cavity of a chambered nautilus *[Nautilus pompilius]*) and will inform activities related to PPE and risk. Numerous texts and online resources, as well as colleagues with species-specific knowledge, are good places to start. It is also worth remembering that, although every taxon and species has unique features, familiarity with one often serves as a helpful reference for another. For example, familiarity with the anatomy of well-described and studied domestic or farmed species is directly applicable to the anatomy of most nondomestic mammals, fish, and birds. Invertebrates provide a greater challenge. This is an incredibly diverse group that includes more than 99% of all animal species on the planet. Over the past decade, there has been increased interest in this group, and expertise and resources are becoming more widely available.

All necropsy examinations should be systematic, follow standardized procedures and protocols, and include checklists for the collection of "standard" sets of tissues (Box 30.2). This ensures that a carcass is thoroughly examined, standardized information for generating baseline and documenting abnormalities is captured, and a complete set (or sets) of data and samples are collected for baseline, diagnostic, archival, and research purposes. When practical, fulfilling sampling/research-associated requests (e.g., biomaterials requests) allows valuable, additional information to be generated. Where applicable, standardized necropsy procedures and/or sampling/research-associated requests that are required by governmental agencies should also be followed.

Even in this age of digital photography, written descriptions of visual observations (animal, physical environment, etc.) remains a critically important component of a necropsy examination. But that does not mean that they need to be overly complicated. Two general rules of thumb during a necropsy: describe what you see and limit descriptions to factual observations rather than interpretations or diagnoses (this occurs later). Keep in mind that you describe all sorts of things every day and the language you use to describe what you see during a necropsy examination is no different than the language you use during everyday conversations. Basic necropsy descriptions include information about location (e.g., organ, body system) number (e.g., 1, 10, more than 20 but less than 100, multiple, many, myriad), color, size, shape, distribution (e.g., focal, regionally extensive, diffuse), consistency and texture (soft, firm, hard), and odor. For



• **Figure 30.1** Mortality Investigation Flow Chart. All mortality investigations include a consistent set of standardized activities. These include collection of information related to the circumstances of an animal's death, gross necropsy and sample collection for ancillary diagnostic testing, and establishing a set of differential diagnoses and case definitions against which test results are applied in order to arrive at final diagnoses and conclusions. Integral in this process is collection of information and documentation of all findings and clear and consistent communication with all relevant stakeholders.

example, "Dozens of pinpoint to 5 mm in diameter, soft, light tan nodules that contain thick, white material on cut section are present throughout the liver." Measurements are useful for documenting lesions, including weights of organs thought to be too large or too small. Size measurements should be taken in three dimensions (length × width × height/thickness). Remember, a description should include those details that allow someone who did not witness the necropsy to create an accurate image of what you saw. Also remember that a description is not an interpretation or a diagnosis. Those are best left to the pathologist to formulate based on your description (in the above description, it is hoped that you imagined multiple hepatic abscesses). For photodocumentation, always include a size reference (e.g., small ruler) and animal-related information (minimum: ID number, date). All information from the necropsy and collected samples should be entered into logs and secure databases developed for this purpose. This is important for a whole host of reasons, including rapid data retrieval and collation during mortality events, and data and sample sharing for current and future research projects. Information should be as detailed as possible to ensure that important information is not forgotten or lost.

Under ideal conditions, a necropsy is performed and samples are collected from freshly dead animals. If necropsy will be delayed, carcasses should be refrigerated or kept cool on ice for several hours to a few days; freezing should be an option of last resort because the freeze-thaw cycle will alter the color of many tissues and kill many pathogens (e.g., bacteria, fungi), and ice crystal formation will significantly limit the value of histology. However, under some circumstances (e.g., when a necropsy cannot be safely, thoroughly,

• BOX 30.2 Sample Collection Checklist

Skin

Umbilicus (neonates)

Skeletal muscle

Peripheral nerve
(e.g., sciatic)

Bone/bone marrow
(e.g., end of long bone, rib)

Salivary gland

Lymph nodes
(e.g., popliteal, bronchial, mesenteric, ileocolic)

Tongue

Thyroid glands (both)

Parathyroid glands

Trachea

Esophagus

Heart
(free walls and septum with valves)

Lung
(each lobe and a bronchus)

Thymus

Diaphragm

Stomach
(cardiac, fundic, pyloric)

Small intestine
(with and without pancreas)

Large intestine

Liver
(with and without capsule)

Gallbladder

Pancreas
(with and without intestine)

Spleen
(with and without capsule)

Adrenal glands
(bisected longitudinally)

Kidney
(bisected longitudinally; include cortex, medulla, pelvis)

Ureters

Urinary bladder

Urethra

Accessory glands
(e.g., prostate)

Testes/epididymis
(bisect longitudinally)

Penis

Ovaries

Uterus

Cervix

Vagina

Tonsil

Brain
(whole or cut longitudinally along midline)

Spinal cord

Eye

*This is a general list that should be modified to reflect the anatomy of the species being necropsied and any additional specific needs (e.g., research requests, specific pathogen screening). For example, collection of tissues from ruminants should include sections from the forestomachs (rumen, reticulum, omasum and abomasum) while samples from fish would include gill and anterior kidney. Collect one to multiple samples of normal and abnormal tissue and junctions between normal and abnormal. For bilateral organs (e.g., kidneys, adrenal glands), samples should be collected from the left and right sides. Multiple parallel cuts should be made in solid organs (e.g., liver) to assess the parenchyma prior to sampling. Tubular organs (e.g., intestine) should be opened along their long axis and examined prior to sampling. Tissue samples should not be greater than 0.5 cm thick and should be placed in 10% neutral buffered formalin (NBF) for fixation (1 part tissue to 10 parts NBF).

or adequately performed in a timely manner), a necropsy can be performed on previously frozen carcasses, although the results will not be optimal.

The *external examination* is an assessment of the tissues outside the main body cavities. This begins with direct measurement or estimation of carcass weight, collection of morphometric data, photodocumentation, and assessment of state of decomposition. Every carcass, regardless of its state of decomposition, may provide valuable scientific data. In many field situations, access to fresh carcasses is the exception rather than the rule. Environmental conditions (e.g., high humidity, high temperatures, or dense foliage in natural or recreated settings) and interval from death to discovery all affect the degree of decomposition. As the body decomposes, all tissues degrade. The speed at which this occurs and the reliability of diagnostic test results that may be obtained from different tissue types during this process vary. It is therefore important to document the degree of carcass decomposition, otherwise known as carcass condition, to contextualize observations and diagnostic test results. This is a subjective assessment, and a relatively common scheme categorizes postmortem carcass condition as excellent (freshly dead) to mildly autolyzed, moderately autolyzed, severely autolyzed, or skeletonized/desiccated/mummified remains. In the marine mammal community, several protocols use a similar carcass condition coding system.[3] Assessment of carcass condition is particularly useful in low-resource or time-restricted settings to identify those carcasses that will be the most diagnostically valuable (e.g., those that are "freshest" or most intact) and to direct sampling and testing.

In vertebrates a systematic external examination of the carcass includes evaluation of normal and abnormal findings in the structures of the head (e.g., eyes, ears, nares, bill, beak, teeth, cere, gills, tonsil); the outer covering of the body (e.g., skin, hair, feathers, scales, claws, hooves); the subcutis, glands (e.g., periorbital, salivary, scent, salt, perianal, uropygial), and skeletal muscles; external reproductive system tissues; the skeletal system (including one to multiple joints); and the peripheral nervous system. It also includes objective (measured) and/or subjective assessment of fat stores (e.g., subcuticular, blubber) and muscle mass and collection of bone marrow (typically femur or rib in nonavian and tibiotarsal bone in avian species). Written and photographic documentation of all notable normal and abnormal findings should be recorded, and representative sample(s) from all tissues and all lesions should be collected and placed into 10% neutral buffered formalin (NBF) for histology or collected as appropriate for ancillary diagnostic testing.

An *internal examination* is an assessment of the organ systems and individual organs in the body cavities. There are a number of common approaches to an internal examination. One is not necessarily better than another, and your preferred method may vary depending on the species and specific circumstances. Regardless of the method, of paramount importance is that you work safely, have an organized plan, and have a systematic strategy for organ and organ system examination to ensure all tissues are examined, lesions documented, and samples collected. This is especially important when working in large teams, on large animals, or in complicated situations (e.g., a mass mortality event). It is also important to work cleanly to minimize, as much as possible, contamination of the worksite and exposure risks to personnel and other animals (e.g., scavengers).

Species/Age-Specific Considerations

There are a number of notable, normal features to be aware of when performing necropsies on neonates. In mammals and birds, these include skeletal muscles that will generally be lighter in color than in adults; soft, light tan/orange liver (due to hepatocellular glycogen stores); low amounts of subcuticular or cavitary fat; and a very soft brain even in very fresh carcasses. In term mammal neonates, the ductus arteriosus and foramen ovale may be probe patent (should be functionally closed) and heal within the first few days of birth, the gonads and adrenal glands may appear relatively large, and if the carcass was refrigerated after death, the lenses may become opaque white (rather than translucent). In birds an egg tooth and (occasionally prominent) pipping muscle are seen. Birds and reptiles will have an internalized yolk sac, which may occupy a majority of the caudal coelom. As in their domestic counterparts, important abnormal findings include the presence of congenital abnormalities or defects (e.g., cleft palate, extra or missing limbs or limb deformities, cardiac septal defects) and excessive numbers of squames, meconium, yolk, or other foreign material in the lungs on histology. They may also have incompletely internalized yolk sacs (suggestive of premature pipping or hatch complications) or subcutaneous edema (e.g., head, neck), which may reflect suboptimal incubation parameters. In eggs the content of the air cell (it is normally empty; cultures of accumulated fluid or membranes may be taken at this point), condition of the membranes (color, intact, pipped, etc.), and assessment for fertilization (e.g., presence of blood spot, embryonic membranes) and the developmental stage, and position of a developing fetus/embryo should be assessed.[6]

Specific precautions should be taken when performing necropsies on venomous snakes, lizards, amphibians, fish, invertebrates, and mammals. Only those with experience and knowledge of the type, strength, and mechanism of actions of venom, location of production, and means of envenomation (e.g., rear fangs, spines, spurs, secretions) should handle venomous animals. Handling, PPE, and emergency protocols should be established before and followed during the necropsy. For example, venomous snakes should be placed in a completely secured, see-through container for transport, information about the species and antivenin should be attached/submitted with the animal/container, and the head should be taped or otherwise secured in such a way that the mouth is not free to open. Guidelines for contacting emergency/first responders, an envenomation center, emergency hospital, zoological institution or others that maintain antivenin stocks, and an emergency hospital with expertise in managing envenomation patients should be included in these protocols. For venomous snakes, the recommended first step in the necropsy procedure after confirming the snake is dead by observation through the see-through container is to remove the head and place it into formalin. This accomplishes two tasks, deactivation of venom and tissue fixation. However, it is not known if formalin deactivates all venom proteins, and tissue fixation may be incomplete in deep tissues, especially in large heads, so continued caution is warranted whenever the head is handled.

Cytologic examination of skin (and external mucus layer), fin tissue, and gill clips to identify ectoparasites, fungus, or other pathogens should be performed as a first step in a fish necropsy. Invertebrates present a challenge at necropsy, due to the myriad species and body types represented by this vast group of animals. Critical to gross and histologic evaluation, as with all species, is first reviewing basic textbooks and journal publications on anatomy, biology, and published diseases[7–12] and, through this, generating a checklist of organs and organ systems for consistent gross and histologic evaluation. In some species, gross evaluation will significantly disrupt tissue architecture. This is particularly true of arthropods and bivalve mollusks. In those cases, limited external evaluation of the ectoskeleton (carapace) and soft tissues, and collection and examination of hemolymph and cytologic (wet mount) evaluation of gills and external abnormalities are recommended prior to fixing the entire animal for histologic evaluation.

For very large animals, heavy equipment may assist in moving the carcass, limbs, and organs, as well as for carcass movement or burial. For very small animals (<5 g), the use of a dissecting microscope or head loupe is an incredibly helpful tool. In some cases the best option is to fix an entire animal. This may be achieved by either making a small incision in the body cavities to allow fixative to contact internal organs or through injection of the body cavities with fixative.

Some diseases, such as anthrax (caused by the bacterium Bacillus anthracis) or Ebola viruses, cause serious zoonotic diseases that can be fatal in humans. The former is of particular importance during ruminant necropsies (though other wild and domestic ruminants, horses, zebra, wild pigs and carnivores can be infected[2]), the latter is of concern during great ape necropsies in parts of Africa. The most common gross finding in either disease can be bloody discharge from body orifices. If either or other life-threatening diseases are suspected upon encountering a carcass or during a necropsy, the necropsy should not proceed and the body should not be moved until testing to confirm or exclude its presence is completed. If possible, guarding the carcass to prevent scavenging is recommended. For anthrax, multiple cytologic preparations of blood (ear nick from the downside ear or coronary band), aspirate from the thoracic cavity, and/or tissue (submaxillary lymph node, spleen) should be air dried, fixed in methanol, and stained with polychrome methylene blue (McFadyean reaction) or Giemsa stain. The bacteria are large bacilli with square ends and a clear or pale halo; bacterial culture can also be performed for confirmation, but cytology will typically provide the quicker preliminary result. If positive, the necropsy should not proceed and the carcass should be burned and buried or buried.[4,5]

Outbreak Investigations

As in all necropsies, a systematic approach is absolutely essential in managing the increased complexity, due to

expanded scope and scale, that is inherent in an outbreak/mortality event. In many countries, local and national plans or other strategies have been developed and are activated in outbreak situations. Familiarity with those that exist in your region is important because valuable resources that aid or are necessary in your response and investigation may be available through these programs. For example, the Incident Command System (ICS) is a management concept that was created to address the demands and complexities inherent in emergency response or natural disaster events. In the United States the ICS is used by many federal agencies, and its adapted use may provide organizational structure to veterinary outbreak and mass mortality event response and investigations. Main components of the ICS are: Unified Command, Operations, Planning, Logistics, and Finance and Administration; subdivision units (e.g., incident commander; safety, information and liaison officers) can be developed depending on the needs for the particular event (see https://training.fema.gov/EMIWeb/IS/ICSResource/index.htm; accessed 16 Feb 2018).

Critical components in mortality events and outbreak investigations include (1) identifying an investigative team and resources, (2) verifying that an outbreak is occurring, (3) establishing a case definition(s) and categorizing cases using results of necropsy examination, histology, ancillary diagnostic test results, etc., to establish the scope of the outbreak, (4) establishing baseline information about the event and disease (e.g., temporospectral, species, gender, age class), (5) examining the descriptive epidemiologic features of the cases to generate hypotheses, (6) testing hypotheses and performing additional analysis as needed, (7) implementing control measures (when possible), and (8) communicating findings and maintaining surveillance.[12,13] For any event, it is also important to quickly determine if a disease (e.g., infectious, toxic) with significant zoonotic or animal health impacts (e.g., anthrax, Ebola) or illegal activities is present in order to engage and work together with the appropriate agencies and authorities in the mortality investigation. It is also important that the investigative team has a broad range of expertise (e.g., epidemiology, population biology, pathology, clinical medicine, genetics, bacteriology, virology, toxicology, and species- and environmental specific expertise) to help guide the investigation. Pathologists often work closely with epidemiologists during outbreak or mass mortality event investigations to construct case definitions, develop hypotheses on the cause of the event, analyze data, recommend ancillary diagnostic testing, and ultimately when making recommendations for control measures or long-term surveillance.

Epidemiologic characteristics of an outbreak, along with historic data and necropsy findings, guide decisions related to first tier diagnostic testing (those deemed most relevant and immediately important). First tier diagnostic tests and histopathology findings often guide further ancillary testing. Approaches to necropsy evaluation and sampling during an outbreak or large-scale mortality event are like those used during routine, systematic, and thorough diagnostic necropsies. Establishment of a sampling and necropsy protocol as soon as possible at the outset of the outbreak or mortality event is crucial to obtaining the best data to aid the investigation. Sampling and necropsy protocols should incorporate appropriate tissue collection for evaluation of not only the first tier differential etiologies but also a broad range of alternative etiologies. Often with large-scale mortality events, it is not practical or logistically possible to examine or sample every carcass in a reasonable postmortem interval. As such, focusing thorough diagnostic efforts on the freshest carcasses, especially those that die earliest in the event, may yield the most reliable data. In addition, plans should be established at the outset of the investigation for carcass management, database and sample management and storage, and sharing of data. It is also valuable during the course of the event to reevaluate the plan in order to make modifications based on what is being learned during the event (e.g., recognition of broader geographic scale or additional affected species) that increase efficiency and investigation outcomes.

Clean-Up and Carcass Disposal

As occurs during a necropsy, PPE should be worn during clean-up and carcass disposal. Blood and residual tissues should be removed from instruments and work surfaces with soap and water, after which they should be disinfected. The best choices for disinfection have broad antimicrobial properties (e.g., 0.5% sodium hypochlorite; 10% bleach, 70% alcohol, borax; see also references [14 and 15] for more information about common disinfectants) and are effective against many common pathogens. Depending on the disinfectant (e.g., bleach), instruments may need to be rinsed after disinfection to prevent corrosion. It should be noted that prions are resistant to a number of common disinfectants and other forms of destruction.[16] Current, best practice protocols for disinfection and carcass management should be obtained and implemented in consultation with governmental agencies for suspected or confirmed cases.

Whether in a laboratory or a field setting, necropsy examinations and disposal of carcass, necropsy waste, sharps, and infectious materials should be performed in a manner that minimizes environmental contamination and exposure of the necropsy team or domestic or wild animals to infectious, toxic, or other disease agents. Efforts should be made to minimize the amount of nonanimal waste (e.g., aprons, masks, gloves, plastic bags) that is generated and burned, buried, or transported. It is important to be aware of and adhere to relevant organizational, local, national, and international regulations and protocols for carcass and medical waste disposal, which may involve consultation with local environmental and health agencies. Options may be limited due to a number of factors, including personal safety, location, size of a carcass(es), environmental conditions, location relative to water sources/catchments/wells etc., ability to safely move/relocate a carcass for disposal generally and within a reasonable timeframe (this may be logistically and politically complex and challenging),

financial limitations, animal welfare and environmental considerations, and willingness/ability/permission of contracting facilities/landfills, etc. to accept the carcass/waste.

Options for carcass, tissue, and waste disposal include incineration/burning and burial, burial, rendering, landfill, fermentation, and biocremation (alkaline hydrolysis), which may be possible on-site or by external contractors, as well as mounding, composting, and natural decay at the site of death/necropsy. A number of detailed descriptions of several of these options are available and include those of national and international governmental and nongovernmental regulatory agencies.[4,5,17] On-site incineration/burning and burial or burial alone may be the most practical option in some cases (e.g., in mass mortality events). In known or suspected anthrax cases, incineration/burning and burial or burial, or other methods that prevent sporulation and destroy the bacteria, should be used.[2] If prion disease is suspected, a 2001 report suggested rendering, incineration, and alkaline hydrolysis (all under certain conditions) as the most reliable technologies at that time for reducing the infectivity of the organism.[4] In addition, remember that pathogens may persist, proliferate, and pose extended human and animal exposure risks in unburned, buried or closed plastic bags.

Sample Collection and Management

Even when the cause of death seems obvious, it is important that interpretations and conclusions drawn during a mortality investigation on an individual or group of animals are informed by all relevant information. This process is sequential and iterative, with gross necropsy typically followed by histology. Both directly affect decisions about ancillary diagnostic tests that are needed to confidently arrive at conclusions and are subsequently performed. In addition to a thorough, systematic necropsy examination, collection of a set of tissues for histology and ancillary diagnostic testing and tissue archiving (Table 30.1) should be a standard in all necropsy examinations. It may not always be possible to collect all of the recommended samples from each animal, but the more consistently these goals can be achieved and reports generated, the greater the chance that we will accurately identify diseases and disease trends. Pathologists are often asked about the minimum set of tissues that should be collected or that are necessary to make a diagnosis. However, in situations in which collection of a complete set of tissues is not possible, a should be collected. This will vary by species and circumstance but should include those tissues that are most likely to be most diagnostically relevant (e.g., common sites of pathogenic infection). In general, these are the major organs and include heart, lung, liver, kidney, spleen, brain/spinal cord, stomach, and small and large intestine. Targeted tissue testing can also be performed for specific suspect diseases (e.g., brain, lung, tracheobronchial lymph nodes for suspected canine distemper testing; liver, kidney, fat, stomach content, spleen, hair, brain for general toxicology screening; urine for domoic acid testing). Collecting a limited set of tissues may provide a diagnosis. However, this may reflect an incomplete picture of the cause and contributing or predisposing factor(s) in death. In a worse-case scenario, this can lead to misinterpretations and false conclusions that drive inappropriate husbandry, medical management, or other animal- or outbreak-related activities and mitigation strategies.

Sample handling and storage for histology and ancillary diagnostic testing are critically important to optimize their diagnostic value and contributions to animal health, management, surveillance, and conservation outcomes (see Table 30.1). With the exception of formalin-fixed tissues, most diagnostic samples need to be refrigerated or frozen to prevent degradation and maintain the viability of pathogens, toxins, etc. if immediate on-site testing is not available, for transport to an external diagnostic laboratory, or when archived. This often includes establishing and maintaining a cold chain, which can be particularly challenging in field settings.[18] This involves appropriate, consistent temperatures using one or a combination of materials and equipment. Storage containers/equipment holding diagnostic samples should not be used for holding food, beverages, or other medical materials such as pharmaceuticals. Basic equipment typically includes refrigerators (4°C ice chests/boxes, ice/gel packs, etc.), freezers (e.g., 0°C, −20°C, −80°C; non "frost free"), and containers appropriate for standard and ultralow storage (e.g., cryovials). Other options include dry ice and liquid nitrogen (LN_2) (e.g., dewars [vacuum flasks], dry shippers [vapor shippers]). Use of and handling samples stored at ultralow temperatures should be carried out with extreme caution because exposure can cause severe burns. In additional, dry ice and LN_2 vapor can cause asphyxiation and can be explosive so should be used only in ventilated spaces or containers; handling should be limited to trained personnel. For electrical equipment, a consistent source of power is an absolute necessity. In all settings, backup sources of electricity, often in the form of a generator, are essential. In less-developed settings, multiple generators may serve as both the primary and backup power sources. Scheduled monitoring and documentation of temperatures should be standard operating procedures to ensure consistent and continual storage temperatures. Whenever possible, samples should be immediately stored and frozen samples handled in ways that minimize freeze-thaw cycles.

Two significant sample management challenges are space and sample curation. With proper storage, tissues are a valuable diagnostic resource for many years. Managing space, cost, power needs, ventilation, duration of research projects, chain of custody, and other aspects of short- and long-term management of samples all need to be considered and strategies developed. Plans and protocols for the curation of sample storage and movement (e.g., for diagnostic testing, archiving, to fulfill research requests) must also be developed and implemented. Sample information has historically been maintained in paper logs; however, most people, even in remote locations, have access to computers and use electronic databases or software, which need to be

TABLE 30.1 Diagnostic Tests and Recommended Sample Collection, Handling, and Storage

Diagnostic Tests	Sample Collection	Sample Handling	Sample Storage
HISTOLOGY[‡]: The microscopic examination of tissue to study the manifestation of disease. It is a valuable adjunct to gross observation because many diseases may look similar macroscopically.	• Collect samples from all organs and any tissue that looks abnormal (relative to adjacent tissue) • Collect a representative sample from each part of the organ/lesion • Limit sample thickness to 0.5 cm (1 cm^3) for good fixation • For abnormal tissue, collect junction between normal and abnormal • Label important items or samples that came from a specific location • All samples from a necropsy may be placed in a single container	• Place tissue and 10% NBF in leak-proof container • Need 1 part tissue to 10 parts formalin for good fixation	• Short term: room temperature • Long term: room temperature DO NOT FREEZE
ELECTRON MICROSCOPY[§]: Higher magnification to resolve pathogens and tissue structure than may be achieved with routine light microscopy. Options include transmission, scanning, or negative stain electron microscopy.	• Tissue should be no greater than 1 mm^3 • Common fixatives include glutaraldehyde or osmium tetroxide	• Place tissue into fixative (1 part tissue to 10 part fixative) in leak-proof container	• Short term: refrigerate • Long term: refrigerate DO NOT FREEZE
CYTOLOGY: The microscopic examination of fluids and cells. It may be performed immediately and aids in making a preliminary diagnosis. Cytologic review may occur locally or at a distant laboratory.	• Fluids: direct smear of fluid in thin film onto slide OR spin sample to make cell pellet (cell poor sample) that is then smeared onto a slide • Tissues: touch prep: gently blot on a paper towel to remove blood and then gently touch tissue to slide surface • Tissues: crush prep: collect small tissue sample onto slide; cover with coverslip/cover glass; flatten tissue	• Fluids, touch prep: Air dry, fix (methanol), stain (e.g., Diff-Quik®, Gram, Wright Giemsa, acid-fast) • Crush prep: immediately directly view unstained tissue/cells	• Short term: clean, sturdy, crush-proof, bug/vermin-proof slide box or container • Long term: clean, sturdy, crush-proof, bug/vermin-proof slide drawer DO NOT EXPOSE AIR-DRIED SLIDES TO FORMALIN
MICROBIOLOGY: The study of microorganisms, including bacteria and fungi. Typical tests include bacterial or fungal culture or PCR. The most commonly sampled sites are obvious lesions (e.g., abscess) or the intestinal tract.	• Clean and sterilize sampling site (e.g., alcohol, heated blade) • Use sterile instruments/techniques; minimizes risk of contamination • Instruments: swab, needle + syringe, scalpel blade, skin punch, trochar, etc.	• Place the sample/swab in appropriate medium for the desired test (e.g., bacterial culture media for aerobic or anaerobic bacteria bacterial culture)	• Short term (<1 week): room temperature OR refrigeration • Long term (>1 week): –80°C, liquid nitrogen (but may kill target pathogens) DO NOT FREEZE BLOOD OR SAMPLES FOR VIBRIO, LEPTO CULTURE
VIROLOGY: The study of viruses. Typical tests include viral culture or PCR. Commonly sampled items are lung, liver, spleen, lymph nodes, intestinal tract/feces, brain.	• Clean and/or sterilize sampling site (e.g., alcohol, heated blade) • Use sterile instruments/techniques; minimizes risk of contamination • Instruments: swab, needle + syringe, scalpel blade, skin punch, trochar	• Place swab/tissue in VTM • No transport media, no refrigeration available: place 1 part tissue: 5–10 parts 50% buffered glycerine	• Short term (<48 h): refrigerate • Long term: fresh tissue, VTM: –80°C or liquid nitrogen DO NOT EXPOSE SAMPLES TO DRY ICE REFRIGERANT

TABLE 30.1 Diagnostic Tests and Recommended Sample Collection, Handling, and Storage—cont'd

Diagnostic Tests	Sample Collection	Sample Handling	Sample Storage
PARASITOLOGY: The study of parasites and parasitism. Depending on the species, a wide variety of external (ectoparasites) and internal parasites (endoparasites) may be present. Collection allows for parasite identification and documentation. Some parasites also harbor infectious agents like bacteria and viruses and may be used for PCR analysis or virus isolation.	• Ectoparasites: Collect grossly visible parasites. Swab or scrape ears or skin for small parasites (may not be grossly visible) and smear material as a thin film onto a slide • Endoparasites: Collect grossly visible parasites. Collect fresh feces in a clean container or bag	• Place grossly visible parasites in 70% alcohol (ethanol) or formalin for identification • Examine scrapes under oil immersion microscopy immediately • Examine feces using standard procedures (e.g., gross appearance, direct smear, fecal floatation [for metazoan parasites, glycerin sedimentation for ameba])	• Short term: Fresh feces: refrigerate • Short and long term: Visible parasites: 70% ethyl alcohol or formalin; room temperature

TOXICOLOGY:
Toxins are important agents to consider, particularly in the acute death of an animal or group of animals with few clinical signs. Toxins may be natural (e.g., poisonous plants, algae), agricultural (e.g., herbicides, pesticides, rodenticides), or industrial (e.g., lead, arsenic, waste from mining). Because many toxins are unstable and degrade with time, rapid testing or appropriate handling and short and long-term storage are essential.
	• A large volume of fresh tissue is needed (50–500 g of sample)
	• Tissues useful in a general toxin screen:
	  • Liver
	  • Kidney
	  • Brain
	  • Stomach or intestinal content
	  • Fat
	  • Eye (aqueous humor)
	  • Blood
	  • Urine
	  • Hair, flight feather
		• Place tissues in clean, leak-proof containers with no chemical preservatives
		• Note: aluminum and plastic may leach and interfere with tests, so collection of two sets of tissues in different container types is recommended when possible
		• Filter paper (e.g., Whatman 903) for heavy metal analysis
			• Refrigeration or freezing for transport is essential
			• Long term: –20°C, –80°C, liquid nitrogen

MOLECULAR DIAGNOSTICS: Typically performed for genetic or pathogen testing or discovery. Target tissues usually include: lesions and/or liver, kidney, lung, spleen, brain, pancreas, gonads, lymph nodes, conjunctiva, mucocutaneous junctions of the oral cavity or genital tract, fluids/secretions, hair or skin.	• Small sample volume needed (1 g or less of tissue, 0.1 mL of blood or a clean dry swab) • Sample site should be clean and sterile • Wear gloves; use sterile instruments and techniques to collect samples and minimize risk of contamination • Instruments typically used for sampling: swab, needle and syringe, scalpel blade, skin punch, trochar	• Place sample in sterile container • Options: fresh frozen; VTM, RNAlater®, RNAlater-ICE, ethanol, TRIzol® (check preference of your lab, research project for others) • Blood or fluids: may be dried on a piece of filter paper (e.g., Whatman FTA for DNA-based infectious disease; Whatman 903 for proteins)	• Short term: fresh frozen: –80°C; RNAlater®: (<1 day) room temperature (37°C), (<1 week) refrigeration (4°C), (>1 week) –80°C; filter paper (<1 m) room temperature • Long term: fresh frozen, VTM, RNAlater®, filter paper: –80°C

*This list includes several common test categories and general recommendations for sample collection, handling, and storage. Specific best practice protocols should be consulted whenever possible, in development of your in-house standard operating procedures for sample management.
†Recommended for labeling is pencil for glass slides, permanent marker or other appropriate inks for bags, tags, and labels that are resistant to alcohol, formalin, other solvents, refrigeration, ultralow temperatures (e.g., –20°C, –80°C, liquid nitrogen) routinely used in necropsy or cytologic procedures or tissue storage/handling/transport. It is also useful, in addition to labeling the container/bag, to place a labeled tag (labeled ink) in the container; cut pieces of Tyvek® may be used.
‡Tissues collected for histology are typically placed in 10% neutral buffered formalin (NBF). For NBF and tissues placed in other fixatives, it is critical to maintain a minimum ratio of 1 part tissue to 10 parts fixative to ensure proper fixation. Salinity and pH of fixative are important considerations for marine invertebrates. For invertebrates, 10% formalin or sea-water formalin are adequate fixatives in most cases. Specialized fixatives are preferred for some species such as zinc-formalin (Z-fix) for corals and Davidson solution for soft-bodied invertebrates (cnidaria). Decalcification can cause significant artifactual changes in the heavily mineralized tissues of some species, particularly corals and echinoderms. If the structure of the skeleton of these animals is critical for evaluation, the pathologist should consider submitting tissues for processing in a mineralized state (such as through plastic embedded and sectioning at a bone pathology laboratory) or a slow method of decalcification should be chosen (using the acetic acid in Davidson solution or pH-balanced EDTA). Enrobing the delicate tissues of coral species in alginate prior to decalcification has proven an excellent method for protecting the delicate surface polyps while retaining skeletal structural features. Formic acid and hydrochloric acid may be used for decalcification, but tissue disruption through gas bubble formation should be recognized and not interpreted as a lesion. For species with a chitin-based ectoskeleton, Pyreni solution may be used to soften the chitin and provide soft supple tissue for sectioning.
§Recipes for marine invertebrate specific electron microscopy fixative are recommended.
NBF, Neutral buffered formalin; *VTM*, viral transport medium.

regularly updated and backed up to prevent catastrophic data loss. In some situations, for example, large active sample repositories or zoo collections with large sample inventories, dedicated staff may be needed to curate and manage sample acquisition and disposition information and databases, as well as sample retrieval and shipment. Archived inventories should be reviewed every 6 months to update additions and remove tissues that become redundant.

Most facilities do not have the in-house resources or expertise to perform the broad range of tests that are run as components of a complete necropsy/postmortem examination. Shipment of samples to external laboratories is therefore a requisite of many necropsies. All sample shipments must comply with all local, state/provincial, federal/national, and international laws and regulations to ensure human and animal safety and sample viability. Special packaging, handling, and labeling may be required (e.g., for shipments containing dry ice or LN_2). For shipment to local laboratories, contacting the lab to discuss specific package labeling, submission forms, test requests, and minimum sample volumes is of value. For international shipments, export and import permits to ship samples and compliance with treaties and regulations concerned with the international movement of samples from endangered or managed species (e.g., Convention on International Trade in Endangered Species of Wild Fauna and Flora [CITES], US National Oceanic and Atmospheric Administration [NOAA for marine mammals], CDC [primates, rodents, civets, bats]) may also be required (see Chapter 4). The regulations and paperwork differ in every country and can be quite complicated, and processing can be excruciatingly time consuming. However, it is essential to understand all steps in the process, be in compliance, and pay attention to all of the many details. Failure to do so knowingly or unknowingly may result in significant fines, import/export permit refusal, confiscation and return of samples to their country of origin, or confiscation and destruction of samples by border patrol agents. Because of the latter, it is often a good idea, whenever possible, to maintain a duplicate set of samples at your facility as an assurance against problems that might occur during the shipping process. In addition, many diagnostic labs will discard tissues, glass slides, or paraffin blocks after a certain retention time, and many claim ownership of all submitted specimens (which they may or may not have the legal authority to claim). Written agreements with laboratories may be required to ensure return, retention, or ownership of your samples.

Data Management and Sharing

All historical data, pathology reports, ancillary diagnostic test results, photographic documentation, and sample archiving and curation must be organized and managed. In many settings the records exist as/in a combination of paper and electronic documents and databases. However, technologic advances will likely push all activities related to documentation, even that done in the field, onto electronic platforms in the not too distant future; robust systems for secure data storage and backups will therefore continue to be a necessity. These databases contain a treasure trove of baseline and disease-related information and available samples, all of which are hugely valuable in retrospective and prospective projects focused on wildlife ecology, health monitoring and disease surveillance, and conservation projects in zoos and other managed and free-range settings. Shared access to these valuable resources should be the rule rather than the exception (e.g., collaborative research projects, biomaterials requests, contributions to SSP/EEP and TAG programs). Whether retrospective or prospective, inclusion and participation of wildlife veterinary pathologists, who can appropriately interpret and contextualize data to prevent misinterpretation (a common problem in data review by nonpathologists) and contribute to and guide the development of protocols that involve necropsy and sample collection from free-ranging wildlife or zoo animals, are essential.

Communication

Communication during a necropsy or active mortality investigation and after are critical, both practically and politically. Communications should be directly coordinated first with managers and/or managing authorities (institutional, local, national, etc.) before being more widely disseminated. Information is generally of interest and should be shared regularly and transparently with stakeholders (e.g., clinical veterinary staff, keepers, curatorial staff, collaborators, indigenous farmers, public) as results become available and interpretations and conclusions are drawn. Immediate communication of test results is also specifically important for medical treatment and/or mitigation of disease in contact with wild or domestic animals, staff, or others (e.g., public health networks) and for directing additional ancillary diagnostic testing. As mentioned previously, suspicion or confirmation of listed or reportable diseases must be immediately shared with appropriate local, national, and international authorities (see above), who may need to participate in disease management. The format for the communication varies depending on need and may include sharing of complete or summary pathology reports; peer-reviewed publications; presentations at professional conferences or community meetings; development or iterative revision of protocols, practical technical manuals, and other teaching materials; or other forms of communication with the media, including interviews and articles in the lay press. For high-profile cases or outbreaks with high public interest, it may be of value to identify a communications lead or team to field questions and provide comments that are informed by the pathologist and necropsy team. Creating a list of key talking points to be used by the communications team is beneficial to guide messaging, especially when conveying complex scientific information to the public. Care should also be taken to communicate only confirmed data or to qualify all results as preliminary until they are confirmed.

Acknowledgments

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31

Use of Computed Tomography/ Magnetic Resonance Imaging in Zoological Medicine

MICHAEL J. ADKESSON AND MARINA IVANČIĆ

Introduction

The value of cross-sectional imaging (computed tomography [CT] and magnetic resonance imaging [MRI]) in zoological medicine was quickly recognized following the first emergence of the technology in the 1970s, but widespread application was restricted due to cost and equipment availability.[1] Currently, CT and MRI scanners are installed in veterinary facilities around the globe, providing many zoos with ready access. As the technology continues to rapidly evolve, imaging once reserved only for complex, high-profile cases has now become standard, routine care in some zoological facilities.

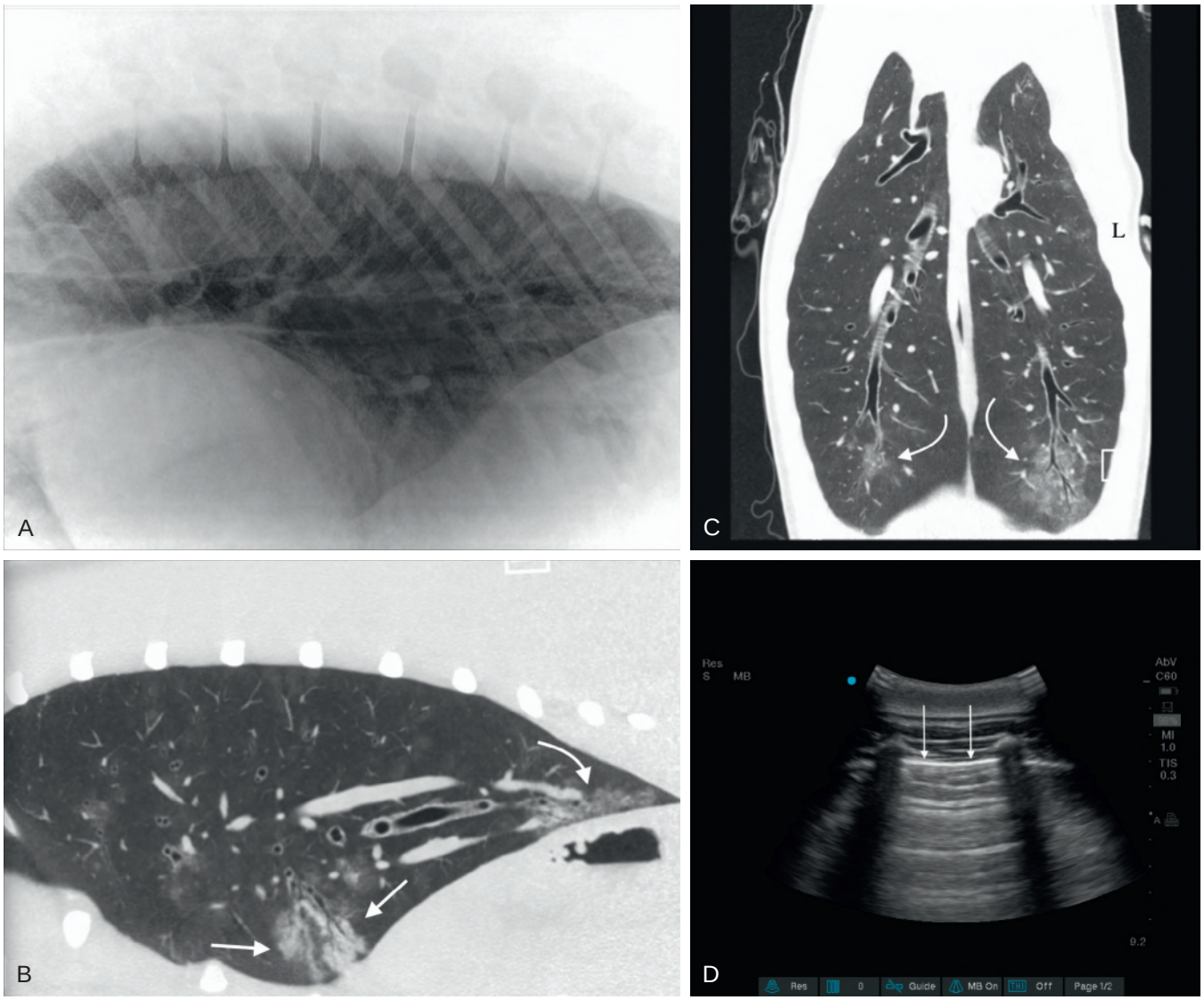
Entire textbooks are devoted to the veterinary applications of CT and MRI, and the reader is directed to these sources, as well as theoretical medical imaging texts, for discussion of the underlying physics and principles of image acquisition, image interpretation, minimization of artifacts, and advanced applications.[2–5] Technologic evolution is rapid, and readers are also referred to other sources to best assess current equipment options. The aim of this chapter is to provide an overview of the value of cross-sectional imaging in nondomestic species and the optimization of image acquisition in a zoo setting. Discussion is heavily focused on CT because its use in zoological medicine is far more widespread (see also Chapter 32).

Benefits of Cross-sectional Imaging in Zoological Medicine

Cross-sectional imaging is by definition superior to radiography due to the elimination of superimposition artifact (i.e., three-dimensional [3D] data of a 3D patient, instead of 2D data of a 3D patient). It is not susceptible to critical ultrasonography artifacts such as shadowing, which obliterates an operator's ability to see tissues deep to bone, the surface of the lung, and gas within the gastrointestinal tract. Modern CT is also best suited to accommodate large patient sizes because CT x-ray photons are less attenuated by large patient tissues than ultrasound waves, resulting in less deterioration of image quality while maintaining excellent spatial resolution.

The superiority of CT over radiographs and ultrasound for detection and characterization of innumerable conditions is well established in domestic animals. For instance, the diagnostic value of CT in tumor and metastasis detection is broadly recognized,[6] and CT broadly allows for characterization of orthopedic lesions that are otherwise undetectable. It also allows superior assessment of soft tissue and bony structures in the sinuses, pulmonary parenchyma, abdominal viscera, vasculature, and lymph nodes, among many others. Studies specifically characterizing the superiority of CT diagnostic sensitivity in nondomestic animals are limited, but extrapolation is valid. In birds, CT has been shown to be superior to radiographs for detection of the early stages of upper airway disease and other soft tissue disorders of the head.[7] Radiographic evaluation of birds in many zoos is performed on a regular basis as a screening tool for aspergillosis, but mild abnormalities may be detectable only with CT.[8,9] Similarly, pulmonary parenchymal lesions in cetaceans that are silent on both ultrasonographic and radiographic evaluation can be detectable on CT (Fig. 31.1).[10,11]

Radiographs and abdominal ultrasound are commonly performed in zoos as part of preventative medical examinations, and an additive integration of CT into such routine practice offers many benefits for preventative care. For example, traditional three-view radiographs in great apes present many positioning challenges under anesthesia. Their size results in marginal diagnostic quality for fine scale lesion assessment and often requires hemithoracic views. In contrast, CT imaging provides a comprehensive study that allows detection of pulmonary lesions undetectable

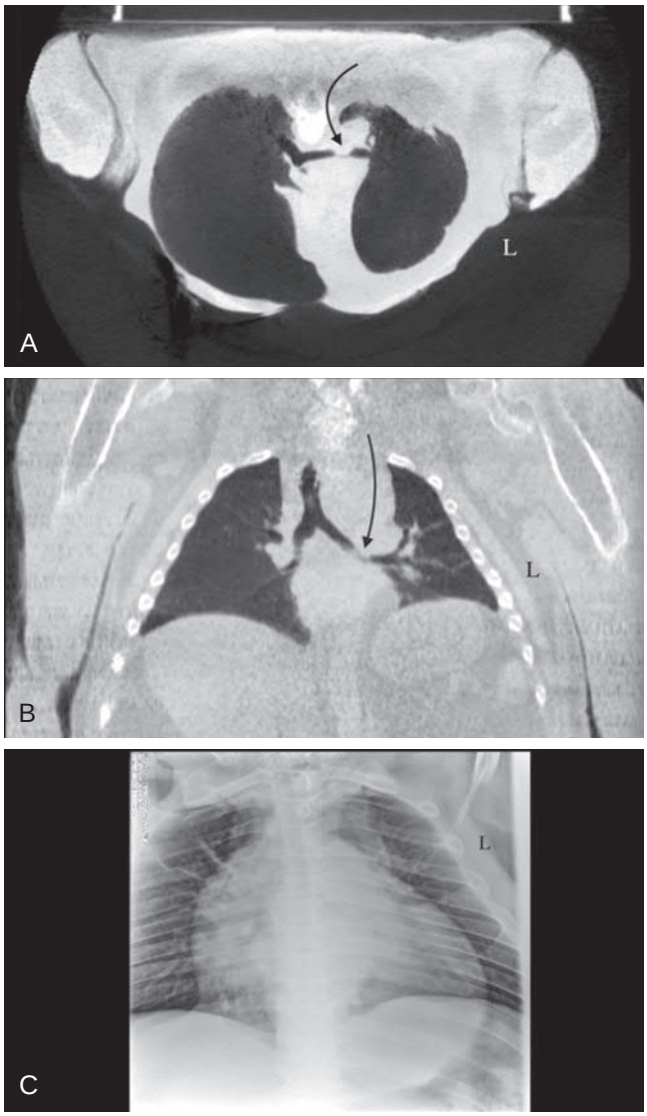


• **Figure 31.1** Computed tomography (CT) provides superior detection of many diseases in zoo species. Shown are a lateral thoracic radiograph (A) and a parasagittal CT reconstruction (B) of a bottlenose dolphin. Note the CT projection reveals sites of peribronchiolar consolidation *(arrows)* not detectable radiographically. Also shown is a coronal reconstruction (C) demonstrating central peribronchiolar lesions and foci of ground-glass opacification *(arrows)* not visible on ultrasonographic evaluation of the normal peripheral lung (D).

radiographically (Fig. 31.2), superior detection of sinusitis and air sacculitis,[12] and complete assessment of degenerative changes in spinal and thoracic joints.[13]

In many species, anatomic structures limit the value of other diagnostic imaging modalities. Protective features (e.g., plates, shells, scales, quills) are superimposed over internal structures on radiographic images, drastically decreasing diagnostic utility. These same features can also render ultrasonography impractical. Cross-sectional modalities are generally not impeded by these features, and they can be removed from view in a digital environment. The use of CT is widely recognized as a best practice for imaging chelonians and respiratory disease in reptiles.[9,14] CT provides great value in assessing general health of species with anatomy that precludes easy palpation. For instance, it has proven valuable for objective body condition assessment and establishment of weight goals by monitoring adipose stores (Fig. 31.3).

Cross-sectional imaging allows unparalleled assessment of dentition and is of particular value in species with a narrow oral gape, such as aardvarks *(Orycteropus afer)* and macropods. Integration of CT into examinations in these species allows for better preventative care through earlier detection of periodontal disease and superior monitoring of response to treatment.[15] CT provides unobstructed views of the jaw and tooth roots to allow lesion localization (Fig. 31.4) and precise serial monitoring. Integration of CT into preventative healthcare for such species has been greatly beneficial. The authors have also found value in using CT to localize foramina for dental nerve blocks



• **Figure 31.2** Computed tomography (CT) provides superior diagnostic capabilities in preventative medical care. Axial MinIP (minimum intensity projection) (A) and coronal CT reconstruction (B) from a preventative medical examination on a gorilla reveal external compression and narrowing of the left mainstem bronchus (*curved black arrows*) and hypoinflation of the left lung. These signify important considerations for patient positioning and anesthesia. A ventrodorsal radiograph (C) fails to diagnose bronchial constriction. Radiographs also require acquisition of multiple hemithoracic views due to patient size.

and for measuring canal depth in endodontic repairs (see Fig. 31.4).

The large size of many zoo species can restrict imaging success with ultrasound (limited penetration capability) and radiography (practical and technologic limits). Advancements in human bariatric medicine have resulted in greater availability of CT scanners with a large gantry bore (≥90 cm) and robust table design (≥300 kg) that offer immense versatility for large zoo species (Fig. 31.5).

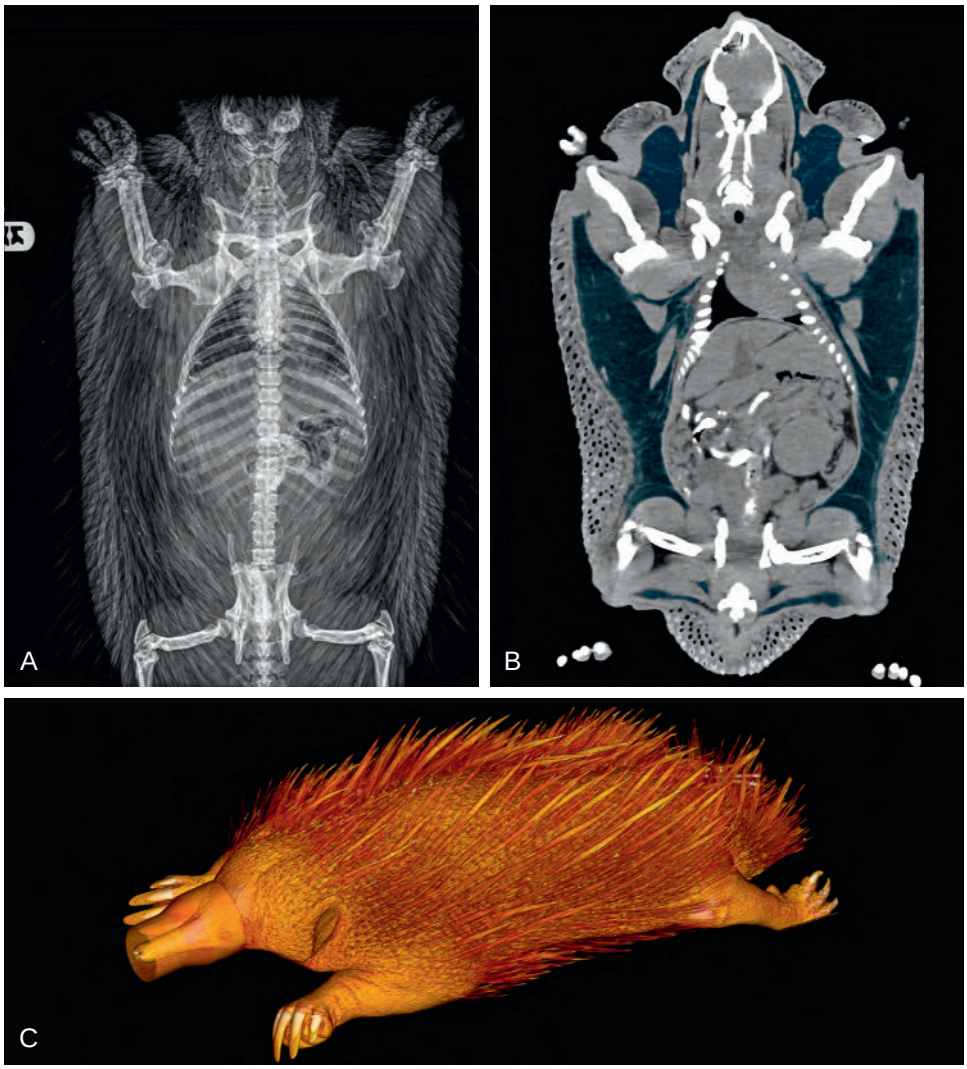
Three-dimensional renderings created from cross-sectional data provide further value in assessing species that lack thorough anatomic descriptions and medical illustrations. The manipulation of 3D images in virtual space is an excellent tool for procedure planning and education. Assessment of 3D vasculature can facilitate surgical precision, allowing minimally invasive techniques that are of tremendous value for species with challenging postoperative care. The advancement of 3D printing adds the ability to create customized equipment (e.g., custom-sized orthopedic implants) and to practice procedures on realistic models. Software programs can also create extremely valuable virtual endoscopic guides. For instance, creating a virtual "roadmap" of a cetacean's respiratory tract to a precise location for bronchoalveolar lavage has proven tremendously useful when speed is paramount (Fig. 31.6).

Equipment Considerations

Equipment Access

In the past, access to CT and MRI equipment posed a challenge to many zoos. Currently, arrangements with veterinary referral clinics, veterinary colleges, and human imaging centers often provide zoo clinicians with access to cross-sectional imaging, but routine use at an off-site facility remains challenging. Dangerous and large animals must be maintained under anesthesia during transport, increasing anesthetic risk for the patient due to prolonged procedure time and inability to maintain an ideal anesthetic environment in a moving vehicle. Human safety considerations also exist when transporting dangerous zoo animals off-site into less-controlled environments. Logistical problems may exist when maneuvering large animals through facilities designed for small animals or people, and scheduling considerations often necessitate "after hours" use. Even when animals can be placed in a crate and anesthetized off-site, having access to appropriate emergency equipment and staff can be challenging. Bringing zoo animals into a veterinary facility in proximate contact with companion animals creates infectious disease risks. Use of human facilities leads to both veterinary concerns of anthropozoonoses and liability concerns related to zoonotic diseases. Public relations and privacy considerations must be considered before transporting zoo animals, and the expenses associated with facility use and transportation may be considerable.

The immense value of on-site equipment at a zoo cannot be understated. Safety and collection health considerations are dramatically decreased. Time under anesthesia is minimized to the extent that a full body multidetector CT scan can be performed more quickly than a radiographic study. New concerns noted during an exam can be addressed immediately with advanced imaging without the need for an additional anesthetic event. Complete imaging studies can be performed during preventative healthcare exams, increasing early detection rates. Considerations of cost per case are eliminated, encouraging frequent, routine use that results in higher-quality medicine and a new standard of care. The controlled zoo environment also facilitates the ability to perform interventional CT procedures that enhance diagnostics and treatment. In-house equipment



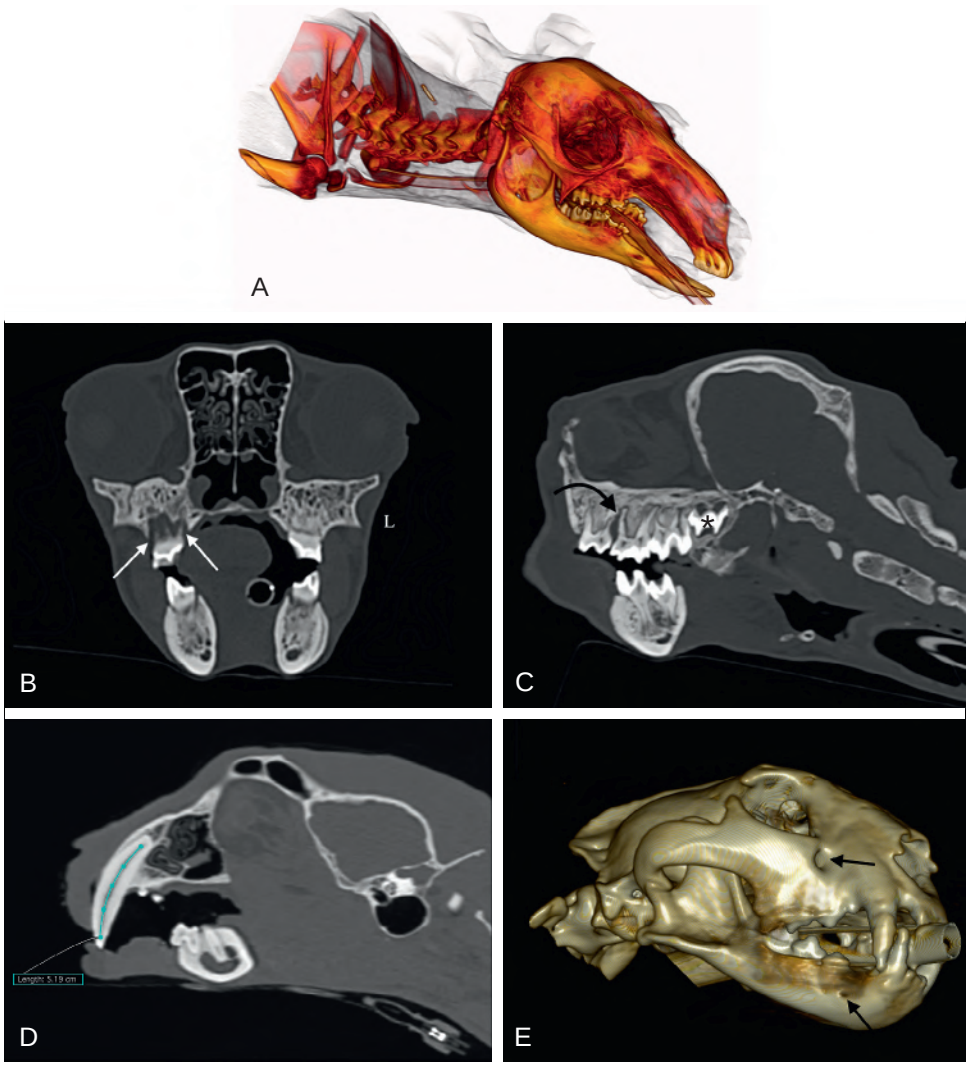
• **Figure 31.3** Computed tomography (CT) provides versatility in imaging patients with protective outer coverings. A ventrodorsal radiograph (A) of a short-beaked echidna provides limited diagnostic information due to superimposition of quills. A coronal soft tissue CT reconstruction (B) of the same animal reveals superior detail. Body condition can also be evaluated using CT to quantify adipose stores *(shaded blue)*; this provides more accurate characterization than radiographs or visual examination (represented by a three-dimensional reconstruction of the animal [C]).

allows clinicians to easily maintain advanced anesthetic monitoring equipment, intravenous access, patient thermoregulatory support, and ventilatory support (Fig. 31.7).

Selection of Computed Tomography Equipment

The investment required for a scanner is substantial and remains the principal limitation to the widespread application of CT in zoological medicine. Installation into an existing facility may require upgrades to electrical utilities, surge suppression equipment, ventilation modifications for adequate cooling, and installation of lead-lined drywall and windows. Special care should be taken to ensure adequate floor space is available for maneuvering gurneys with large patients, anesthesia equipment, and interventional supplies. If building infrastructure is designed appropriately, future upgrades to newer CT scanners incur relatively little installation cost. As technology advances, refurbished equipment is broadly available at steeply reduced prices. Many zoos also have broad community support, which may assist in securing a CT scanner from a local hospital upgrading to a newer unit.

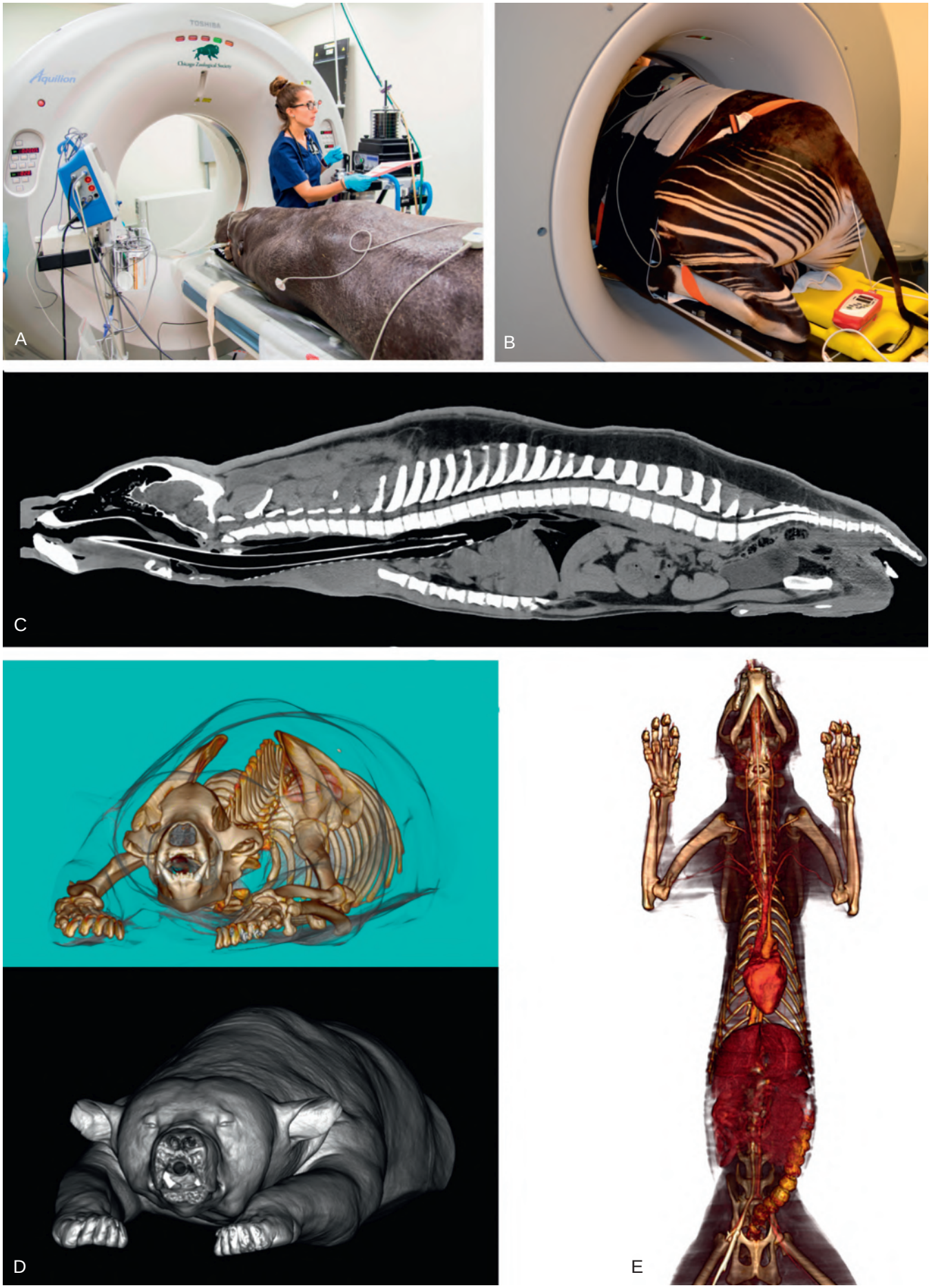
Preventative maintenance and repairs represent considerable ongoing costs. A service contract can help to keep these annual costs consistent and avoids unexpected fluctuation in operating budgets. Facilities must also be willing to invest appropriately in staff training to ensure proficiency in CT operation and rudimentary image assessment. Human CT equipment is generally operated by registered CT technicians, but most veterinary facilities use veterinary technicians with advanced training. Veterinarians must become familiar enough with CT principles to articulate their study goals and corresponding scan parameters.



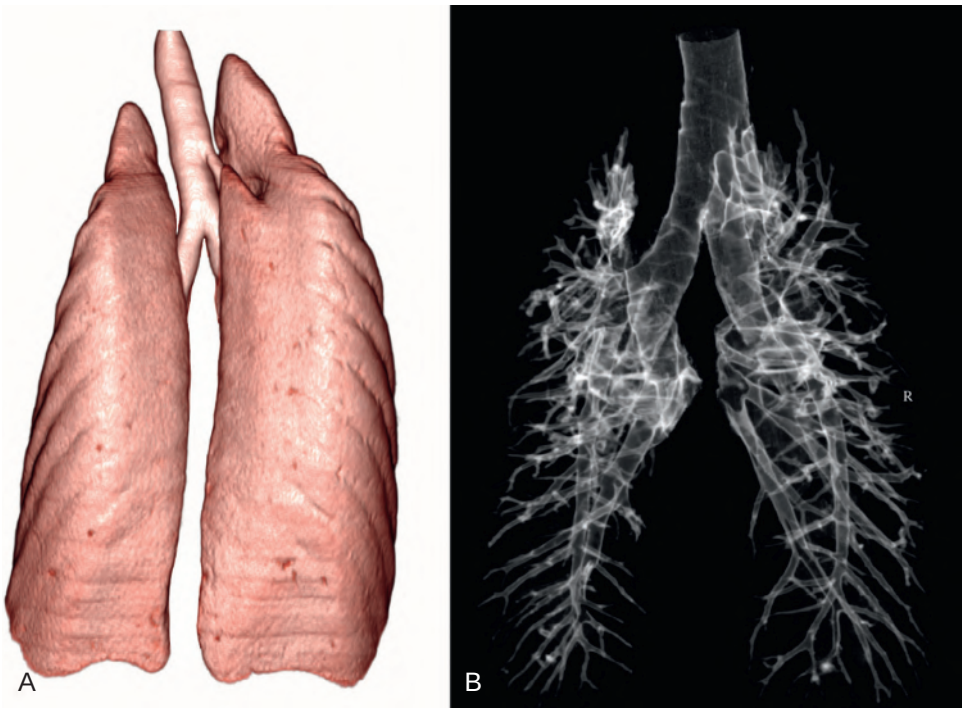
• **Figure 31.4** Dental evaluation with computed tomography (CT). Three-dimensional (3D) (A), axial (B), and parasagittal (C) CT images of a western grey kangaroo illustrate the value of CT for evaluating dentition in species with a narrow oral gape. Note the severe focal lysis of root apices and alveolar bone of this right maxillary molar (*arrows* in B, C). A normal unerupted molar is also seen *(*)*. Sagittal (D) and 3D (E) CT reconstructions of the teeth of an Amur leopard illustrate the value of CT in efficient endodontic repair in large animals by providing precise pulp cavity measurements and foraminal locations for nerve blocks *(arrow)*.

Conventional (fan-beam) multidetector CT operates by simultaneously scanning multiple slices, thereby improving image quality (spatial resolution) and reducing scan time. Scanners are routinely referred to by their number of detector arrays (e.g., 64-slice CT) and the smallest achievable slice thickness (e.g., 0.5 mm). Dual-source CT is a generational technology step that combines two x-ray sources and detectors that rotate simultaneously to further improve resolution and speed, while decreasing radiation dose. Such advanced machines (>320 slice, 0.3 mm) allow imaging of the entire human heart within one beat and other specialized applications. Although not yet broadly available in veterinary medicine, such technology will become increasingly accessible to zoos over time.

Commonly used in human maxillofacial imaging, cone beam CT (CBCT) has emerged in small animal and equine veterinary medicine over the past decade, driven by its lower cost and installation ease. CBCT operates on standard electrical supply and requires fewer safety considerations due to lower radiation output. Although superior to traditional radiography with regards to lack of superimposition, CBCT has numerous drawbacks compared with conventional CT. CBCT studies are more sensitive to patient motion, have poor soft tissue contrast resolution, and are prone to windmill streaking artifact (Fig. 31.8).[16,17] Given the intended use in human medicine for small structures of the head, these artifacts are amplified in larger veterinary patients. Furthermore, tissue attenuation cannot be quantified reliably in Hounsfield units with CBCT, leading to significant restrictions in diagnostic interpretation relative to conventional CT.[18]



• **Figure 31.5** Bariatric computed tomography (CT) scanners offer enhanced diagnostic capabilities for large zoo species. (A) Pygmy hippo CT scan. (B) Okapi pelvic CT scan. (C) Sagittal soft tissue CT reconstruction of a sloth bear. Three-dimensional CT volume renderings of a sloth bear (D) and an Amur leopard (post contrast) (E).



• **Figure 31.6** Thoracic three-dimensional volume renderings of a bottlenose dolphin; dorsal view. (A) Lung surface. (B) Bronchial tree with digital excision of pulmonary tissue. The CT data enable virtual endoscopy in a digital environment to create an efficient endoscopic route to a precise location in the live animal for sample collection. ([A], Ivančić M, Solano M, Smith CR: Computed tomography and cross-sectional anatomy of the thorax of the liver bottlenose dolphin *(Tursiops truncatus)*. *Anat Rec* 297:901–915, 2014.)

• **Figure 31.7** On-site installation of a computed tomography scanner at a zoo provides a safe, controlled environment that allows easy use of multiparameter anesthetic monitoring, ventilatory support, intravenous fluids, and thermoregulatory support.

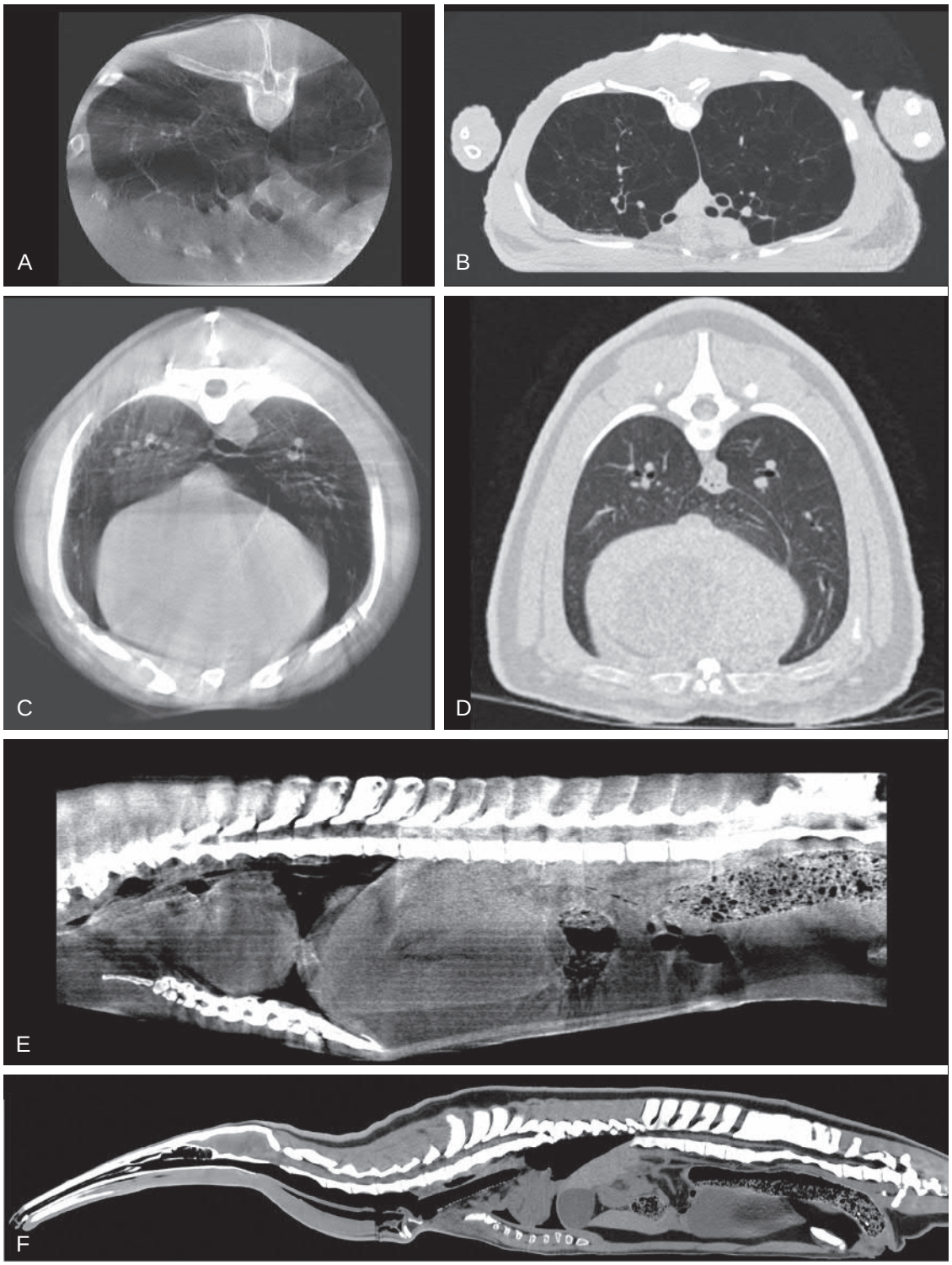
Review of Cross-sectional Images

Data Management

Digital Imaging and Communications in Medicine (DICOM) is a medical diagnostic imaging standard that refers to the handling, storing, printing, and transmitting of information. It includes a file format and network protocol that enables medical imaging devices (hardware, servers, and workstations) from multiple manufacturers to communicate. CT and MRI images are stored as DICOM files in a picture archiving and communication system (PACS) database, alongside images acquired by other modalities. A PACS network generally consists of a central server that stores the local database and allows multiple local and remote users to retrieve and display images using medical imaging software.

Communication between veterinary and information technology staff is vital to ensuring proper data storage. Development of a proper PACS database requires meticulous loading of all scan data and standardized patient data entry across modalities to facilitate data retrieval. Manual editing of PACS metadata (patient and scan parameter data) is generally required for scans performed off-site to ensure proper database integration. The PACS database is a vital part of medical record management, and a redundant local or cloud-based copy should be maintained. The appropriate development of robust, high-speed network infrastructure is also needed for the efficient transfer and storage of large cross-sectional studies.

Raw data are stored on the CT scanner for a period of time and are gradually overwritten with new data. This raw data can be used to create retrospective reconstructions that minimize artifacts, change algorithms, collimate display field of view (DFOV), and perform a variety of other manipulations. Timely review of images by a radiologist is important to ensure reconstructions are of diagnostic quality and no postcapture data manipulation is needed.



• **Figure 31.8** Cone beam computed tomography (CBCT) compared with conventional CT (CCT) in large zoo species. (A) Axial CBCT image of the lungs of an American alligator. (B) Axial CCT image of the lungs of an Orinoco crocodile. (C, E) Axial and sagittal CBCT reconstructions of a giant anteater in lung and soft tissue reconstruction algorithms. (D, F) Axial and sagittal CCT reconstructions of a giant anteater in comparable reconstruction algorithms. Note that CBCT provides a limited field of view and poor soft tissue contrast resolution compared with CCT and is prone to motion and windmill streaking artifacts (visible as radiating and semicircular lines in [C], and horizontal lines in [E]).

Radiology Expertise

Review of cross-sectional imaging requires time and a disciplined, systematic approach because the volume of data can quickly become overwhelming. A detailed whole body scan in multiple reconstruction algorithms, before and after iodinated contrast administration, can easily generate more than 10,000 images. Thorough review of cross-sectional imaging requires more advanced medical imaging software (e.g., 64-bit OsiriX) than that needed for radiograph review.

Such software should at minimum allow for the generation of multiplanar reformats (sagittal and coronal planes), minimum/maximum intensity projections, synced review of multiple axial series, and 3D reconstruction.

Expertise in image interpretation is critical to proper diagnosis. As medical knowledge continues to grow exponentially, veterinarians cannot be experts in all fields. In much the same way most clinical veterinarians are not adequately trained to evaluate histopathology, most veterinarians are not adequately trained to form detailed diagnoses from cross-sectional imaging. The diagnostic knowledge and expertise a veterinary radiologist provides cannot be understated. A radiologist's expertise is reliant on familiarity with the anatomy of a given species. Medical radiologists may be of great value in interpretation of images from an ape, but their expertise diminishes in other taxonomic clades. Veterinary radiologists possess a wider breadth of expertise, particularly in species with a close domestic animal counterpart. Normal anatomic structures, such as the avian glycogen body, can be easily misinterpreted as pathologic lesions by radiologists unfamiliar with a given species. Radiologists with specialized expertise in nondomestics are highly desirable within zoological medicine and provide tremendous clinical guidance.

Optimization of Computed Tomography for Zoological Applications

Procedure Planning

Advanced imaging technology necessitates a degree of technical knowledge and prescan planning far more complex than radiography or ultrasonography. The added logistical challenges posed by anesthetized zoo patients demand maximal preparation, coordination, efficiency, and expertise. By having practical knowledge of CT acquisition parameters, clinicians can greatly enhance the diagnostic quality of a study. A mutual understanding of the primary clinical concern by both the clinician and radiologist is critically important for appropriate planning.

Patient Positioning and Anesthesia

With large patients, positioning on the CT table is paramount to ensure unimpeded movement through the gantry. Weight limits on the cantilevered portion of the table may necessitate splitting a study and craniocaudally rotating the patient between scans. In such instances, scanned regions should overlap to avoid data loss. The anatomic region of interest (ROI) should be horizontally and vertically centered in the scan field of view (SFOV), but vertical centering may not be possible in large patients, depending on bore size. In zoo species with limited reference data, the use of a wide SFOV avoids cropping of anatomy that may be of diagnostic or future reference value but can adversely impact image resolution. The DFOV for reconstructed images is spread across a fixed matrix (generally 512^2 pixels), determining resolution. A tightly collimated DFOV thus provides optimal resolution. Retroactive reconstruction of raw data can be performed during postprocessing to address this issue, allowing enhanced DFOV resolution within a wider original SFOV.

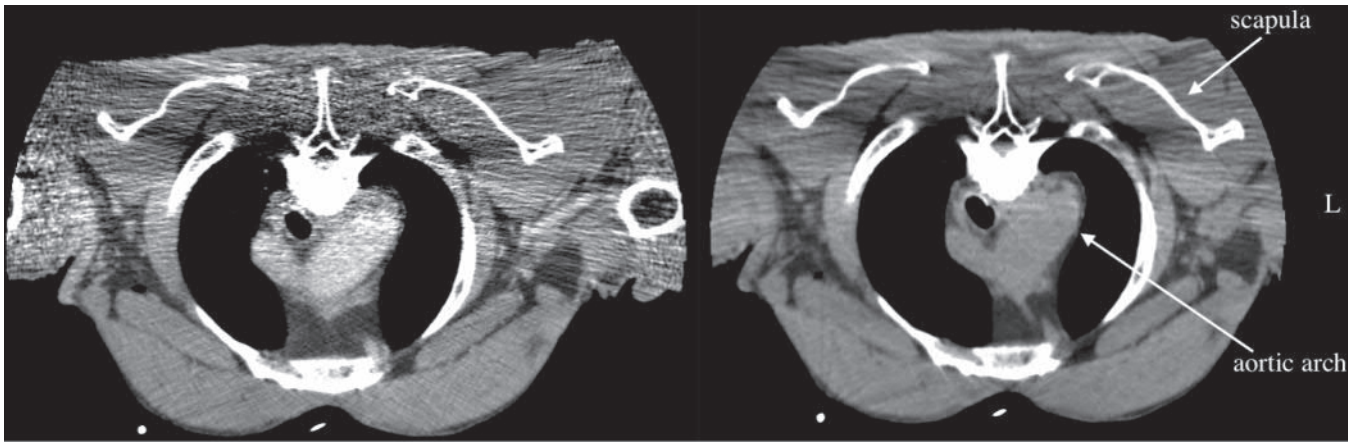
Placement of patient limbs is important to maximize image quality of specific organs. To decrease tissue penetration depth by the beam, forelimbs generally should be extended cranially for imaging of the thorax or the limbs themselves and positioned caudally for imaging of the head or neck. Orthopedic implants should be positioned away from the ROI to reduce streaking from scatter, beam hardening, and edge effects. Patient recumbency can influence the degree of motion artifact that occurs with respiration. Positioning has been shown to also significantly affect lung volume in some species (e.g., red-eared slider turtles *[Trachemys scripta]* and Humboldt penguins *[Spheniscus humboldti]*).[19,20]

Appropriate anesthetic management during a scan is paramount. Minimally, an ECG and capnograph should be visible from outside the CT room during the scan. Monitoring equipment may be left in place during scout scans used for study planning, but any wires or metal equipment that passes through the gantry should be removed prior to full diagnostic scans, to avoid artifacts. Intubation and ventilator use are recommended for long, complex scans. Patient temperature should be monitored closely because CT rooms are cooled to maintain the equipment. Small patients can become hypothermic quickly, and patient warmers should be used proactively.

Scan Settings

Consultation with a radiologist is recommended prior to any CT scan to ensure image acquisition settings are set appropriately. Slice thickness, interval, pitch, rotation speed, and other settings all impact the number of axial images acquired during a scan. This, combined with the length of the ROI, determines the scan duration. Acceptable scan duration varies based on the species, health status, anesthetic considerations, and other factors. Contrast enhancement adds additional time. In large patients, high tube current may necessitate a wait period between scans to allow for tube cooling. It is important that clinicians and CT operators communicate closely regarding acceptable scan duration.

Clinicians should have a general understanding of reconstruction algorithms. Images should be acquired using the proper algorithm (e.g., soft tissue, bone, lung) for a given ROI. For instance, lung tissue should not be evaluated using a soft tissue algorithm. Acquisition with the wrong algorithm severely limits diagnostic quality because window width and level adjustments during viewing can only partially compensate for an incorrect algorithm. Multiple reconstruction algorithms can be applied simultaneously during scanning or retrospectively reconstructed from raw data. As a general guide, scans in zoo species are best acquired using multiple algorithms.



• **Figure 31.9** Postprocessing of raw computed tomography (CT) data. Shown are original *(left)* and postprocessed *(right)* axial CT reconstructions from a gorilla thorax at the level of the shoulder. Note that reducing the display field of view and application of an adaptive noise reduction algorithm to the raw CT data greatly decrease image noise, increase resolution, and improve image quality in large zoo species.

Collecting CT data beyond the specific region of clinical concern is valuable, given the relative lack of reference images in many species. Modern scanners allow for efficient acquisition of whole body studies. However, clinicians should remain cognizant of dose efficiency (achieving diagnostic image quality at the lowest possible dose), particularly in young animals and long-lived species exposed to repeated scans.

Contrast Enhancement

Pre- and post-contrast imaging is routine in veterinary CT studies, unless scan duration is a concern or there are contraindications to intravenous contrast administration (e.g., patient dehydration).[21] There is still much to be learned about contrast use in zoo species due to variations in anatomy and physiology. Nonionic, iodinated contrast agents (e.g., iohexol) are safer for young, geriatric, and debilitated patients than ionic solutions (e.g., iothalamate, diatrizoate). Timing of image acquisition is critical for meaningful angiography, excretory imaging studies, and assessing tissue enhancement. Large-bore catheters facilitate the necessary rapid administration of contrast, but a power injector is beneficial in large species. Iodinated contrast can also be administered enterally for luminal gastrointestinal enhancement.

Artifact Detection and Prevention

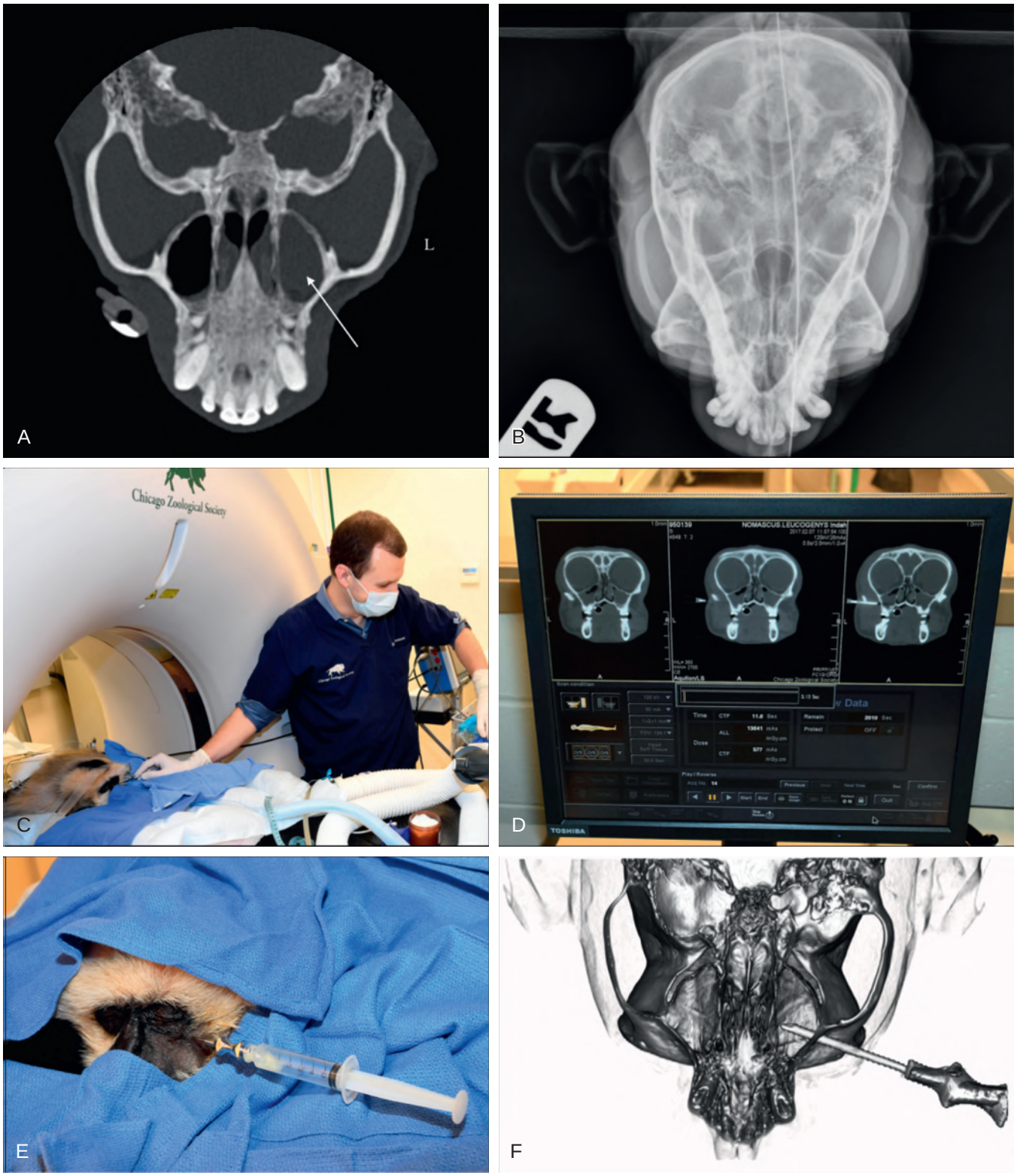
Imaging artifacts can severely impact the quality of a scan by depicting a structure on an image that is not present within the patient. Detailed discussion of artifact formation is available in other sources.[2,5,16,22] Recognition of some common artifacts (e.g., beam hardening, ring detector, partial volume, metal streaking) can be partially corrected with software applications or recalibration.

Motion artifact from respiration can significantly affect thoracic imaging in zoo animals, particularly when using slower CT scanners. Respiratory gaiting eliminates this artifact by syncing image acquisition with breathing but necessitates the use of a ventilator in veterinary patients that do not breathe on cue. Alternatively, decreasing slice thickness, increasing slice overlap, and adjusting rotation speed may minimize the impact of individual breaths. Recognition of significant motion artifact during a scan may necessitate image reacquisition.

Streaking artifacts can occur in highly attenuating skeletal regions (shoulders and pelvis) due to photon starvation (i.e., an insufficient number of photons reach the detector). Proper patient positioning can decrease this artifact. Inadequate dose can also result in increased noise and diminished image quality. Automatic mA adjustment in modern scanners minimizes artifacts by increasing tube current for highly attenuating sections within a ROI. Photon starvation is a notable challenge in large species because further increases in tube current may not be possible. In such cases, postprocessing of raw data with adaptive noise reduction algorithms can greatly enhance image quality (Fig. 31.9). Given the rise in bariatric imaging and emphasis on dose efficiency in humans, future technology will likely continue to aid image quality for zoo species.

Interventional Computed Tomography Applications

Interventional radiology refers to the use of imaging procedures to guide minimally invasive diagnostic and therapeutic techniques. Software features on CT scanners facilitate the collection of precise biopsies (full-core and aspirates). CT-assisted biopsies can be obtained using laser guidance, trajectory assessment, and repeated short-range scans. CT-fluoroscopy in modern scanners allows for real-time visualization of needle placement and advancement. Using this technology, highly precise procedures in delicate anatomic regions are possible (Fig. 31.10).



• **Figure 31.10** Interventional computed tomography (CT). A coronal CT reconstruction (A) reveals soft tissue attenuating material filling the left maxillary sinus (*white arrow*) in a white-cheeked gibbon, not apparent radiographically (B). On-site CT equipment at a zoo facilitates interventional CT procedures (C). Using CT laser guidance, detailed measurements, and focal repeated scans (D), a Rosenthal needle is precisely directed into the sinus to obtain diagnostic aspirates while avoiding delicate adjacent anatomic structures (E). A three-dimensional volume rendering illustrates needle placement (F).

Conclusions

The advantages of cross-sectional imaging in zoological medicine have been long recognized, but historical application has been limited. However, recent advances in technology and increased access to equipment are now enabling zoo clinicians to use the full spectrum of opportunities afforded by the technology. As equipment costs continue to decrease, cross-sectional imaging will continue to play a bigger role in routine veterinary care at zoos. Radiologists with specialized expertise in zoo species will provide further enhancement to the standards of care in zoological medicine.

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32

Moving Beyond Survey Radiographs

ELIZABETH MARIE RUSH AND BRETT FUNDAK

Introduction

Radiology is one of the best noninvasive diagnostic tools used in zoological medicine. Examination of many individuals in zoos or wildlife collections is recommended or required to determine and maintain health, and radiology is considered an integral (and often required) part of patient examination.[1–10] In some species it has been determined that there is benefit to advanced imaging modalities over conventional radiographs for routine examination and determination of abnormalities.[6,8,11,12] Although currently there are some physical, financial, and potentially location-related limitations to the use of more advanced imaging such as computed tomography (CT), magnetic resonance imaging (MRI, also referred to simply as MR), and ultrasonography (US), especially in larger, living patients, there is tremendous diagnostic value in using these modalities when possible. While they require special planning and accommodation, some of these modalities provide better images with minimal increase, and potentially decreased or elimination of time required for anesthesia than radiographs.[13–21] New technology continues to improve the opportunity to utilize advanced imaging equipment in species where this was previously difficult or impossible (Figs. 32.1–32.4).[14,19,22–25] See also chapter Use of Computed Tomography/Magnetic Resonance Imaging in Zoological Medicine in this volume (see Chapter 31).

Because of the importance of imaging for examination of our zoological specimens, it is important to understand the advantages and disadvantages of different modalities and requirements to properly obtain diagnostic images.

Much of the information presented within this chapter is based upon practical clinical experience that has been gained over years of practice within our respective fields of specialty. This chapter suggests a new paradigm for imaging of exotic species. Rather than attempt to cover all possible imaging modalities, we will focus on a possible imaging acquisition scheme that would contribute to improved diagnostics for cases, and the establishment and implementation of a digital database for all practicing zoological veterinarians.

It is fundamental to know what is normal for each species before recognizing abnormalities. This applies to all imaging modalities: radiography (preferably digital radiology [DR], as will be expanded upon), US, and advanced cross-sectional imaging studies of CT and MR.

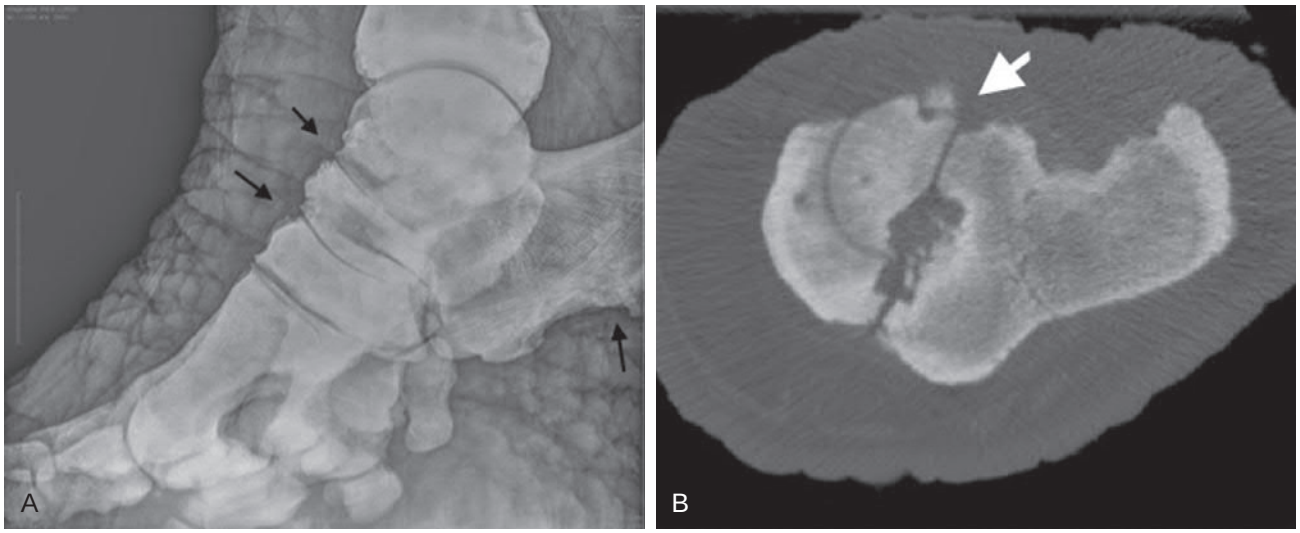
Imaging Paradigm—Starting Point

Radiography may be utilized both as a diagnostic tool during the workup of medical conditions of animals and to provide a baseline assessment of animals during a wellness examination. This modality is in common usage and well known to practicing clinicians. It is a quick screening test that may rapidly provide needed clinical information or determine if additional testing, including more imaging, is required.

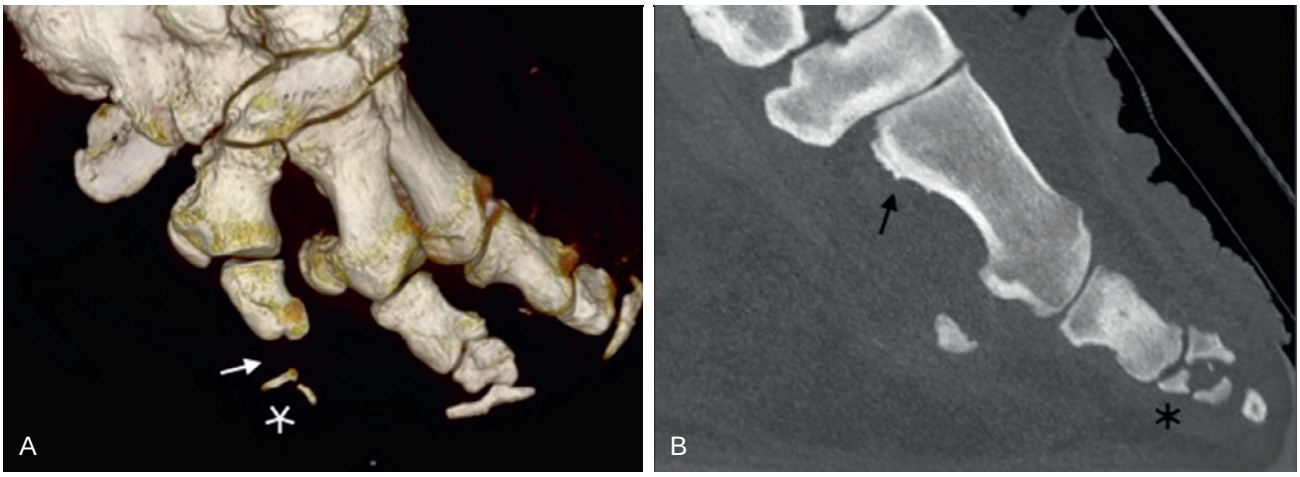
Radiographs provide a fast review of anatomic structures with regard to the radiographic variation of number, size, shape, opacity, location, and margination (changes of these entities are the Roentgen [radiographic] signs upon which radiographic interpretation is based). If adipose tissue is present within the abdomen, then organ size and shape may be readily ascertained. If detail is absent or decreased, then it may be necessary to transition to different imaging modalities to resolve structures within the abdomen or thorax for better visualization of the changes.[26–29] This is where alternative imaging modalities may be considered: US, CT, and MR are modalities that may improve detail and the opportunity for diagnosis of pathology in some cases (Figs. 32.5–32.10).[18–28]

In radiology, image detail is based upon the five basic radiographic opacities that range from minimal attenuation for gas, with increasing attenuation of x-ray beam for adipose, then soft tissues, bone, and finally metal (e.g., lead and metallic surgical implants). The difference in attenuation between adipose tissue and soft tissues provides detail within the abdomen to access soft tissue boundaries. When detail in the abdomen is decreased due to weight loss or failure to thrive (loss of intraabdominal adipose tissue), peritoneal fluid, or peritonitis, the ability to define margins of soft tissue organs is decreased, or completely lost where only a pendulous soft tissue opacity and distended abdomen are apparent outside of gas within the bowel.[26–30]

Osseous structures are well evaluated with radiographs. Moreover, use of mirror-image symmetry of extremities



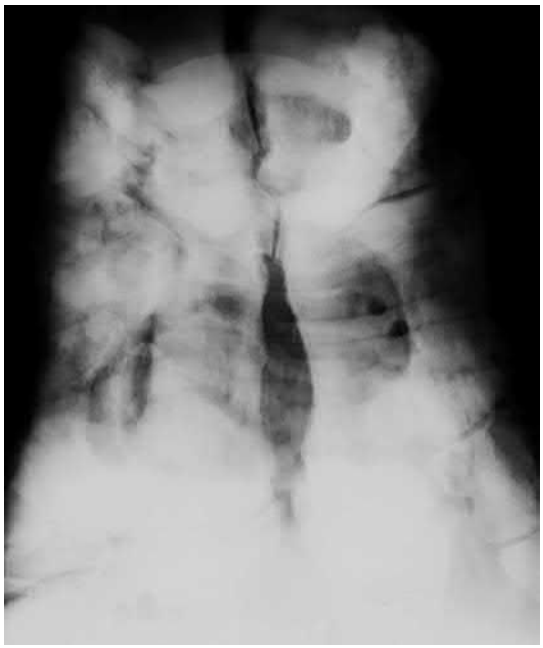
• **Figure 32.1** (A) and (B) African elephant *(Loxodonta africana)* lateral digital radiology (DR) image of necropsy specimens along the dorsal margin of the proximal and distal inter-tarsal joints of the left limb, with mild to moderate osteophyte formation *(black arrows)* present consistent with osteoarthrosis and degenerative joint disease (Fig. 32.1A). Computed tomography image of the left tarsus, seen in Fig. 32.1A, of the African elephant showing severe erosions and fragmentation of the articular margins and subchondral bone is present *(white arrow)*, which was not apparent in the DR images (Fig. 32.1B). (Images courtesy of Ajay Sharma, University of Georgia College of Veterinary Medicine.)



• **Figure 32.2** (A) and (B) 3D reconstruction from computed tomography (CT) of the dorsolateral view of the right hind foot of an Asian elephant *(Elephas maximus)*, showing a bipartite distal phalanx *(white star)* and absence of the middle phalanx of digit 5 *(white arrow)* (Fig. 32.2A). Sagittal slice CT image of an Asian elephant, showing remodeling of the metacarpal *(black arrow)* and fracture of the middle phalanx digit 4 of the right hind foot *(black star)* (Fig. 32.2B). (Courtesy PeerJ, Inc 2017. Regnault, et al.)

allows for built-in controlled comparison between abnormalities localized to a specific region. This is then based on physical examination, observation, and/or history, and compared to those without mirror-image structures that are of normal appearance. For this comparison to occur within the given patient or within a given species, it is important that positioning be performed in a consistent and reproducible manner for all regions of interest. There are standard views used to complete survey radiographic examination of normal structures with all requiring a minimum of two orthogonal images (taken at 90 degrees to one another) to complete the study.[12,26–30]

The purpose of two orthogonal images is to visualize the region(s) of interest in two different projections and determine how the abnormality silhouettes on these radiographic views. Because this compresses a three-dimensional structure into a two-dimensional image, we use the second radiograph to ascertain the location of an object within the third plane due to orthogonal nature. Some changes are only apparent on a single view due to superimposition or absence of radiographic changes within the opposite image from the beam not striking the interface at a tangent; thus not demonstrating attenuation of the beam in the projected image (no changes in radiographic signs are apparent on

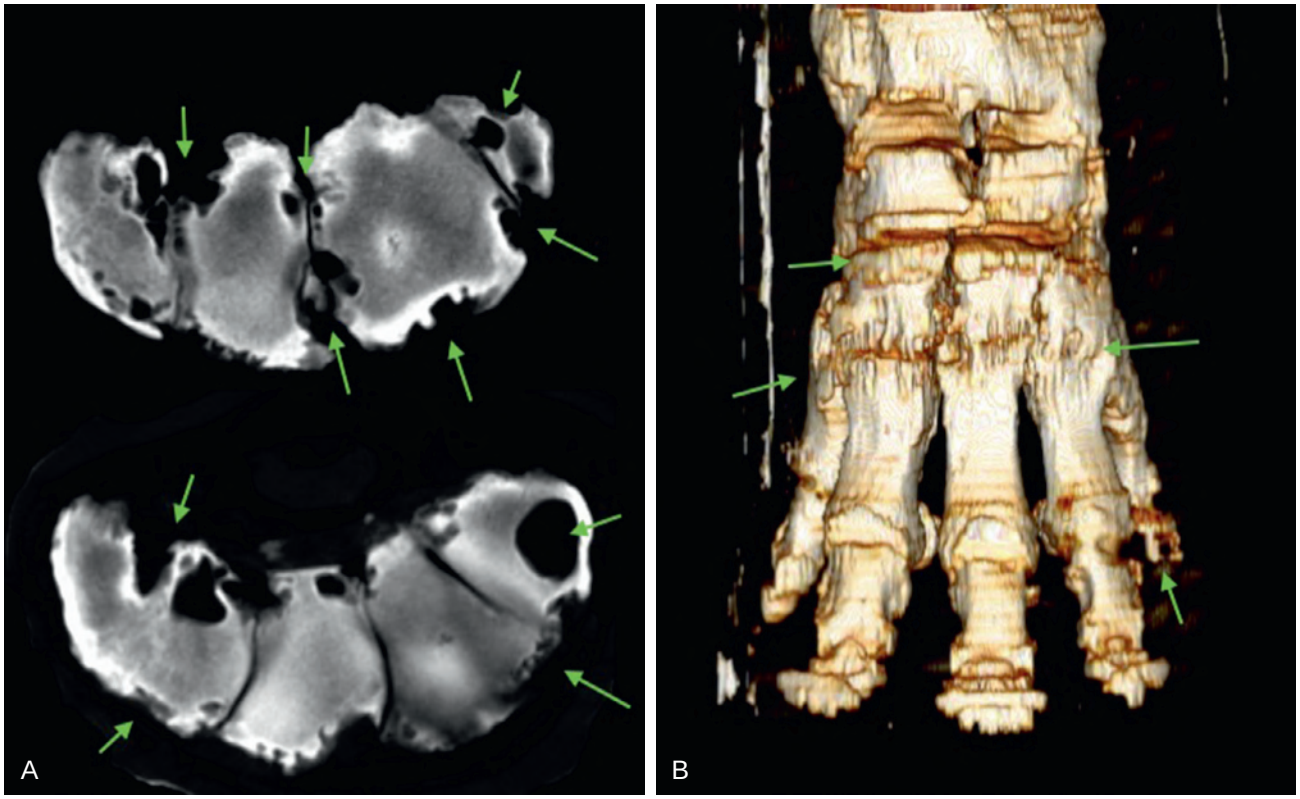


• **Figure 32.3** Asian elephant *(Elephas maximus)* right carpus digital radiology, demonstrating the difficulty in discerning details of the joints and individual bones of the metacarpal and carpal region, or any distinct lesions present. (Courtesy Rush EM, et al.)

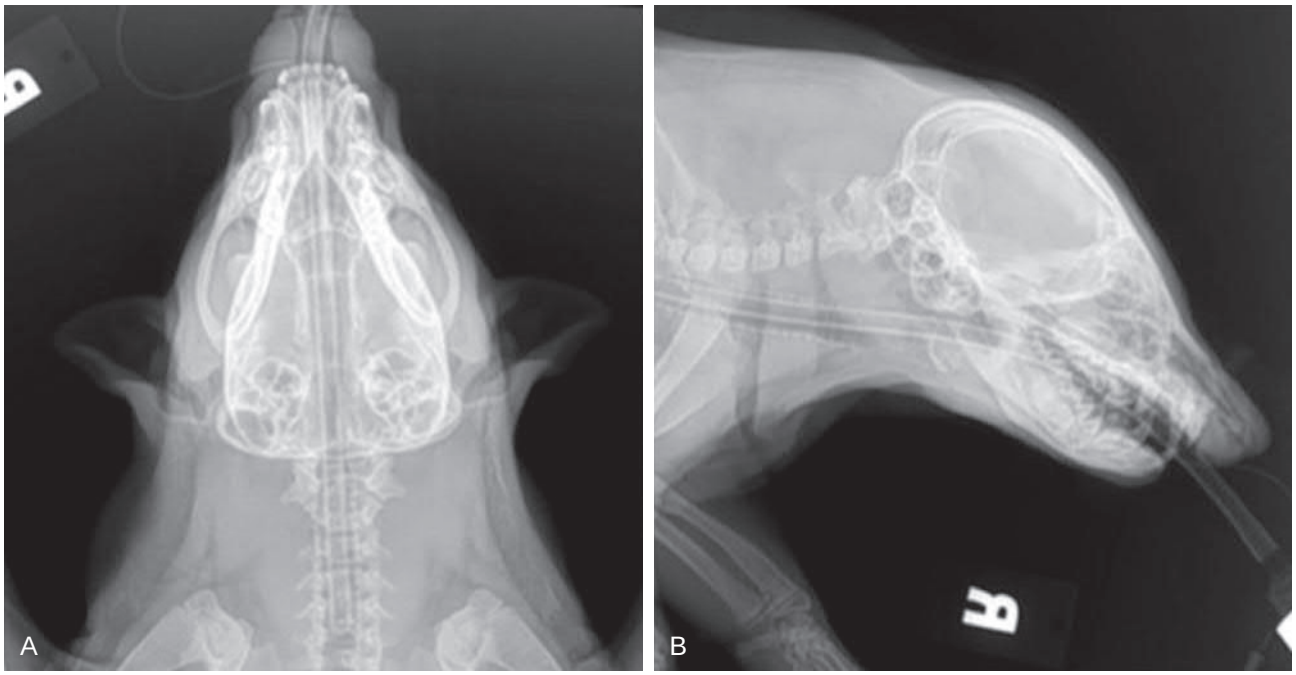
one of the views). Ideally any lesion should be confirmed in two imaging planes to reduce the possibility of erroneous interpretation due to artifacts or missed Roentgen signs (Fig. 32.11).

Good detail (detail describes the ability to discern edges and margins of structures within the imaging plane) within our radiographic images produced during a radiographic examination depends on multiple factors. Motion of region of interest (voluntary movement and involuntary motion such as breathing, panting, shivering, convulsing) degrades image detail and blurs margins of structures of interest. This blurring of edge perception is only reduced by minimizing voluntary movement with sedation/anesthesia and decreased time of exposure to attempt to freeze motion during the time of exposure. In most cases, there is limited control over exposure time (mAS); however, stabilization of the patient and cessation of motion may be controlled with sedation or anesthesia. In zoo and wildlife species, this is less commonly a concern, due to the nature of the patients and necessity for immobilization in many cases for diagnostics and procedures to be performed. Positioning and restraint of these patients, and time under anesthesia, may be an imposition to certain specific projections, or modalities used for diagnostics.[26–33]

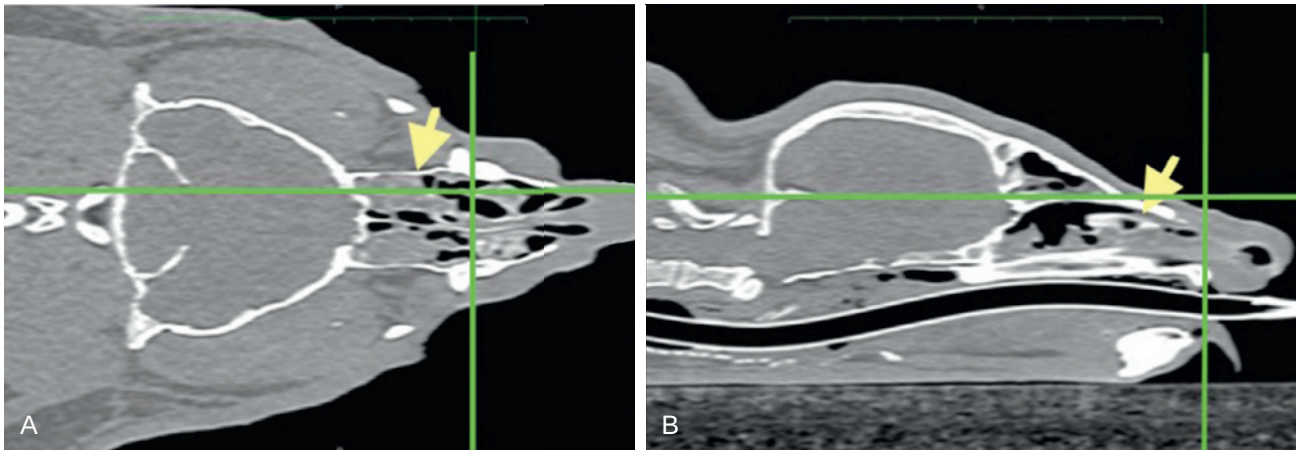
DR is considered the best method for radiographic acquisition. This is based on the following rationale:



• **Figure 32.4** (A) and (B) Computed tomography (CT) of the right carpus of Asian elephant *(Elephas maximus)*. CT cross section (Fig. 32.4A) through the proximal and distal carpus (Fig. 32.1A and 32.2), and 3D reconstruction (Fig. 32.4B), showing the obvious presence *(arrows)* of osteoarthrosis and degenerative change in the metacarpus and carpus. (Images by Rush EM.)



• **Figure 32.5** (A) and (B) Orthogonal skull digital radiology of a juvenile badger *(Taxidea taxus)* with neurologic signs and chronic sinusitis and rhinitis. (Courtesy Antech Imaging Services, 2017.)

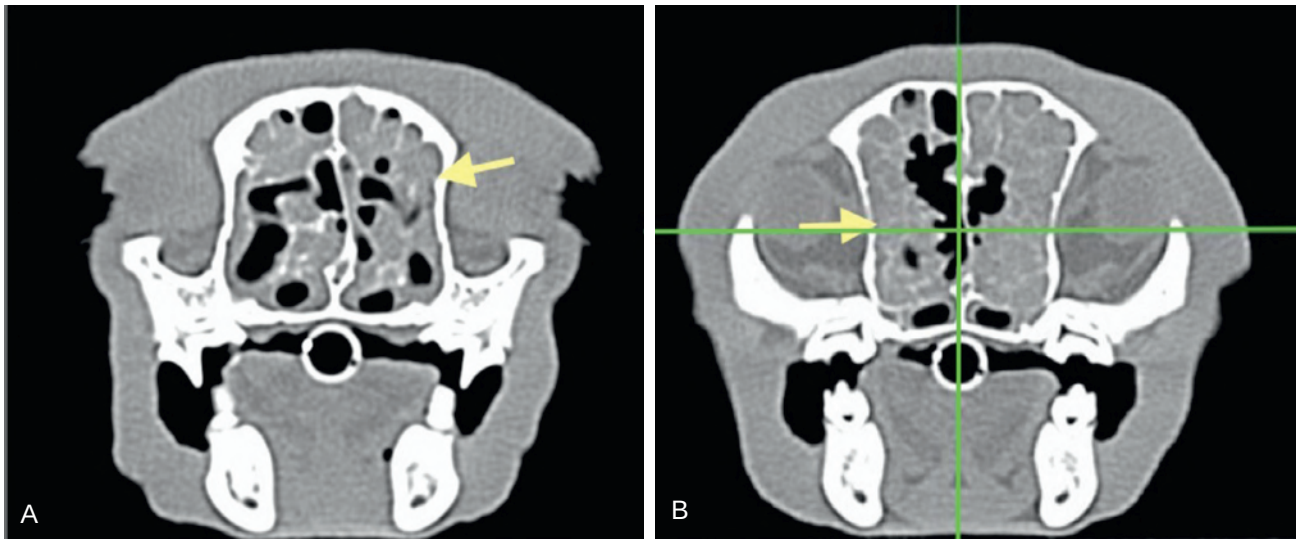


• **Figure 32.6** (A) and (B) Computed tomography image with multiplanar reconstruction of the badger *(Taxidea taxus)* skull, ventrodorsal (Fig. 32.6A) and lateral (Fig. 32.6B) views. Note the deviation of the rostral sinuses from the midline. Infiltrative change in the sinuses is designated by the arrows. The *green crosshairs* indicates the location in the skull with respect to sections taken in Fig. 32.7. (Courtesy Antech Imaging Services, 2017.)

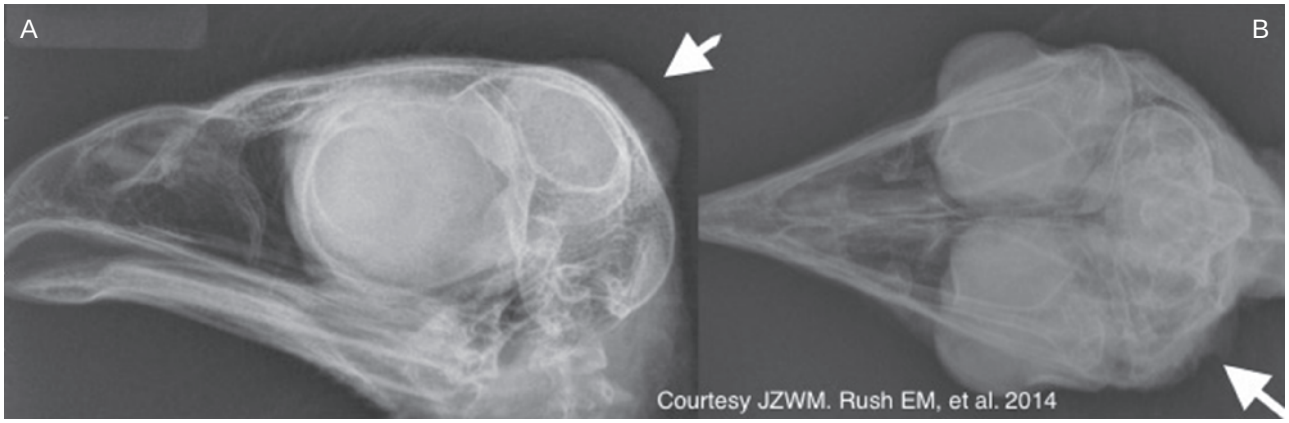
1. DR images may be reviewed immediately after exposure on a local monitor to determine if views are correctly positioned and include the entire region of interest. Both analog radiographs and computed radiology (CR) require a processing step before an image may be observed. The patient cannot be moved from the location of image capture (most often a specified room or table) before the study is completed. DR allows for decreased anesthesia time required to obtain images in comparison to analog and CR. DR removes the processing step and provides an instant view of the produced image for quick evaluation of technique and positioning.
2. Images produced by DR and CR are typically produced in Digital Imaging and Communications in Medicine format (DICOM) for transfer to digital database.[17,29] Analog films must be digitized with a scanner or possibly a camera before they are available on a DICOM network. Each time an image is copied into an alternate format, some of the information and detail will be lost. Often this will produce an acceptable image, which retains diagnostic detail; however, other times it may reduce the copied image to nondiagnostic with regard to quality and detail.[17]

Imaging Paradigm—What Next

After radiographs of the region of interest have been obtained and reviewed for abnormalities, consideration for



• **Figure 32.7** (A) and (B) Multi-planar reconstructed computed tomography of the badger *(Taxidea taxus)* skull, axial projection showing the severe destructive and infiltrative changes in the maxillary sinuses at different levels of the sinus *(arrows)*. (Courtesy Antech Imaging Services, 2017.)



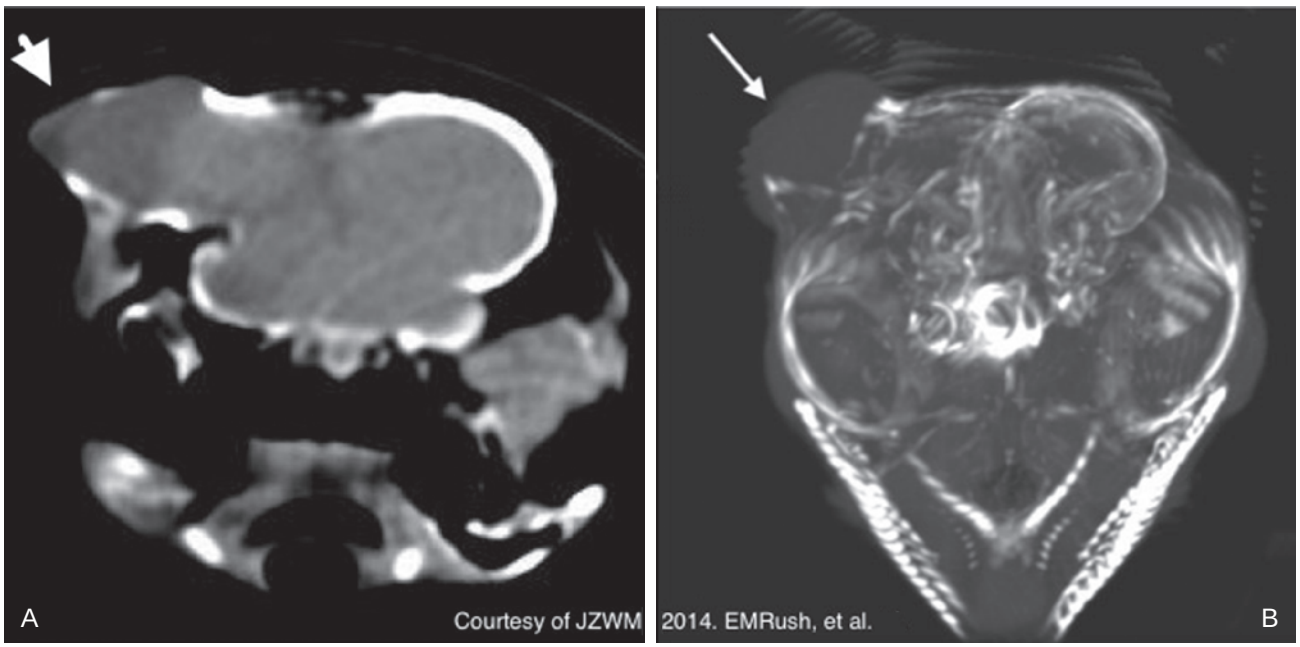
• **Figure 32.8** (A) and (B) Orthogonal digital radiology projections of a red-tailed hawk *(Buteo jamaicensis)* skull with a large growing fracture not visible on radiographs. Note the soft tissue enlargement and poor definition of the lesion *(arrows)*. (Courtesy JZWM, Rush EM, Shores A, Meintel S, et al: Growing skull fracture in a red-tailed hawk *(Buteo jamaicensis). J Zoo Wildl Med* 45(3):658–663, 2014.)

additional noninvasive diagnostic imaging may be needed to further evaluate an area or organ system. If detail is poor or absent in the abdomen, then progression to an alternate imaging modality (e.g., CT or US) may be needed to see the soft tissues within the abdomen that are surrounded by fluid or have loss of regional adipose tissue. Ultrasound is ideal for focal examinations but may be difficult for evaluation of an entire abdomen due to superimposed gas, underlying pain, and/or pathology, or the massive size of a lesion that is contiguous with multiple organs, limiting ability to determine site of origin. If US is nondiagnostic, then whole body CT examination to image the entire thorax and abdomen may be warranted.[14,17,20–26]

Just as in radiography, movement by the patient during CT image acquisition decreases image detail and could mask pathology. Patient positioning is critical to allow obtainment of CT axial images in the standard imaging plane for review. The patient must be positioned in the center of the CT gantry without rotation or bending of the skull. The hard palate of the skull should be parallel to the scan table to allow direct acquisition of true axial slices of the skull and brain.

Slices generated by the CT machine should be accompanied by scout views (similar to radiograph and produced in two orthogonal planes) and used in planning the slice acquisition with regard to start and finish of axial slices. The scout views are linked with the axial slices at time of review on the computer monitor so that relative position of the slice within the thorax, abdomen, or extremity is readily apparent by superimposition of the slice on the scout image. With these, it may be difficult to determine which ribs and vertebrae are present within a given axial slice when reviewing axial images.

The major advantage of CT images is production of slices of body tissue (determined by slice thickness set by operator before scan) with removal of superimposition artifact, and



• **Figure 32.9** (A) and (B) Cross sectional computed tomography (CT) image made prior to surgery of the red-tailed hawk *(Buteo jamaicensis)* demonstrating the defect in the skull with slight soft tissue protrusion and mild cerebral herniation. This image also demonstrated the smooth (chronic) nature of the edges of the skull and the site of the defect which is notated with an *arrow* (Fig. 32.9A). Sagittal and 3D reconstruction images of preoperative CT scan clearly define the skull defect with the smooth, lipped edges that were noted in surgery. The *arrow* clearly notes the defect in the skull (Fig. 32.9B). (Courtesy JZWM, Rush EM, Shores A, Meintel S, et al: Growing skull fracture in a red-tailed hawk *(Buteo jamaicensis). J Zoo Wildl Med* 45(3):658–663, 2014.)

increased ability to differentiate tissues and organs due to differential attenuation of the x-ray beam within the body (detected by the CT during image acquisition).

Another advantage of CT is multiplanar reformatting. This is where cross-sectional images from a region of interest are reconstructed in the other two orthogonal planes: sagittal and coronal (sometimes referred to as dorsal plane). In order to produce high-resolution reformatted images in the desired plane of interest, thinner axial slices are needed and patient motion must be minimized.[17,29,34]

Contrast-enhanced images should be obtained following standard noncontrast enhanced CT imaging. This increases conspicuity of vessels, regions of increased blood flow, or abnormal vasculature permeability that results in regional contrast enhancement. This enhancement is often needed to determine size and location of lesions observed during a scan. Some lesions may not be visible, or size may be underestimated/overestimated due to contrast enhancement.

Once CT images have been obtained, interpretation of the images should produce a reasonable list of differential diagnoses based on radiographic findings, clinical history, and possible signalment of patient (species, age, sex, geographic location). The location of the lesion as well as the effect on adjacent structures (including invasion, displacement, or encapsulation/encasement) may be used for planning the next course of action. Changes seen are often nonspecific, and the CT machine may be used to guide aspiration and biopsy of lesions for pathologic assessment, or to guide surgery approach and resection/debulking options.[17,29,34]

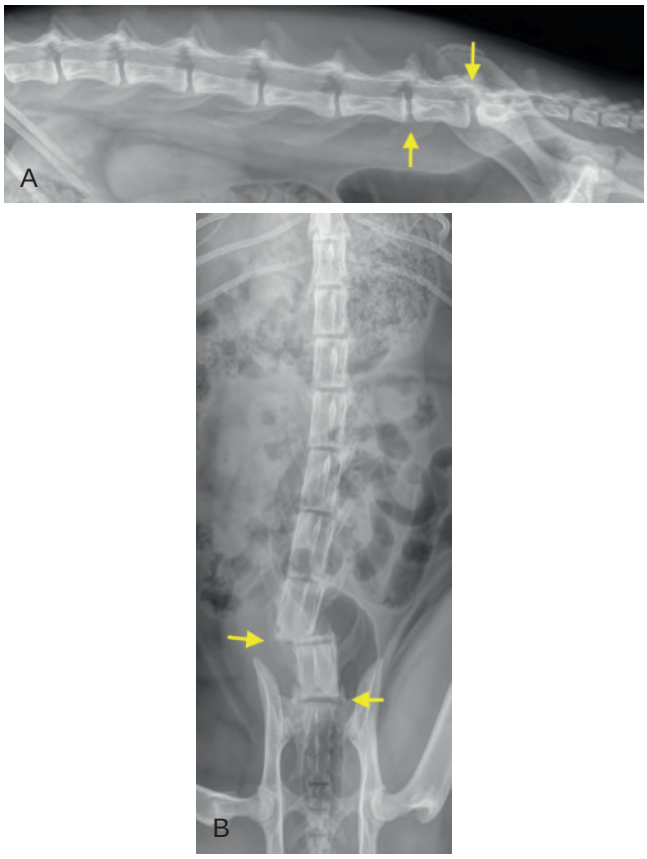
Summary of Imaging Planning

1. Standardized positioning of anatomic region for all species.
2. Minimum of two views to complete the radiographic study.
3. Sedation/anesthesia to minimize movement and control respiration.
4. Transfer images to data storage center for storage and possible review by specific radiologist and/or clinicians.

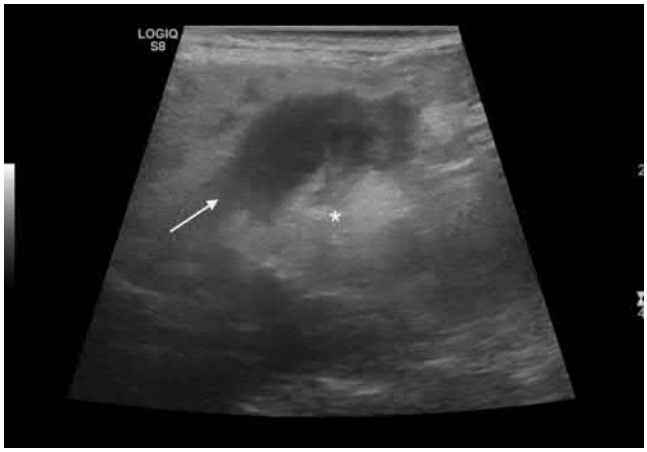
Referral of images to an imaging expert may help to interpret the diagnostic images and help set up protocol for further assessment of the lesion via additional imaging modalities. This relationship may be very productive and advantageous as radiologists are trained to consider a next imaging option or step in the continued evaluation of patients, based upon findings of initial survey radiographic images.

Future Directions

Both zoological and radiographic knowledge of multiple species could significantly increase over time with a shared database. The ability to predict which possible disease processes are present based on location, appearance, and



• **Figure 32.10** (A) and (B) Lateral and ventrodorsal (VD) projection of a lumbar spine, demonstrating the need for orthogonal projections to identify pathology. While the lateral view appears unremarkable, the fracture of the lumbar spine is clear in the VD projection at L6–L7, as well as angular widening at the lumbosacral junction *(yellow arrows)*. (Courtesy Antech Imaging Services, 2017.)



• **Figure 32.11** Ultrasound image of an edematous left limb of the pancreas, with a regional peritonitis. This is not visible on radiographs. The left limb of the pancreas is hypoechoic *(arrow)* and thickened with regional hyperechogenicity of the surrounding mesentery/adipose tissue *(star)*. Appearance is consistent with moderate to severe pancreatitis. (Courtesy Antech Imaging Services, 2017.)

signalment would be accomplished with much better accuracy than is currently possible due to the deficits in our basic normal anatomic knowledge and the variation of normal in a given individual. The availability of this database to zoological institutions to provide images for specific species comparison, both normal and pathologic, would provide an opportunity to better assess each patient and consider treatment options. Additionally, this could allow for collegial consultation on cases with similar lesions, pathology, or treatment needs for individuals, based on diagnosis when compared to stored images.

There have been numerous advances to medicine, radiology, and computer technology to allow for broad sharing of information and images for the benefit of our patients. At this time, a universal storage of information and normal references to aid in the imaging diagnosis of all species does not exist. Most current veterinary programs teach several different species through gross anatomy, preserved specimens, skeletons, and exposure to radiographic imaging. Some species are better described than others; however, the ranges of sizes and morphologic variation of a zoological collection is far beyond our ability to master all normal radiographic appearances and anatomic variations, and thus enable recognition of abnormalities that are present within species of interest.

To meet this goal of learning normal radiographic anatomy to facilitate radiographic diagnosis, consideration should be given to creation of a universal shared database of images. Storage should be based upon a medical standard format that unifies systematic storage of patient radiologic information for all human and veterinary medical images (as well as digital microphotographs to review pathological, cytologic, and histopathologic specimens on a local computer) in a retrievable digital format. This DICOM standard has been in place for many years.[17,21,26,29,34]

It is essential that all future imaging studies be performed in the DICOM format for this collection of information to occur. A web-based image storage facility must be accessible to anyone with a reasonable network connection speed to facilitate uploading and downloading large images. This digital storage facility could serve as a repository for studies that document both normal and abnormal radiographic anatomy for these valuable species.

Acknowledgments

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SECTION 8

Emerging and Changing Infectious Diseases

33

Equine Herpesviruses and Interspecies Infections

ALEX DAVID GREENWOOD AND KLAUS OSTERRIEDER

Equine Herpesvirus Background

The *Herpesvirales*, as a viral group, have a remarkably wide host species range and can infect countless vertebrate and invertebrate species.[1] While not among the most aggressive and virulent viruses known, within each of the herpesvirus families, subfamilies, and genera are pathogens, which may cause a range of clinical signs and have serious consequences for the host. Despite the wide distribution of herpesviruses among the metazoa, comfort has been taken in the widely accepted tenet that herpesviruses are generally host-specific and, therefore, zoonotic infections are not a general consideration for this viral group.[2] However, this tenet is eroding as examples of historical and recent cross-species (and even cross-ordinal) infections have been described, many associated with severe symptoms or fatalities; for example, malignant catarrhal fever (MCF) is caused by a member of the *Gammaherpesvirinae* that is not restricted to a specific species and can cause outbreaks with high mortality in mixed-species zoological collections.[3]

Equine herpesviruses (EHVs) were recently shown to also cause interspecies infections. There are nine EHVs identified to date, three of which (EHV-2, EHV-5, EHV-7) are classified within the *Gammaherpesvirinae* and six of which (EHV-1, EHV-3, EHV-4, and EHV-6, EHV-8, EHV-9) are classified within the *Alphaherpesvirinae*.[4] EHVs classified as *Gammaherpesvirinae* are rarely associated with disease. However, the gammaherpesviruses in particular appear to have historically violated the tenet of expected host specificity, with most viruses in this group deriving from bats and primates.[5] The lack of direct or frequent association with negative health outcomes (MCF, Epstein-Barr virus, and Kaposi sarcoma-associated herpesviruses as exceptions with few equine associated health effects) is reflected in less research focused on equine gammaherpesviruses and the long- or short-term health consequences of interspecies infection are unclear.

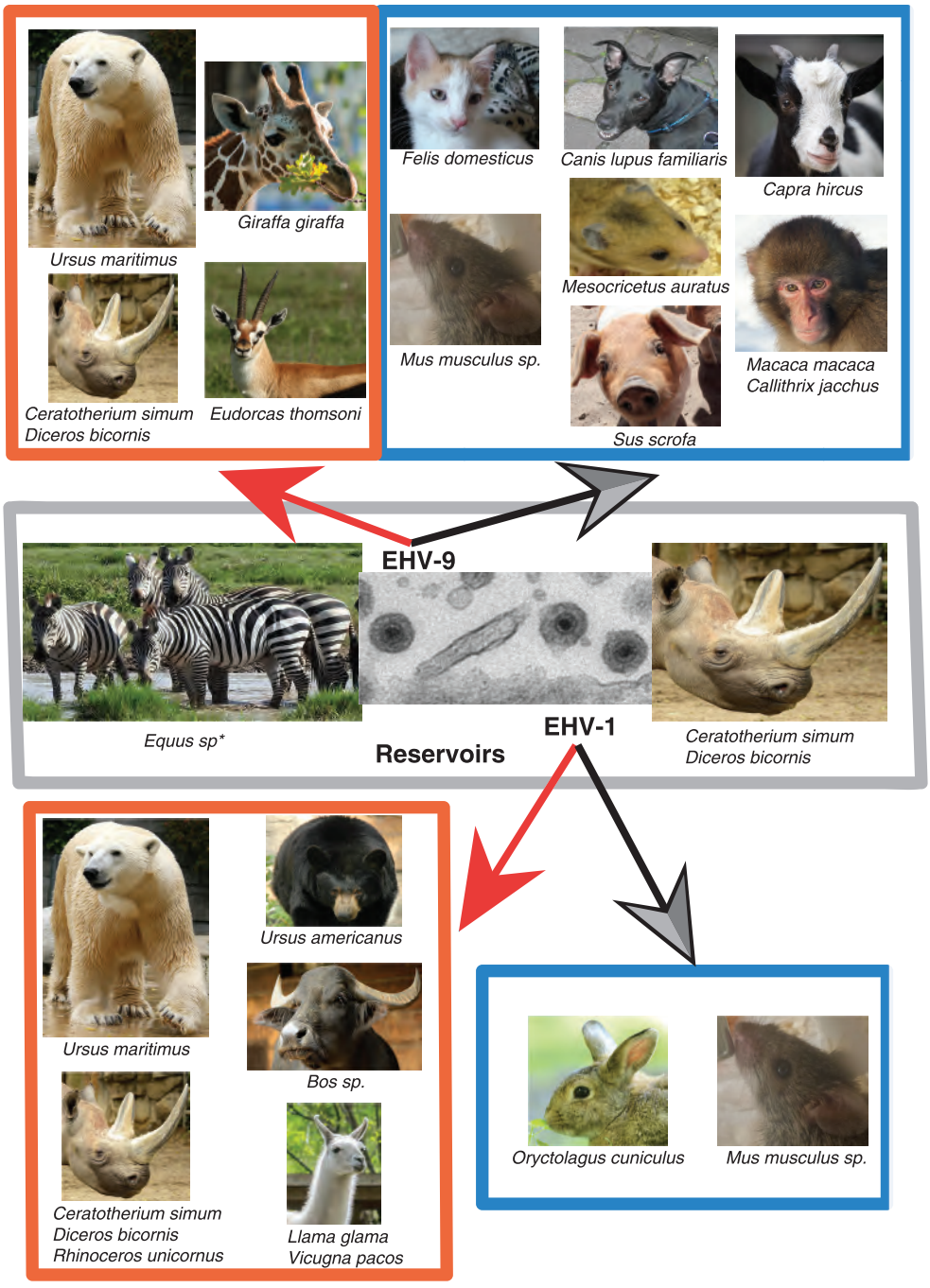
The *Alphaherpesvirinae* EHV-1, EHV-4, and EHV-9 are all associated with abortion, neonatal death, and respiratory and neurologic disorders in domestic equids, albeit with different frequencies and severities. EHV-1 is one of the most consequential pathogens of domestic horses *(Equus caballus)* worldwide.[6] In horses there are serious consequences of EHV-1 infection as show events, races, and transport may be affected, with the economic consequences often devastating due to movement restrictions, and cancelling of events.[7]

Considered a horse pathogen, recent discoveries in both zoo and wildlife strongly suggest that EHV-1 and its close relative EHV-9 lack strong species specificity and can both infect and cause disease in a wide variety of mammalian taxa (Fig. 33.1).[8–13]

Diagnostics

Because of the lack of species specificity of EHV-1 and EHV-9 and clinical consequences, the rest of this chapter will focus on these two virus species. Before discussing the specifics of interspecies EHV-1 infections, it is worthwhile reviewing the typical clinical signs and the diagnostic tools available for confirming infection. Neurologic manifestation such as epileptiform seizures are an indicator of encephalitis, which in captive settings could be taken as evidence for EHV-1 or EHV-9 involvement.[7] However, the list of known encephalitis-causing bacterial and viral pathogens is quite long. In polar bears *(Ursus maritimus)*, domestic cats, and humans, autoimmune encephalitis against glutamate receptors has been documented.[14] Therefore, while encephalitis is a commonly observed symptom in EHV-1 and EHV-9 interspecies infections, it is not a basis from which to make a diagnosis. In addition, the neurologic disease induced by EHV-1 in horses is an encephalopathy, a stroke-like syndrome following endothelial cell infection and vasculitis, not a bona fide encephalitis.

There are some considerations in suspected EHV-1 and EHV-9 cases for facilitating diagnostics and helping to ensure that a conclusive diagnosis can be reached. In nonfatal cases where abortion occurs or neurologic symptoms are detected, fetal tissues should be frozen for diagnostic



• **Figure 33.1** Equine herpesviruses (EHV-1 and EHV-9) infect a broad range of mammal species experimentally, in captivity, and in nature. Infected host species are indicated by picture or by vector graphic. Reservoir species for EHV-1 and EHV-9 are boxed in grey. The asterisk "*" indicates that while EHV-1 and EHV-9 circulate among wild and domestic equids, their distributions are not uniform. For example, the domestic horse breeds are not reservoirs for EHV-9. Experimentally infected animal groups are boxed in blue and nonexperimental infections (zoological collections or in the wild) are boxed in orange. EHV-1 and -9 have been detected in both wild and captive zebra and rhinoceros species. However, the only known fatal EHV-associated rhinoceros case was an Indian rhinoceros *(Rhinoceros unicornis)*. The black bear *(Ursus americanus)*, Thompson's gazelle *(Eudorcas thomsonii)*, zebra, domestic cat, domestic dog, house mouse, and hamster photographs were kindly provided by Arne Lawrenz, Oliver Höner, Marion East and Peter Seeber, Kristin Mühldorfer, Emanuel Heitlinger and Satoko Izume, respectively. The black bear, macaque, and rabbit photographs were kindly provided by the Tierpark Berlin and the goat, llama, giraffe, pig, rhinoceros, and water buffalo photos by the Zoological Garden Berlin.

purposes. From adults, nasal discharge, nasal swabs, blood, and serum should be collected. In fatal cases, lung, blood, serum, nasal epithelia, and brain tissue should be removed and stored at −80°C if possible and not at −20°C. Of critical importance, samples should be collected and stored, and preferably frozen, as quickly as possible to prevent sample degradation, which complicates or prevents further downstream analysis. In many cases it is better to just refrigerate samples if they can be transported to a diagnostic laboratory within a few days.

In equids, pathology and histology usually present as the myeloencephalopathy, mostly affecting the spinal cord. In non-equids, neurologic symptoms are often characterized by severe seizures. Pathologic findings generally include nonsuppurative encephalitis and gliosis, but Barr bodies are mostly absent.[8,10] DNA extracted from tissues can be tested for presence of herpesviruses using pan-herpesvirus (qPCR) protocols,[15] which can distinguish between herpesviruses normally associated with a given species or evidence for a cross-species transmission following DNA sequencing of amplified PCR products. More sensitive and EHV-specific quantitative PCR assays[16] can be applied in cases of faint signal or to quantitate viral loads in a given sample with such services provided by many laboratories in Europe and the United States. In the case of EHV-1, there are a number of World Organisation for Animal Health (OIE) reference laboratories that provide state-of-the-art diagnostic services (http://www.oie.int/).

Symptomatic individuals in a population, and even viremic individuals, are only a fraction of animals that have been infected at some point in their lifetimes. Even for highly pathogenic viruses, many infected individuals will be asymptomatic and will clear the infection. However, infection generally results in the production of specific antibodies that may persist in the individual long term or, in some cases, lifelong. Therefore, exposure of different species to different EHVs can be measured indirectly, irrespective of whether the individual is currently productively infected or not. Serology-based approaches measure antibodies against a given virus or viral antigen. Assays based on whole viruses, for example, serum neutralization tests (SNTs), examine whether viral replication can be neutralized by serum from a given animal, indicating antibodies against the virus are produced by the individual. However, SNTs will fail to discriminate closely related viruses, for example, EHV-1 from EHV-4 or EHV-9, because they are genetically and antigenically very similar. Recently, synthetic peptides that are designed to specifically identify viral epitopes that are as distinct as possible have been developed and tested for their discriminatory power for the closely related alphaherpesviruses EHV-1, EHV-4, and EHV-9.[17,18] The test used is an antigen enzyme-linked immunosorbent assay (ELISA) and can be performed on 96 samples in a single experiment in microwell plastic plates, allowing for the simultaneous screening of a large number of individual animals. The peptide antigens are used to coat the plates and serum is applied followed by a secondary antibody, which, when bound to the primary antibody, initiates an enzymatic reaction that can be detected by determining substrate conversion using an optical scanner that measures optical density in the wells of the plate. If no antibody is present in the serum, there is nothing for the secondary antibody to bind to and no signal is emitted. In the case of EHV-1 and EHV-9, discrimination was achieved, although peptides for EHV-1 cross-react somewhat with EHV-9-specific sera. However, the developed EHV-9 peptide does not react with EHV-1 positive sera.[17] The peptides were shown to be specific and applicable to blood samples from a variety of wild and captive animals. They were also substantially more sensitive to low titer antibody than SNT analysis. Of note, due to issues of background and secondary antibody cross reaction, application to carnivore sera has not been successful to date, restricting analysis in these animals to SNT. Nonetheless, specific peptide-based ELISA systems provide a very efficient method for screening serum from animals for past exposure to EHVs. Both methods allow for the determination of EHV-1 and EHV-9 exposure, whether the individual is currently infected or not.

Experimental Interspecies Infections

Evidence for the general ability of EHV-1 and EHV-9 to cause disease comes in part from experimental infection studies (Fig. 33.1). Recent work has shown that much like gammaherpesviruses, EHV-1 and EHV-9 can infect non-equine species. Several experimental infections have demonstrated that a wide range of species in most mammalian orders are susceptible to EHV-1 and EHV-9. Relatively few experimental infections in non-equid species have been performed for EHV-1. Rabbits experimentally infected with EHV-1 developed both respiratory and neurologic symptoms.[19] Laboratory mice infected with EHV-1 could clear lytic infection but latency was established. However, this depended on the EHV-1 strain with some strains (Brazilian strains A4/72 and A9/92) able to induce neurologic clinical signs.[20,21]

EHV-9 experimental infection studies are broader with respect to representatives of different mammalian orders tested. Laboratory mice were able to clear experimental infection after 72 hours.[22] However, lesions were induced in suckling hamsters.[23] Neurologic signs were observed in both dogs and cats infected with EHV-9.[24,25] Lethal encephalomyelitis was induced by EHV-9 in goats.[26] Conflicting results were observed for pigs. In one study, no clinical signs were observed, although neurologic lesions were observed, while in a second experimental infection meningoencephalitis was induced.[27,28] It is not clear why the outcomes were variable. Among primates, macaques *(Macaca macaca)* appeared to be resistant to EHV-9 infections, whereas marmosets exhibited severe neurologic symptoms.[29,30] The conclusions that can be drawn are that EHV-1 and particularly EHV-9 are able to infect and cause clinical symptoms in a broad diversity of mammalian orders, including primates, in experimental infections.

Nonexperimental Interspecies Infections

Experimental infections involved controlled and often very high viral doses that may not reflect the conditions required for natural viral transmission. In addition, progression of infection is often very specific to the tissues infected at the point of entry for the virus, for example, intranasal administration of viruses. Therefore, while many species can potentially be infected by EHV-1 and EHV-9, it remains somewhat unclear what the full species susceptibility spectrum of infected may be in natural settings or in zoological collections. We make the distinction between natural settings and zoological collections because natural infections would involve contact among members of the same species or among species that are sympatric in nature. In contrast, zoological collections bring together many species at high densities, many of which have never existed in sympatry and are potentially mutually naïve to pathogens carried by species in adjacent enclosures. It should also be pointed out that it is far easier to observe clinical signs resulting from interspecific infections in zoological collections in general, because there is little predation that would result in the culling of sick animals. Individual animals are also under a level of surveillance that is rarely achieved in the wild, facilitating the observation of even minor alteration in behavior indicative of disease.

The lack of host specificity of EHV-1 and EHV-9 is not restricted to experimental infection and extends to both zoological collections and the wild. Among equids, fatal cases of cross-species transmissions in zoos have been observed frequently in a diverse set of taxa. Among non-equid species, EHV-9 was isolated from fatal cases in captive Thompson's gazelles *(Eudorcas thomsonii)*, giraffes, and a polar bear.[9,11,12] Although identified in non-equid species, EHV-9 does not appear to be a naturally occurring virus of domestic horses though it can be isolated from several wild equine species, particularly zebras.[31–33] Captive gazelles, antelopes, cattle, alpacas *(Vicugna pacos)*, llamas *(Lama glama)*, and black bears *(Ursus americanus)* have also been shown to be infected with EHV-1.[13,34–36] More recently, an EHV-1–EHV-9 recombinant virus was associated with both lethal and nonlethal polar bear encephalitis cases and with a fatal encephalitis case in an Indian rhinoceros *(Rhinoceros unicornis)*.[8,10]

In the wild, EHV-1 and EHV-9 also exhibit cross-species circulation where they have been tested (which to date has been relatively limited). Serologic evidence exists that black and white rhinoceros (*Diceros bicornis* and *Ceratotherium simum*, respectively) are seropositive for EHV-1 and EHV-9.[37] A recent study of 277 animals in the wild or kept in captivity, including 151 wild samples from 41 wild plains zebras *(Equus quagga)* and 17 wild black rhinoceros sera, indicated significant prevalence of EHV-1 and EHV-9 in both captive and wild populations.[17] The results demonstrated very high prevalence of EHV-1 and EHV-9 in zebras and black and white rhinoceros in both captivity and the wild. Statistical analysis demonstrated that captive zebras have a significantly lower prevalence of seropositivity than wild zebras. Zebras in general are more likely to be serologically positive for EHV-1 when compared to EHV-9; in contrast, rhinoceros are more likely to be serologically positive for EHV-9 than EHV-1. The results suggest that African rhinoceros may be reservoirs species for EHV-1 and particularly EHV-9. This may have significant management repercussions as even in zoological collections that do not maintain zebras or other equids in captivity, the presence of rhinoceros could potentially result in cross-species transmission of EHV-1 or EHV-9. Although diagnosed fatal cases are not frequent, in captivity and in the wild, EHV-1 and -9 exhibit a broad host range and broad prevalence in perissodactyls.

Modes of Transmission

EHV-1 and EHV-9 infection is known to be spread from individual to individual as a respiratory smear infection. However, this route of transmission applies to equids and may not be generally applicable to interspecies transmissions. In the majority of observed cross-species transfers, there was no obvious physical contact between equids and non-equids. For example, in a 2010 fatal EHV-1 infection of a polar bear, the zebra enclosure was 200 m from that of the polar bear, and the zookeepers were not shared between the enclosures.[10] Nor were the polar bears fed equine meat or carcasses, which could expose them to remaining infectious virus if the culled equids were virus positive at the time of killing. The conditions were similar in a fatal polar bear case in 2009 with involvement of EHV-9.[12] Transmission was indirect inasmuch as neither equids nor rhinoceros were co-housed in close proximity. In a recent case, a polar bear and Indian rhinoceros exhibited neurologic signs. The polar bear recovered and was found weakly positive for EHV-1 in nasal swabs.[8] Several weeks later, the rhino aborted an 8.5-month-old fetus, exhibited neurologic clinical signs, and was euthanized after failure to respond to treatment. An EHV-1–EHV-9 recombinant very closely related to that found in the 2010 polar bear case was identified in the lung and brain of the rhinoceros. A clear route of spread or connection, if there was any, between the polar bear enclosure and rhino enclosure is not obvious. Clearly, direct respiratory transmission from equid to non-equid has not been likely in the majority of reported cases.

Fomites, defined as nonliving objects (anything from dust to clothing to shared instruments) are often responsible for transfer of pathogens.[12] Supporting this mode of transmission, Saklou et al. (2013) demonstrated that in "barn conditions" and at certain temperatures, infectious EHV-1 could persist on a variety of surfaces including stall bedding, shavings, and leather.[38]

Another potential source of contamination is water. EHV-1 remains stable for several weeks in water, even at high temperature.[39,40] Many zoological collections have enclosure-connecting water sources or noncaptive animal populations (rodents, birds) that move among enclosures

and/or water sources. Thus, it is possible that water contaminated with the viruses could expose multiple enclosures to various EHVs. Alternatively, secondary reservoirs or fomite carrying rodents and birds could transfer infected materials from one water source to the next.

The mode of transmission from environmental sources or intermediate hosts remains an unsolved mystery and there may be more than one route. This is a critical area of research because, without a clearer picture, developing management strategies will remain difficult. Regardless, stringent hygiene controls should be applied to prevent EHVs from spreading from equid (or rhinoceros) enclosures to other areas of the zoo. Equid meat should be fed only to carnivores naturally sympatric with equids. For example, lions hunt and consume zebras in nature but polar bears do not. Therefore, it is likely less problematic, from a health standpoint, to feed zebra or other equid meat to lions than to non-African carnivores. This applies to mixed species enclosures where natural sympatry should be considered when placing species together.

To Vaccinate or Not?

While EHV-1 has been intensively studied in domestic horses, there are still sufficient unknowns about the virus, for example, its capacity to evade hosts immune responses, to preclude a highly effective vaccination strategy.[7] Among the recommendation for horses are a commercial live viral vaccine (Rhinoimmune) and two inactivated viral vaccines (Pneumabort K-Fort Dodge and Prodigy-Intervet).[7] There is some conflicting evidence as to whether live or inactivated vaccines can lower incidence of EHV-1 induced abortions, but the majority of publications suggest efficacy. There is very little evidence, however, that vaccination can prevent neurologic disease. It is clear, however, that vaccination does not guarantee the complete prevention of viral shedding. Vaccination trials will have to be performed in different equid species to determine the efficacy of the commercial vaccines and determine if the benefits of a vaccination program are worth the financial cost. Regardless of whether vaccination regimens are implemented or not, consistent monitoring of viral shedding, isolation of shedding individuals, and decontamination of areas exposed to shedding animals will be key to managing cross-species transmission. If live virus escape can be reduced at the source, the chance of interspecies transmission should also be reduced.

Conclusions

EHV-1 and EHV-9 have the ability to infect a very wide range of mammals in experimental, captive, and natural settings. The full "natural" host range of these viruses is not fully determined and the modes of transmission need further exploration. It seems evident that the two viruses can threaten the health of a variety of equid and non-equid mammals in zoological collections. Vaccination efficacy in nondomestic equid species needs research, but there are other management and hygiene options available. Diagnostics for viremic and nonviremic are rapid, sensitive, and specific. If used for regular monitoring, shedding can be detected early enough to isolate individual animals and prevent the build-up of virus in the environment that would promote interspecies transfer. Implementation of such monitoring is strongly recommended.

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Ebola Virus Disease in Great Apes

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Introduction

All species of great apes are classified as either endangered or critically endangered by the International Union for Conservation of Nature (IUCN); worldwide, great ape populations are in decline, threatened by habitat degradation and destruction, poaching, climate change, and disease.[1] Among disease threats, Ebola virus disease (EVD) is cited as a major cause of population declines of western lowland gorillas *(Gorilla gorilla gorilla)*.[2]

Ebola Virus Disease

EVD is an acute, severe disease caused by viruses of the genus *Ebolavirus*, family Filoviridae.[3] *Zaire Ebolavirus* (ZEBOV), the species most associated with EVD in apes, was first discovered in 1976. Since then, four additional species of *Ebolavirus* have been identified: *Sudan Ebolavirus* (SUDV), *Taï Forest Ebolavirus* (TAFV), and *Bundibugyo Ebolavirus* (BDBV) in Africa and *Reston Ebolavirus* (RESTV) in Asia.[3] Multiple Ebola virus species have caused more than 30 EVD outbreaks in humans throughout sub-Saharan Africa. Eight of those—all caused by ZEBOV—have occurred in Gabon and the Republic of Congo (Congo) and were linked to human contact with gorilla or chimpanzee meat and/or carcasses.[4,5] A prolonged EVD outbreak caused by ZEBOV in West Africa from 2013 to 2016 infected more than 28,000 people, and more than 11,000 people died.[6] One epidemiologic study proposed that the index case of that epidemic may have been exposed to ZEBOV from an insectivorous bat; however, no definitive link could be demonstrated.[7]

Impact of Ebola Virus Disease on Great Apes

Although the precise degree of impact of EVD on great ape populations is difficult to quantify, there is little doubt that it has been significant. Only ZEBOV and TAFV have been identified in great apes.[3,5,8,9]

Ebola Virus Disease in African Apes

In contrast to human EVD outbreaks, which have occurred throughout tropical Africa, those involving apes have been restricted to West and Central Africa. In 1994 a single EVD outbreak occurred in chimpanzees in Taï National Park, Ivory Coast. Mortality was estimated at approximately 25% of the chimpanzee community.[10] The pathogen involved was identified as a new species of filovirus, subsequently named *Taï Forest Ebolavirus*.[11] One person, who contracted EVD after performing a postmortem examination on a chimpanzee that died during the outbreak, survived infection.[11]

In Central Africa, Gabon and Congo are home to the vast majority of western lowland gorillas *(Gorilla gorilla gorilla)* and central chimpanzees *(Pan troglodytes troglodytes)*.[2] In the late 1990s and early 2000s, EVD outbreaks involving ZEBOV occurred in two clusters in northeastern Gabon and northwestern Congo. Between 1994 and 1996, multiple reports of gorilla, chimpanzee, and other wildlife carcasses accompanied the human EVD outbreaks in Gabon's Minkébé Forest region. Most of the human outbreaks there were linked to contact with chimpanzee carcasses.[4,12] Wildlife abundance surveys conducted in the region before and after the outbreaks showed up to a 98% decline in gorilla and chimpanzee populations.[12]

Five human EVD outbreaks that occurred in the Gabon-Congo transborder region from 2001 to 2005 were also associated with contact with infected carcasses, mostly gorillas and chimpanzees.[5,8,13] A large number of wildlife carcasses had been reported in this region just prior to the human outbreaks and, in each, the human index case (usually a hunter) was linked to infection from a gorilla, chimpanzee, or duiker carcass.[5,8] Large mammal abundance estimates conducted in 2000 and 2003 (pre- and post-EVD outbreaks) in the Gabon-Congo transborder region showed a 59%, 89%, and 53% decline in gorillas, chimpanzees, and duikers, respectively. Based on this information, investigators estimated that hundreds or thousands

of gorillas there were lost to EVD.[8] Between 2001 and 2003, investigators recovered 98 wildlife carcasses in this region, including 65 great apes. Of 21 carcasses tested for Ebola virus by polymerase chain reaction (PCR), antigen detection, and, in some cases, immunohistochemical staining, 14 tested positive by at least one diagnostic test, including 10 gorillas, 3 chimpanzees, and 1 duiker.[5] From 2002 to 2003, researchers in the Lossi Gorilla Sanctuary in northwestern Congo witnessed a mortality event in free-ranging wild gorillas and chimpanzees under study. Over a period of several months, dozens of gorilla and chimpanzee carcasses were discovered. Overall mortality, based on nest counts and observations of known individuals, was estimated at 90%–95%. Gorilla mortality there was estimated to have exceeded 5000 individuals.[14]

Then in 2005 an EVD outbreak occurred in great apes in Odzala-Kokoua National Park (OKNP), northwestern Congo, concurrent with a nearby human outbreak. Wildlife abundance surveys conducted in the park in 2005, 2008, and 2012 estimated a nearly 50% reduction in gorillas there, representing about 20,000 individuals.[15] This decrease in gorilla populations in the absence of observed outbreaks supports the assertion that, given the extreme difficulty in monitoring wild great ape populations in this region, additional outbreaks may have gone undetected. In fact, an unusual ape mortality event was noted in early 2007 along the eastern edge of OKNP, during which local villagers reported dozens of gorilla carcasses. One chimpanzee and nine gorilla carcasses were sampled over a 4-month period.[16] Despite the lack of confirmatory testing, the mortality event appeared to be consistent with an EVD outbreak (K. Cameron, P. Reed, personal communication, June 30, 2017).

Although multiple human EVD outbreaks have occurred in the Democratic Republic of Congo (DRC), none has been reported in bonobos *(Pan paniscus)* or eastern gorillas *(Gorilla beringei)*. To date, BDBV and SUDV have not been detected in wild apes.

Based on observations of EVD impacts on known wild gorilla populations, ZEBOV-associated mortality in apes may reach 95%, exceeding that in humans.[14,17] In summary, although the precise number of apes killed by EVD is impossible to determine, overwhelming circumstantial evidence points to massive EVD-associated gorilla and chimpanzee mortality in the Central Africa region.

Ebola Virus Disease in Asian Apes

RESTV, the only Ebola virus species known to occur in Asia, has been identified in laboratory nonape primates imported to the United States from the Phillipines.[18] RESTV is not believed to be pathogenic to humans or Asian monkeys and no cases of EVD have been reported in Asian apes.[19] One study did report ZEBOV antibodies in captive Bornean orangutans *(Pongo pygmaeus)*, but these findings have been called into question.[20,21]

Clinical Signs

In humans, the incubation period of EVD is typically 2–21 days, and victims are not considered infectious until they develop symptoms. Once symptoms develop, they are variable but include a sudden onset of fever, fatigue, muscle pain, headache, and sore throat, followed by vomiting, diarrhea, rash, and, in a minority of cases, both internal and external bleeding (e.g., oozing from the gums, blood in the stool).[22] Sequelae in many EVD survivors—including musculoskeletal pain, headache, ocular symptoms, abdominal pain, and sexual dysfunction—constitute what is referred to as post-Ebola syndrome. These symptoms may persist for months to years after recovery.[23]

In apes, clinical signs are not specifically known, but there have been observations of wild chimpanzees exhibiting severe diarrhea and emaciation, and other nonhuman primates exhibiting vomiting, diarrhea, hair loss, emaciation, and epistaxis in association with known ZEBOV-associated EVD epizootics.[24] Necropsy results on a TAFV-positive chimpanzee reported signs of hemorrhage and nonclotting blood.[11]

In one study, nonape primates experimentally infected with ZEBOV or SUDV exhibited a wide variety of clinical signs, typically beginning within 3 days postinfection. These included fever, anorexia, cachexia, dehydration, diarrhea, intermittent melena, rectal bleeding, and petechial rash.[25] In another study, nonape primates died within 8 days of being experimentally infected with ZEBOV and within 12–14 days after being infected with TAFV.[26]

Apes may survive Ebola virus infection, as evidenced by the detection of antibodies in wild-born chimpanzees and other primates, in feces of wild gorillas, and by the survival of individuals in groups that suffered mortality during an EVD outbreak.[14,24,27,28]

Transmission

In humans, Ebola viruses are highly infectious. Infection usually occurs through direct contact of infected bodily fluids with mucous membranes or broken skin. An increased risk of transmission occurs in the acute phase of infection, when patients are viremic. Prior to the onset of clinical signs, and once the virus is cleared, there is little risk of transmission.

However, ZEBOV may persist in urine as well as in the placenta, amniotic fluid, and fetus in women who became infected while pregnant; it may also persist in breast milk in women infected while breastfeeding.[22] Viral persistence has also been demonstrated in "privileged" sites, such as the eyes, brain, and testes in both convalescing patients and laboratory monkeys.[29,30] ZEBOV has been isolated from human semen up to 7–9 months postonset of EVD.[31] Such viral persistence raises the possibility of transmission or reemergence postoutbreak.[31]

Routes of transmission in apes have not been definitively established, including routes of spillover from putative

reservoir species. A predominant theory proposes that apes may consume food items contaminated with bodily fluids (saliva, urine, feces) from reservoirs (e.g., fruit bats) at common feeding sites.[32,33] However, viral shedding of ZEBOV in wild bat urine or feces has not been reported.[34]

Transmission within ape groups presumably occurs through physical contact with a sick or deceased group member.[5] Transmission between ape groups may occur to varying degrees by a variety of mechanisms, including contact with a deceased member of another group, dispersion of females upon the death of the group leadership, cofeeding encounters between groups, intergroup aggressive interactions, or intergroup breeding.[5,35–37]

Evidence suggests that EVD outbreaks in ape populations may extend over large geographic areas, though how this occurs is a matter of debate. Two theories predominate in the literature.[5,12,14] One proposes that transmission of the virus continues from group to group, moving as a "wave" of transmission across the landscape.[38] Transmission in this scenario would be largely dependent on the incubation period and speed of disease progression in individual apes, on group and individual movement, and on intra- and intergroup social dynamics.[39,40] But detection of different strains of ZEBOV in gorilla carcasses located in close proximity during a given outbreak argues against a major role of such transmission.[5]

A second theory proposes that such wide geographic spread results from multiple spillover events into an ape population from a reservoir host species. Transmission in this scenario would be more dependent on reservoir species dynamics.[8] The fact that large distances between ZEBOV-positive ape carcasses found during a short period and the existence of physical barriers to ape movement, such as roads and rivers, tend to support this mode of transmission.[5] These proposed transmission mechanisms are not mutually exclusive, however, and could occur concurrently during a given outbreak.

Reservoir

Great apes, like humans, are considered dead-end hosts of Ebola virus. African fruit bats are often cited as putative reservoir hosts.[22,41] In studies in which dozens of plant, vertebrate, and invertebrate species were experimentally inoculated with ZEBOV, viral amplification occurred only in bat species.[42–47] Bats in one study became infected, replicated the virus, seroconverted, and shed virus in feces but without developing clinical signs of disease.[42] Ebola virus RNA and/or ZEBOV-specific IgG antibodies have been found in several species of African fruit bats.[47–49] Insectivorous African bats cannot be ruled out as potential reservoirs. Emerging diagnostic and epidemiologic data suggest they may also harbor ZEBOV.[7,50] Yet despite thousands of wild species having been tested since 1976, live virus has never been isolated from a wild-caught animal, the first step in defining the natural reservoir host.[34,48,51] Interestingly, live *Marburgvirus*—another member of the family Filoviridae closely related to *Ebolavirus*—has been isolated from Egyptian fruit bats *(Rousettus aegyptiacus)*, supporting the theory of bats as filovirus reservoirs.[52,53]

Diagnostics

The diagnosis of Ebola virus disease in humans is based on the presence of viral RNA, antigen, or antibodies. Diagnostic assays employed in suspected human cases include antibody-capture enzyme-linked immunosorbent assay (ELISA), antigen-capture detection tests, serum neutralization test, reverse transcriptase polymerase chain reaction (RT-PCR) assay, electron microscopy, and virus isolation by cell culture.[22] Preferred diagnostic specimens in humans include whole blood in ethylenediaminetetraacetic acid (EDTA) from live symptomatic patients or an oral fluid specimen in universal transport medium collected from a deceased patient or when blood collection is not possible.[22]

Collection of blood samples from wild apes is impractical and diagnostics are usually conducted on carcass samples. Obtaining an accurate diagnosis may be particularly challenging in tropical field settings, where carcasses degrade quickly. Degraded tissues may be unsuitable for many Ebola virus diagnostic assays and more prone to produce false-negative results.[5] In fact, in field settings, attempts at viral isolation from decomposed carcasses have generally been unrewarding.

Differential Diagnosis

Differential diagnosis is dependent on the epidemiologic context and may include bacterial, viral, fungal, and parasitic causes.[32] In humans, significant diseases to rule out include malaria, typhoid fever, shigellosis, cholera, leptospirosis, plague, rickettsiosis, relapsing fever, meningitis, hepatitis, yellow fever, and other viral hemorrhagic fevers.[22] In African apes, anthrax may also be considered.[54,55]

Control Measures

Limited options have been identified for control of Ebola virus infection in great ape populations.

Vaccination

If a vaccine becomes available (see later), vaccination of wild apes against Ebola virus will gain momentum as a possible EVD control measure for ape populations.

A number of Ebola virus vaccines are under development, mostly intended for human use. Two in particular, both vector-based vaccines expressing ZEBOV antigens, have been proposed for use in apes. The monovalent recombinant chimpanzee adenovirus type-3 vector-based vaccine (ChAd3-EBO-Z) and recombinant replication-competent vesicular stomatitis virus-based vaccine (rVSV-EBOV) both provide immunity following a single dose and have undergone human clinical trials.[56,57] A third vaccine, under

development specifically for use in great apes, is based on an ape-specific cytomegalovirus (CMV).[58] This is a self-replicating vaccine, designed to spread from ape to ape, stimulating immunity against ZEBOV as is does so. The proposed advantages of such a vaccine are (1) that it could, once administered to relatively few individuals, immunizing a much larger portion of the ape population and (2) that it could do so with reduced effort and in a logistically easier and more cost-effective, manner. The predominant potential disadvantage of this self-replicating vaccine relates to concerns about safety in target and nontarget species.[59]

Practical concerns about vaccinating wild apes include the route of vaccine administration (e.g., by injection versus orally), the ease of access to apes (e.g., habituated to close human presence versus unhabituated), the number of doses of the vaccine needed to achieve adequate immunity in an individual (i.e., a single dose versus the need for booster doses), the purpose of the vaccination campaign (e.g., to provide immunity from future infection or to halt an ongoing epizootic), the relative risk of an EVD outbreak to a given ape population, and more.[59] A given vaccine may have more or less application to various ape populations, their level of habituation, and the degree and nature of the Ebola virus threat. Given that many human EVD outbreaks are closely associated with contact with wildlife carcasses, reducing occurrence of EVD in ape populations could also have protective benefits for humans.

This issue of ape vaccination against Ebola virus has stimulated lively discussion among conservationists. Moral issues about intervening in natural processes in "wild" populations versus human obligations to do so, potential unintended consequences of the use of genetically modified vaccines, and other vaccine safety issues dominate these discussions.[59,60] First and foremost, any vaccine must be shown to be efficacious and safe for target (ape) and nontarget (nonape, including wildlife and human) species prior to deployment.[60]

Carcass Disposal

In the event of detection of a great ape carcass that may signal an EVD epizootic, programs have been established to collect samples for Ebola virus diagnostic testing.[5,16] Whether or not to dispose of these potentially Ebola virus–infected carcasses in order to limit transmission potential has been debated and remains a practical issue in EVD surveillance efforts. Carcass degradation is likely to vary with environmental conditions. One study showed that carcasses are likely to remain infective for 3–4 days in a tropical environment.[8] In another study, in which carcasses were not exposed to insect scavengers, viable Ebola virus was isolated from infected laboratory macaques up to 7 days or more postmortem.[61]

Burial and/or incineration of carcasses on site has been proposed (K. Cameron, P. Reed, personal communication, June 30, 2017). The high risk of breach of personal protective equipment that burial of a ZEBOV-infected carcass—particularly in a wooded environment—would put investigators at additional risk of exposure. Incineration using vegetation and/or diesel fuel, which has been employed in more accessible regions, is very time-consuming, impractical in many remote regions, and carries additional risk. Transporting the carcass to another location for incineration or burial is highly ill-advised, as it carries significant risk and goes against basic principles of pathogen containment. Spraying the carcass with a 5% sodium hypochlorite solution has also been proposed but carries the risk of human poisoning in the event that local inhabitants attempt to scavenge meat (K. Cameron, P. Reed, personal communication, June 30, 2017).

Given these concerns, one program left carcasses to decay unburied following sampling (K. Cameron, P. Reed, personal communication, June 30, 2017). Concerns about human infection in the meantime were addressed by conducting outreach education in local communities before departing the region (K. Cameron, P. Reed, personal communication, June 30, 2017).

Future Directions

Although laboratory and field studies over the past 4 decades have greatly improved our understanding of the epidemiology and pathophysiology of Ebola virus in humans, our understanding of its ecology as well as its precise impact on wild apes remains relatively poor. This lack of basic knowledge makes designing potential mitigation strategies for both apes and humans more challenging. More investigation is needed to better characterize the basic ecology of Ebola viruses, importantly regarding potential reservoir species, including bats; the identification of potential vector species that may complicate the transmission chain and obfuscate the reservoir host; elucidation of the ecology of those reservoirs and vectors; and identification of environmental factors that may affect the virus's sporadic reemergence. Greater understanding of the virus's transmission in a natural setting is needed, including the route or routes of spillover from reservoir to susceptible hosts; both interspecific and intergroup transmission among apes; and the potential roles of other susceptible species, such as wild pigs and duikers. As this knowledge improves, there will be a need for updated and more refined projections of the long-term impacts of EVD on ape populations. Improved surveillance, allowing early detection of EVD outbreaks in apes, would help to facilitate much of the needed research. In the meantime, work should continue on the potential use of vaccination as a control measure to protect ape species against EVD, with a particular focus on vaccine safety for both target and nontarget species. In order to accomplish this and maximize future successes in preserving these iconic ape species, the issue of EVD's impact on wild ape populations will require improved open, rational, and constructive dialogue within the scientific and conservation communities.

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35

Chagas Disease: Wildlife Infection With *Trypanosoma Cruzi* in a One Health Context

SARAH HAMER AND CAROLYN HODO

Background

Trypanosoma cruzi, the etiologic agent of Chagas disease (American trypanosomiasis), is a zoonotic vector-borne protozoan capable of infecting animals from virtually all mammalian orders.[1] It is transmitted via the feces of hematophagous insects of the subfamily Triatominae (Hemiptera: Reduviidae), commonly known as kissing bugs or conenose bugs, and is endemic across the Americas from Argentina and Chile to the southern United States. Diverse mammalian wildlife species are critical in the ecology of Chagas disease as both a source of blood to the triatomine vectors and also as reservoirs of the *T. cruzi* parasite in sylvatic transmission cycles.[2,3] The most common manifestation of Chagas disease is an infectious myocarditis leading to acute or chronic heart failure, with gastrointestinal megasyndromes (megacolon, megaesophagus) being less common, and many infected hosts may remain asymptomatic. Chagas disease is well described in humans, nonhuman primates, and domestic dogs, while pathologic effects in wildlife reservoirs are relatively understudied.

From the earliest days in Chagas disease research, a One Health approach was used to describe the etiology, transmission, and epidemiology of disease. In 1909, Dr. Carlos Chagas of the Instituto Oswaldo Cruz-Fiocruz in Brazil first discovered *T. cruzi* in triatomine bugs in Brazil and then implicated the parasite as the cause of disease in a febrile child.[4] Chagas then searched for the animal reservoirs of the parasite, first identifying trypomastigotes in a domestic cat,[5] then describing the armadillo *(Dasypus novemcinctus)* as a wild reservoir.[6] Chagas' comprehensive approach allowed for the rapid characterization of the etiology of this new disease, and the continued integration of the study of wildlife, domestic animals, and human health is necessary to address the many unanswered questions about *T. cruzi* that persist over 100 years after its discovery.

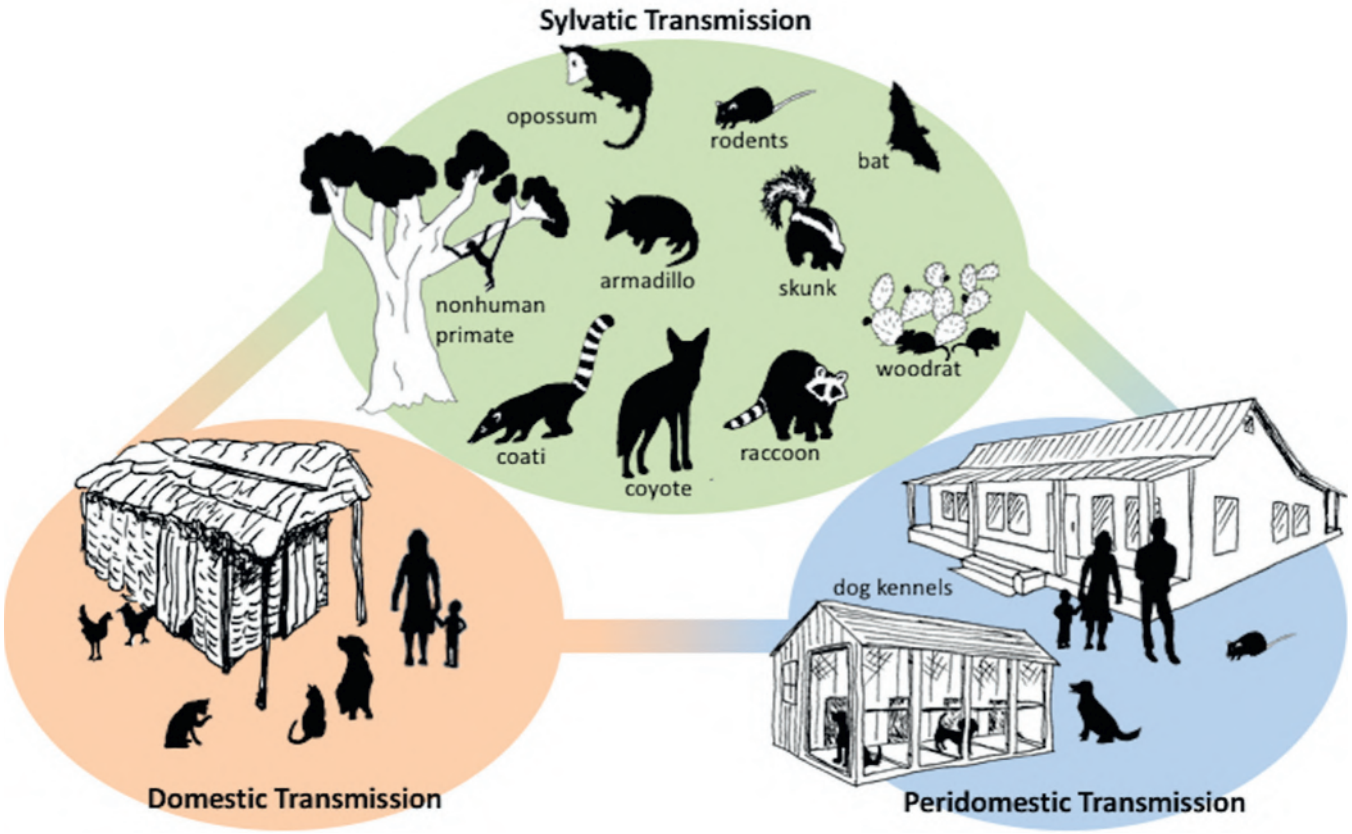
Chagas disease presents a significant human health burden across Latin America, where an estimated 6 million persons are infected.[7] In these regions, human disease is most commonly associated with domestic or peridomestic transmission settings where poverty and housing conditions facilitate vector colonization, or spillover from sylvatic transmission cycles (Fig. 35.1) in areas where domestic transmission has been controlled. Human Chagas disease outside of Latin America occurs predominantly in immigrants from endemic countries.[8,9] Nevertheless, there is increased recognition of autochthonous transmission to humans in the southern United States,[2,10,11] likely reflecting spillover from robust sylvatic transmission cycles (see Fig. 35.1) that have been documented for decades. Accordingly, knowledge of the distribution and infection prevalence in wildlife species provides an important basis for not only managing animal health, but also human health.

Epidemiology

Hosts

Mammals of virtually all orders are susceptible to *T. cruzi* infection, and the parasite has been documented in 150–200 different species.[12] Birds, amphibians, and reptiles are refractory, "dead-end" hosts for *T. cruzi*, although they provide blood meals to triatomine vectors and therefore may contribute to sustaining vector populations. The most important reservoir species vary widely on a spatiotemporal scale. Given that triatomine vectors feed dozens of times on diverse host species throughout their life, a complex network of host species that together comprise a "reservoir system" is responsible for maintaining the parasite in nature (see Fig. 35.1).

Dogs, cats, commensal rodents, and domesticated guinea pigs serve as predominant reservoirs in the peridomestic and



• **Figure 35.1** Key reservoir hosts in the ecology of Chagas disease across the Americas differ in domestic, peridomestic, and sylvatic transmission settings, with marked geographic variation in vertebrate reservoir community and triatomine vector distribution. However, there is considerable overlap between cycles, with dogs playing important roles in both domestic and peridomestic transmission, and certain wildlife species being important as both sylvatic and peridomestic reservoirs. True domestic transmission involves vectors that colonize homes, feeding on human and domestic animal inhabitants, while peridomestic and sylvatic transmission occur in the outside environment and involve different vector species.

domestic settings.[3,13] Dogs can develop acute or chronic cardiac disease, whereas the clinical implications of infection in other domestic reservoirs have been relatively understudied. In the United States, *T. cruzi* has been detected in at least 26 wildlife species across 15 southern states, as far north as Missouri.[2,14] Among the most well-studied and highly infected reservoirs in the United States are carnivores including raccoons *(Procyon lotor)*, striped skunks *(Mephitis mephitis)*, coyotes *(Canis latrans)*, gray foxes *(Urocyon cinereoargenteus)*; woodrats (*Neotoma* spp.); opossums *(Didelphis virginiana)*; and nine-banded armadillos *(D. novemcinctus)*. In Brazil, key wildlife reservoirs include opossums (*Didelphis* spp. and *Philander* spp.); xenarthrans including armadillos, anteaters, and sloths; nonhuman primates especially tamarins (*Leontopithecus* spp.); carnivores especially coatis *(Nasua nasua)*; and bats; while diverse rodent species generally have overall low *T. cruzi* infection prevalence and seem to play a secondary role as reservoirs.[15] In Mexico, opossums (*D. marsupialis* and *D. virginiana*), rodents, bats, raccoons, and xenarthrans are among the key reservoir hosts in nature.[16]

Zoological parks or areas with captive animals are potentially high-risk areas for Chagas disease if they coincide with the distribution of triatomine vectors, because animals are housed in outdoor areas that are accessible by blood-sucking arthropods. Furthermore, many zoo animals have extensive travel histories, providing opportunities for the importation or exportation of parasite infection to and from the park, including exotic parasite strains that may differ from those maintained by local wildlife. Disease has been reported in a number of wild and captive New and Old World nonhuman primates.[17–21] For example, heart abnormalities consistent with Chagas disease were found at a nonhuman primate center in Louisiana, where 1.6% of over 2000 individuals had been exposed to the parasite.[22] Additionally, 8.5% of cynomolgus macaques *(Macaca fascicularis)* in outdoor housing in central Texas were infected or exposed to the parasite, many of which harbored cardiac lesions from infection.[19] At a nonhuman primate facility in central Texas with ongoing *T. cruzi* infections in macaques, we found high levels of parasite in the blood and tissues of native wildlife trapped at the facility, including raccoons, opossums, and skunks, suggesting these wild animals serve as a source of infection that can be transmitted by vectors to the primates.[23] A polar bear *(Ursus maritimus)* at a zoo in Mexico developed fatal acute cardiomyopathy due to *T. cruzi* infection.[24] Chagas disease was implicated as the cause of sudden death of a 7-year-old female wolf-hybrid at a zoo in central Texas where triatomine vectors are commonly

found in and around the animal enclosures (Clark P, personal communication, February 27, 2017). Because Chagas disease often presents with nonspecific signs or sudden death, it is likely that it is underreported in wildlife and zoo animal species.

Vectors

Across the Americas, insects of the family Reduviidae, subfamily Triatominae, including species of the genera *Rhodnius*, *Panstrongylus*, and *Triatoma*, are the most important vectors of *T. cruzi* (Fig. 35.2) to humans and animals. These nocturnal insects are blood feeding throughout all five nymphal stages and as adults. Eleven species of triatomine insects have been recorded across the southern United States,[2] where they are colloquially known as "kissing bugs" or "conenose bugs." In Mexico, 19 of the 31 local triatomine species have been consistently found to invade human houses and all have been found to be naturally infected with *T. cruzi*.[25] In the Amazon Basin, at least 10 of the 16 triatomine species that occur in the region have been found to be infected with *T. cruzi*.[26] Both adults and nymphs have been collected from zoos,[24,27,28] nonhuman primate centers,[29] and domestic animal enclosures.[30] Blood meal analyses of triatomines have revealed a generalist opportunistic feeding strategy appearing to reflect local, easily-accessible blood sources,[13] including nidicolous mammals such as woodrats and armadillos.[2] Because infection prevalence[31] and sylvatic host associations[2] are known to vary among triatomine species, risk of parasite transmission to humans and animals likely varies according to the vector species found in a local area.

• **Figure 35.2** Triatomine vectors, commonly known as kissing bugs or conenose bugs, are large nocturnal insects that are dark brown to black in body color, often with distinct, reddish- to cream-colored stripes visible along the edges of the abdomen. Their head is stick-like and tapering, and their six legs are relatively thin and tapering. Left to right; *Triatoma protracta*, the most common species in the western United States; *Triatoma gerstaeckeri*, the most common species in Texas; *Triatoma sanguisuga*, the most common species in the eastern United States. Scale bar represents 25 mm or approximately 1 inch. (Photo credit: Gabriel Hamer, PhD, Texas A&M University Department of Entomology. Originally printed in Curtis-Robles R, Wozniak EJ, Auckland LD, et al: Combining public health education and disease ecology research: using citizen science to assess Chagas disease entomological risk in Texas. *PLoS Negl Trop Dis* 9:e0004235, 2015.)

Transmission Routes

Transmission of *T. cruzi* is predominantly through the infected feces of the triatomine vector. The insect vector acquires the blood form trypomastigote stage of the parasite while feeding on an infected mammalian host, and the parasite replicates as the insect form epimastigote stage in the digestive tract of the bug. Epimastigotes mature to infective metacyclic trypomastigotes in the insect hindgut, which are passed in the feces and may be deposited onto a susceptible host. The parasite does not penetrate intact skin but can enter microlesions at the site of feeding, other broken skin, or a mucous membrane; this mode of transmission is termed stercorarian, or "vector-fecal" and is thought to be the most important route accounting for human infections across the Americas where vectors colonize homes. Transmission via the oral route occurs through a variety of means and may be the most important route in free-ranging mammals.[5] Oral transmission can occur following the ingestion of an infected bug, ingestion of food contaminated with bug feces, or ingestion of infected blood or tissue from a parasitemic host. Additionally, at least one species of opossum *(Didelphis marsupialis)* has been demonstrated to shed the infective form of the parasite in anal sac secretions,[32] presenting a method for oral transmission via ingestion of parasite from opossum feces in contaminated foodstuffs. Oral transmission has been demonstrated in skunks, raccoons, opossums, and wood rats,[33–36] and suggested in dogs.[37] Additionally, congenital transplacental transmission has been described in humans and dogs,[38] but its importance as a route of infection to wildlife or other animals in natural environments has not been studied. Congenital transmission was not demonstrated in experimentally infected opossums *(D. marsupialis)*.[39]

It has been suggested that hematophagous (sucking) lice may serve as alternative vectors of *T. cruzi* in nonhuman primate facilities, though transmission via this route has not been proven.[40] Bed bugs *(Cimex lectularius)*[41] and bat bugs *(Cimex pilosellus)*[42] have been shown to be competent vectors for *T. cruzi* in laboratory settings, but the importance of other blood-sucking vectors in the transmission of *T. cruzi* in natural settings has yet to be demonstrated.

Implications of *Trypanosoma cruzi* Genetic Diversity

T. cruzi is a genetically heterogeneous species and comprises at least seven strain types or discrete typing units (DTUs): TcI-VI and TcBat. TcI has been divided into TcIdom and TcIsyl, representing domestic and sylvatic isolates.[43] The parasite DTUs provide a framework for understanding

associations of the parasite with different geographical locations, reservoir host species, and clinical manifestations in hosts infected with different parasite strain types.[3,44]

The general distribution of DTUs includes TcI across the Americas; TcII, TcV, and TcVI in the Southern Cone; TcIII in central South America; and TcIV in the southern United States and northern South America.[45] In some geographic areas, parasite DTUs are closely associated with certain vector or host species; for example, the vast majority of *T. cruzi* in raccoons in the southern United States is TcIV,[46,47] and opossums across the Americas harbor TcI.[2,3] TcIII occurs almost exclusively where the vector *Panstrongylus geniculatus* is distributed in central South America.

Growing evidence suggests that certain *T. cruzi* strain types may be associated with different clinical outcomes in humans.[13,45,48] Similarly, experimental studies in dogs have demonstrated differing clinical, pathologic, and immunologic outcomes resulting from infection with different strains. For example, dogs infected with *T. cruzi* isolates from an armadillo and opossum developed acute and chronic myocarditis, while dogs infected with an isolate from another dog did not develop disease,[49] and increased numbers of inflammatory cells were observed in the heart in dogs infected with TcI compared to TcII.[50] Few studies have assessed the degree to which different *T. cruzi* strain types are associated with differential clinical outcome in infected wildlife species.

Pathogenesis

Although few specific studies have examined *T. cruzi* pathogenesis in wildlife species, in humans and dogs, infection with *T. cruzi* is characterized by acute, indeterminate, and chronic stages. During the acute stage, parasitemia can be high and the parasite is found in many organs. A small percentage of individuals experience severe cardiac disease during this stage, but the majority will have only mild nonspecific clinical signs or be completely asymptomatic. An effective immune response reduces parasite numbers but is unable to eliminate the parasite completely. During the indeterminate phase, clinical signs are absent, but the parasite persists in target organs, most often the heart, and antibodies can be detected in the serum. Approximately 30% (in humans) of indeterminate stage patients will progress into the chronic stage characterized by cardiomyopathy or rarely, megasyndromes of the gastrointestinal tract. The proportion of infected nonhuman animals that develop chronic disease is relatively unknown and likely species-dependent.

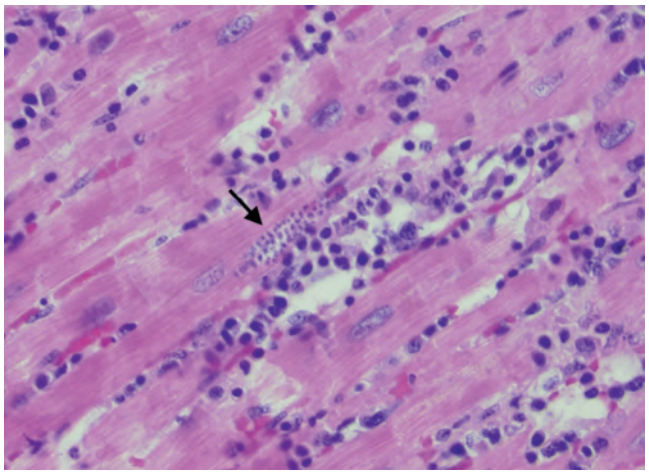
Clinical Signs

Clinical signs during the acute phase, if present, are generally nonspecific. In the chronic stage, clinical signs are those of heart failure (exercise intolerance, lethargy, coughing, pleural effusion, ascites, dependent edema), and not specific to *T. cruzi* infection. Cardiac arrhythmia may be a prominent feature, especially right bundle branch block. Dependent edema may be a feature, and scrotal edema was described in an infected chimpanzee.[20] In some cases, acute death occurs with no premonitory clinical signs.

Pathology

The typical *T. cruzi* pathologic changes in the heart are characterized by mononuclear cell inflammatory infiltrates composed of lymphocytes, plasma cells, and macrophages, together with myocardial degeneration and necrosis, with fibrosis in the chronic stages. The heart may be grossly enlarged and/or pale depending on the severity and chronicity of the disease. The amastigote tissue stages ("leishmanial forms") of the parasite are found in intracellular tissue pseudocysts, are approximately 4 µm in diameter, and contain a round nucleus and rod-shaped kinetoplast. Amastigotes are most commonly found in the heart (Fig. 35.3) but have been reported in many other tissues, such as skeletal muscle, lymph nodes, central nervous system tissue, and tissues of the digestive tract. Atypical manifestations of *T. cruzi* infection may include acute lymphadenopathy[51] or neurologic disease as described in dogs[52,53] and a horse.[54]

While studies that document wildlife infection are common, those that characterize the pathology associated with infection are rarer. Experimentally infected South American opossums *(D. marsupialis)* developed only mild inflammation associated with scattered parasites, while more intense inflammation and tissue destruction was observed in naturally infected opossums.[55] Mild to moderate inflammation was observed in tissues of experimentally infected Virginia opossums *(D. virginanus)*, while experimentally infected raccoons *(P. lotor)* developed moderate to severe



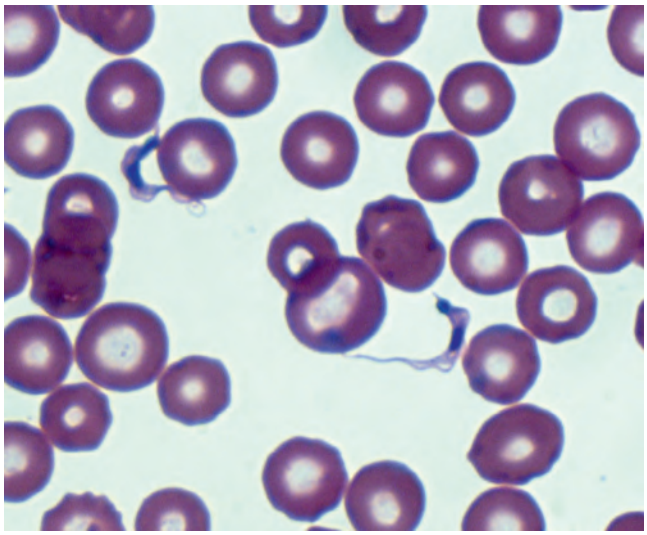
• **Figure 35.3** Photomicrograph of the heart of a naturally infected rhesus macaque from a nonhuman primate facility in central Texas. A cardiac myocyte contains a pseudocyst filled with *Trypanosoma Cruzi* amastigotes *(arrow)*. The myocardium is infiltrated by inflammatory cells, predominantly lymphocytes and plasma cells with few macrophages, and there is multifocal myocardial degeneration and loss (hematoxylin and eosin stain, 20× magnification). (Photo credit: Wallace Baze, DVM, PhD, DACVP, Keeling Center for Comparative Medicine and Research, MD Anderson Cancer Center.)

inflammation.[56] No histopathologic lesions were observed in a survey of infected raccoons, coyotes, foxes, and bobcats in Texas,[46] while a more detailed pathologic investigation revealed inflammation and myocardial degeneration in some infected Texas coyotes.[57]

Diagnosis

Diagnosis of *T. cruzi* presents significant challenges, especially for zoo and wild animal species for which diagnostic tests have not been validated. Diagnosis of Chagas disease is based on clinical suspicion and supporting diagnostic test results. The parasite may be directly observed via microscopic examination of blood smears during parasitemic stages or of infected tissues during chronic stages. Parasitemia has been shown to occur within days to 4 weeks after infection in dogs and mice but becomes undetectable after a short period of time.[58] On light microscopy, the blood form of the parasite is C- or S-shaped, 16–22 µm in length, with a rod-shaped kinetoplast, an undulating membrane, free flagellum, and a round nucleus located in the central or front portion of the body (Fig. 35.4). Histopathologic examination of tissues can demonstrate the presence of amastigotes and/or characteristic lesions (described previously). Amastigotes may be rare in chronic stages of the disease and their absence does not preclude diagnosis if characteristic myocardial inflammation is present. Immunohistochemistry would be helpful to confirm infection when amastigotes are not seen but is not widely available.

Numerous indirect methods are used to determine infection, many of which have not been properly validated for use in wildlife species or domestic animal species, given the absence of a gold standard diagnostic test. Sensitivity and specificity of different existing diagnostic tests may vary widely across tests and species, and dynamics of local transmission vary by geographic location, affecting positive predictive values. Serologic tests detect the presence of antibodies; antibody-positive animals are generally considered to also be currently infected because self-cure is thought to be rare. Serologic testing, namely, indirect fluorescent antibody assay (IFA), is a common tool for detecting anti-*T. cruzi* antibodies in dogs but is not commonly available for zoo and wildlife species. Rapid immunochromatographic lateral flow assays designed for human diagnostics have been used on dog and wildlife samples in research settings. Limitations of serology include the difficulty of finding species-specific controls for many wildlife and zoo species, lack of validation in these species, and potential for cross-reaction with other trypanosome species in areas where those are endemic (e.g., *T. rangeli*, *T. vivax*, *T. evansi*, *T. equiperdum*, and *T. theileri*). Further, very acute infections may fail to be detected using serologic methods alone; anti-*T. cruzi* antibodies are first detected an average of 3 weeks postinfection in dogs.[58] Polymerase chain reaction (PCR) allows direct detection of the parasite, is generally considered highly sensitive and specific, and does not necessitate species-specific reagents or controls, thereby increasing its utility as applied to wildlife or zoo animals. However, PCR of blood is a useful diagnostic tool only during a period of parasitemia, the level and duration of which may vary between host species. PCR of the heart or other tissues is useful for postmortem diagnosis. Often, several diagnostic methods must be used to make a diagnosis, combining clinical suspicion, serologic status, pathology, and molecular results.



• **Figure 35.4** *Trypanosoma cruzi* trypomastigotes in a canine blood smear, with undulating membrane, free flagellum, apical nucleus, and large kinetoplast (100× magnification). (Photo credit: Karen Snowden, DVM, PhD, DACVM, Texas A&M University College of Veterinary Medicine and Biomedical Sciences.)

Treatment, Management, Prevention

Treatment options for *T. cruzi* infection are extremely limited. Benznidazole and nifurtimox are the antiparasitic drugs most commonly used to treat humans but are not readily available in veterinary medicine (especially not in the United States) and are associated with significant side effects. Clinical case management of diseased animals typically focuses on the symptomatic treatment of cardiac complications, and case management is complicated by the absence of a vaccination or approved antiparasitic treatment against *T. cruzi*. Although many infected animals may remain asymptomatic, there are not currently prognostic data available to evaluate which animals will develop disease.

Major recommendations for reducing risk of transmission in domestic and captive animal settings generally focus on integrated vector control methods (Box 35.1). Vector control recommendations include insecticides, screening animal enclosures, reducing use of night lighting (which may attract flying adult triatomines), removing brush piles and other microhabitats conducive to wildlife nests that serve as sources of blood meals for kissing bugs, and sealing crevices that may serve as hiding places for triatomines. Additionally, because of the possibility of transmission via carnivory, efforts should be made to limit the exposure of captive animals to potentially infected wildlife. While

• BOX 35.1 Recommendations for Triatomine Vector Control

- Use of residual insecticides and physical barriers such as diatomaceous earth
- Screening animal enclosures and human housing
- Reducing use of night lighting (which may attract flying adult triatomines)
- Removing brush piles and other microhabitats conducive to wildlife nests or bug harborage sites
- Sealing crevices within animal enclosures that may serve as hiding places for triatomines

rats captured at Texas nonhuman primate facilities with documented local transmission were not infected with *T. cruzi*,[59] rodents have been implicated as reservoirs in other areas.[13,60] Because of the ability of the opossum to shed the infective stage of the parasite through anal secretions,[32] care should also be taken to prevent the contamination of animal feed by opossum feces.

While direct animal-animal transmission is considered unlikely in the absence of predation, there is a potential risk that captive infected animals may serve as reservoirs to infect local vectors. However, this level of risk is not well characterized, and an individual animal's reservoir potential is variable on a species-level and individual-animal basis. Additionally, in endemic areas including the southern United States, local wildlife populations are frequently highly infected, likely serving as the primary parasite reservoirs, with captive animal reservoirs playing secondary roles. Nevertheless, vector control activities could help to mitigate risk by limiting the access of vectors to infected captive animals.

Zoonotic Potential

The main risk of human exposure to *T. cruzi* is from contact with infected vectors. The efficiency of the stercorarian (vector-fecal) route of transmission is extremely low, and estimates are that several hundred or more contacts with infected bugs are necessary for vector-borne parasite transmission,[61] and so human transmission is greatest in areas where there are frequent contacts between vectors and people. Direct transmission from infected animals to humans is possible via exposure to infected blood, which may occur through broken skin (needle stick, existing skin wounds) or through contact with mucous membranes (oral, conjunctival). As such, care should be taken when handling the blood of infected animals. Additionally, because of the ability of infected opossums to shed infective parasites in their anal secretions, care should be taken when handling feces and cleaning animal enclosures to avoid contact with broken skin or mucous membranes. The presence of infected animals may increase local risk to other uninfected animals or humans if the infected animals maintain a parasitemia that allows them to serve as reservoirs to infect vectors, as has been shown for dogs and cats in villages in Argentina.[62] However, this contribution must be considered in light of the presence and infection status of other local reservoirs and vectors.

Conclusion

T. cruzi is a zoonotic parasite with a wide host range including hundreds of wild and domestic mammals, and a One Health approach to assess human, wildlife, domestic animal, and vector infection data has been useful in defining transmission networks and assessing disease risk. The clinical impacts of infection across most wild taxa are largely unknown. Because most diagnostic tests are validated for human or canine use, diagnosis of Chagas disease in zoo and wild animals is a challenge; further, antiparasitic treatments are not widely available especially in the United States. Vigilance for triatomine vectors and integrated vector control are the key for Chagas disease control. Enhanced awareness for vectors and infected animals among veterinary practitioners working with zoo animals and wildlife will afford the protection of both human and animal health.

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36

The Effects of Climate Change on Disease Spread in Wildlife

ERIK HOFMEISTER AND CAROLINE VAN HEMERT

Introduction

A growing body of evidence indicates that climate change alone,[1–3] or acting synergistically with current anthropogenic threats,[4] is affecting the health of wild populations of aquatic and terrestrial wildlife. Measurable by-products of climate change include elevated atmospheric concentrations of greenhouse gases, higher average global temperatures, variations in global precipitation patterns, rising and warming oceans, altered hydrographs of rivers, and increased mid-continental drying during summer.[5] These consequences affect the terrestrial environment through shifts in phenology, vegetation cover, and fire regimes. Warmer ocean temperatures, increased acidification, rise in sea levels, and reduction in sea ice cover are also leading to widespread ecologic changes in marine systems.[6] Wildlife populations face a variety of climate-related pressures, such as changes in animal distribution or density, limitation of food resources, and alteration to critical habitats.[2,3,7]

The increased potential for emergence and resurgence of diseases that are responsive to environmental conditions also has implications for wildlife populations. Shifts in temperature or other climatic factors may directly affect the incidence of disease in wildlife by altering host-pathogen interactions, promoting vector populations or allowing new ranges for vectors, or reducing development times for parasites.[2,3,7] A number of examples from both field and laboratory studies have demonstrated a clear link between warming and disease spread.[3,7,8] Many climate-related environmental changes also influence wildlife health indirectly. For example, increasing temperatures, in combination with shifts in rainfall and humidity, may aggravate current trends for water resource limitation and habitat degradation or destruction and lead to increased crowding of animal populations, thereby promoting transmission opportunities of pathogens within populations or across species.[9,10]

Although it may be difficult to disentangle the influences of other anthropogenic changes from the direct effects of warming, some ecosystems provide especially useful models for studying climate-related disease spread in wildlife. For example, the effects of climate change on parasite dynamics may be easily observed in the Arctic, where environmental changes are occurring rapidly, anthropogenic influences are relatively limited, and biodiversity is generally low.[11–13] Marine ecosystems are also undergoing rapid rates of change and may be vulnerable to a variety of natural and anthropogenic perturbations. Although many factors affect the health of organisms in ocean environments, temperature has been clearly linked to an increase in disease prevalence among sessile organisms such as corals.[2,6,14]

In this chapter, we discuss observed and predicted changes to wildlife health resulting from climate change. Our review will not include all aspects of wildlife health but will instead focus on established or suspected links between climate drivers and disease spread and discuss examples from the current literature. Here, we define disease spread to include: (1) change in geographic or altitudinal distribution of pathogens, parasites, and vectors and the diseases they cause; (2) change in prevalence or severity of disease; and (3) emergence of novel diseases. Additionally, because wildlife species serve as reservoirs for zoonotic diseases that affect both animals and humans, we include select examples of the effect of climate change on the capacity of wildlife to harbor and spread these disease agents.

Distribution of Pathogens, Parasites, and Vectors: Geographic and Altitudinal Spread

Temperature and precipitation are among the primary factors that influence distribution of pathogens or parasites and their vectors. Changing environmental conditions may make some areas more suitable for establishment of disease organisms and result in increased geographic or altitudinal range. Parasites, particularly those with a free-living stage in the environment, and vector-borne diseases may be especially responsive to warming and changing precipitation regimes (see also Chapter 92).[2,11]

Physical changes in the environment, such as higher ambient or soil temperature, can release thermal constraints that affect the life cycle of parasites, thereby allowing for invasion and expansion of a parasite's geographic range. One such example is the recently documented expansion of parasitic nematodes in the Canadian Arctic.[15] The lung nematode *Umingmakstrongylus pallikuukensis* infects muskoxen *(Ovibos moschatus)* in the Arctic and has recently been detected beyond its endemic range on Victoria Island. The invasion and expansion of *U. pallikuukensis* have likely been facilitated by more permissive climatic conditions and increased animal movements.[15] Higher summer temperatures allow the larvae of *U. pallikuukensis* to develop to the infectious stage more rapidly within its intermediate host, the marsh slug *(Deroceras laeve)*, thus reducing the parasite's life cycle from 2 years to 1 year.[16] Increased rate of larval development results in a higher density of infectious larvae and a longer period of time that muskoxen are at risk of exposure.[15,16] Historically, movements of muskoxen populations between the Canadian mainland and Victoria Island occurred sporadically but have recently become more common in association with icing events.[15] Thus, changing environmental conditions affect not only the life cycle of the parasite but also movement patterns of hosts, leading to increased distribution of this lungworm. Similarly, *Varestrongylus* sp., a lung nematode that infects both muskoxen and caribou *(Rangifer tarandus)*, has also been described on Victoria Island, and available data suggest recent invasion and expansion.[15]

Climate change may also affect the development and associated geographic spread of disease vectors. Epizootic hemorrhagic disease (EHD) virus is vectored by a biting midge *(Culicoides sonorensis)* and causes disease in North America among populations of white-tailed deer *(Odocoileus virginianus)* and other wild cervids.[17] As with other vector-borne infectious diseases, higher summer temperatures often result in increased vector abundance, higher viral load, and a faster rate of viral replication in infected vectors.[18] Lower summer rainfall may also lead to increased vector abundance due to the greater exposure of mud flats, the preferred breeding sites for midges. A record number of EHD cases in wild ungulates were reported in 2012 from 27 US states, including states that had not previously reported the disease.[19] In 2012, the states reporting record outbreaks of EHD all recorded above average or record annual temperatures and, with few exceptions, below to much below average precipitation. In addition, midges capable of transmitting EHD were recorded for the first time in southern Ontario,[20] indicating a potential range expansion of the vector.

Environmental changes have also recently influenced the distribution of tick-borne diseases, for which wildlife species often serve as reservoirs or play an important role in transport of vectors. Climate warming is contributing to the northward expansion of the range of the blacklegged tick *(Ixodes scapularis)*, the vector of Lyme disease and several other tick-borne zoonoses in North America. *Ix. scapularis* has been expanding northward from the United States into Canada, affecting southern and western Ontario, Manitoba,[21] and Quebec.[22] Distribution of Lyme disease is predicted to continue its northward expansion to match the shifting ranges of *Ix. scapularis* and the white-footed mouse *(Peromyscus leucopus)*, a highly efficient reservoir host that is constrained by winter climatic conditions.[23] A similar northward expansion of *Ix. ricinus*, the vector of Borreliosis, tick-borne encephalitis, and other diseases in Europe, has been reported in Norway[24] and Sweden.[25] Migrating birds carrying feeding ticks are a likely source of tick range expansion, with higher summertime temperatures facilitating tick establishment in more northerly regions.[24,26]

The spread of other wildlife diseases and disease vectors to higher elevations has also been linked to climate change.[5] Currently, avian malaria, caused by *Plasmodium relictum*, is limited to elevations below 1500 m on the island of Hawai'i. This is due to a reduction of mosquito activity at higher (cooler) elevations[27] and also a longer development time for the parasite within mosquitoes.[28] Even though the forests of the island of Kaua'i are below 1500 m, native Hawaiian avian communities have been maintained because the island is cooler than comparable elevations on other Hawaiian islands.[29] However, recent surveys demonstrate an increase in avian malaria in native birds on Kaua'i, the presence of mosquitoes at higher elevations, and a precipitous decline in native forest birds on the island.[29] Native Hawaiian birds are already threatened by introduced diseases,[30] and climate-induced elevational shifts in avian malaria transmission could result in further extinctions (Fig. 36.1).[31,32]

It is also important to note that, although less commonly reported, climatic changes can lead to range contractions for select pathogens or parasites. Such a case has been predicted in North America for the Lone Star tick *(Amblyomma*

• **Figure 36.1** 'Apapane *(Himatione sanguinea)*, a native Hawaiian forest bird, is currently threatened by avian malaria, a disease spread by mosquitoes that occur at higher elevations due to climate change (*insert* shows stained *Plasmodium relictum* parasite in avian red blood cells). (Photographs by © Jack Jeffrey Photography and C. Atkinson, USGS Pacific Islands Ecosystems Research Center.)

americanum) that serves as a vector for several human diseases with white-tailed deer as the primary reservoir. Models based on using climate variables to identify suitable habitat predict continued northward range extension, but contraction of the range along the US Gulf coast and lower Mississippi River basin.[33]

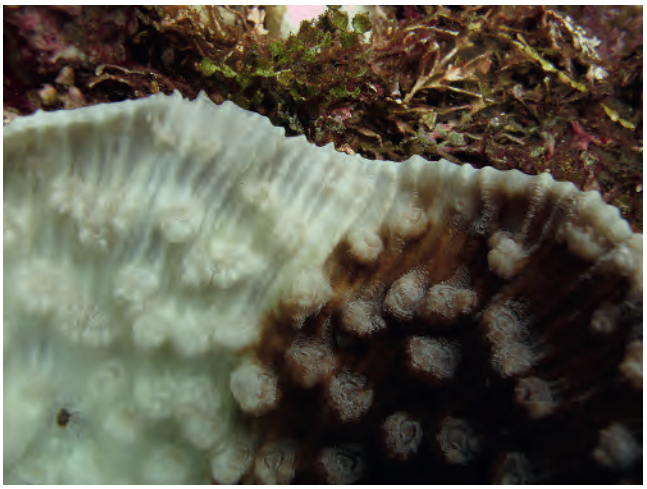
Prevalence or Severity of Disease

Climate-mediated changes also influence the prevalence or severity of some endemic diseases in wildlife populations. Increased morbidity and mortality may result from changes in the life cycle of a pathogen or parasite, altered distribution or density of vectors, compromised immunity of the host, or a combination of such factors.

Environmental conditions favorable to parasite survival may increase prevalence of certain diseases. For example, survival of the nematode *Parelaphostrongylus tenuis*, a brainworm of white-tailed deer, is likely to increase as a result of warmer temperatures and milder winters in the north-central United States and southern Canada. Like the lungworm of muskoxen, *P. tenuis* overwinters as larvae in snails and causes neurologic disease in white-tailed deer, moose *(Alces alces)*, and other ungulates. In North Dakota, a notable increase in prevalence was observed among white-tailed deer between the 1990s and early 2000s.[34] In that study, in which climate and habitat variables were examined relative to the increase in prevalence, increased precipitation in the growing season was the most important predictor of disease in white-tailed deer. Climate change also contributes to heat stress[35] and other diseases among moose,[36] potentially making them more susceptible to parasitism by the brainworm.

In Fennoscandia (the region including Finland, Norway, Sweden, and parts of northwestern Russia), warmer temperatures promote infection with the mosquito-borne filarioid nematode *Setaria tundra*, which may cause morbidity and mortality in reindeer and moose.[37] Although *S. tundra* persists at low prevalence in reindeer populations in Finland, severe outbreaks leading to widespread mortality have occurred only during periods in which average summer temperatures exceed 14°C for 2 consecutive years.[37] Models suggest that warmer temperatures lead to shorter development time of *S. tundra*, increased abundance of the parasite within mosquito vectors, and altered herding behavior, all of which contribute to disease emergence in reindeer.[37]

Earlier melting of snowpack in North America is believed to result in increased survival of winter ticks *(Dermacentor albipictus)* that feed upon moose and *Rangifer* subspecies, often leading to a weakened, emaciated state.[38] Moose acquire larval winter ticks from the vegetation in the early fall, at which time the larvae burrow into the animal's fur and consume a blood meal, then remain on the host through nymphal and adult stages. Parasitized moose often carry tens of thousands of feeding ticks, causing the animal to rub off fur in response to the irritation. Female ticks that drop into snow cover after a blood meal are less likely to survive than those falling onto bare leaf litter exposed by earlier snowmelt. In addition to increased individual survival, the range of winter ticks is advancing northward. Historically limited by temperature to areas far south of the Arctic tundra, the winter tick substantially expanded its geographic range from 2000 to 2012 and is now approaching the tundra-boreal forest ecotone.[16] Tundra microhabitat temperatures may also be adequate for egg development[39] and the potential for winter ticks to establish and amplify on migratory tundra caribou is of considerable concern given the significant health impacts on hosts.[16]



• **Figure 36.2** Bleached *(left)* and healthy *(right) Mycedium robokaki*. Coral bleaching, which leaves corals susceptible to infectious disease, has been observed worldwide and is linked to increased ocean temperatures and other anthropogenic pressures. (Photograph by T. Work, USGS National Wildlife Health Center.)

Diseases among marine organisms may be especially responsive to changes in temperature and pH. Recent widespread decline of corals has been linked to climate-related pressures (Fig. 36.2). Although coral bleaching is not a new phenomenon, more severe and frequent outbreaks associated with warmer water temperatures have led to large mortality events across a broad geographic area.[14,40] Coral bleaching refers to the process of heat-induced expulsion of the symbiotic algae (zooxanthellae) that live within corals, a process that may cause direct mortality or increase a host's susceptibility to infectious disease. Laboratory and field studies have demonstrated a positive relationship between seawater temperature and outbreaks of coral diseases.[7,41] Other stressors such as ocean acidification, which results from the increase of carbon monoxide concentrations in the ocean, further threaten the health of calcifying organisms.[40,42] Many marine invertebrates, including corals, are important foundational species, and widespread mortality or disease can have cascading effects on community structure and function. For example, degradation of coral reefs may lead to impaired reproduction of urchins[43] that play a critical role in keeping algal overgrowth of reefs at bay.[44]

The influence of climate on prevalence and severity of wildlife disease is not restricted to infectious agents. For example, harmful algal blooms (HABs) are being increasingly detected worldwide and many of these events have

been linked to climate warming.[45–47] Toxicity resulting from exposure to HABs that are caused by dinoflagellates, cyanobacteria, or, in some cases, diatoms[48] affect a variety of vertebrate and nonvertebrate marine species. Specific drivers behind HAB events are often not well understood but are thought to be related to nutrient levels, ocean currents, upwellings, and ocean temperature.[49] The largest recorded outbreak of domoic acid, produced by the diatom *Pseudo-nitzschia australis*, occurred in 2015 along the west coast of North America and resulted in extensive strandings of marine mammals and closures of clam and crab fisheries.[45] This event was apparently initiated by anomalously warm ocean conditions, and subsequent laboratory and field experiments demonstrated maximum growth rates of *P. australis* with increased temperature and higher toxin production with nutrient enrichment.[45] In Scandinavian countries bordering the North Sea, regional climate warming has led to increased fresh water input to marine environments, causing stratification of the marine waters by salinity. Stratification, along with increased nutrients contained in the run-off, has been associated with changes in the diatom: dinoflagellate ratio and bloom formation.[46] Current predictions indicate that the influences of warmer ocean temperatures, ocean acidification, vertical stratification, extreme weather patterns, and continued anthropogenic influences will lead to an increase in severe HAB events.[8,45,47,50]

In some instances, prevalence or severity of disease may also be reduced as a result of climate change, especially for cases in which warmer temperatures hinder pathogen or parasite development. Such an example has been described in the Arctic for *Ostertagia gruehneri*, a common abomasal nematode of caribou and muskoxen.[3,39] Contrary to expectations, experimentally warmed tundra plots resulted in reduced development rates of *O. gruehneri*, a pattern that was verified in the laboratory.[39] Although cold is typically a more limiting factor than heat in tundra environments, *O. gruehneri* appears to be highly adapted to Arctic conditions and warming temperatures will likely decrease the exposure of Arctic ungulates to this parasite.[12]

Emerging Diseases

Here we define emergence of novel pathogens to include newly detected pathogens as well as occurrence of known pathogens in previously unaffected taxa. The precise causes of emerging diseases are often not known, but climate-driven environmental changes may be contributing factors. Recent shifts in ecosystem composition and population dynamics have important implications for disease spread as severe and erratic disease outbreaks may occur if formerly isolated species or populations come into contact.

Rapid environmental changes, leading to new interactions between species at key ecologic transition zones, are predicted to lead to an increase in novel disease outbreaks among Arctic wildlife.[51,52] For example, reduction in sea ice along the Arctic coastline has led to more frequent use of land by polar bears and walruses, thus promoting contact between marine and terrestrial species and creating new opportunities for disease transmission.[51,52] Additionally, loss of sea ice allows for greater mixing of pathogen communities as eastern and western marine species come into contact, such as is projected for the Canadian Arctic.[10] Loss of sea ice may also result in seasonal changes in animal movements, with potential impacts on disease transmission. The first known occurrence of avian cholera in Alaska occurred in 2013 and affected several marine bird species that had not previously been documented with the disease.[13] Although the cause of the outbreak is unknown, it occurred coincident with unusually warm water temperatures and reduced sea ice cover in the Bering Sea, conditions that allowed birds to remain in open water around the island for a longer duration (K. Kuletz, personal communication, December 23, 2014).

Direct and indirect effects of climate change may also alter transmission patterns of disease in wildlife through changes in population density or population structure. Unlike infectious diseases that result in low pathogenicity and a more chronic course, infectious agents that cause epizootics are often potentiated by increases in the density or proportion of susceptible animals in a population. In North America, drought, one possible extreme outcome of climate change, has been associated with transmission of St. Louis encephalitis to birds.[53] Using models to predict past and future cases of West Nile virus (WNV) in humans, drought was identified as a more reliable predictor of human cases than temperature or precipitation alone.[54] Drought is believed to promote congregation of birds at available water sources and increase the rate of host and vector contact. Wild birds are variably susceptible to WNV, and population declines have been reported in several North American species.[55]

In the marine environment, warmer water temperatures may be contributing to sea star wasting disease (SSWD), an emerging aquatic disease thought to be caused by a densovirus (Fig. 36.3).[56] The virus has been detected in museum specimens dating back nearly 70 years, but a major outbreak of SSWD occurred in 2013–2014 and resulted in widespread mortality among a number of sea star species from northern California to Alaska. The disease appears to be more prevalent and more severe in warmer water,[57] and ocean temperature anomalies were strong during the outbreak,[57] although this pattern was not consistent across all affected areas.[58] The role of climate change in SSWD is still being investigated, but the frequency of outbreaks may increase with rising ocean temperatures.

In addition to landscape-level impacts, climate change has the potential to affect a variety of microclimates. Such changes may be related to snake fungal disease (SFD), a newly emergent disease in North America, caused by the fungus *Ophidiomyces ophiodiicola* (Fig. 36.4; see Chapter 56).[59] The fungus, which infects the epidermis of an animal and can also invade the subcutaneous tissue, muscles, and organs, has been identified since 2006 in wild snakes in the eastern United States.[60] Infection can lead to death of the

• **Figure 36.3** Diseased *(left)* and healthy *(right)* ochre sea stars *(Pisaster ochraceus)*. A large outbreak of sea star wasting disease occurred in 2013–2014 on the northern coast of Washington and was correlated with higher ocean temperatures. (Photograph by K. Lafferty, USGS Western Ecological Research Center.)

• **Figure 36.4** Northern water snake *(Nerodia sipedon)* with crusty and thickened scales overlaying raised blisters resulting from snake fungal disease (SFD) caused by the fungus *Ophidiomyces ophiodiicola*. Reports of SFD in N. America have increased since 2006 and may be related to microhabitat changes caused by climate change. (Photograph by D. Green, USGS NWHC.)

animal or, in less severe cases, visual impairment, inability to sense or prehend food, and emaciation. Increased detection efforts have identified affected snakes across a growing geographic area. Although the disease could have been introduced from captive snakes independent of climate change, it is also plausible that the disease was present in wild snakes historically and that shifting environmental conditions led to its apparent emergence. Microhabitat changes cause the animals to seek cooler temperatures and humidity in borrows and be more at risk of exposure to fungus contained in the soil or in shed epithelial crusts.[59]

Conclusions and Recommendations

As discussed previously, some infectious diseases are responsive to climate-induced environmental changes and may have important ramifications for wildlife health and biodiversity. Significant population declines and extinctions have been associated with infectious disease outbreaks in wildlife,[2,61–63] and current projections suggest that warming will further compromise wildlife health.[3,10] There is considerable urgency to address these complex matters because of the unprecedented rates of climate change[5] that coincide with the increased emergence of infectious diseases evident from the last quarter of the 20th century.[64–66] In addition, awareness has been growing about the close relationship between diseases maintained or circulating in wildlife and zoonotic diseases resulting from contact with diseased animals or infected vectors.[67] Thus, the effects of climate change on wildlife health also have implications for the health of domestic animals and humans.

Despite the clear importance of this topic, many information gaps currently remain. There are large regional disparities in our understanding of climate change and wildlife health. A majority of studies have been conducted in the temperate regions of Europe and North America and the conclusions regarding climate change may not readily transfer to tropical, subtropical, and Arctic regions.[68] For example, there is currently little empirical evidence available to assess the scope or magnitude of changes in wildlife disease occurrence in the Arctic, in part due to the paucity of baseline data. It is therefore challenging to distinguish truly emerging pathogens from those that may have been present, but not previously documented.[52] Further complicating the matter, the effects of climate change may be more regional than global and may be most important in the context of local ecologic changes.[69] For example, two-thirds of the human population is expected to reside in urban areas by the middle of the century, a shift that will likely result in a reduction of wildlife diversity and a concomitant increase in populations of urban-adapted wildlife.[70] Other stressors,

RECOMMENDATIONS

- Long-term interdisciplinary projects to study environmental drivers and transmission dynamics of disease
- Focus on regions subject to rapid environmental change (e.g., Arctic and marine ecosystems)
- Identification and mapping of current ranges of vector species
- Baseline studies on wildlife disease in currently underrepresented regions such as the Arctic and the tropics
- Collaboration across political and geographic boundaries and development of continent-wide databases to track emerging diseases
- Consideration of other anthropogenic or landscape-level factors (besides climate change) that may influence disease spread

such as habitat destruction, the introduction of exotic and invasive species, and pollution, are important determinants of wildlife health and often confound or exacerbate the effects of climate change. From a conservation standpoint, mitigation of specific, identifiable threats may also be more tenable than the cessation of large-scale warming. An extensive review of nearly 250 papers revealed that the potential effects of climate change on wildlife health were often considered independently from other relevant anthropogenic factors,[68] suggesting that a comprehensive understanding of the most critical factors relevant to disease spread may be lacking.

To address existing information gaps, we recommend the initiation of long-term, interdisciplinary projects focused on disease ecology in representative ecosystems subject to current or foreseeable climate change. As discussed previously, both Arctic and marine ecosystems may provide useful models for understanding direct impacts of warming and other environmental changes. Collaboration across political and geographic boundaries is necessary to identify and track emerging diseases, and information sharing could be facilitated by the establishment of continent-wide morbidity and mortality databases. Finally, predictive models based on existing information are necessary to guide management efforts to minimize the threats of wildlife and zoonotic diseases.

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Prion Diseases in Wildlife

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Prion diseases, also known as transmissible spongiform encephalopathies (TSEs), are infectious and fatal neurodegenerative disorders that include bovine spongiform encephalopathy (BSE), TSEs of zoo animals, scrapie of sheep and goats, transmissible mink encephalopathy (TME), and chronic wasting disease (CWD) of cervids (Table 37.1).[1–8] To date, outbreaks in zoos have been limited to small numbers of animals, however, infectious prions can incite large-scale, multispecies epidemics. For example, within the United Kingdom, BSE infected more than 180,000 cattle,[6,9] as well as zoo bovids, felids, and primates.[10–16]

The infectious agent in prion disease is composed of the pathogenic misfolded and aggregated prion protein, known as PrP^{Sc} ("Sc" denotes scrapie, the prion disease of sheep and goats).[17,18] The primary amino acid sequence of the prion aggregate is determined by the host cellular prion protein, PrP^{C} ("C" denotes the cellular, physiologic form of the prion protein), encoded by the prion gene, *Prnp*.[19] Following transmission to the host, prions seed the misfolding of PrP^{C} in an autocatalytic process. Therefore prions are infectious proteins and do not contain any specific nucleic acids; the term *prion* is derived from the words "proteinaceous infectious particle."[18] Over a period of months to years, prions accumulate to high levels in the brain and spinal cord, resulting in spongiform degeneration with vacuolation, neuronal death, and activated astrocytes and microglia. Although the incubation period can be many years, even decades, the clinical phase is typically rapidly progressive (weeks to months) and may include behavior abnormalities, motor dysfunction, cognitive impairment, and ataxia, depending on the prion and species affected.

Here we review the transmission and epidemiology, clinical signs and diagnosis, and the treatment and prevention of prion diseases in zoo animals and in free-ranging cervids.

Transmission and Epidemiology

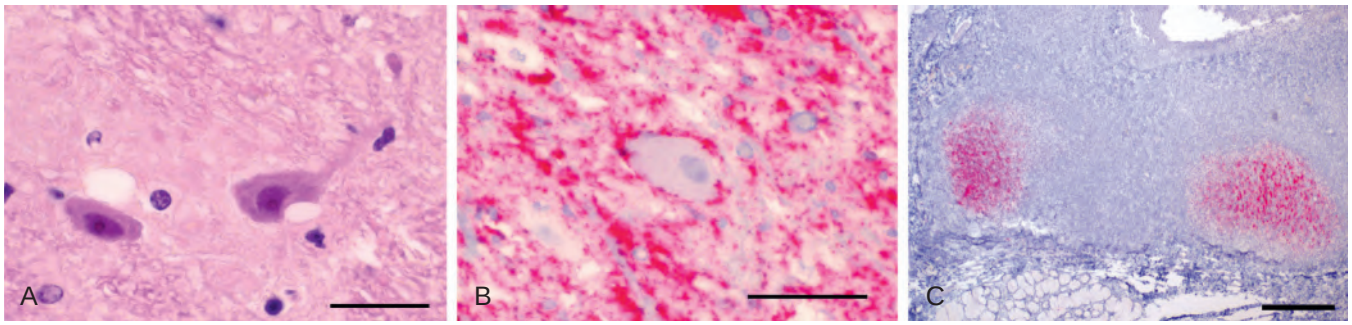
Most prion diseases of animals are acquired by exposure to prions, however prions can also arise sporadically in aged animals, for example, atypical scrapie in sheep and goats and atypical BSE in cattle.[20–22] For the acquired TSEs, the most plausible route of transmission is through ingestion of prion-contaminated feed.

The efficiency of prion transmission between individuals varies widely depending on the prion. BSE in cattle shows little evidence of direct transmission from one individual to another and prions are mostly constrained to the central nervous system (CNS). Zoo animals were likely infected through consuming BSE-contaminated meat or meat and bone meal (MBM). In zoo collections, prion infection was diagnosed in 19 species, including 8 antelope and bovid species [greater kudu *(Tragelaphus strepsiceros)*, eland *(Taurotragus oryx)*, nyala *(Tragelaphus angasi)*, gemsbok *(Oryx gazella)*, scimitar-horned oryx *(Oryx dammah)*, Arabian oryx *(Oryx leucoryx)*, ankole cows *(Bos taurus)*, and American bison *(Bison bison)*], 7 felid species [cheetah *(Acinonyx jubatus)*, puma *(Felis concolor)*, ocelot *(Felis pardalis)*, Asian golden cat *(Catopuma temminckii)*, leopard cat *(Prionailurus Bengalis)*, tiger *(Panthera tigris)*, and lion *(Panthera leo)*], and 4 nonhuman primates species [Mayotte brown lemurs *(Eulemur fulvus)*, white fronted brown lemur *(Eulemur albifrons)*, mongoose lemur *(Eulemur mongoz)*, and Rhesus macaque *(Macaca mulatta)*] (see Table 37.1).[23] Prion disease outbreaks also occurred in exotic animals in France, Ireland, Germany, and even Australia, although almost all of the infected animals originated from zoological collections in the United Kingdom.[1]

In contrast to BSE in cattle, scrapie and CWD are highly transmissible between animals. In scrapie-infected sheep and CWD-infected deer, prions accumulate in lymphoid tissues, including tonsils, lymph nodes, and Peyer patches (Fig. 37.1).[24–26] CWD prions are shed in the saliva, urine, and feces directly into the environment,[27,28] contaminating grazing areas and water sources. CWD prions can be transmitted via fomites; for example, feed buckets and bedding used by CWD-infected deer can transmit the infection to uninfected deer.[29] Additionally, direct animal-to-animal contact or contact with prion-contaminated secretions and excretions, such as feces, urine, saliva, blood, placenta, and milk, are possible routes of infection.[30–32] Vertical transmission to offspring has also been demonstrated for prion-infected sheep, goats, and deer.[33,34]

TABLE 37.1 Prion Diseases in Wild and Captive Animals

Disease	Host	Etiology	Year of Description
Transmissible mink encephalopathy (TME)	Mink	Ingestion of bovine spongiform encephalopathy (BSE) or scrapie-contaminated meat and bone meal	1965[3,4]
Feline spongiform encephalopathy (FSE)	Domestic cat, cheetah, ocelot, puma, lion, Asian golden cat, leopard cat, tiger	Ingestion of BSE-contaminated meat and bone meal	1990[1,5]
Exotic ungulate spongiform encephalopathy	Kudu, eland, gemsbok, nyala, oryx, bison		1989[2]
Transmissible spongiform encephalopathy in nonhuman primates	Lemur, rhesus macaque		1996[11]
Chronic wasting disease (CWD)	Mule deer, white-tailed deer, moose, reindeer, sika deer, Rocky Mountain elk	Unknown source; highly transmissible through the ingestion or direct contact with pastures, soil, feces, urine, saliva, or blood	1967[7,65]
Bovine spongiform encephalopathy (BSE)	Cattle	Ingestion of BSE-contaminated meat and bone meal	1986[6]
Bovine amyloidotic spongiform encephalopathy (BSE-L)	Cattle	Unknown source; probably spontaneous origin	2004[66]
Atypical spongiform encephalopathy Type-H (BSE-H)	Cattle	Unknown source; probably spontaneous origin	2004[67]
Scrapie	Sheep, goat, mouflon	Unknown source; transmissible through the ingestion or direct contact with pastures, feces, placenta, or blood	1732[68]
Atypical scrapie	Sheep and goat	Unknown source; probably spontaneous origin	1998[69]



• **Figure 37.1** Brainstem and tonsil sections from a chronic wasting disease-infected mule deer. (A) Hematoxylin and eosin stain of brain shows neurons and adjacent vacuoles *(arrows)*, lesions typical of a spongiform encephalopathy. (B) Immunohistochemical (IHC) stain for PrP shows abundant PrP^{Sc} deposits *(red)*, here shown on the neuronal plasma membrane *(center)*. (C) IHC of the tonsil reveals PrP^{Sc} deposits in the germinal center of lymphoid follicles. Scale bars = 50 μm (A), 100 μm (B), or 500 μm (C).

TME was first documented in 1947 in Minnesota and Wisconsin, and sporadic outbreaks have appeared in farmed mink *(Neovison vison)* in the United States, Canada, Finland, Russia, and Germany,[35,36] with the last case occurring in the United States in 1985.[35,36] Epidemiologic and experimental studies suggested that TME in mink arose from the ingestion of prion-contaminated meat from cattle, possibly infected with an atypical, sporadic form of BSE.[37]

Clinical Signs of Prion Disease in Zoo Animals and Farmed Mink

Prion diseases have long incubation periods, yet once clinical neurologic signs develop, the disease typically progresses rapidly. In zoo ruminants, the clinical course varies depending on the species. Acute onset of clinical signs followed by

a slow disease progression over weeks is common. The main signs reported include ataxia, tremor, abnormal head and ear posture, and weight loss. Myoclonus, dullness, excessive movements of the lips and tongue, nibbling of the tail, decreased rumination, hyperesthesia, and anxiety may also be observed.[1,38]

The incubation period for feline spongiform encephalopathy (FSE) in cheetahs ranges from 4.5 to 8 years. For domestic cats, the incubation period is unknown, although all affected animals were at least 2 years old. Clinical signs rapidly progress over 1–3 months.[23] Domestic cats initially display behavior changes such as unusual timidity, hiding, and aggressiveness, followed by ataxia, gait abnormalities (mainly affecting the hind legs), hypermetria, and hyperesthesia to sound and touch. Decreased grooming, excessive salivation, polyphagia, polydipsia, and dilated pupils have also been reported. In later stages of the disease, animals are somnolent and may develop tremors. Similar behavior and motor signs have been described in zoo cat species.[39]

Prion-infected nonhuman primates initially show behavioral signs of depression, nervousness, voracious appetite, teeth grinding, yawning, and altered grooming activity, which progresses to truncal ataxia with tremors. In end stages of disease, animals display myoclonic jerks, ataxia, and aggressiveness.[23,40]

In TME-infected mink, the incubation period ranges from 7 to 12 months. Progression of the disease is similar to FSE, as mink show altered behavior with hyperesthesia, increased aggressiveness, and hyper-irritability, followed by ataxia, circling, tremors, and compulsive biting of self or objects. Death occurs one week to several months after disease onset.[23,41]

Chronic Wasting Disease

CWD is among the most infectious prion disease in animals, affecting captive and free-ranging Cervidae including mule deer *(Odocoileus hemionus)*, white-tailed deer *(O. virginianus)*, Rocky Mountain elk *(Cervus canadensis nelsoni)*, moose *(Alces alces)*, sika deer *(Cervus nippon)*, and reindeer *(Rangifer tarandus tarandus)*. Reported prevalences of up to 35% in free-ranging animals and 90% in captivity are a testament to the efficient transmission of CWD prions. Importantly, CWD prions can cross species barriers and infect other cervids, including Reeves' muntjac *(Muntiacus reevesi)*. No cervid species should be assumed to be CWD-resistant. As CWD prions are typically shed into the environment, outbreaks of CWD are best identified early and controlled expeditiously. Two CWD-positive animals have been reported in zoological collections.[42]

Epidemiology

CWD was initially discovered in captive mule deer in northern Colorado in 1967.[43] Soon thereafter, captive Rocky Mountain elk in Wyoming as well as free-ranging cervids were diagnosed with CWD. For the next two decades, CWD was thought to exist exclusively in Colorado and Wyoming. In 1995, BSE prions were discovered to have spread from cattle to humans, and with a zoonotic risk of prions recognized, CWD surveillance surged throughout North America and revealed isolated, noncontiguous populations of CWD-infected cervids from Utah to New York, and south to Texas and Arkansas, as well as in two Canadian provinces (Fig. 37.2). Highly reported prevalences in certain states, including Wisconsin and Arkansas (>20%), suggests that CWD has been established in some regions for more than a decade. To date, CWD-infected cervids have been reported in 25 US states, 2 Canadian provinces, South Korea (ranched elk imported from North America), and most recently in Europe. In 2016, free-ranging reindeer and moose in Norway were diagnosed with CWD,[44] raising questions of how CWD prions spread to free-ranging reindeer and moose populations in northern Europe. Possibilities include spread by commercially available cervid urine used as a hunting bait, CWD-contaminated fomites, or horizontal spread from sporadic prion disease.

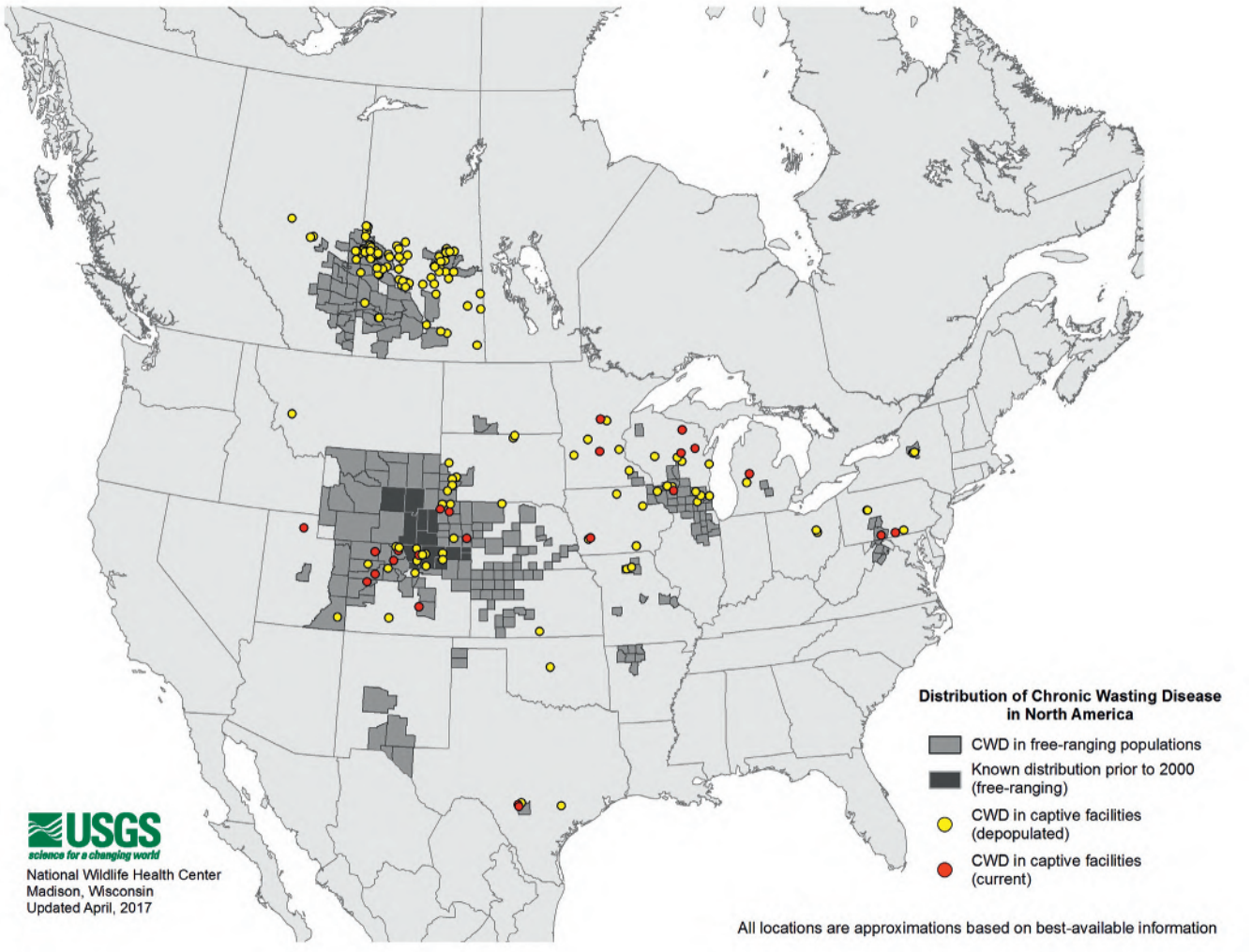
Is interspecies prion transmission a risk, given that predators, scavengers, rodents, ungulates, and birds are likely exposed to CWD-infected cervids? There are no known naturally occurring cases of CWD in noncervid species, suggesting a high transmission barrier, yet cross-species transmission remains a possibility.

Clinical Disease

The initial stages of CWD are clinically subtle and include weight loss and behavior changes, such as depression and isolation from the herd. Animals often appear thin and unthrifty, and may develop polyuria and polydipsia, hypersalivation, ataxia, frequent regurgitation, and esophageal distension.[42] The cause of death is commonly due to aspiration pneumonia. Given that the clinical signs are often subtle and nonspecific, surveying cervid populations is critical to avoid large-scale epidemics within a collection and to prevent transmission of CWD among cervid populations.

Transmission

CWD prions are not restricted to the CNS and also accumulate in systemic organs beginning in early disease. Few other prion infections induce such extensive deposits of extraneural prions. In CWD-infected deer, prion aggregates accumulate in all lymphoid tissues including tonsils (see Fig. 37.1), lymph nodes, Peyer patches, and spleen, as well as in adrenal glands, pancreatic islets, pituitary, and skeletal muscle.[25,26] Such an extensive extraneural reservoir may underlie the efficient horizontal spread of CWD prions. Prions are shed in saliva, urine, and feces, and these excreta, as well as blood, can transmit CWD to naïve animals.[27,28] Shed prions bound to soil contaminate the environment,



• **Figure 37.2** Map of chronic wasting disease in North American cervids.

and exposure to prion-contaminated fomites, that is, water sources or bedding, can initiate an infection.

Diagnostic Tools for Prion Disease

Extraneural prion accumulation presents opportunities for antemortem screening of cervid populations. In deer, prion immunolabelling of recto-anal mucosa-associated lymphoid tissue biopsies are a sensitive and specific method for testing live animals for CWD infection.[45,46] CWD-infected elk cases may be missed, however, as not all elk develop prions in lymphoid tissues. Similarly, scrapie can be detected by prion immunolabelling of the third eyelid lymphoid follicles, although this test is only highly sensitive by 10–14 months or later after infection.[47]

Postmortem tests for prion disease are numerous and include immunohistochemical staining of brainstem, or western blot, or commercial ELISA assays to detect aggregated prions. Prion diseases are diagnosed postmortem by detection of PrP^{Sc} aggregates in the CNS with immunoblotting, ELISA, or IHC (see Fig. 37.1). These diagnostic methods allow the discrimination of the pathologic aggregated isoform, PrP^{Sc}, from the normal cellular isoform, PrP^{C}, as PrP^{Sc} is relatively resistant to protease digestion and is detected with anti-PrP antibodies. ELISA-based diagnostic kits are available for testing brain samples.

Histologic examination of the brain typically reveals neuronal vacuolation and astrogliosis (see Fig. 37.1). Each prion disease produces a different disease phenotype in terms of brain areas affected, size and morphology of prion plaques, and level of gliosis and vacuolation. For example, FSE is characterized by spongiform degeneration in the neuropil of the brain and spinal cord with the most severe lesions localized to the medial geniculate nucleus of the thalamus as well as the basal nuclei. TME-infected mink develop extensive neuropil vacuolation, as well as neuronal degeneration and astrocytosis in the cerebral cortex, particularly in the frontal cortex, as well as the corpus callosum.

New extraordinarily sensitive techniques are under development for large-scale population testing of body fluids or tissue samples. Protein misfolding cyclic amplification (PMCA) and real-time quaking induced conversion (RT-QuIC) assays amplify low levels of aggregated prions in early stages of the disease with high sensitivity in a number

of tissues and body fluids, including cerebrospinal fluid (CSF), urine, saliva, blood, brain tissue, and lymph node tissue.[48–51]

Treatment and Control

Currently, there is no treatment to cure prion diseases, although glycosaminoglycans have been successfully used to slow[52–54] PrP^{Sc} formation in scrapie-infected rodents.[55–57] Precautions should be taken among zoo staff working with a suspected prion-infected animal, as certain TSEs, such as BSE, are zoonotic.

The BSE epidemic was rapidly controlled by banning the feeding of cattle tissues to ruminants. Annual monitoring programs for BSE were based on active and passive surveillance designed to identify animals at risk. Positive animals were euthanized as well as birth cohorts and offspring in some cases. These regulations sharply decreased the incidence of BSE in ruminants and TSEs in zoo animals.

For scrapie in sheep and goats, similar control and eradication measures were established. Susceptibility of small ruminants to scrapie is strongly modulated by the host prion protein genotype, and selective breeding programs were developed to select for the prion protein allele associated with resistance, $A_{136}R_{154}R_{171}$, in sheep herds. This selective breeding strategy has resulted in the rapid control of scrapie outbreaks[58] and has lowered the risk of scrapie infection even in animals with susceptible genotypes.[59–61] Similarly, the prion resistance genotype K_{222} is currently being selected within goat herds.[62,63]

In the case of CWD, prions accumulate in tonsil, lymph nodes, and Peyer patches by 3 months and are detectable in the brainstem by 6 months post-prion exposure.[26,64] The disease incubation period varies but can be as short as 15 months. Nevertheless, there is a long period of minimally 14 months in which prions may be shed into the environment or transmitted by direct contact. In captive cervids, the prevalence of CWD can be as high as 90% of the animals. Because CWD is clinically subtle and can spread surreptitiously in zoo cervid populations, CWD testing of all cervids at necropsy should be a management priority.

Prions deposited in the environment through saliva, urine, feces, or prion-contaminated carcasses remain highly stable and infectious for years. Thus control measures to prevent prion contamination of the environment are crucial. Risks for CWD entry into zoo populations include inadvertent contact with free-ranging cervids or acquisition of asymptomatic prion-infected animals from prion-infected herds. Acquisition of free-ranging cervids from North American sources risks introducing CWD into the collection.

Because prion diseases can be clinically subtle, postmortem surveillance of zoo bovids, ovids, and cervids for prions in the brain will ensure early detection and prevention of prion spreading among the more at-risk zoo species. Diligent CWD monitoring of cervid populations by postmortem testing of brain is highly recommended. A CWD information sheet is available from the American Association of Zoo Veterinarians Infectious Disease Committee at http://www.aazv.org/?754.

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38

Avian Influenza: A Brief Overview of the Pathobiology and Current Status in Domestic and Nondomestic Species

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Introduction

Influenza is a highly adaptable and genetically mutable viral infection of both birds and mammals. Although many influenza viruses are relatively host-restricted, others have shown the potential to cross species lines, adapt, and expand their range. Avian influenza viruses (AIVs) display such host plasticity, where select strains pose significant risk to domestic poultry, wild birds (including captive species), and some mammalian species. Some avian influenza strains have even demonstrated zoonotic potential (e.g., Eurasian HPAI H5N1 and LPAI H7N9).[1] This chapter provides the reader with an overview of influenza virology and disease dynamics with a focus on avian influenzas and their impact on domestic and captive wild species.

Influenza viruses are classified in the family Orthomyxoviridae and are enveloped, single-stranded negative-sense RNA viruses with segmented genomes consisting of 8 gene segments coding for 11 known proteins.[2] Influenza viruses are broadly subdivided into four known types, specifically: A, B, C, and D. Type A influenza viruses, which include avian influenza and human seasonal influenza, are further classified into subtypes based on the primary antigens embedded in the viral envelope, namely the glycoproteins hemagglutinin (H or HA) and neuraminidase (N or NA). These glycoproteins provide the basis for the subtype nomenclature (e.g., H5N1, H3N2, H7N9) and are the major antigens against which the immune response is directed. For avian influenza there are 16 recognized HA and 9 NA subtypes that circulate in waterfowl and shorebirds, which are considered to be the natural hosts for influenza viruses.[2,3] Dabbling duck species (especially those found in the family *Anatidae*) appear to be the preferential natural hosts for the virus, where it exhibits great genetic diversity.[4] However, recent reports described two novel influenza strains found only in bat species (i.e., H17N10 and H18N11).[5,6]

The host range for type B influenza viruses are humans and seals, although laboratory animals can be experimentally infected.[7] Type C influenza viruses are found predominantly in humans but may also affect swine. Type D influenza viruses are largely restricted to cattle but have also been reported in swine.[8]

Arguably both type A and B viruses are the most clinically important in regard to animal and public health. Type A influenza viruses are antigenically diverse and may affect a number of different avian species as well as a broad range of mammals, including but not limited to humans, swine, horses, dogs, ferrets, bats, and marine mammals. Clinically, influenza A viruses are responsible for outbreaks, epidemics, and pandemics.

Type B influenza viruses are also classified into subtypes but express a more restricted host range including humans, seals, and, experimentally, ferrets. Infection with influenza B viruses typically results in outbreaks and epidemics but not pandemics. Type C influenza viruses are the least antigenically diverse and are largely confined to humans, although both canine and swine infections have been reported, and ferrets have been infected experimentally. Type C influenza viruses cause outbreaks and highly localized epidemics but are not involved in pandemics. The clinical relevance of Type D viruses for cattle and swine is still being investigated.

Virology of Avian Influenza

Type A influenza viruses are thought to have their origins in waterfowl and shorebirds, which are the natural hosts for AIVs. AIVs have demonstrated the potential to adapt to mammalian species and become established in those populations, but how this occurs has not been well elucidated. Mammalian influenza viruses are largely transmitted via respiratory routes, whereas AIVs are mainly transmitted via a fecal-oral route in their natural hosts.

Type A influenza viruses infect host cells through attachment of the hemagglutinin protein to glycoprotein receptors located on the host's respiratory or gastrointestinal epithelial

cells. The conformation of these host glycoprotein receptors helps to determine species and tissue tropism depending on how a terminal sialic acid moiety is linked to a penultimate galactose. AIVs prefer this terminal linkage to be in an α-2,3 orientation, but mammalian adapted influenza viruses prefer an α-2,6 orientation. This specificity helps to partially explain the host preferences of these viruses. Birds have a greater density of α-2,3 receptors on their relevant epithelial surfaces, whereas mammals have a greater concentration of α-2,6 receptors. However, mammals do possess α-2,3 receptors; in humans and swine, these are typically found in the lower respiratory tract. This is one of the possible pathways for AIVs to infect mammalian hosts. Swine have been postulated to serve as "mixing vessels" for avian and mammalian strains by generating novel influenza viruses that have components of each species-adapted virus. Such novel viruses may be prima facie mammalian viruses but contain avian genes.[9] Quail have been reported to play a similar role in this type of adaptation scheme; however, how this might occur has not been elucidated.[10]

After HA has been bound to the sialic acid moiety of the receptor, the virus is taken into the host cell through endocytosis. The endosome containing the virus fuses with a lysosome, resulting in acidification of the endosome and precipitating an essential enzymatic cleavage of the hemagglutinin protein, which must occur for the virus to fuse with the endosome membrane and release its contents into the cytoplasm. This sequence and the types of amino acids associated with this HA cleavage site have important implications for which cells can support viral replication and the virus's inherent pathogenicity. This is discussed at length later.

The manner in which novel type A influenza viruses evolve is governed largely by two mechanisms: antigenic drift and antigenic shift. Antigenic drift introduces mutations at a predictable rate and occurs because the virus's RNA polymerase has no proofreading function; therefore base substitutions are introduced at an error rate between 1×10^{-3} and 8×10^{-3} substitutions per site per year.[9] Antigenic drift may contribute to changes that facilitate the virus's ability to elude the host's immune response and reduce the efficacy of vaccines; however, it generally does not result in significant changes in the virus's virulence. By contrast, antigenic shift may result in radical changes in pathogenicity and even host preference. The most significant mechanism by which antigenic shift occurs is reassortment, which is a mixing of the gene segments of two heterosubtypic viruses in a coinfected host, thus resulting in the generation of novel viruses.[9] For example, if a host is coinfected with an H5N1 and an H3N2, new reassortant viruses such as an H5N2 and an H3N1 can result. Another mechanism that results in antigenic shift is recombination with segments of the host's genetic material. Recombinant events with chicken ribosomal RNA have resulted in a shift in virulence from low pathogenicity to highly pathogenic behavior.[11] Antigenic shifts may greatly increase virulence or host adaptation in a single viral generation.

Pathogenicity: Highly Pathogenic Avian Influenza and Low-Pathogenicity Avian Influenza

AIV subtypes are further classified by their inherent pathogenicity, namely highly pathogenic avian influenza (HPAI), such as HPAI H5N2, and low-pathogenicity avian influenza (LPAI), such as LPAI H4N6. This pathogenicity is predicated on the clinical behavior in domestic poultry, so HPAI infection typically causes severe illness and death, but the clinical signs of LPAI range from subclinical to mild, depending on the species infected, age, and the strain of virus. It should be noted that it is possible to have strains with the same nomenclature; for example, both LPAI and HPAI H5N2 strains have been identified. It behooves researchers, clinicians, and students to understand the importance of identifying the pathogenicity in describing these viruses.

Waterfowl and some shorebirds are the natural hosts for LPAI, which is a normal commensal infection of waterfowl, most often juvenile birds. LPAI subtypes encompass the full range of HAs (1–16) and NAs (1–9). Occasionally LPAI strains spill over from the waterfowl compartment and infect land-based poultry, such as chickens or turkeys. Such events typically result in mild or asymptomatic infection and may be of little to no clinical consequence. However, LPAIs of the H5 and H7 subtypes are subject to mutation in land-based poultry and may evolve into highly pathogenic strains. The axiom is that all currently known HPAI viruses are restricted to subtypes H5 and H7 (although not all H5 and H7 are highly pathogenic; in fact, most are LPAI viruses). HPAI virus infections may cause high flock mortality, up to 100%. When an H5 or H7 subtype is detected in poultry, it is often referred to as "notifiable avian influenza" because of this potential to evolve into a more pathogenic form. Such detections must be reported to both state and federal regulatory authorities, who may take actions necessary to prevent dissemination of the disease.

Commercial poultry in the United States are subjected to rigorous surveillance for notifiable LPAI/HPAI; infection is usually detected through routine serologic surveillance for subclinical infections or by clinical illness and production losses (e.g., weight loss and lethargy in broilers and turkeys, reduced egg production in layers, or increased mortality rates). Commercial flocks showing clinical signs consistent with AIV may be immediately depopulated to prevent spread. LPAI is most frequently detected through routine serosurveillance, and—even though virus may be detected by polymerase chain reaction (PCR), depending on the timing of the infection—it is often not possible to isolate the virus. With HPAI, birds often die before antibodies can be generated; therefore diagnosis is often by PCR, virus isolation, and antigen capture tests.

LPAI infection of land-based poultry is largely confined to the respiratory tract unless exacerbated; in waterfowl it is largely subclinical and confined to the intestines.[12] This

tissue tropism results because of the presence of HA-specific enzymes needed to cleave the hemagglutinin protein (an essential step for viral infection of the cell) after attachment to the cell in the avian intestinal or respiratory tract. As alluded to earlier, an important cell tropism and pathogenicity determinant of AIVs is the sequence and types of amino acids associated with the HA cleavage site. If a single basic amino acid is strategically located at the cleavage point within HA, this restricts the enzyme or enzymes (and thereby the cell types) that may successfully cleave the HA protein to allow fusion with the host cell membrane and completion of the replication cycle. LPAI viruses possess a single basic amino acid at the cleavage point within their HA protein; this permits action only by enzymes found in the intestinal tract of waterfowl. By contrast, HPAI viruses have multiple basic amino acids located in the cleavage region; this permits an array of enzymes of multiple cell types to actively cleave the HA protein. Therefore HPAI viruses are systemic in nature and may infect multiple organ systems, whereas LPAI viruses are limited to single organ systems.

To meet the regulatory definition for HPAI, the viral HA must first be sequenced to ascertain the composition of the cleavage region. If multiple basic amino acids are detected in this region, the virus by definition is considered to be HPAI irrespective of its clinical manifestation. Most HPAI viruses are indeed highly pathogenic, but some may be subclinical in behavior depending on the species and inherent characteristics of the virus.[12]

The Changing Ecology of Avian Influenza Viruses

For many years, the long-held dogma was that low-pathogenicity viruses were found in wild waterfowl and occasionally spilled over into the domestic poultry compartment but that highly pathogenic viruses were almost exclusively limited to domestic poultry, with very rare exceptions.[13] However, a novel AIV emerged in Asia that was to change that paradigm. The first detection of Asian lineage HPAI H5N1 (HPAI H5N1) was in 1997 in Hong Kong, where it caused severe disease in domestic poultry and exhibited a zoonotic potential resulting in illness and death in humans.[14] Rapid response to the outbreak seemed to eradicate the virus, but HPAI H5N1 did not disappear; in fact, it continued to persist and reassort in wild migratory waterfowl and domestic poultry. HPAI H5N1 spread throughout East and Southeast Asia and became endemic in that region. However, in 2005, the virus was carried by wild migratory waterfowl into the Middle East, Europe, and Africa.[4] This was the first HPAI virus that appeared to successfully infect wild and domestic waterfowl (predominantly in the genus *Anas*) without causing significant clinical disease in all cases. Hence, these birds could move HPAI H5N1 long distances along migration routes and precipitate outbreaks in domestic poultry without manifesting severe clinical illness themselves. In the past two decades, HPAI H5N1 has continued to diversify, and the lineage has given rise to multiple sublineages designated in phylogenetic terms as *clades*.

The New Paradigm

The HPAI H5 clade 2.3.4.4 of avian influenza (first reported in domestic ducks in China in 2008) burst forth from Asia in 2013–2015 to create an intermittent global epiornitic, even reaching North America in 2014–2015.[15] This clade of H5 viruses achieved what their progenitor HPAI H5N1 could not—namely global distribution—and it appears to be uniquely adapted to the dabbling duck (genus *Anas*). H5 clade 2.3.4.4 includes multiple subtypes, but the most notable are Eurasian lineage HPAI H5N8, H5N6, and H5N2.[16] Despite descending from the zoonotic HPAI H5N1, few of these novel subtypes have demonstrated the same zoonotic potential with the exception of EA HPAI H5N6.

Eurasian HPAI H5N8 (EA HPAI H5N8) has achieved a global distribution through movement by wild migratory waterfowl, whereas H5N6 and H5N2 have largely been limited to Asia to date. This clade is now well established throughout Asia and possibly other areas. H5 clade 2.3.4.4 viruses do not appear to cause clinical disease in many species of wild or domestic dabbling ducks but have been shown to be lethal to other wild bird species such as raptors, geese, and swans.

Beginning in January 2014, H5N8 spread rapidly through domestic poultry operations in South Korea and even resulted in mass mortalities of some wild birds such as Baikal teal *(Anas formosa)*. Then, in the fall of 2014, H5N8 reached the Middle East and Europe. By November 2014, H5N8 was detected in North America and presumably arrived through viral exchange in Beringia between infected Eurasian ducks and North American ducks.[17] H5N8 reassorted with an endemic North American LPAI virus to give rise to novel reassortants including Eurasian/North American HPAI H5N2 and HPAI H5N1 (EA/NA HPAI H5N2 and H5N1). Both H5N8 and H5N2 were carried south by wild migratory waterfowl, predominantly dabbling ducks. There was only a single detection of EA/NA HPAI H5N1, and it did not appear to persist or spread in the waterfowl population. However, H5N8 did spread in wild ducks and precipitated outbreaks in domestic poultry in the United States, largely in the fall of 2014 and early winter of 2015 along the Pacific flyway. H5N2 caused serious outbreaks in domestic turkeys in British Columbia, Canada, in late 2014 but did not result in significant outbreaks in the United States until the spring of 2015. Starting in March 2015 and coinciding with the northern migration in the Mississippi flyway, H5N2 precipitated the largest cluster of HPAI outbreaks in domestic poultry in the history of the United States, mainly in the central and upper Midwest. These outbreaks continued and were not resolved until June 2015.

Wild bird surveillance has shown that neither H5N8 nor H5N2 appeared to persist long-term in the wildlife compartment, with only two geographically independent detections in single mallards *(Anas platyrhynchos)* in the summer and fall of 2015 and a single detection in a mallard in the summer of 2016. Similar observations have been made after incursions into Europe from 2013 to the present. This suggests that H5 clade 2.3.4.4 does not readily persist within the wild migratory waterfowl compartment at prevalence rates detectable by the various surveillance systems in place globally. This notion is supported by the fact that outbreaks in domestic poultry diminish and then disappear outside of the migration season. Moreover, active surveillance within the US wild bird compartment shows that these H5 viruses circulated at a low prevalence in that population. Hence, one might speculate that to maintain infection of the wild waterfowl compartment with subtypes of this clade of H5 viruses requires frequent spillover events from infected domestic poultry. Indeed, domestic ducks have been shown to be important subclinical reservoirs for Eurasian H5 HPAI viruses.[18] Additionally, there is evidence suggesting that residual cross-protection generated from natural infection by endemic LPAI viruses provides some heterosubtypic immunity, which may be another factor contributing to the failure of EA H5 HPAI clades to persist in wild waterfowl populations.[19] Last, one might hypothesize that EA H5 HPAI viruses are less virally "fit" in wild waterfowl as compared with LPAI strains that naturally circulate in this population. Therefore the LPAI viruses are more competitive in the host.

It is essential to recognize that as this clade of EA H5 HPAI viruses maintains wide circulation in Asia, it will continue to evolve and pose an ongoing threat to domestic poultry and wild birds globally. Whether and when this clade of H5 viruses will return via migration to either Europe or North America depends on a number of factors that are not well defined but may include weather patterns and storms affecting migration and the prevalence of infection in domestic poultry (especially domestic ducks of the genus *Anas*).

Biosecurity of Collections and Vaccination of Nondomestic Avian Species

Zoos and exhibitors face a number of issues when trying to safeguard collections against incursions from potentially infected wild migratory waterfowl, and zoos have been affected by HPAI H5N8 clade 2.3.4.4.[20] The water features present in many zoological parks are attractive to wildlife and serve as a ready stopover point, replete with food and shelter, for migrants. This makes management of outdoor collections challenging. It is probably best for zoos to consult experts on wildlife deterrents; these can be found in many state or federal wildlife agencies. However, there are basic precautions that may be taken to minimize risk of infection to collections. It is helpful to consider biosecurity concepts and to translate them into site-specific actions depending on risk of infection. First, conceptual biosecurity should include a site evaluation relative to surrounding risk factors such as wetlands (HPAI reservoir habitat) or poultry operations. Structural biosecurity measures are the "physical" barriers to disease. In the 2014–2015 HPAI outbreaks in the Midwest, specimens in high-value collections were housed indoors until the disease threat had been minimized. Other facilities were able to install temporary "covers" to minimize the entry to certain exhibits by wild waterfowl. Operational biosecurity should describe how the site is managed to prevent the introduction of pathogens. Personnel protocols, personal protective equipment (PPE), and procedures for movement, cleaning, and disinfection of equipment and how these materials are moved through the facility are elements of operational biosecurity. Examples include changing footwear when entering animal enclosures and ensuring that any free-roaming fowl (e.g., peafowl and pheasants) may be caged when there is a threat.

Vaccination of a collection is a last resort, and any threat from infection must be severe to warrant implementing this countermeasure. There are many impediments to vaccinating nondomestic species. First, any such procedure must be cleared with the national and state/provincial animal health authorities because there are many regulations surrounding the use of avian influenza vaccines directed against notifiable subtypes. Second, the types of vaccines available for use in nondomestic birds is largely limited to inactivated products, because the efficacy of modern vectored or modified live-vectored vaccine products (those routinely used in domestic poultry) is largely unknown and such products may not be efficacious for nondomestic avian species. Third, the performance of even inactivated products may be highly variable across species lines in terms of efficacy and duration of immunity. Lastly, vaccinated birds may have to be appropriately identified by some type of permanent marker (e.g., a tag, microchip), and the movement of vaccinated birds may well be restricted both domestically and internationally by trade law owing to the risk of imperiling commerce in poultry products. Nevertheless, vaccination of nondomestic avian species has been accomplished successfully in countries that are under constant or intermittent threat from HPAI.[21,22] It is important to recognize that vaccination is a tool and that it should not supplant any of the tenets of biosecurity.

Summary

Avian influenza evolves in unpredictable ways that will continue to challenge the health of both wild and domestic species. The dynamics of viral exchange between recognized host species such as ducks and land-based poultry may contribute to the ongoing genetic destabilization of the virus and increases in virulence. The recently acquired ability of these novel influenza viruses to be subclinically carried and transmitted by select species of wild waterfowl increases the threat to captive collections globally. However, the best

tools to defend zoological parks and collections against this continuing threat are vigilance and practical biosecurity.

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39

Emerging Reptile Viruses

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In reptile virology, it may be difficult to recognize the true emergence of a pathogen, as detection of previously unknown organisms has occurred rapidly in recent years. This has been due both to the use of new technologies in reptile diagnostics and to the increased interest of researchers from various backgrounds in reptiles as virus hosts. The increasing use of next-generation sequencing (NGS) methods has led to many of these discoveries and appears likely to increase our knowledge of viruses in zoo, aquarium, and wildlife species significantly in the coming years. The development and commercial availability of sensitive diagnostic tests has increased, and changes in detection rates may not always reflect a true increase in viral prevalence among reptiles. This underlines the importance of additional studies using a wide range of disciplines and techniques to understand both the clinical significance of many viruses in various reptile species as well as their host ranges, the pathogenesis of associated disease, and epidemiology.

Numerous factors influence the emergence of viral infections in reptile populations. These include effects of climate change, which may influence the spread of viral infections in reptiles in multiple ways, including increasing the range of invertebrate vectors, especially for arboviruses (Table 39.1); influencing the reptile immune system; and the replication rates of viruses capable of infecting reptiles. Direct human interaction has been shown to affect the spread of multiple diseases in wild animals, and the pet trade plays a large role in the international spread of viruses (e.g., for ranaviruses,[1,2] herpesviruses,[3] and reptarenaviruses) in pet reptiles.[4] In addition, the prevalence of viruses in pet reptiles and possibly also wild reptiles appears to undergo a fluctuating pattern in many cases, so that study over multiple years or decades may be necessary in order to understand the natural dynamics of viral infection in these animals.[5] This may be in part due to functions of reptile immunity as well as, in some cases, the long time span that may occur between infection and the development of disease.

A wide range of viruses has been described in reptiles (Table 39.2). This chapter focuses on select viruses that have been recently described or for which there is evidence that their epidemiology has changed in recent years. Ranaviruses are important emerging pathogens in chelonians and squamates as well as in amphibians and fish; they are covered in Chapter 52.

Adenoviridae

Adenoviruses are large, nonenveloped double-stranded (ds) DNA viruses. Members of three different genera have been described in reptiles: atadenoviruses, siadenoviruses, and the proposed "testadenoviruses."[6] The atadenoviruses have been hypothesized to have evolved in squamate reptiles,[7] and some of the viruses found in these reptiles appear to be host species–specific. Atadenovirus infections in squamates are not always associated with disease, although they may be involved in multipathogen disease processes. There is increasing evidence that numerous squamate atadenoviruses are able to switch between various squamate hosts, with unknown clinical and epidemiologic consequences[8] as well as ramifications for viral detection methods. Although atadenoviral infections in squamates are well documented, reports of adenoviral infections in chelonians are relatively rare and the genetic diversity of the detected viruses is relatively high, indicating that at least in some cases adenoviruses have recently switched to chelonians as hosts. The first report of a disease outbreak associated with adenoviral infection in chelonians was in a group of Sulawesi tortoises *(Indotestudo forsteni)* that were illegally imported into the United States.[9] Affected animals developed severe multisystemic disease with clinical signs including anorexia, lethargy, mucosal ulcerations, nasal and ocular discharge, and diarrhea. The group experienced a mortality rate of 82%. An adenovirus was detected by polymerase chain reaction (PCR) as well as by electron microscopy in cells of affected tissues. The virus was determined to belong in the genus *Siadenovirus*. Siadenoviruses had previously been described in birds but are hypothesized to have evolved in amphibians.[10] The same virus was later also found in impressed tortoises *(Manouria impressa)* and a Burmese star tortoise *(Geochelone platynota)* with systemic disease.[11]

Other adenoviruses found in chelonians have differed genetically from previously described genera. These viruses have been described in a wide range of terrestrial and aquatic species in the United States and Europe. They have been found in both diseased and clinically healthy animals.

TABLE 39.1 Arboviruses Described in Reptiles

Virus Family	Virus Name	Invertebrate Vectors	Reptilian Hosts	Geographic Distribution*	Associated Disease in Reptiles	Zoonotic Potential	Ref.
Bunyaviridae	*Orthobunyvirus*: Cache Valley virus, Tensaw virus, Kowanyama virus	Mosquitoes, *Anopheles* spp.	Texas soft-shelled turtle *(Apalone [Trionyx] spinifera emoryi)*, Skink *(Cryptoblepharus [Ablepharus boutonii] virgatus)*	North America, Australia	None	Yes	55, 56
	Nairovirus: Crimean-Congo hemorrhagic fever virus	Ticks *(Hyalomma aegyptium)*	Horsfield's tortoise *(Testudo horsfieldii)*	Middle East	None	Yes	57
Togaviridae	Eastern equine encephalitis virus (EEEV), Venezuelan equine encephalitis virus (VEEV), western equine encephalitis virus (WEEV)	Mosquitoes	Various squamates, most often in various snake spp.; chelonians, crocodilians	North and South America	None	Yes	58–60
Flaviviridae	Japanese encephalitis virus, St. Louis encephalitis virus, Powasan virus, West Nile virus (WNV), Zika virus	Mosquitoes	Crocodilians, squamates, chelonians; WNV especially in crocodilians	Asia, North and South America	Neurologic and skin lesions in crocodilians	Yes	59–62
Rhabdoviridae	Vesicular stomatitis virus, Charlesville virus, Almpiwar virus, Marco virus, Timbo virus, Chaco virus, Sena Madureira virus	Mosquitoes (e.g., *Aedes aegypti*), sandflies (*Phlebotomus* spp.), midges (*Forcipomyia* spp., *Culicoides* spp.)	Redbelly water snake *(Nerodia [Natrix] erythrogaster)*, Australian house gecko *(Gehyra australis)*, teiid lizards: giant ameiva *(Ameiva ameiva ameiva)*, Striped forest whiptail *(Kentropyx calcarata)*, Caiman lizard *(Dracaena guianensis)*, skink *(Cryptoblepharus virgatus)*	Australia, North and South America	None reported	Yes	55, 56, 63, 64
Iridoviridae	Hemocytiviruses	Unknown	Various squamate and chelonian spp.; sequence data available from: Bearded dragon *(Pogona vitticeps)*, peninsula ribbon snake *(Thamnophis sauritus sackenii)*, Iberian mountain lizard *(Iberolacerta [Lacerta] monticola)*	Africa, Asia, Europe, North America,	Anemia, systemic disease	Unlikely	65–69

*Geographic areas in which detection in reptiles has been reported. The geographic range of individual viruses may be greater.
This table includes descriptions of detection of viruses by various methods as well as serologic evidence of previous infection (without proof of viremia).

TABLE 39.2 Virus Families and Genera Described in Orders of Reptiles

		Nonavian Reptile Host Order		
Virus Family	**Virus Genus**	**Testudines**	**Squamata**	**Crocodylia**
Adenoviridae	*Atadenovirus*	X	X	
	Siadenovirus	X		
	"Testadenovirus"	X		
	Unclassified			X
Herpesviridae	*Scutavirus*	X		
	Unassigned		X	X
Iridoviridae	*Ranavirus*	X	X	
	Iridovirus		X	
	"Hemocytivirus"*		X	
Papillomaviridae	*Dyozetapapillomavirus*	X		
	Unclassified	X	X	
Poxviridae	*Crocodylipoxvirus*			X
	Unclassified	X	X	
Circoviridae	Unclassified	X	X	
Parvoviridae	*Dependoparvovirus*		X	
Hepadnaviridae	Unassigned	X	X	
Retroviridae	*Gammaretrovirus*		X	
	Unassigned	X	X	X
Reoviridae	*Orthoreovirus*	X	X	
Bornaviridae	Unassigned		X	
Paramyxoviridae	*Ferlavirus*	X	X	
Sunviridae	*Sunshinevirus*		X	
Rhabdoviridae	Unassigned	X	X	
Orthomyxoviridae				*
Arenaviridae	*Reptarenavirus*		X	
Bunyaviridae	*Orthobunyavirus*	X		
	Unassigned		X	
Coronaviridae	Unassigned		X	
Picornaviridae	*Torchivirus*	X		
	"Rafivirus"	X		
	Unclassified		X	
Caliciviridae	*Vesivirus*		X	
Flaviviridae	*Flavivirus*	X	X	X
Togaviridae	*Alphavirus*	X	X	X

*There is some evidence of infection with these viruses in this group of reptiles.

The viruses detected in these cases have been preliminarily named "testadenoviruses," based on the hypothesis that they have coevolved in chelonians.[6] In another case in a spur-thighed tortoise *(Testudo graeca)* with stomatitis and esophagitis, a virus in the genus *Atadenovirus* was detected by PCR and sequencing.[12] There are therefore a multitude of different adenoviruses belonging to at least three different genera that have been discovered in chelonians within the past decade. The viruses hypothesized to have recently switched hosts into chelonian species (the siadenovirus and atadenovirus) appear to have been associated with more severe pathologies.

Herpesviridae

Herpesviruses are large, enveloped dsDNA viruses that are known to cause latent infections in hosts surviving acute infection. Herpesviruses have long been described

as pathogens in a wide range of reptilian hosts, especially chelonians. Detections in various orders of reptiles have increased in recent years. In many cases, virus detection is based on histologic detection of intranuclear inclusion bodies in infected cells, followed by electron microscopy and on detection of viral DNA using a panherpesviral PCR targeting a portion of the DNA-dependent DNA polymerase.[13]

Crocodilian Herpesviruses

Recent studies in Australia in farmed saltwater crocodiles *(Crocodylus porosus)* have led to the description of three different crocodilian herpesviruses.[14] Infections have been associated with three disease syndromes: conjunctivitis and/or pharyngitis (CP), lymphoid proliferation and nonsuppurative encephalitis (SLPE), and multifocal lymphohistiocytic infiltration of the dermis (LNS).[5] CP was found primarily in hatchlings, SLPE in juveniles and growers, and LNS in harvest-sized animals. The causative nature of the herpesviruses for each disease syndrome has not been fully established, although herpesviruses were found significantly more often in diseased crocodiles than in controls.[5]

Squamate Herpesviruses

Herpesviruses have been described periodically in various squamate species. Genetically, little information is available on these viruses, but what is available indicates that they are diverse and not closely related to chelonian or crocodilian herpesviruses. In lizards, herpesviruses have been associated with stomatitis,[15,16] papillomas,[17] hepatitis, and enteritis.[18] In a disease outbreak associated with a herpesvirus infection in captive adult horned vipers *(Vipera ammodytes ammodytes)* in Europe, animals developed widespread hemorrhage, coelomic and pericardial effusion, and hepatitis. All of the horned vipers in the collection died, whereas common European vipers *(Vipera berus)* housed in the same facility remained unaffected.[19]

Chelonian Herpesviruses

Herpesviruses have long been described as important pathogens in various chelonian species, mostly in sea turtles, where they are considered the cause of fibropapillomatosis (see Chapter 57), as well as grey patch disease,[20] lung, eye, and trachea disease,[21] loggerhead genital-respiratory herpesvirus-associated disease, and loggerhead orocutaneous herpesvirus-associated disease,[22] and in tortoises, in which they have mostly been associated with stomatitis, rhinitis, and conjunctivitis. The chelonid fibropapillomatosis–associated herpesvirus, officially known as *Chelonid alpha herpesvirus 5*, has been placed in a separate genus, *Scutavirus*, in the subfamily *Alphaherpesvirinae*.[23] All reported herpesviruses of chelonians appear to cluster in this genus.[24] Recent studies on herpesviruses in a wide range of chelonian species have led to the description of numerous new virus types and have also indicated shifts in prevalence of specific viruses in some pet chelonians.

There are four known genetically distinct herpesviruses that can infect tortoises, named testudinid herpesvirus 1 through 4 (TeHV1-4). TeHV1, 2, and 3 have all been associated with severe stomatitis and glossitis as well as rhinitis, conjunctivitis, and hepatitis. TeHV4 was originally detected in a clinically healthy tortoise during quarantine screening.[25] TeHV3 has been associated with higher morbidity and mortality rates than TeHV1.[26] Recent studies using molecular techniques to investigate genomic differences between TeHV3 strains as well as transmission studies have provided evidence that there may also be differences in pathogenicity between different isolates.[24,27] In a study in Europe almost 20 years ago in which samples from tortoises kept as pets were tested for the presence of herpesviruses, only TeHV1 and TeHV3 were detected, with TeHV3 making up greater than 80% of the detected herpesviruses.[28] In a recent study screening samples from over 1000 chelonians in Europe for herpesviruses and other pathogens, approximately half of the herpesviruses detected were categorized as TeHV1, indicating that the prevalence of this virus in pet tortoises in Europe has increased over the past years, possibly due to changes in the pet trade.[3] It might also reflect a change in the popularity of specific tortoise species in the pet trade. During that same study, a TeHV4 was detected for the first time in Europe in an African tortoise species, the leopard tortoise *(Stigmochelys pardalis)*.[29]

Detections of herpesviruses in other families of chelonians has also increased rapidly in recent years.[30–36] In the family Emydidae, herpesviruses have been detected in a freshwater turtle *(Pseudemys concinna concinna)*, a northern map turtle *(Graptemys geographica)*, wild bog turtles *(Glyptemys muhlenbergii)*, wood turtles *(G. insculpta)*, spotted turtles *(Clemmy guttata)*, and box turtles (*Terrapene* sp.). Clinical signs associated with infection in these animals have ranged from detections in clinically healthy animals to stomatitis, papillomatous skin lesions, rhinitis, and sudden death. Histology has demonstrated hepatic lipidosis, pneumonia, and both hepatocellular and splenic necrosis. Intranuclear inclusions have been found in cells in the liver, lung, and spleen.[31] Infected animals have included both captive and wild turtles.

In the order Pleurodira, herpesviruses have been described in a captive Krefft's river turtle *(Emydura macquarii krefftii)* (family Chelidae) in Australia with ulcerative lesions of the skin and shell associated with orthokeratotic hyperkeratosis with intranuclear inclusions in keratinocytes.[35] In the family Pelomedusidae, a herpesvirus was detected in West African mud turtles *(Pelusios castaneus)* that were imported into Europe from Africa and were clinically healthy.[36]

Picornaviridae in Tortoises

Picorna-like viruses have been known to occur in tortoises in Europe since the mid-1990s. They were originally isolated in cell culture and, proving difficult to characterize,

were sometimes called "virus x." Viruses in this category have been sequenced and shown to represent a new genus in the family *Picornaviridae*, with the name *Torchivirus*.[37,38] This group of viruses have been detected mostly in *Testudo* spp. but also in several African tortoise species. They have been associated with softening of the plastron in juvenile tortoises and with rhinitis, stomatitis, and ascites in adult tortoises. They have also been isolated from clinically healthy animals.[28,39] Transmission studies with *T. hermanni* and *T. graeca* showed that the kidneys were most severely affected.[40] Serologic testing has shown that some wild-caught tortoises in Europe and Africa have antibodies against these viruses. Epidemiology of these viruses in Europe appears to undergo some fluctuation over time. A recent study showed that torchiviruses were detected by PCR using mostly oral swabs as samples in 2.2% of over 1000 chelonians tested over a 1½-year period in a commercial laboratory in Europe.[3] Another picornavirus with the suggested genus name "Rafivirus" was described from Sulawesi tortoises that were also infected with an adenovirus.[41] Animals died with severe systemic disease,[9] but the role of the picornavirus in the disease is not known.

Nidovirales

Coronaviruses belong to the order *Nidovirales*, and viruses in this family have been among the most prominent emerging viruses in a wide variety of mammalian species in recent years, causing various respiratory disease syndromes including severe acute respiratory syndrome (SARS) and Middle East respiratory syndrome (MERS). NGS of samples from reptiles displaying signs of respiratory disease has led to the description of viruses in the order *Nidovirales*, family *Coronaviridae*, subfamily *Torovirinae* in various python species and in boas.[42–45] A new genus name, "Barnivirus", has been suggested for these viruses.[42] Infections have been reported most commonly in ball pythons *(Python regius)* but also in Indian pythons *(P. molurus)*, Burmese pythons *(P. bivittatus)*, green tree pythons *(Morelia viridis)*, carpet pythons *(M. spilota)*, and boa constrictors *(Boa constrictor)*. A genetically related but distinct virus has been described in shingleback lizards ("bobtails," *Tiliqua rugosa*) in Australia.[46] In ball pythons, the "Barnivirus" was associated with a proliferative interstitial pneumonia. In some cases, tracheitis, stomatitis, esophagitis, and/or rhinitis were also associated with infection. In individual cases, lesions were also observed in other parts of the body and included encephalitis, acute nephritis, salpingitis, hepatic lipidosis, keratitis, and colitis. The greatest viral load was detected in the lungs of affected snakes.[42] A similar disease syndrome of unknown etiology was reported to have been observed in ball pythons since the 1990s.[42]

The shingleback lizards, in which a related virus was detected, were wild caught in western Australia and found to have a disease syndrome called "bobtail flu," with clinical signs including mucopurulent discharge from the eyes and nose, lethargy, lack of appetite, pale mucous membranes, depression, and loss of body condition.[46] Following detection of a barnivirus-like virus in affected animals, a real-time PCR was developed and used to detect virus in oral secretions. Virus was detected significantly more often in diseased than in healthy animals, although virus was also detected in some of the lizards that were considered healthy at the time of sampling.[46]

Arenaviridae in Snakes

Inclusion body disease (IBD) was originally described in the early 1980s and 1990s[47] and was defined by the presence of characteristic eosinophilic to amphophilic intracytoplasmic inclusions in neurons and in epithelial cells of a wide range of tissues.[48] The inclusions are made up of a specific protein known as inclusion body disease protein (IBDP).[48] The disease affects various species of boas and pythons and is associated with a wide variety of clinical signs. Central nervous system (CNS) signs are most often described, but animals may develop anorexia, pneumonia, various skin lesions, mouth rot, and other problems. However, a large number of snakes (especially boa constrictors) may remain clinically healthy despite the presence of inclusions and/or virus. In 2012, NGS showed the probable cause of IBD to be newly discovered viruses in the family *Arenaviridae* in the new genus *Reptarenavirus*.[49–51] These viruses are closely associated with the disease and can cause the development of similar inclusions in cell culture. Antibodies against reptarenaviruses isolated in cell culture bind to typical inclusions in the cells of affected animals.[49,50] IBDP is believed to be accumulated viral nucleocapsid protein.[49] Additional studies on snakes infected with arenaviruses have demonstrated a huge amount of genetic diversity in these viruses, with multiple species and possibly different genera detected.[4,52] In many cases, snakes were infected with multiple viruses, and the two genomic segments of the reptarenaviruses, L and S, appeared to reassort readily.[4] It has been hypothesized that snake importation and husbandry practices may have created this diversity among reptarenaviruses, with capture of wild snakes, importation into various countries, mixing of animals for breeding purposes, and overcrowding all being part of an "anthropogenic disruption of pathogen ecology."[4] It has been hypothesized that coinfection or superinfection with multiple reptarenaviruses could play a role in disease development.[52]

Other Newly Described Viruses of Reptiles

There are many other examples of recently described viruses in reptiles, with new reports coming out regularly. Recent developments include a description of the new family *Sunviridae* for *Sunshinevirus* in the *Mononegavirales*. *Sunshinevirus* is associated with CNS and respiratory disease in pythons, mostly in Australia.[53] Bornaviruses have also recently been detected in snakes in several cases,[54] and there is some indication that infections may be associated with

neurologic disease (T. Hyndman, personal communication, March 30, 2016).

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Emerging Diseases in Bats

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Introduction

The majority of emerging infectious diseases are zoonotic, and most originate in wild animals.[1] The rate of emerging disease has increased significantly over the past few decades, and the majority of emerging pathogens are RNA viruses. These are distinctive in that they have the ability to mutate rapidly compared with DNA viruses and bacteria, allowing them to adapt to new hosts and spread more effectively.[2] Little is understood about the dynamics of zoonotic viruses in their natural reservoirs, yet it is becoming clear that anthropogenic environmental change is driving the spillover of pathogens from wildlife populations into domestic livestock and humans. Activities such as urbanization, agricultural intensification, and global travel and trade are expanding the interface between people, livestock, and wildlife, providing continuous opportunities for pathogens to spill over into human populations and then move around the world.[3] Viral spillover between wildlife and domestic animals or humans probably occurs more frequently than is recognized, owing to limited or poor surveillance systems.[4] Zoonotic disease emergence is most likely to occur in regions where biodiversity and human population density are high and where human activities that alter the environment—such as urbanization, agricultural expansion, and deforestation—are most intensive.[2,5]

Among mammalian taxa, bats (order Chiroptera) carry more zoonotic viruses than other mammalian groups.[6] Why bats are special is not completely understood, but there appears to be a combination of ecologic, genetic, and immunologic factors, the last two of which have only recently begun to be explored.[6,7] Bats have been associated with several zoonotic viruses that have recently been discovered and linked to significant human and animal disease, including severe acute respiratory syndrome (SARS), Middle East respiratory syndrome (MERS), Ebola and Marburg viruses, and Nipah virus (NiV)[8] (see also Chapters 19, 34, and 42). There are more than 1200 species of bats in the world, forming the order Chiroptera, which makes them the second most speciose taxonomic group of mammals after rodents, representing 20% of mammalian diversity.[9] Bats are found on every continent and in every environment in which humans live. They successfully exploit human dwellings, constructs, and food resources, which creates opportunity for direct and indirect contact with people and domestic animals. Although bats typically avoid direct contact with people, indirect exposure to excreta created by bats roosting within households, buildings, mines, and caves may lead to human infection with bat-borne pathogens.[10] Frugivorous bats are often found roosting in trees in rural and even urban environments.[11,12] They will eat cultivated fruit such as mangos, rambutan, and guava, as well as and other human-provided food resources, which, when contaminated with bat excreta, may also serve as route of infection for people or animals. Despite their potential to carry zoonotic viruses, bats are overwhelmingly beneficial to people and plants, performing vital ecosystem services in the form of agricultural pest control[13,14] and seed dispersal and pollination.[15] In many parts of the world, bats are hunted for food, sport, or traditional medicine.[16] The butchering and consumption of bats provides an opportunity for the transmission of blood-borne pathogens.[8]

Many novel viruses or viral sequences have been identified in bats, but in most cases their ability to infect other species remains unknown. One of the major challenges to predicting zoonotic disease emergence is our inability to translate viral genotype into phenotype (clinical presentation and pathogenicity of a virus). Viral discovery has, however, significantly expanded our understanding of the phylogenetic breadth of important viral families such as filoviruses (e.g., Ebola virus), paramyxoviruses (e.g., NiV), and coronaviruses (e.g., SARS coronavirus [CoV]), which is necessary for both better understanding what makes viruses pathogenic and also for recognizing wildlife reservoirs of viral pathogens, once they do emerge, more rapidly.[17]

The following is a review of recently emerging zoonotic viruses that have bats as a natural reservoir. These examples highlight viruses whose emergence has been linked to human behaviors and that have caused significant morbidity and mortality in people, but have also involved other species in the transmission chain between bats and people, making them relevant to both human and animal health.

Emerging Viral Zoonoses Carried by Bats

Henipaviruses (Nipah and Hendra Viruses)

NiV is a zoonotic paramyxovirus (genus *Henipavirus*) first recognized in Malaysia in 1998 as a respiratory and neurologic disease in domestic pigs; it subsequently infected farm workers.[18] The initial spillover occurred because mango orchards were planted next to pig enclosures. The mangos attracted frugivorous bats that carried NiV; the proximity to the pig enclosures allowed contaminated fruit to be dropped and consumed by pigs. The size of the farm created an environment that could support a sustained NiV outbreak in pigs over the course of a year, which fueled the broader epidemic.[19] The outbreak in Malaysia spread via the movement of infected pigs from farm to farm, ultimately leading to the depopulation and closure of thousands of farms and the infection of 265 people in Malaysia and Singapore, of whom 105 died.[18] After NiV was stamped out by the systematic depopulation of pig farms, policies were put into place that required a buffer zone between orchards and livestock enclosures on commercial farms. This solution has proven effective in removing the key interface that led to NiV spillover and emergence on the index farm, and there has not been an outbreak since, despite the continued presence of the two pteropid host species and continued livestock production.

Four years prior to the discovery of NiV in Malaysia, Hendra virus was discovered as the cause of an outbreak of severe respiratory and neurologic disease in horses in racing stables in Hendra, a suburb of Brisbane in the eastern Australian state of Queensland. Fourteen horses were affected with respiratory and neurologic signs, and the horses' trainer became sick and died after being exposed to the horses. Hendra virus was ultimately traced back to flying foxes (*Pteropus* spp., of which there are four in Australia) as the natural reservoir.[20] NiV's genetic relationship to Hendra led to the investigation and confirmation of the two endemic pteropid bat species in Malaysia as reservoirs for NiV.[21]

In Bangladesh, outbreaks of NiV encephalitis in people have been reported on a near annual basis since 2001, with some causing case fatality rates of 100%.[22,23] Outbreaks in Bangladesh are seasonal and spatially clustered within the western half of the country.[22] The consumption of raw date palm sap has been the primary exposure associated with infection, and the timing of date palm sap harvesting (November–April) aligns with human NiV encephalitis outbreaks.[22] NiV, like Hendra virus, is excreted by *Pteropus* bats in saliva, urine, and feces; in experimental infections it does not cause visible clinical signs or severe pathology despite widespread viral infection of endothelial tissue.[24,25] Contamination of date palm sap likely occurs when the Indian flying fox *(Pteropus medius)* feeds from the sap flow or from sap collection pots.[26] In Bangladesh and India, antibodies against NiV as well as viral RNA have been detected in *P. medius*, which is the only pteropid bat on the Indian subcontinent.[27–30] In addition to NiV, RNA sequences from closely related paramyxoviruses have been identified in this bat.[31] Nonneutralizing antibodies reactive to NiV have been found in domestic animals in Bangladesh, including pigs, goats, and cattle, suggesting that spillover of Nipah-like viruses has occurred, although no human cases have been linked to domestic animal infections in Bangladesh.[32]

Hendra virus has continued to cause outbreaks in horses across Queensland and the adjoining state of New South Wales since 1994, and evidence of infection has been detected in each of the four species of flying fox present in this range.[33] Although the definitive mode of transmission between bats and horses remains uncertain, it is hypothesized that infected bats feeding or roosting in trees within horse enclosures contaminate the area beneath, and horses are exposed either by direct exposure to excreta or by ingesting contaminated feed or water.[33] Infected horses are then able to transmit the virus to other horses and to humans. Outbreaks in horses are sporadic; however, since 2006 there has been a marked increase in the frequency and number of equine cases identified.

Pteropus species are the primary natural reservoirs for henipaviruses throughout Asia and Australia[24,34]; however, the full geographic extent and host diversity for henipaviruses is still being studied. Antibodies against a Nipah-like virus were recently detected in the straw-colored fruit bat *(Eidolon helvum),* a migratory pteropodid bat, and in hunters in Cameroon, suggesting that related viruses may be circulating in Central Africa.[35,36] Antibodies against Nipah-like viruses have also been detected in insectivorous bat species in China.[37] To date, human infections have been identified in relatively few countries compared with the distribution of henipaviruses in bats (India, Bangladesh, Malaysia, the Philippines, Singapore, and Australia).[8] Although no treatment or vaccine for NiV currently exists, the advent and commercial production of a Hendra virus vaccine for horses in 2014 have offered an effective tool for limiting HeV cases in Australia.[38] Currently NiV is listed as a priority by the World Health Organization (www.who.int/blueprint/priority-diseases/en/) and the Coalition for Epidemic Preparedness Innovations (CEPI; www.cepi.net) for the development of a vaccine. Experimental Nipah vaccines that utilize soluble G proteins, like the Hendra vaccine, have been found to be effective in nonhuman primate models.[39]

NiV's broad geographic host range, its ability to infect multiple domestic animal species and humans, its repeated spillover in populous areas and ability to spread among people, and its association with high mortality rates make NiV a significant threat to human and animal health.[8,40] Because of the potential severity of henipavirus infection in people and livestock, improved surveillance systems are needed to both ensure rapid detection and response to outbreaks as well as to identify high-risk areas where host, virus, and an interface that promotes spillover exist so that effective interventions can be implemented.

Filoviruses (Ebola and Marburg Viruses)

Ebola virus was first discovered in 1976; since then there have been more than 26 outbreaks of Ebola virus disease.[41] Over the past 40 years, the natural reservoir for Ebola virus has remained a mystery. Although some of the outbreaks were epidemiologically linked to contact with wild animals, few had evidence directly linking cases to contact with bats.[8] Human infections in Central Africa have been associated with such contact and with the consumption of infected animals such as gorilla *(Gorilla gorilla)*, chimpanzee *(Pan troglodytes)*, or duiker (*Cephalophus* spp.) carcasses.[42] In December 2013, an outbreak of Ebola Zaire virus, of unprecedented magnitude in West Africa, began in Guéckedou, Guinea, following a single introduction from an unknown animal reservoir (hypothesized to be a bat) into the human population.[43] Importantly, human social dynamics, rather than repeated introductions from an animal reservoir, were responsible for the rapid and uncontrolled spread of Ebola virus disease through Guinea, Sierra Leone, and Liberia, underscoring the importance of human-wildlife interaction in spillover and the triggering of epidemics and pandemics.

However, over the past decade there has been a growing body of evidence suggesting that multiple bat species carry Ebola viruses, whereas Marburg virus appears to be primarily carried by the Egyptian fruit bat *(Rousettus aegyptiacus)*, a common frugivorous bat found throughout the African continent and in the Middle East.[44] Marburg virus infection occurs seasonally in *R. aegyptiacus*, with peak infection rates occurring during the birthing season.[45] As with henipaviruses, experimental infections with Marburg virus in *R. aegyptiacus* suggest that there is minimal pathology and no visible signs of disease in these bats when infected and that they may shed virus for up to 19 days postinoculation.[46] Ebola virus Zaire has been detected in several different bat species in Central Africa, including the hammer-headed fruit bat *(Hypsignathus monstrosus)*, Franquet's epauletted fruit bat *(Epomops franquetti)*, and the little collared fruit bat *(Myonycteris torquata)*.[47] Ebola virus has not yet been isolated from bats; however, viral RNA and antibodies have been detected in several species. Ebola Reston virus, a species causing disease in macaques but *not* humans or pigs, was detected in the common bent-wing bat *(Miniopterus schreibersi)*, a common insectivorous bat, in the Philippines.[48] Antibodies reactive to Ebola Zaire antigen have been found in Leishenault's fruit bat *(Rousettus leishenaulti)* in Bangladesh, and although a filovirus has not yet been identified, these findings suggest that an immunogenically related filovirus is circulating in these bats.[49] Novel filoviruses, yet to be characterized, have been found in the cave nectar bat *(Eonycterus spelea)* and *R. leishenaulti* in China.[50] These viruses may be closely related to those causing the immune response detected in the same species in Bangladesh. The NPC-1 receptor, used by filoviruses for cell entry, is conserved across several bat species, which further supports a broad bat species range for Ebola viruses.[51] As with CoV and henipaviruses, filoviruses appear to be geographically widespread in bat hosts in both Africa and Asia. Although some Ebola viruses and Marburg virus have been associated with high mortality rates in people, Ebola Reston virus illustrates how genetic diversity within a viral group can influence pathogenicity in humans or domestic animals. Until there is an a priori method for determining pathogenicity from genetics, filovirus surveillance and ecologic research in bats and other wildlife—including work done at the International Centre for Medical Research, Franceville (CIRMF)[42,47]; the US Centers for Disease Control[44,45,52]; and the US Agency for International Development (USAID) under its Emerging Pandemic Threats: PREDICT program[53]—will help to provide a better understanding of filovirus host ecology and viral genomics and inform strategies to reduce the risk of Ebola virus disease and outbreaks of Marburg virus disease (see also Chapter 19).

Coronaviruses (Severe Acute Respiratory Syndrome and Middle East Respiratory Syndrome)

CoVs comprise a large viral family known to infect a wide variety of animals, including humans. Prior to the emergence of SARS and MERS, only four CoVs were known to infect humans.[54] The SARS pandemic of 2002–2003 infected more than 8000 people in 27 countries and had a case fatality rate of ~9%.[55,56] MERS-CoV (as of June 2017) has infected more than 2000 people in 27 countries and had a 35% case fatality rate.[57] These two epidemics solidified CoVs as a viral family of concern for human health. SARS-CoV emerged from bats through the live animal markets of southern China in 2003.[55] The close caging of various mammalian species, including bats, and the general lack of effective biosecurity practices in handling and butchering animals in live animal markets facilitated the infection of multiple species, including civets *(Paguma larvata)*, raccoon dogs *(Nyctereutes procyonoides)*, and ferret badgers (*Melogale* spp.), all of which were initially suspected as being the primary source of the virus in early investigations.[58] Initially, civets were implicated as the source of SARS-CoV, and markets and farms were depopulated of civets as a control measure. Importantly, farmed civets outside the marketplace did not have evidence of SARS-CoV infection, suggesting an alternate reservoir.[59,60] The eventual discovery of SARS-like CoVs in bats was an important step in understanding the natural reservoir, although early bat viral isolates did not use the same cell entry mechanism as SARS-CoV and therefore were not able to cause SARS in animal models. In 2012, nearly 10 years after the initial discovery of bat SARS-like CoVs, a CoV much more closely related to SARS-CoV and capable of directly infecting humans was identified among Chinese horseshoe bats *(Rhinolophus sinicus)* in Yunnan, China.[61] Although bats are no longer legally sold in live animal markets in China, there are still communities,

including some in Yunnan, that hunt and eat *Rhinolophus* bats, raising the possibility that SARS could reemerge.

In 2012, another novel CoV was discovered in people in Saudi Arabia.[62] Ultimately named MERS-CoV, its genetic relationship to SARS-CoV and other beta CoVs found in bats in Hong Kong led early investigations to focus on bats as a potential reservoir. A short RNA fragment matching MERS-CoV was found in an Egyptian tomb bat *(Taphozous perforatus)* in Saudi Arabia, although epidemiologic studies have not confirmed this species as a reservoir.[63] MERS-related CoVs have been found in other bat species in Asia and Africa[64]; however, dromedary camels are the most likely animal source of infection for people.[65] Juvenile camels shed MERS-CoV more frequently than adults, and infection is associated with a mild respiratory disease.[65] Nosocomial transmission has also been a significant risk factor for human MERS-CoV infection.[66] In 2015, an outbreak involving 81 people occurred in South Korea and was linked to hospital-based transmission.[67]

The discovery of SARS-like CoVs in bats fueled further investigation and the discovery of a large diversity of CoVs in bats and the hypothesis that all human CoVs originated in bats.[64,68] It is estimated that 1200–6000 CoVs are carried by bats worldwide, some of which will also have the potential to emerge in human or domestic animal populations.[64] Porcine epidemic diarrhea virus (PEDV) is an alphacoronavirus that in 2013 emerged in the United States and reemerged in Asia, causing economically significant disease outbreaks in domestic pigs.[69] Although PEDV has not been directly linked to bats, it does cluster phylogenetically with other alphacoronaviruses that have been found in bats.[64,70] CoV diversity and richness correlate with bat species richness, but as with all categories of novel viruses, it is not currently possible to determine which viruses have zoonotic potential.[64] Hospital- or community-based severe acute respiratory infection (SARI) surveillance in regions with high bat biodiversity and high-risk bat-human interfaces (e.g., guano mining[71]) should consider CoV screening as part of a diagnostic approach to investigating respiratory disease clusters in people or diarrheal disease in animals. Identification of novel CoVs as etiologic agents in humans or domestic animals will provide additional insights into genetic determinants of pathogenicity and their relationship with bat CoVs.

Discussion

Within each of the groups of viruses discussed, it is likely that there are still many as yet undiscovered species, strains, and genetic variants comprised by the genetic diversity of nature. In addition, the high mutation rate of RNA viruses—and in the case of CoVs the ability to recombine—means that new genotypes are continuously being created. This presents a serious challenge to cataloging viral diversity, but doing so may ultimately pay off by allowing for the identification of genetic determinants of pathogenesis. Also, having a library of sequences from all bat CoVs, filoviruses, or henipaviruses may provide insight into where surveillance should be targeted based on viral diversity hot spots. Currently there are geographic regions such as Latin America where there is a disproportionately low number of zoonotic viruses that have been characterized in bats, relative to bat species richness, making this a region where surveillance efforts could potentially bring a high yield.[6] Ultimately the integration of host ecology with viral discovery will be important for understanding the risk of viral spillover. NiV is an important reminder that simply the presence of a host and virus is not sufficient for zoonotic transmission to occur. A viable "interface" or mechanism of transmission is also needed for spillover (provided that people or domestic animals are susceptible to infection). Experimental studies will also be vital to clarify the pathogenesis and transmissibility of novel viruses. Reverse engineering has made it much easier to "rescue" or recreate viruses in the laboratory from sequence data. Genetically modified mice provide an important model for the study of susceptibility to infection and pathogenesis in human physiology.

The recent deluge of new viruses found in bats globally warrants a degree of caution against overstating their threat to human or animal health when communicating findings to the public or policy makers. In the majority of instances, there is no evidence that any newly discovered virus in bats has infected any other animal or person, thereby making it simply a bat virus until proven otherwise. When newly identified viruses are related to known zoonoses, they are often presented as potential threats to human or animal health, but there is the potential to cause undue public alarm when reporting these findings. Given the potential for negative and scientifically unsupported actions against bats that include extermination, messaging to the public should provide appropriate context where there is a lack of evidence for human or animal health impact and emphasize that bats are ecologically invaluable animals. Extermination of bats should *not* be considered an effective response to an outbreak of a bat-borne pathogen or a control measure to prevent outbreaks. This approach actually enhanced the local transmission of Marburg virus among bat populations following the extirpation of bats from a mine in Uganda.[72]

There will continue to be a large research and surveillance focus on bats as hosts for zoonoses. Data are mounting to support bats as important reservoirs compared with other mammals, and large-scale surveillance efforts like PREDICT and the recently launched Global Virome Project, a 10-year effort to identify the majority of viruses in key wildlife species in emerging disease hot spots,[73] will shed more light on the total diversity of viruses in bat species and the types of human-animal interfaces that exist in different geographic and cultural contexts. Understanding specific human behaviors that promote contact with bats and developing strategies that limit bat-human-domestic animal contact without harming bats is key to reducing the risk of viral spillover while also preserving bats and the ecologic services they provide.

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Zika Virus: A Real Threat to Wildlife?

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Introduction: Arboviruses and Epidemiology

Arthropod-borne viruses (also known as arboviruses) normally circulate in nature through biological cycles of transmission between susceptible vertebrate hosts and blood-feeding arthropods, such as mosquitoes (Culicidae, Psychodidae), biting midges (Ceratopogonidae), black flies (Simuliidae), and ticks (Ixodidae and Argasidae).[1] Arboviruses have RNA genomes and are classified within the families *Peribunyaviridae*, *Flaviviridae*, *Reoviridae*, *Rhabdoviridae*, *Phenuiviridae* e *Togaviridae*.[2] With few exceptions, they are zoonotic and depend on vectors and wild and/or domestic animal interactions for maintenance in nature.[1,3]

Birds, nonhuman primates (NHPs), and rodents are the main reservoir hosts and mosquitoes and ticks the most common vectors for the significant arboviruses.[1,3] "Spillover" of arboviruses from enzootic cycles to humans by enzootic or "bridge" vectors may also occur under appropriate ecologic conditions.[4] For most arboviruses, humans are dead-end or incidental hosts; however, there are several viruses—such as dengue, Zika, and chikungunya—that may use humans as their amplification hosts during outbreaks.[3–9]

The infection caused by Zika virus (ZIKV) in humans often presents with mild or nonspecific symptoms, similar to other prevalent viral diseases in Brazil such as dengue and chikungunya, hampering attempts to perform an accurate diagnosis based solely on clinical grounds. The disease is usually characterized by an acute onset of fever, nonpurulent conjunctivitis, headache, arthralgia, myalgia, asthenia, and a maculopapular rash.[10,11] Guillain-Barré syndrome and other central nervous system (CNS) anomalies have also been associated with the Zika epidemic in the Americas.[7,10,12]

Zika Virus: Molecular

ZIKV is a member of the *Flavivirus* genus, which belongs to the Flaviviridae family. This family includes other agents of clinical significance, such as dengue virus (DENV), yellow fever virus (YFV), West Nile virus (WNV), and Japanese encephalitis virus (JEV).[7,8,13] Flaviviruses are small, spherical in shape, and contain a positive single-stranded nonsegmented RNA of approximately 11 kb.[14] The genome is flanked by two noncoding regions (NCRs): a 5′ NCR of approximately 100 bp and a 3′ NCR of about 450 bp.[15] ZIKV is phylogenetically divided into three lineages: two African (eastern or western African) and one Asian genotype. Only the Asian lineage is directly related to human cases of congenital CNS changes causing microcephaly and other alterations in adults including Guillain-Barré syndrome.[10,11,16,17] Recent bioinformatics and phylogenetic analyses suggest that ZIKV isolates currently circulating in the Americas constitute a new clade of the Asian genotype.[7,16]

Epidemiologic studies in humans indicate widespread distribution of ZIKV in many countries in Southeast Asia, the Americas, and Africa,[18] reaching approximately 84 countries with reports of human illness (World Health Organization [WHO], Situation Report Zika Virus, March 20, 2017).

Epidemiology and Wild Hosts

The ZIKV prototype was obtained from a febrile sentinel rhesus monkey *(Macaca mulatta)* and *Aedes africanus* mosquitoes in the Zika Forest near Entebbe, Uganda, in 1947.[12,19] The virus was subsequently detected in tropical Africa in monkeys during serosurveys and human surveillance of febrile disease, followed 60 years later by its emergence in people in the Pacific Islands and the Americas, including the Caribbean region.[7,20,21] In February 2016, the WHO declared it a public health emergency of international concern.[22,23]

In the sylvatic environment, ZIKV is maintained between mosquitoes and wild hosts (Table 41.1).[5,8,9,13,24–26] The main route of ZIKV infection is through bites by an infected female hematophagous mosquito, after a 10-day incubation period. Many known mosquito species have been detected with ZIKV, but *A. africanus* and *Aedes aegypti* were the most competent vectors.[8,12,13,24] Moreover, in mosquitoes the virus may also be sexually and vertically transmitted.[20,27]

TABLE 41.1 Wildlife Species That Have Tested Positive for Zika Virus Antibody or Antigen by Serologic, Molecular,and/or Viral Isolation Testing in Experimental and/or Natural Conditions

Order	Family	Common Names	Scientific Name
Artiodactyla	Bovidae	Hartebeest	*Alcelaphus buselaphus*[†,‡]
		Wildebeest	*Connochaetes taurinus*[†,‡]
		African buffalo	*Syncerus caffer*[†,‡]
		Gazelle	Not mentioned[†,‡]
		Impala	*Aepyceros melampus*[†,‡]
	Hippopotamidae	Hippo	Not mentioned[†,‡]
Carnivora	Felidae	Lion	Panthera leo[†,‡]
Charadriformes	Scolopacidae	Ruff	Philomachus pugnax[†,‡]
Chiroptera	Vespertilionidae	Little brown bat	Myotis lucifugus[*,NA]
Lagomorpha	Leporidae	Rabbit	Not mentioned[*,‡]
Pelecaniformes	Ardeidae	Cattle egret	*Bubulcus ibis*[†,‡]
	Threskiornithidae	African sacred ibis	*Threskiornis aethiopicus*[†,‡]
Perissodactyla	Equidae	Zebra	Not mentioned[†,‡]
Primates	Aotidae	Owl monkeys	*Aotus nancymaae*[*,‡,§,¶]
	Callitrichidae	Marmoset	*Callithrix jacchus*[†,§]
		Callimico	*Callimico goeldi*[*,‡,§,¶]
		Capuchin monkey	*Sapajus flavius*[†,‡]
	Cebidae	Capuchin monkey	*Sapajus libidinosus*[†,‡,§]
		Squirrel monkey	*Saimiri collinsi*[*,‡,§,¶]
		Squirrel monkey	*Saimiri boliviensis*[*,‡,§,¶]
		Squirrel monkey	*Saimiri sciureus*[*,‡,§,¶]
	Cercopithecidae	Rhesus monkey	*Macaca mulatta*[*,†,‡,§,¶]
		Red-tailed monkey	*Cercopithecus ascanius*[*,†,‡]
		Grivet monkey	*Chlorocebus aethiops*[*,†,‡]
		Mangabey	*Lophocebus albigena*[†,‡]
		Mona monkey	*Cercopithecus mona*[†,‡]
		Putty-nosed monkey	*Cercopithecus nictitans*[†,‡]
		Olive Baboon	*Papio anubis choras*[†,‡]
		Patas	*Erythrocebus patas*[†,‡]
		Pigtail macaque	*Macaca nemestrina*[*,‡,§,¶]
		Cynomolgus macaques	*Macaca fascicularis*[*,¶]
	Hominidae	Bornean orangutan	*Pongo pygmaeus*[†,‡]
Proboscidea	Elephantidae	Elephant	Not mentioned[†,‡]
Rodentia	Caviidae	Guinea pig	*Cavia* sp.[*,NA]
	Cricetidae	Cotton-rat	*Sigmodon hispidus*[*,NA]
	Muridae	Swiss albino mouse	*Mus musculus*[*,NA]
		Abyssinian grass rat	*Arvicanthis abyssinicus*[†,‡]
		African grass rat	*Arvicanthis niloticus*[†,‡]
		Kaiser's rock rat	*Aethomys kaiseri*[†,‡]
		Indian gerbil	*Tatera indica*[†,‡]
		Indian desert jird	*Meriones hurrianae*[†,‡]
		Sind rice rat	*Bandicota bengalensis*[†,‡]
	Soricidae	African giant shrew	*Crocidura occidentalis*[†,‡]
Squamata	Lamprophiidae	Brown house snake	*Boaedon fuliginosus*[†,‡]
	Varanidae	Water monitor	*Varanus niloticus*[†,‡]

*Experimental infection; †natural infection; ‡serologic diagnostic; §molecular diagnostic; ¶virus isolation diagnostic; *NA*, not mentioned.

Data from Bueno MG, Martinez N, Abdalla L, et al: Animals in the Zika virus life cycle: what to expect from megadiverse Latin American countries. *PLoS Negl Trop Dis* 10, 2016; Haddow AD, Schuh AJ, Yasuda CY, et al: Genetic characterization of Zika virus strains: geographic expansion of the Asian lineage. *PLoS Negl Trop Dis* 6:e1477, 2012; and Vanchiere JA, Ruiz JC, Brady AG et al: Experimental Zika Virus Infection of Neotropical Primates. *Am J Trop Med Hyg* 98:173–177, 2017.

Few studies have focused on the role of animals as hosts for ZIKV.[16,24] In 1956, for the first time, Boorman and Porterfield were able to confirm the transmission of ZIKV from *A. aegypti* to animals, especially mice and monkeys during experimental studies.[28] Multiple monkey species in the Zika Forest were found to be seropositive for ZIKV, suggesting that they may become infected and support viral replication.[26] However, other mammals in the Zika Forest (including squirrels, tree rats, giant pouched rats, and civets) did not show serologic evidence of ZIKV infection,[19] although a subsequent study in Kenya detected ZIKV antibodies in small mammals including rats and shrews.[29] A study by Bueno and collaborators (2016) presented wildlife species that were antibody-positive to ZIKV, both in situ and experimentally. Most primates identified as ZIKV-positive in the wild or in sentinel studies are Old World species.[24] To date, there has not been solid evidence of an Asian or American sylvatic cycle of ZIKV, but such a sylvatic cycle could be widespread and still go undetected due to the lack of surveillance for sylvatic arboviruses.[9,24] The presence of animals seropositive for ZIKV does not necessarily mean that they are viremic or may be able to transmit the virus to a mosquito (as a reservoir). Further studies are required to properly understand the role of vertebrates in the ZIKV dynamic transmission.[9,18,24] Recently ZIKV has been of significant concern for conservationists, and there is a lack of studies in wildlife species.[1,13,30] Free-living and semicaptive orangutans *(Pongo spp.)* were evaluated during translocations from forest fragments or degraded habitat in eastern Sabah (Malaysia) in 2003 and were found to have anti-ZIKV antibodies.[30] But how this virus could be pathogenic for wildlife that already suffer anthropogenic and natural pressure remains unknown. Is the ZIKV capable of leading to NHP population declines, as has occurred for howler monkey *(Alouatta spp.)* due to yellow fever outbreaks?[9,18,24] The role of neotropical primates in the maintenance cycle of ZIKV is still unclear.[31] Could other vectors—such as *Haemagogus* sp. or *Sabethes* sp.—serve as bridges facilitating the reverse spillover and establishment of an enzootic ZIKV transmission cycle in the Americas?[9,24,31] Based on the huge biodiversity in the Americas, the proximity of wild vertebrate species to urban and rural areas, and the wide distribution of *A. aegypti* and other mosquito genera, ZIKV spillover to wild primates or another wildlife vertebrate is a potentially real scenario.[9,18,24]

In northeastern Brazil, studies showed that 29% (7/24) of free-living and asymptomatic New World primates, *Callithrix jacchus* and *Sapajus libidinosus*, were infected with ZIKV.[31] Using real-time reverse-transcriptase polymerase chain reaction (qRT-PCR), they detected the virus and showed that the ZIKV genome sequence from monkeys was 100% similar to the ZIKV circulating in humans in South America.[31] To our knowledge, no other neotropical species were detected with ZIKV in natural conditions. However, Oliveira-Filho et al. (2018) found the presence of neutralizing antibodies for ZIKV in *S. libidinosus* (1/49) and *S. flavius* (1/49) in natural conditions in Brazil.

In response to the ongoing epidemic, new studies on ZIKV have been carried out in model animals, mainly to address research and development needs for novel methods of diagnosis, vaccination, and therapy for human populations. Nevertheless, research is still greatly needed to better understand the risk of ZIKV for wildlife.

Pathogenesis of Zika Virus

Mosquito-borne flaviviruses are thought to replicate initially in dendritic cells near the site of the infectious bite and then to spread quickly to lymph nodes, where they replicate and thereafter reach the bloodstream.[26] In vitro, ZIKV has been shown to infect fibroblasts and keratinocytes.[26] Despite recent progress in our understanding, the pathogenesis of ZIKV in vivo remains largely unknown.[26] Similarly, ZIKV distribution in anatomic tissues, the duration of infectious shedding, and the immune response to primary ZIKV in humans and other animals remain unclear.[21]

Several species of immunocompromised mice[29] and NHPs, such as rhesus, pigtail *(Macaca nemestrina)*, and cynomolgus *(Macaca fascicularis)*, were used for studies of the natural behavior and pathogenesis of ZIKV infection.[5,12,21,26] Studies also have been conducted to better understand the serologic behavior and pathogenesis caused by the Asian lineage in neotropical primates *(Callimico goeldi, Saimiri collinsi, Saimiri boliviensis boliviensis, Saimiri sciureus sciureus* and *Aotus nancymaae)*.[32–36] These studies evaluated the experimental infection by ZIKV, the inoculation routes, and the viral load in the viremic period in an effort to evaluate these NHPs as ZIKV study models.[32–36] All assays of rhesus and cynomolgus monkeys have shown to be permissive for infection by the Asian strain of ZIKV. Virus RNA was detected in several body fluids, and it was possible to detect different concentrations of this nucleic acid in different body fluids such as plasma, urine, saliva, cerebrospinal fluid, semen, seminal fluids, and vaginal fluids 1–10 days postinfection (DPI).[5,12,25,26] Cynomolgus monkeys presented higher viral loads of ZIKV in testis 8 DPI. After 2–3 weeks of infection, neutralizing antibodies to ZIKV were detected in most animals.[5,12,26] Cynomolgus monkeys were also challenged with the African lineage, but they were not permissive.[5] Dudley et al. (2016) and Osuna et al. (2016) reinfected rhesus monkeys and did not observe viral replication, which could mean that the monkeys may acquire immune protection after the first ZIKV exposure.

According to Vanchiere et al. (2017), viremia and viruria were detected in two *(Saimiri boliviensis boliviensis, Saimiri sciureus sciureus)* of the four total animals by RT-qPCR. Peak plasma viremia occurred between 5–10 DPI as extrapolated from qPCR. Viruria was detected at 9 and 14 dpi; salivary ZIKV secretion was detected in three animals as late as 14 dpi.

Two of the four owl monkeys *(Aotus nancymaae)* had ZIKV viremia at 2 and 4 dpi by PCR. However, ZIKV was

not detected in urine, salivary secretions, or excretions from owl monkeys by culture or qPCR assays.[36]

Clinical Manifestations

As occurrence of ZIKV infections in wildlife was mainly found accidentally during serosurveys searching for other pathogens, there is almost no information on clinical signs in wild animals.[35] In a sentinel study in Uganda in 1947, one primate showed mild pyrexia.[12] Typically ZIKV infection in humans has been associated with a self-limiting febrile illness often including rash, arthralgia, and conjunctivitis, although most infections are asymptomatic.[13,29] Wild mammals with ZIKV infection apparently have few clinical signs.[24]

A study by Dudley et al. (2016) in rhesus macaques infected with ZIKV showed mild to moderate inappetence resulting in mild weight loss in four animals. Two animals also developed a very mild rash around the inoculation site at 1 DPI that persisted for 4–5 days. No other abnormal clinical signs were noted. Two other studies in rhesus macaques demonstrated that within 8–10 DPI animals displayed fever (axillary temperature 38.9°C), with peak temperatures of 40.1°C[23] and 39.5°C.[21] All experiments used almost the same infectious dose of ZIKV (approximately 10^5 plaque-forming unit [PFU]) but from different strains, which may explain the difference in results.

One of the six cynomolgus monkeys that had been challenged with the ZIKV African lineage developed a slight erythema around the injection site (Draize dermal score of 1 on study days 8 and 10 DPI). The erythema had resolved by day 14. No clinical signs were observed in any of the other animals during the course of the study.[5] As this individual did not present viremia of the ZIKV African lineage, the erythema was probably not related with the virus.

None of the neotropical primates infected by the Vanchiere et al. (2017) exhibited signs of clinical disease after ZIKV inoculation.

No evidence of neurologic complications, such as the Guillain-Barré syndrome observed in human adults, was found in the experiments with monkeys. However, congenital birth defects were demonstrated in one experiment.[33] Ten days after inoculation with a ZIKV Asian lineage strain in a pregnant pigtail macaque at 119 days of gestation, the fetal brain developed a periventricular lesion and evolved asymmetrically in the occipitoparietal lobes. However, in this experiment, five subcutaneous infections in the pregnant animal were initiated using 10^7 PFU each.[33] This amount of ZIKV inoculated is higher than that used in the other experiments and may not represent natural infection.

Complete Blood Count and Blood Chemistry Panels

Blood chemistry was monitored in three ZIKV infection assays.[21,25,26] According to the observations made by the investigators, aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase, and creatinine phosphatase increased and peaked by day 3 and day 21.[21,25,26] Creatine kinase increases may be due to viral myositis or repeated sedation hemolysis and endocrine abnormalities.[25]

The pattern of white blood cell counts remained within normal variation ranges,[25,26] although increases were found by Osuna et al. (2016). However, the counts returned to baseline in most animals within 2 days of infection.[21]

All neotropical primates infected by Vanchiere et al. (2017) remained clinically well after inoculation. The routine hematology and serum chemistry analyses were normal at 4 dpi, with the exception of a modest increase in ALT over baseline for one animal.[36]

Zika Virus Targets Various Organs and Causes Pathologic Damage

Necropsy data from rhesus and cynomolgus macaques demonstrate that ZIKV strains may invade and replicate within the CNS (including cerebrum, cerebellum, brain stem, and spinal cord) of macaques following subcutaneous infection despite the fact that no neurologic signs are observed.[21,26] ZIKV RNA has also been detected in visceral fragments (e.g., liver, kidney, spleen, parotid glands, large intestine, small intestine, cecum, bladder, testes, lymph node, heart, and stomach) in rhesus macaques. Male genital tracts were identified with persistent foci of ZIKV-infected cells localized in the testes, prostate, and seminal vesicles in the two species studied.[21,37] This observation may have negative implications for conservation programs if it is demonstrated that ZIKV can be transmitted sexually.

Collecting Biological Materials to Investigate Zika Virus in Wildlife

Once collected, blood samples should be divided into two aliquots. A whole-blood sample is immediately stored in liquid nitrogen or dry ice; the other is maintained at 4°C for 3 hours until it is centrifuged for the collection of serum, which is then placed in liquid nitrogen. Samples should be sent on dry ice to the diagnostic reference center. A minimum of 0.5 mL of whole blood is required to perform molecular assays for the detection of virus and 0.5 mL of serum is the minimum necessary to conduct antibody screening. For the detection and isolation of ZIKV, whole blood should be collected in the viremic period (1–5 DPI); it may be detected by qRT-PCR up to 14 DPI depending on the inoculated viral load. After 7 DPI, antibody testing is prioritized. Molecular detection in other fluids such as urine, saliva, and tears and also tissues such as CNS and placenta occurs well during the viremic period and should be stored in liquid nitrogen and transported on dry ice. Tissues for histopathologic processing should be stored in 10% formaldehyde and sent at room temperature.[38]

Diagnosis

Demonstration of ZIKV infections in wildlife may be difficult because of the cross-reactivity of diagnostic flavivirus antibody assays. One of the current tests most frequently performed is the hemagglutination inhibition (HI) test, which has high sensitivity but low specificity among arboviruses belonging to the same genus.[10,39] This test detects both IgM and IgG as well as total antibodies. All studies should be repeated after 6 months in the same animal to evaluate the variation of antibody titers.[40] Another test to detect specific antibodies against ZIKV in samples is the enzyme-linked immunosorbent assay (ELISA) to detect IgM antibodies. If IgM is positive, it suggests that the animal was exposed to the virus in the previous 3 months.[10,30] The plaque reduction neutralization test (PRNT) generally has better specificity than immunoassays but may still yield cross-reactive results to other flavivirus infections. All serologic tests may be conducted on samples obtained after 7–10 days of clinical signs.[13] Rapid tests, although available for humans, are not yet available for NHPs or other animals. More studies to discover alternative diagnostic tests in the field or in settings of poor laboratory conditions need to be performed.

Molecular tests for ZIKV detection are the best option for detecting ZIKV RNA. This includes qRT-PCR tests on acute-phase serum samples, tissues, or whole blood. PCR tests can be conducted on samples obtained less than 7 days after disease onset.[13]

The inoculation of acute-phase serum samples, tissues, or whole blood in the permissive cells lineage Vero or C636 cells has been shown efficient at ZIKV isolation. This technique may be conducted on samples obtained less than 7 days after disease onset.[13]

The use of techniques for the detection of viral antigens by immunohistochemistry and observation of morphological changes by histopathological technique (Hematoxylin Eosin- HE) are other important tools for the elucidation of ZIKV infection. In both methods, the CNS, liver and reproductive tissues should be collected.[21,33,34]

Necropsy

Children and human fetuses infected during gestation period demonstrated that ZIKV is a neurotropic pathogen, similar to West Nile, St. Louis, and other encephalitic Flaviviridae.[41,42] Computed tomography scans and transfontanellar cranial ultrasound have shown a consistent pattern of widespread brain calcification, mainly in the periventricular, parenchymal, and thalamic areas and in the basal ganglia. In addition, lissencephaly, hydrocephalus, periventricular necrosis, and diffuse astrogliosis with activated microglia have also been found.[41–43]

Experimental assays in NHPs—Old and New World primates—have demonstrated injuries similar to those found in humans.[21,26,32,33]

Histopathologic processing in rhesus and cynomologus macaque samples allows for the observation of hepatic and CNS lesions as long as 23 weeks after infection, and immunohistochemistry can detect viral antigens in various tissues.[21] Because ZIKV is neurotropic, the CNS should be targeted during necropsy. In pregnant primate females, the placenta and fetus should be the main tissues to be evaluated.

A necropsy of a pigtail macaque fetus infected in utero revealed ZIKV in the brain and significant cerebral white matter hypoplasia, periventricular white matter gliosis, and axonal and ependymal injury.[37]

In the 17 neotropical primates *Saimiri collinsi* infected with 10^5 PFU/50 µL ZIKV Asian Lineage, the necropsy of the fetuses demonstrated gross evidence of a congested brain with dilation of the 4th cerebral ventricle (Fig. 41.1A). Microscopically, the frontal cortex had significant gliosis, satelitosis, neurophagocitosis congestion and Minimal infiltration inflammatory cells was found (Fig. 41.1B). The presence of immunohistochemical marking on neurons and glia cells indicates antigen challenge anti-ZIKV antibody (Fig 41.1C and D).[33,34] These findings suggest the fetuses were susceptible to congenital infection followed by maternal subcutaneous infection.

Treatment, Prevention, and Control

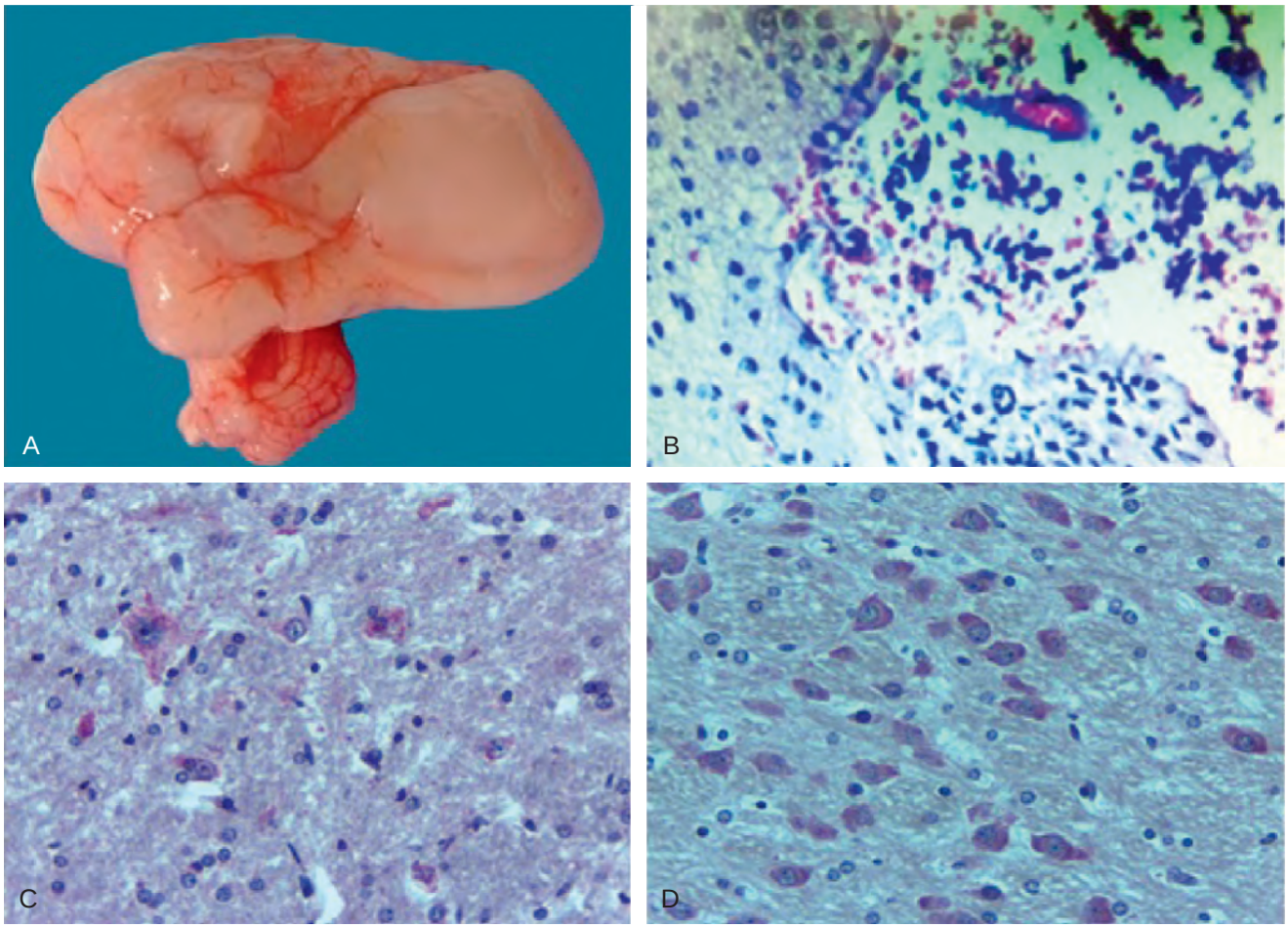
Currently no vaccines or specific antiviral drugs are available to prevent or treat ZIKV infection in humans or other animals, including wildlife. Public health policy efforts have mainly focused on vector control and personal protection measures. Due to the high number of wild species with the potential to establish a sylvatic cycle, it would be extremely difficult, even impossible, to control ZIKV if competent hosts were present in the wild to maintain infection.[9,24]

For monkeys living in zoo collections or other captive/semicaptive settings, vector and virus exposure may be prevented by fitting screens to doors and windows, removing debris that provide breeding sites for mosquitoes (i.e., freestanding water in discarded tires, broken bowls, flower vases), and avoiding the placement of vegetation or yards around the animals' enclosures.[22]

Final Considerations: Wildlife as Sentinels and Public Health—A Conservation Medicine Approach

Infectious diseases have important implications for animal and human health as well as biodiversity. Monkeys may be considered sentinels for pathogens of human health concern and seem to be the major potential wildlife reservoir for ZIKV.[19,24,30,31,35,44]

Given its recent history of rapid spread in human populations, it is anticipated that ZIKV will continue to spread for the foreseeable future in the Americas and globally in regions where competent *Aedes* mosquito vectors are present.[20] Added to this, the risk of ZIKV introduction in a new sylvatic environment—such as tropical rainforests—may establish permanent viral reservoirs in an enzootic cycle, thus increasing the risk of constant outbreaks in the



• **Figure 41.1** (A) Fetal necropsy in *Saimiri collinsi*; (B) *Brain infected with Zika virus, showing lisencephaly and dilation of the 4th cerebral ventricle*; (C and D) Frontal cortical area with vasocongestion, focal hemorrage with gliosis, satelitosis and neuroniophagia, besides of immunostaining in neurons and glial cells. (Alcantara BN personal file). (Images courtesy Bianca Alcantara.)

newly affected areas, similar to the sylvatic cycle of yellow fever in South America.[9,24]

Finally, the following research priorities are recommended[9,24] to better understand the dynamics of ZIKV in wildlife: (1) laboratory studies of host competence of wild species, especially NHPs and vector competence for ZIKV; (2) serosurveys to detect spillover and spillback of ZIKV into captive populations of Old World primate species; (3) ecologic long-term monitoring to detect a potential sylvatic circulation of ZIKV in Asia and the Americas; (4) development of rapid diagnostic tests for screening ZIKV and other arboviruses; (5) incorporation of species presence records and environmental changes into distribution maps to assess the overlap of host and vector species with potential for sylvatic ZIKV maintenance; and (6) active surveillance of human populations living close to natural ecosystems.

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42

An Overview of Middle East Respiratory Syndrome in the Middle East

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Introduction

Middle East respiratory syndrome (MERS) is an emerging infectious zoonotic disease caused by a novel coronavirus (CoV). MERS was first reported in 2012 in Jeddah, Kingdom of Saudi Arabia (KSA), and in Jordan, respectively.[1] The disease was considered a potential pandemic threat to public health in the Persian Gulf region.[2] (See also Chapter 19.)

Most known CoVs infect and circulate in animals, mainly bats, but a number of CoVs are known to cause human disease (see also Chapter 40).[3–5] The rapid emergence of MERS-CoV coupled with its limited geographic distribution has led to the suspicion that this is a zoonotic disease with an animal reservoir,[4] and the evidence supports the hypothesis that dromedary camels (DCs) are the reservoir host. In DCs MERS-CoV causes a mild, transient upper respiratory tract (URT) infection.[4–6]

A mild or asymptomatic disease has also been reported in humans, but this is not always the case. MERS-CoV infection in humans often results in a severe, life-threatening disease of the lower respiratory tract (LRT), with high mortality.[1,2,5] Immunocompromised, elderly people and those with comorbidities, usually with a history of close contact with infected DCs, are particularly susceptible.[7,8] In such cases the disease progresses rapidly, resulting in multiorgan failure and acute respiratory distress syndrome. Between 2012 and April 7, 2017, a total of 1936 confirmed human cases were reported to the World Health Organization (WHO), resulting in 690 fatalities (crude case fatality rate of 36%; see Fig. 42.1).[1,9] Potential clinical cases of MERS in DCs must be reported to the World Organisation for Animal Health (OIE) as an emerging infectious disease.[10]

Virology

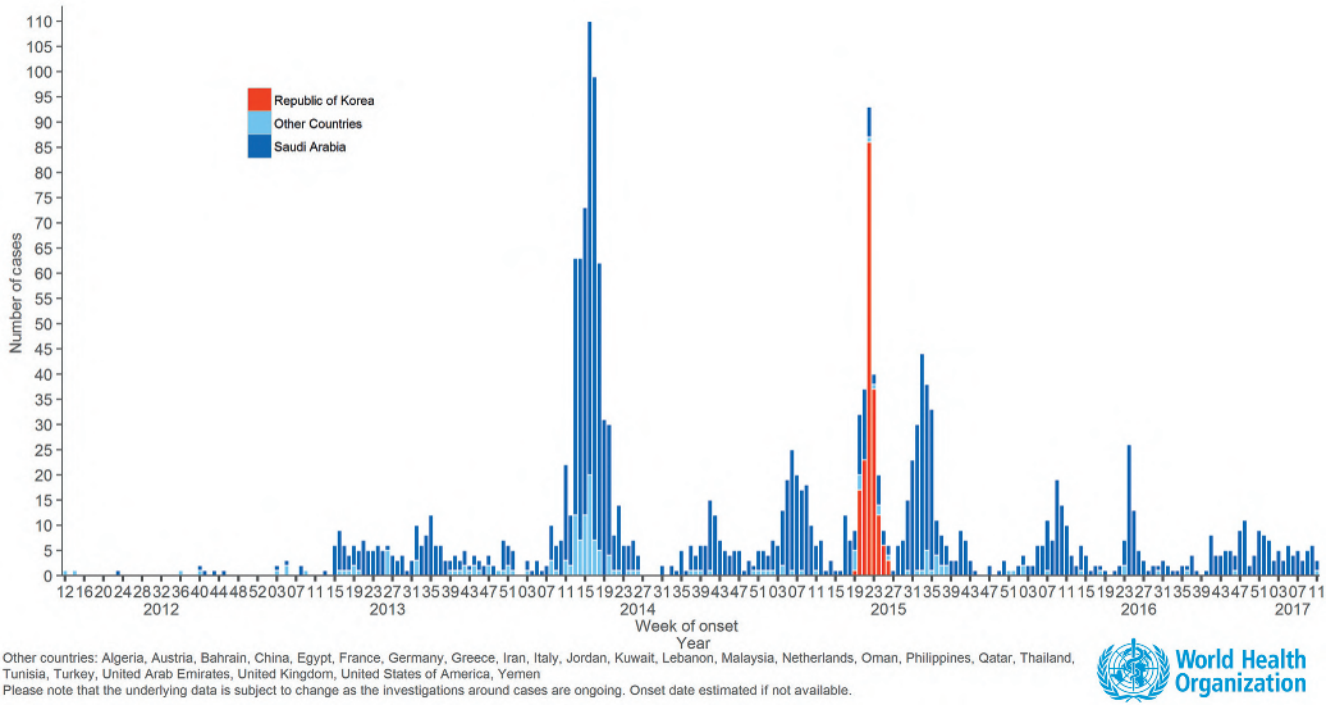
MERS-CoV is a member of the subfamily Coronavirinae, genus *Betacoronavirus*, subgroup lineage 2c.[11] MERS-CoV is a positive-sense enveloped single-stranded RNA virus and is the first lineage of 2c *Betacoronavirus* known to infect humans.[2,8] It is more closely related to bat CoVs HKU4 and HKU5 (lineage 2c) than to the severe acute respiratory syndrome CoV (SARS-CoV, (lineage 2b).[2,3] Recent genome sequencing analysis reported the genomic evolution rate (1.12×10^3 substitutions per site), suggesting that MERS-CoV diverged from its viral ancestor in March 2012.[12]

Analysis of human MERS-CoV sequences has identified several circulating genotypes. These distinct genotypes are phylogenetically classified into clades A, B, and, most recently, C, which correlate with outbreaks of MERS among humans.[4,5,8,12] The emergence of divergent MERS-CoV clades in humans since 2012 is consistent with several independent sporadic introductions into the human population from an animal reservoir, of which the camel was unquestionably the source.[6,8,12,13]

Pathogenesis

Host cell entry of MERS-CoV is mediated by the binding of MERS spike (S) proteins to a specific cellular receptor known as dipeptidyl peptidase 4 (DPP4).[11] DPP4 is expressed on the epithelial and endothelial cells of most human organ tissues in ex vivo studies using human tissue culture lines; this may account for the multisystem clinical spectrum of the MERS-CoV infection.[2,14]

A strain cultured from a fatal human case was experimentally inoculated into three DCs using intratracheal, intranasal, and conjunctival routes.[15] A mild transient disease resulted in submucosal inflammation and necrosis in the URT and LRT, but the alveoli remained unaffected.[15] Experimental inoculation of rhesus macaques *(Macaca mulatta)* and common marmosets *(Callithrix jacchus)* resulted in mild to severe LRT disease causing multifocal interstitial pneumonia in the macaques and extensive fatal pneumonia in the marmosets.[2,14,16]



• **Figure 42.1** Epicurve: global confirmed cases of Middle East respiratory syndrome–coronavirus (MERS-CoV), reported to the World Health Organization as of April 7, 2017 (*n* = 1936). (Reprinted from WHO Emergencies: MERS-CoV. <http://www.who.int/emergencies/mers-cov/en/>.)

Epidemiology

MERS-CoV belongs to a lineage commonly associated with bats, the closest relatives of which lineage were recently identified in Vesper bats (i.e., various species of the family Vespertilionidae) from Europe, Asia, and South Africa. Initial research efforts have focused on establishing an epidemiologic link between bats and humans.[3,4,6,17] There is no conclusive evidence to support the theory that bats are the source of human infection, although there is consensus that bats are the ancestral hosts of the disease.[4,5,17] A related MERS-like CoV virus, isolated from an African pipistrelle bat *(Pipistrellus hesperidus)* in Uganda, has shown high divergence of the S protein nucleotide sequence compared with an index MERS-CoV S protein sequence (46% amino acid identity divergence).[17] This suggests that the two viruses differ significantly in receptor binding properties, implying that the MERS-like CoV virus is not a zoonotic threat and supporting the theory of a common ancestry.[17]

To date the only direct link between bats and human disease was a single instance when an RNA sequence from a fatal human MERS index case showed a 100% nucleotide match of a polymerase chain reaction (PCR)–amplified sequence of a fecal pellet from an Egyptian tomb bat *(Taphozous perforates)* collected in the same area of Bisha, KSA.[18] The human fatality was an owner of four DCs, which also tested positive for the same strain of MERS-CoV.[18] At present, bat-to-human infection by MERS-CoV is considered to be purely speculative.[4]

Surveillance of DCs in KSA has shown that MERS-CoV clade B has been enzootic in the camel population in Arabia since at least 1992.[13] Several MERS-CoV serologic surveys confirm that the disease is not present in domesticated livestock (namely, horses, sheep, goats, and cattle) and is enzootic in the DC population across the Arabian Peninsula as well as in North and East Africa.[6,7,13,15,19–23] Historically, the camel was the mainstay of land-based trade transportation and was used extensively as a food source across the entire region prior to industrialization during the latter part of the 20th century.[5,10] The free movement of humans and animals across the region supports the widespread prevalence and genetic diversity of MERS-CoV in the DC populations of Arabia and East Africa today.[5,10]

The temporal dynamics of MERS infection in DCs in Al-Ahsa, KSA, was examined by collecting nasal swabs and lung tissue during postmortem examination from two independent groups of animals over the course of a year and testing these for MERS-CoV RNA by real-time reverse-transcriptase polymerase chain reaction (RT-rtPCR).[24] Positive samples were typically associated with young immunologically naive animals (<4 years of age) rather than adults (>4 years of age). Seasonal peaks were detected during the winter months and coincided with the calving season, less extreme environmental conditions, cooler ambient temperatures, and higher relative humidity, for the transmission of infection amongst susceptible individuals.[24] This seasonal peak has also been described in epidemic nosocomial outbreaks in humans that occur more frequently during the winter months.[25,26]

Extensive virologic evidence has been accumulated since 2012 supporting the epidemiologic link between DCs and humans in the transmission of MERS-CoV, although

strategic serosurveys of humans using samples collected after 2012 have been infrequent.[4,5,10] There is a paucity of baseline data to describe the proportion of the potentially infected human population for much of the Arabian Peninsula and all of East Africa, including the Horn of Africa.[10]

Transmission

The exact mechanism of transmission from camels to human remains uncertain.[10] Sustained close contact is most probably necessary for transmission by aerosolized droplets, as MERS-CoV viral RNA has been detected in air samples from a barn housing infected DCs in Qatar, and the virus may remain viable in aerosol for up to 45 minutes.[10,27–29] The potential public health risk resulting from aerosol-generating activities ranges from contamination of a room occupied by a symptomatic patient to slaughter practices.[8,24,30]

Aerosolized transmission of MERS-CoV has been attributed to hospital outbreaks in KSA and South Korea.[26,27,29] MERS-CoV spreads inefficiently from human to human, but transmission is effective in a hospital environment, where susceptible individuals are concentrated and the risks are amplified by poor infection prevention and control (IPC) protocols.[8]

In some reported cases of MERS, direct contact with camels was not apparent.[27,29] Camel-to-human transmission through other routes is, however, possible owing to the consumption of unpasteurized camel milk or raw camel meat and in traditional medicine, when camel urine is consumed as a natural remedy for a variety of ailments.[3,10] A recent survey has found that infected camels may shed MERS-CoV virus in milk and urine, and the virus has been shown to remain infectious for 3 days in milk stored at 4°C.[3,10] The transmission risks associated with the handling of camel products, raw milk, urine, and meat during animal slaughter are yet to be fully elucidated. Further studies are needed to demonstrate the potential of camel-to-human transmission.[8]

Diagnosis

Serologic methods with high sensitivity and specificity to detect MERS-CoV antibodies have been developed for use in seroepidemiologic studies. Methods include indirect immunofluorescence assays, enzyme-linked immunosorbent assays (ELISAs), protein microarray technology, and microneutralization (MN) assays.[13,20–22] Pseudoparticle virus neutralization tests (ppNTs) and conventional MN assays have also been used to detect neutralizing antibodies to MERS-CoV.[18]

Validated molecular assays have been developed.[10,13,19] RT-rtPCR is the preferred diagnostic method for the detection of MERS-CoV and has been endorsed by the WHO.[1] Confirmation of MERS in suspected cases requires the screening of samples targeting a number of genes specific to MERS-CoV, namely Up E, ORF 1a, ORF 1b, and N genes.[1,10,13,19]

Genetic deep sequencing methods (i.e., high-throughput sequencing) have been readily available to researchers since the disease was first reported.[12] Sequenced data have been used in these cases to successfully construct the phylogenetic tree between related viruses and hosts.[2,6,7,12]

Direct MERS-CoV antigen detection is possible but has been rarely performed.[10] Immunochromatography assays and monoclonal antibody-based capture ELISAs targeting the MERS-CoV nucleocapsid protein have been described.[20]

Since the virus was first reported in 2012, a range of comprehensive laboratory tests has been developed.[10,30] To better understand the disease, it has been important to collate sampling methodology data, laboratory results, and analyses in combination with clinical and epidemiologic data.[10] Until laboratory assays are fully validated, a combination of molecular and serologic laboratory tests is required to improve confidence in laboratory diagnosis during outbreaks.[30] In cases of mild or asymptomatic infection, full validation of serologic assays is required to rule out false-negative results.[31] Validation is also required to successfully apply newly developed diagnostic serology algorithms to inform public health decisions.[10,30,31]

Treatment

Therapeutic options for MERS-CoV are limited. Supportive treatment is indicated for hospitalized patients, but vigilance for complications is essential.[8] Empirical use of antimicrobial agents or steroids has not succeeded in reversing the progression of severe disease.[8,27,29] No specific drug or vaccine is currently available to treat MERS. Indeed, it has been stated that the complexity and time required for the development and registration process of drugs for human use impedes the ability to counter the rapid threat against an emerging infectious agent.[8] For example, there is no vaccine available against SARS-CoV because of the brevity of the threat to the public health.[2,8] It is likely that a MERS-CoV vaccine for human use may not be developed due to a lack of commercial interest, or if the threat posed by MERS-CoV declines in the meantime.[8]

Nevertheless, given the prevalence of MERS-CoV infection in the Middle East's DC population and due to the potential for spillover to the human population in direct contact with DCs, the development of a vaccine for use in DCs may be feasible.[4,5,32] A recent successful trial of a MERS orthopoxvirus vaccine has conferred mucosal immunity in the URTs of DCs.[32] Eradication of MERS-CoV from herds may be possible, if vaccines are administered to young, immunologically naive camels prior to exposure.[4,5,32]

Control and Prevention

Identification of the zoonotic source of MERS guides control strategies at the human-animal interface.[3,30] By preventing spillover of MERS-CoV from animals to humans, the risk of nosocomial and familial outbreaks in the Middle East could be eliminated.[3]

At present the implementation of intensive IPC measures in human health care is vital, including improving education and awareness among healthcare workers.[1,8] Most human cases have been linked to lapses in IPC, as one-fifth of viral infections have been reported among healthcare workers.[1,8] Stringent precautions while handling suspected MERS-CoV patients include the use of personal protective equipment (PPE) (i.e., disposable gloves, gowns, respiratory protection, and eye protection).[2,8,33] Immunocompromised individuals and those with preexisting medical conditions should avoid close contact with DCs.[2,8]

Public health authorities should adopt a standardized public health response protocol to include standardized case reporting methodology as defined by the WHO.[1,30] Standardization of case definitions aids accurate calculation of a case fatality ratio by including mild or asymptomatic cases.[30] The Health Authority of Abu Dhabi in the United Arab Emirates recently implemented a standardized reporting option for MERS, successfully incorporating it into existing epidemiologic surveillance systems with the aim of enhancing surveillance, educating healthcare workers, and ensuring laboratory capacity.[25]

In countries where MERS-CoV is enzootic in DCs, MERS control at the animal-human interface is unlikely to succeed unless appropriate preventive strategies are implemented.[5] These should include the following:

- Strict regulation of camel movement with imposition of a requirement for MERS clearance prior to the importation and transport of camels, including animals presented for slaughter.
- Camels with detectable MERS-CoV RNA should be quarantined and tested at regular intervals.
- Use of appropriate PPEs while handling DCs.
- Increased awareness among camel owners and the general public of the risks of consuming unpasteurized camel milk and urine. This may prove challenging given the depth of customs and beliefs in some areas.
- Accelerated development of safe and effective MERS vaccines for animal and human use.[5,33]

Conclusions

MERS-CoV has been observed for only 4 years, and vigilance is vital for the containment of the disease due to the high case fatality rate in humans and possible genetic instability of the virus.[8] Continued laboratory testing, genetic sequencing, analysis, timely data sharing, and clear communication are essential if such vigilance is to be effective.[8] Nonetheless, despite the potential for a pandemic outbreak at multiple mass gatherings during the Islamic calendar (Hajj, Eid, and Umrah) there were no reported outbreaks of MERS during or immediately after these events.[10] As such MERS-CoV is not a virus of pandemic concern.[10]

Since 2012 our understanding of MERS has increased greatly although gaps in knowledge still exist. The understanding of the disease's ecology—especially the interplay between camels, humans, and the environment—is still in its infancy. Aside from bats, the role that other wildlife may play in the ecology of MERS-CoV in East Africa and Arabia is yet to be elucidated.

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Disease Risk to Endemic Animals From Introduced Species on Madagascar

FIDISOA RASAMBAINARIVO AND STEVEN M. GOODMAN

Introduction

In different areas of our planet, human activities such as deforestation and hunting are threatening the survival of many wildlife species. Additionally, accidental or intentional introduction of different organisms is exerting additional pressures on native animal species, and in certain cases the former becomes invasive.[1] In natural habitats, introduced animals have been shown to reduce or eliminate populations of endemic species through different mechanisms, such as direct predation or the reduction of available resources.[2] This increase in native-exotic animal interactions also presents a potential for "pathogen pollution," or the introduction of a pathogen into a new geographic area or host species.[3] Invaders may also act as an additional competent host for native pathogens, thereby increasing infection rates of native species via spillback mechanisms.[4] Additionally, invader-borne pathogens may have more subtle and persistent effects and alter the outcomes of trophic or competitive interactions.[5] In fact, a shared pathogen can have considerable impact on the population size and the distribution of native species, even if the introduced species does not compete directly with the native species for resources; this is called "apparent competition" and is illustrated by the success of the invasive gray squirrel *(Sciurus carolinensis)* and the decline of the native red squirrel *(S. vulgaris)* in the United Kingdom—the result of apparent competition mediated by a parapox virus.[6,7]

Species inhabiting island ecosystems tend to be more heavily affected by invasive species because they lack appropriate behavioral traits or immunologic capacity, making them particularly vulnerable to the threat of introduced species and/or their associated pathogens.[8,9] The high rate of bird extinction on Hawaii due to the introduction of arthropod vectors and the transmission of avian malaria and avipoxvirus are cases in point.[10,11]

Madagascar is a well-known biodiversity hot spot with approximately 90% of plant and animal species being endemic, including all nonhuman primates (superfamily: Lemuroidea), all native rodents (subfamily Nesomyinae), a radiation of insectivore-like animals (family Tenrecidae), and 9 of its 10 wild carnivorans (family Eupleridae).[12] Through a series of colonization events, probably during the Tertiary Period via some sort of over-water rafting, the ancestor for each of the four groups of terrestrial mammals occurring on the island (primates, rodents, insectivores, and carnivorans) established initial populations; their subsequent adaptive radiations produced the high diversity and 100% endemism rate observed today.[13] The diversity of the herpetofauna of Madagascar is even more impressive, with 390 species of nonmarine reptile species described on Madagascar up to 2013.[14] Of these reptile species, more than 92% are considered endemic and almost all of the 400 amphibian species are also endemic.[15,16]

Madagascar's fauna is remarkable not only by the diversity of endemic taxa found on the island but also because of the absence of entire families that are otherwise present in nearby continental Africa. For instance, no member of the families Canidae, Felidae, and Bovidae have naturally crossed the 400-km Mozambique Channel separating mainland Africa from Madagascar. The different dispersal filters and subsequent isolation of the animals that were able to colonize the island successfully may have important implications for the sensitivity of Malagasy animals to diseases carried by exotic species.

Human colonization of the island occurred some 4000 years ago and since then was associated with a series of introductions of domestic and peridomestic animals such as zebu, dogs, cats, rats (*Rattus* spp.), and mice *(Mus musculus)*.[17] These introduced animals are negatively impacting endemic species and may even lead some native species to extinction. For instance, a survey has shown that in some areas of Madagascar, black rats *(Rattus rattus)* now constitute 95%–100% of the rodent population, effectively replacing the endemic rodents, even in rainforest habitats away from human habitation (Dammhahn, unpublished data).[18] Likewise, research on carnivorans inhabiting several regions of Madagascar has shown an alarming correlation between increasing numbers of introduced cats and dogs and decreasing detection of endemic euplerids and lemurs.[19] As

elsewhere in the world, the reason for the observed declines is likely a combination of factors, including resource competition, predation, and disease.[20,21] This chapter attempts to review the literature on pathogen introduction from exotic hosts and highlights the risks of disease introduction on the rich and unique fauna of Madagascar.

Disease Risks From Introduced Amphibians

The Asian common toad *(Duttarphrynus melanosticus)* was first reported in Madagascar in 2014.[22] This invasive alien amphibian species is widely distributed across many environmental types in Asia and may competitively exclude Malagasy amphibians. It has also been hypothesized that these toads might affect Madagascar's native carnivorans through their cardiotoxic toxins.[23]

An additional concern regarding this invasive toad species is the cointroduction of pathogens, particularly Ranavirus and the amphibian chytrid fungus *Batrachochytridium dendrobatidis* (Bd). Both have been associated with major declines in amphibian populations elsewhere in the world.[24,25] Madagascar was previously considered to be one of the last major land masses without this amphibian fungal pathogen.[26] Despite several surveys and long-term research, the presence of the pathogen was not confirmed on Madagascar before 2010.[26,27] More recently, the fungus was detected on several amphibians shipped from Madagascar to the United States.[28] Subsequently, and as a result of coordinated efforts, the pathogen was found in wild frogs in multiple areas across the island, where representatives of all anuran families have tested Bd-positive.[26,28] However, results from field surveys from the same sites are conflicting, and there has been no evidence of mass amphibian mortalities, raising the question of whether the pathogen is firmly established or is recurrently introduced.[29]

In its native range, the Asian common toad is also known to harbor potentially zoonotic pathogens such as *Salmonella*[30]; by extrapolation, its introduction to Madagascar may also become a concern for public health or the native Malagasy fauna. In any case, further research is needed regarding the pathogens that this toad may carry.

Disease Risks From Introduced Birds

Approximately 209 of the 283 bird species found on Madagascar are breeding residents and a few have regular seasonal migration between sub-Saharan Africa and islands in the western Indian Ocean. The number of Palearctic migrants, particularly Passeriformes, passing through Madagascar is very limited compared with continental Africa; this has important implications for the potential transmission of pathogens between birds.[31] In addition, several species have been introduced either intentionally for food production (e.g., poultry and other domestic fowl) or pest control (common myna *[Acridotheres tristis]*) or accidentally (house sparrow *[Passer domesticus]* and house crow *[Corvus splendens]*). These nonnative birds may facilitate the introduction of pathogens and pose different threats to the native Malagasy avifauna, as has been the case for bird populations elsewhere.[10,11] For example, in the Galápagos, it is thought that the introduction of the avipoxvirus and *Haemoproteus* blood pathogens in the 19th century was associated with either pet caged birds or natural songbird migration.[32] Some species of *Haemoproteus* are known to reduce the physical condition and reproductive success of captive and wild birds; on Madagascar, this genus and *Plasmodium* are transmitted by numerous species of mosquitoes living sympatrically with native birds.[33,34] Most studies of bird pathogens on Madagascar focus on domestic fowl, and only a few investigations have documented pathogens infecting the island's native birds.[33,35]

West Nile virus (WNV), a member of the family Flaviviridae, is widely distributed in parts of the Old World. During the summer of 1999, this virus was introduced in the Western Hemisphere presumably by the transport of infected humans, birds, or mosquitoes and spread rapidly, causing morbidity and mortality of birds and mammals in the United States and Canada.[36,37] On Madagascar, evidence of WNV exposure was found in both introduced and native animal species including birds, peridomestic rodents, fruit bats *(Pteropus rufus)*, and several lemur species.[31,38–40] However, the phylogenetics of the WNV strains isolated on Madagascar suggest a local WNV transmission cycle with no new, recent introductions of WNV despite the migration and introduction of several bird and arthropod species on the island.[31,39] The introduction to Madagascar of a foreign strain of WNV might have a dramatic effect on the local fauna.

Similarly, Newcastle disease virus (NDV), a member of the Paramyxoviridae, is distributed throughout the world and infects a wide range of birds. Virulent forms of NDV cause widespread and highly contagious disease in both domestic and wild birds.[41] It is responsible for the high mortality of chickens in rural Madagascar, which in turn causes important economic burdens.[42] In most cases, domestic bird infections on the island implicate genotype XI of NDV, a form related to the variant responsible for the first pandemic of Newcastle disease (genotype IV), whereas wild birds are primarily infected by genotype Ib, although some are also infected by genotype XI.[43,44] These results suggest that genotype XI circulates across Madagascar between wild and domestic birds and may potentially affect either the native or exotic bird populations via spillover and spillback mechanisms.[45]

A recent study has disclosed that certain coronaviruses, specifically of the genus *Gammacoronavirus*, are present in wild Malagasy birds, particularly species living in aquatic habitats.[46] Coronaviruses are found in a variety of animals, in which they can cause respiratory, enteric, and neurologic diseases. This particular genus is associated with infectious bronchitis virus, but birds that tested positive did not appear to be affected.[46] The strains of avian coronaviruses detected

on the island were closely related to viruses found in Russia and Cambodia, prompting questions on the origins of these viruses on Madagascar.[46] The implications of these results with respect to the presence of this virus group in domestic and introduced birds, their potential impact on the health of the native Malagasy avifauna, and the emergence of new coronaviruses are in need of further research.

Disease Risks From Humans

On Madagascar, human activities, most importantly associated with habitat destruction and hunting, are threatening a large proportion of native animal species. In addition, accidental events such as the introduction of pathogens and disease outbreaks may further place native animals at risk. Elsewhere, humans have served as hosts for pathogens that subsequently had a substantial impact on native species.[47] Studies on the island have shown that lemurs inhabiting human-disturbed habitats have compromised health conditions compared with those living in more pristine habitats.[48,49] This may affect lemur populations by reducing their fitness or facilitating the transmission of pathogens from alien species. For example, the human-adapted protozoan *Cryptosporidium hominis* is now found in the eastern rufous mouse lemur *(Microcebus rufus)* and the greater bamboo lemur *(Prolemur simus)* living in Ranomafana National Park, as well as the ring-tailed lemur *(Lemur catta)* in southwestern Madagascar.[50,51] This zoonotic protozoan has a high prevalence rate in people, domestic animals, and peridomestic rodents inhabiting villages in the vicinity of protected areas; the parasite was most likely transmitted to lemurs as a result of increased exposure to humans.[52] In captive lemurs, *Cryptosporidium* sp. has caused high morbidity and mortality.[53]

Similarly, lemurs inhabiting anthropogenically disturbed habitats are more likely to be infected with potentially pathogenic Enterobacteraceae, which are commonly found in humans, livestock, and peridomestic rodents; such pathogens include enterotoxinogenic *Escherichia coli*, *Shigella*, *Salmonella*, *Yersinia*, and *Vibrio cholerae*, all major causes of diarrhea and mortality in captive lemurs.[54,55]

Disease Risks From Exotic Carnivorans

Interactions between exotic and endemic animals may have important negative impacts on the latter, including disease transmission. In rural areas of Madagascar, dogs and cats are free-roaming within and outside of villages and occupy areas used by wild and endemic carnivorans as well as primates.[19] This contact may potentially facilitate the transmission of diseases such as rabies and canine distemper. For example, the rabies virus has circulated on Madagascar since the 19th century, and dogs constitute the main source of rabies arising in humans and other animals on the island. Reports of rabies virus (RABV) in wild animals from Madagascar are rare, but the strain of lyssavirus isolated from a case involving the euplerid fossa *(Cryptoprocta ferox)* was confirmed to be RABV, phylogenetically close to the types circulating in dogs on Madagascar.[56] Because rabies is transmitted primarily by bites, this result indicates that dogs and the fossa are interacting directly and in some cases may transmit pathogens.

• **Figure 43.1** Indirect interactions between exotic and endemic carnivores on Madagascar. The interval in this sequence between the passage of introduced dogs *(above)* and *Cryptoprocta ferox (below)*, an endemic species of euplerid carnivore, at a single camera trap station was less than 2 hours.

Endemic animals may also acquire pathogens through indirect interactions, as when an endemic animal visits a location following the visit of an exotic species (Fig. 43.1). These sites of indirect interaction tend to be in the general vicinity of villages with trails that give people and domestic animals direct access to natural habitats or near villages with open collections of trash (Rasambainarivo, unpublished data). The time interval between visits by a domestic or feral animal and a native animal may be sufficiently short to allow transmission of pathogens such as canine parvovirus or other environmentally resistant pathogens (Rasambainarivo, unpublished data). Serologic analyses from dogs living in rural areas of Madagascar suggest an enzootic transmission of the canine parvovirus, which could spill over to the native fauna.[57] In western Madagascar, researchers found that one of five analyzed narrow-striped vontsira *(Mungotictis decemlineata)* was previously exposed to canine parvovirus-2.[57]

The spirurid parasite *Spirocerca lupi* is another pathogen transmitted by introduced dogs that may negatively affect the native fauna of Madagascar.[58] This nematode parasite is prevalent in free-roaming rural dogs on the island and is presumably responsible for the death of several captive lemurs through the formation of aortic aneurysms and their subsequent rupture.[59] Whether these parasites negatively affect endemic population of lemurs and carnivorans is unknown and warrants further research.

Additionally, it has recently been found that some brown lemurs *(Eulemur albifrons)* had antibodies to *Toxoplasma gondii,* a protozoan whose only known definitive hosts are members of the family Felidae, represented on Madagascar by introduced cats.[60] This indicates a disease spillover from exotic felids to the endemic mammalian fauna of the island. Similarly, 95% ($n = 44$) of the *Cryptoprocta* evaluated in two western protected areas had antibodies against *T. gondii.*[57] This parasite was associated with neurologic disease and death in several captive Malagasy species and may potentially affect wild endemic euplerid and lemur populations.[61,62]

Disease Risks From Introduced Rodents

Pathogenic genospecies of *Leptospira* bacteria were also detected in a large proportion of introduced rodents in various parts of Madagascar, notably *Leptospira interrogans* in *Rattus rattus.*[63] *Leptospira interrogans* is of particular concern because it is known to be pathogenic in more than 150 animal species.[64] Infected *Rattus*, then, constitute a threat to both public health and animal conservation.[64,65] One of the interesting results of these studies is the genetic uniqueness of several *Leptospira* forms among native Malagasy terrestrial and volant mammals, indicating probable isolation and evolution in deep time.[66] This suggests little exchange of *Leptospira* between *Rattus* and native small mammals.[66] Exposure of other native species including lemurs and carnivorans to *Leptospira interrogans* from invasive rodents is unknown.

In a similar pattern, native rodents seem to harbor different species of trypanostomid parasites than their introduced counterparts. In the Ranomafana National Park, 30% of *R. rattus* examined during a survey were infected by *Trypanosoma lewisii*, whereas the native and sympatric nesomyines belonging to the genus *Eliurus* were infected with a morphologically different trypanostomid.[67] The authors suggest that native rodents may not be infected by the parasite from introduced species or that the relatively recent invasion of *Rattus* in the interior of the park has not yet resulted in the transmission of these parasites. Elsewhere, on Christmas Island, *Trypanosoma lewisii* carried by *R. rattus* was associated with the infection and subsequent decline of *R. macleari*, a native rodent.[68]

Recent research on Madagascar has found a virus of the genus *Morbillivirus* that is currently considered as unclassified Morbilli-related paramyoxoviruses (UMRVs).[69] This virus is thought to be widespread among endemic rodents, tenrecs, and bats as well as introduced rodents, specifically *R. rattus.*[69] Members of the Paramyxoviridae viral family have been associated with a number of emerging diseases that affect humans and natural animal populations. Evidence indicates that *R. rattus* is an important spreader of UMRVs and that there is considerable lateral exchange of UMRVs between sympatrically occurring mammals.[69] To our knowledge, no data are available on the isolation of *Morbillivirus* among lemurs and carnivorans on Madagascar.

Conclusions

Introduced species are an increasingly dominant part of many natural and human-modified landscapes. It is estimated that invasive species, particularly invasive mammal predators such as dogs and cats, have caused the extinction of at least 87 species of birds, 45 species of mammals, and 10 species of reptiles and that they are threatening many more.[70] Some of these extinctions may be mediated by the introduction of pathogens.[71] On Madagascar, several of the "worst invasive species" were introduced following human colonization.[72] Their impacts, including those of associated pathogens, on the fauna of Madagascar warrant further research and monitoring. It is hoped that the data generated from such research will ultimately pave the way to successful strategies for managing the risks of "pathogen pollution" and disease spillover to the native fauna of Madagascar.

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SECTION 9

Infectious Diseases

44

Techniques for Vaccinating Wildlife

MARTIN GILBERT

Vaccines have been widely used for over 200 years, and have been one of the most effective means of controlling infectious disease in human and veterinary medicine. Despite this long history, vaccines have been relatively underutilized in free-ranging wildlife, for a range of logistical, ethical and legal reasons.[1,2] This chapter will summarize the current understanding of vaccine use in free-ranging wildlife, introducing the approaches and limitations to vaccine delivery, and key considerations in their use (see also Chapters 45 and 79). For the purpose of this chapter, vaccines are defined as products that are administered with the intention of evoking a specific immune response (whether humoral and/or cell-mediated) against invasive microparasites, macroparasites, or neoplasias. Although vaccines are also used for regulating reproduction of wildlife populations through immunocontraception, these techniques have been reviewed elsewhere.[3]

Motivations for Wildlife Vaccination

There are three main reasons for attempting the vaccination of wildlife, which have implications for both the design of vaccine products and the intended outcomes of vaccination programs. Firstly, vaccines may be used to control the transmission of pathogens of public health significance, such as rabies, for which wildlife reservoirs may be an important source of human infection. Wildlife reservoirs may also play a role in maintaining pathogens with agro-economic significance due to their impacts on domestic animals, such as *Mycobacterium bovis*, *Brucella abortus*, or classical swine fever virus. Such vaccination programs target reservoir populations in order to reduce or eliminate spillover into humans or domestic animals. In these situations, priority is given to maximizing vaccine coverage within the reservoir, evoking immunity as reliably and cost effectively as possible, with the objective of reducing transmission to acceptable levels, or eliminating the pathogen entirely. The potential success of reservoir-directed strategies is exemplified by the elimination of rabies from western Europe through the distribution of oral vaccinia-based recombinant vaccines targeting red foxes *(Vulpes vulpes)* (Table 44.1).[4,5] This program relied on the availability of low cost vaccine baits that could be readily produced in large quantities, providing a more cost-effective approach to managing outbreaks and disrupting rabies reservoirs than alternative strategies like culling. Ongoing programs continue to strive for rabies elimination in more diverse wild carnivore communities in eastern North America, where elimination from some species (particularly skunks [*Mephitis* spp.]) remains a challenge.[6] More limited programs have also been undertaken to control *M. bovis* and CSFV in wild boar *(Sus scrofa)*, while experimental trails are being pursued to manage *M. bovis*, and *B. abortus* in other species (see Table 44.1).

Vaccines are also used as a tool to promote the conservation of endangered species, either by raising the immune status of the threatened population itself or by preventing disease-mediated losses of important prey species.[7,8] The field use of vaccines for conservation purposes remains limited (see Table 44.1), although a number of novel products are under development.[9–11] In most contemporary examples, field delivery has occurred in a reactive manner, when an outbreak is already underway,[2] although vaccines may also be used prophylactically as part of reintroduction or translocation projects.[12,13] For instance, field trials are now underway using a recombinant canine distemper virus vaccine in Hawaiian monk seals *(Neomonachus schauinslandi)* to manage potential outbreaks of phocine distemper virus.[14] In this case, epidemiologic models suggest that reactive strategies may have little impact on population survival due to an extended period required to achieve protective immunity, whereas prophylactic approaches may be more successful.[15] Recent trials have also shown some promise in reducing the impact of outbreaks of *Yersinia pestis* on populations of prairie dogs (*Cynomys* spp.) in the United States using widespread prophylactic delivery of an orally available raccoon poxvirus-based recombinant vaccine.[16] Although the trial did not eliminate cases of Yersinia infection in vaccinated populations, it was associated with a greater abundance of prairie dogs than unvaccinated control populations. The vaccine therefore could benefit both the prairie dogs themselves, as well as their ecologic dependent, the black-footed ferret (*Mustela nigripes*; a species that has also been a focus of vaccination to reduce the impact of canine distemper virus).[17]

TABLE 44.1 Examples of Vaccines Used to Control Infectious Disease in Wildlife Populations in the Field

Target Species		Pathogen	Country/ Region	Delivery Route	Circumstances	Citation
Mountain gorilla	*Gorilla beringei beringei*	Measles virus	Rwanda	IM	OUTBREAK	51,52
Black-footed ferret	*Mustela nigripes*	Canine distemper virus	United States	SC	CONTROL	17
Island fox	*Urocyon littoralis*	Canine distemper virus	United States	IM, O	OUTBREAK	53,54
Red wolf	*Canis rufus*	Canine parvovirus, canine distemper virus	United States	IM	RELEASE	13
Hawaiian monk seal	*Neomonachus schauinslandi*	Phocine distemper virus	United States	IM	TRIAL	14
Reservoir species	—	Rabies virus	Europe	O	CONTROL	4,5
Reservoir species	—	Rabies virus	North America	O	CONTROL	6
African wild dog	*Lycaon pictus*	Rabies virus	Tanzania, South Africa	IM	OUTBREAK, RELEASE	12,55†
Ethiopian wolf	*Canis simensis*	Rabies virus	Ethiopia	IM	OUTBREAK	19,56
European rabbit	*Oryctolagus cuniculus*	Viral hemorrhagic disease virus, myxomatosis virus	Spain	SC	TRIAL	7
Wild boar	*Sus scrofa*	Classical swine fever virus	Germany	O	OUTBREAK	57
Florida puma	*Puma concolor coryi*	Feline leukemia virus	United States	IM	OUTBREAK	58
Cheetah	*Acinonyx jubatus*	*Bacillus anthracis*	Namibia	PAR	TRIAL	59
Black rhinoceros	*Diceros bicornis*	*B. anthracis*	Namibia	IM	CONTROL	59
Indian one-horned rhinoceros	*Rhinoceros unicornis*	*B. anthracis*	India	IM	OUTBREAK	60
Black rhinoceros, white rhinoceros, roan antelope, kudu, waterbuck, African buffalo hippopotamus	*D. bicornis*, *Ceratotherium simum*, *Hippotragus equinus*, *Tragelaphus strepsiceros*, *Kobus ellipsiprymnus*, *Hippopotamus amphibius*	*B. anthracis*	Zimbabwe	IM	OUTBREAK	22
Prairie dog spp.	*Cynomys* spp.	*Yersinia pestis*	United States	O	TRIAL	8
Black-footed ferret	*M. nigripes*	*Y. pestis*	United States	SC	CONTROL	17,61,62
Common brushtail possum	*Trichosurus vulpecula*	*Mycobacterium bovis*	New Zealand	O	TRIAL	63
Eurasian badger	*Meles meles*	*M. bovis*	United Kingdom	IM	TRIAL	64
Eurasian badger	*M. meles*	*M. bovis*	Ireland	O	TRIAL	65,66
Wild boar	*S. scrofa*	*M. bovis*	Spain	O	TRIAL	67
Bison, elk	*Bison bison*, *Cervus elaphus*	*Brucella abortus*	United States	IM	TRIAL	68,69

TABLE 44.1 Examples of Vaccines Used to Control Infectious Disease in Wildlife Populations in the Field—cont'd

Target Species		Pathogen	Country/ Region	Delivery Route	Circumstances	Citation
Bighorn sheep	*Ovis canadensis*	*Pasteurella multocida*, *P. trehalosi*, *Mannheimia haemolytica*	United States	IM	OUTBREAK	70†
White-footed mouse	*Peromyscus leucopus*	*Borrelia burgdorferi*	United States	O	TRAIL	40,41
Koala	*Phascolarctos cinereus*	*Chlamydia pecorum*	Australia	SC	TRIAL	71

Delivery route is indicated as oral (O), intramuscular injection (IM), subcutaneous injection (SC), or unspecified parenteral route (PAR). Circumstances of vaccine use are summarized as outbreak response (OUTBREAK), long-term control (CONTROL), prophylaxis of released animals (RELEASE), or field trial (TRIAL). Interventions reported by authors as unsuccessful are denoted by †.

In declining populations each individual becomes increasingly important to maintaining overall genetic diversity, and strategies that maximize vaccination coverage may be desirable, as a means of slowing the depletion of unique alleles. However, maintaining this level of vaccine coverage is challenging and may be cost prohibitive in conservation programs that lack an economic or public health incentive. But for highly threatened populations, where the objective may be to minimize the probability of disease-induced extinction, it may be possible to meet program goals through low coverage vaccination strategies that do not attempt the elimination or eradication of the pathogen. This is particularly applicable in the case of multi-host pathogens such as rabies or canine distemper virus, where abundant populations of domestic dogs or wild mesocarnivores can act as pathogen reservoirs and perpetual sources of infection for threatened hosts. Eliminating infection in the reservoir may be impractical, but low coverage strategies that aim to vaccinate small numbers of a threatened population may be sufficient to limit the spread of infection during outbreaks, and avert extinction through the persistence of immune survivors.[18] This strategy was successful in halting the spread of rabies virus during an outbreak affecting Ethiopian wolves *(Canis simensis)*.[19] An initial dose of an inactivated vaccine (Nobivac Rabies, Intervet, Milton Keynes, United Kingdom) was given to trapped wolves, which elicited an antibody response by 1 month post inoculation, although a booster dose at 1–6 months was required to maintain antibody titers.

Methods of Delivery

The availability of vaccines that can be delivered orally is hugely beneficial for wildlife vaccination programs, particularly those that must attain high coverage to achieve the elimination of the pathogen. Oral products have been successfully delivered in meat (e.g., chicken heads or rabbit legs), or via packets embedded in a flavored matrix designed to be palatable for the target species.[1,20] Oral delivery may also be desirable for programs with a conservation objective, but the smaller numbers of animals involved raises the possibility of other delivery methods, particularly when the species is readily observed, or where handling is feasible. However, it should be recognized that vaccines that require parenteral delivery introduce additional risks associated with capture, handling, and/or immobilization.[1] Alternatively, vaccines have been delivered remotely via projectile systems such as darts, or bio-bullets that enable the intramuscular release of a vaccine immunogen.[21,22] Vaccines have also been delivered experimentally via sprays that introduce the immunogen via the nasal or conjunctival mucosa.[23,24] This might have potential applications for simultaneously vaccinating large numbers of animals, particularly for species that congregate when breeding or roosting.

Considerations for Vaccine Delivery

Fundamentally vaccines intended for use in wildlife must meet the same standards of safety and efficacy of products intended for humans or domestic species. However, the circumstances of delivery to free-ranging animals raise a distinct and additional set of challenges.

Safety

As with any vaccine, products used in wildlife should not induce disease, or lead to other deleterious side effects in target species. This presents a challenge in the case of threatened hosts where ethical or practical constraints prohibit safety trials in target species, and it may be necessary to draw inference from trials in a closely related model species. Furthermore, it may also be necessary to consider vaccine safety in nontarget hosts (including humans), particularly when using oral baits or other nonparenteral methods, where there is no assurance that a vaccine product will be delivered exclusively to the intended target species.

Efficacy

A vaccine product must be able to evoke a protective immune response in a target species that will continue for a period that meets the needs of the vaccine program. Experiments to demonstrate a humoral and cell-mediated response in captive animals are indicators of an immune response, but experimental challenge studies are required to demonstrate protection. Once again, this presents an ethical challenge in the case of endangered species and may require the use of a closely related model as a basis for inferring protective immunity. The duration of protective immunity is also an important consideration, particularly in free-ranging species where it may not be possible to deliver booster doses to augment protection. In the case of vaccines where immunity wanes over time, simulation models may have a role in determining whether the period of likely protection is sufficient to meet the objectives of the program, or whether additional measures are required to boost immunity.

Additional factors may interfere with the efficacy of oral vaccine preparations even where immunogenicity has been demonstrated in experimental settings. Oral vaccines must be delivered in a manner that is behaviorally acceptable to foraging hosts and be suitably palatable to ensure ingestion. Biomarkers can be used to quantify rates of vaccine uptake, such as the inclusion of tetracycline in rabies baits, the deposition of which can be measured in teeth and bone,[25] or the use of fluorescent rhodamine dye which can be monitored less invasively in hair using an ultraviolet lamp.[26] Oral vaccine baits degrade relatively quickly, particularly when live vaccines are used. Therefore measures must be taken to maximize environmental stability such as the use of more stable recombinant products, or packaging capable of resisting ultraviolet radiation, extreme temperatures, or moisture. When target animals ingest vaccine baits, appropriate adjuvants can also be used to enhance contact and absorption with immunologic induction sites. For example, viscous suspensions prolong mucosal contact,[27] while abrasive adjuvants promote uptake by scarifying oral tissue.[28] In the case of bacille Calmette-Guérin (BCG) vaccines used in controlling *M. bovis* in brushtail possums *(Trichosurus vulpecula)*, the use of a lipid matrix resists gastric digestion ensuring the delivery of immunogen to target cells in the Peyer patches.[29]

Cautionary Principles

As with any intervention, the vaccination of wildlife carries risk of negative outcomes. These risks may be significant, such as the simultaneous loss of immunity that can occur if vaccine coverage is suddenly withdrawn in event of financial or other constraints interrupting a vaccine program.[2] In these circumstances herd immunity could drop below precoverage levels, leading to larger outbreaks, particularly if vaccination had enabled the size and density of the target population to increase.[2] But just as the public health controversy falsely linking the measles, mumps, and rubella (MMR) vaccine to autism led to reduced coverage and the resurgence of measles,[30,31] any perception of failure in wildlife vaccination may have widespread consequences, even if based on flawed assumptions. As a cautionary example, during the early 1990s the immunization of African wild dogs (AWDs, *Lycaon pictus*) against rabies in the Serengeti received high-profile criticism, following the subsequent extinction of remaining packs.[32–37] Critics proposed that immunosuppression related to the stress of capture may have activated latent rabies infections and drew a direct link between the vaccination program and the extinction of AWDs in the park.[32,36] Although these claims lacked scientific support,[33,35,37,38] the negative perceptions introduced a culture of risk-aversion among wildlife managers in many AWD range states and elsewhere.[39] Securing permission for wildlife captures became increasingly difficult in many areas, and there has been a marked resistance to further wildlife vaccination initiatives.[39] Some of these concerns may have been averted with improved stakeholder engagement before vaccine delivery to achieve more realistic a priori expectations. These would explore the likely consequences of inaction, and both hoped for positive and potential negative outcomes of intervention. The inclusion of unvaccinated control groups would provide a means for assessing the impact of vaccination on survival, and careful monitoring prior to, during, and following vaccine delivery would enable the establishment of health baselines, as well as an opportunity to determine the cause of any unforeseen outcomes.[39] Then again, leaving a cohort group unvaccinated represents a risk in itself when the species facing an infectious disease threat is endangered.

The Future

Advances in the development of new vaccine products, delivery mechanisms, and program design suggest an encouraging future for wildlife vaccination. Public health agencies may soon benefit from an oral Borrelia vaccine that has been shown experimentally to achieve protection in Peromyscus mice that act as a reservoir for Lyme disease, while also clearing infections in feeding ticks.[40,41] In the agricultural sector, refinements in the oral delivery of BCG vaccines and baiting regimes for badgers *(Meles meles)* could make an important contribution to the management of bovine tuberculosis.[42] Vaccines could aid in the conservation of tigers *(Panthera tigris)*,[43] with trials underway in captivity as a precursor to protecting wild populations against the impact of canine distemper virus,[44] while vaccines may also represent the best hope of preventing Tasmanian devil facial tumor disease from causing the extinction of its namesake *(Sarcophilus harrisii)*.[9] Progress is also being made in the development of vaccines to control fungal disease, an area of medicine traditionally ignored by vaccinology,[45] and while results so far have been mixed, researchers are pursuing vaccines to combat perhaps the greatest disease threat to species extinction: chytridiomycosis.[46,47] If experimental vaccines

can be incorporated into more cost-effective "off the shelf" oral delivery systems, wildlife vaccines may move beyond the theoretically possible, to the economically feasible.[48]

Just as technologic advances open new opportunities, they may also introduce new challenges, and following the experiences of the AWD vaccination in the Serengeti, perceived or actual failure can have far-reaching consequences. One conceptual development is the proposed release of self-disseminating vaccines, such as an experimental Ebola virus vaccine intended for use in protecting great apes in Central Africa. The proposed recombinant vaccine uses a cytomegalovirus (CMV) that is assumed to be safe and host-specific, to carry Ebola virus nucleoprotein, and has been shown to protect both mice and rhesus macaques from experimental challenge.[11,49] While this approach could overcome the logistical difficulties of vaccinating rare and cryptic species in remote areas, it requires a high level of confidence in the safety and host-specificity of the product, as containment would be impossible once released into the ecosystem. However, the pivotal assumption that CMV is clinically inconsequential is debatable, as the virus is considered the main infectious cause of human birth defects in the United States (with approximately twice the incidence of Down's syndrome).[50] There could be considerable public health ramifications of releasing a recombinant and transmissible CMV, not to mention the untested susceptibility of our close relatives, and primary vaccine target, the great apes. Even if safety could be ensured, the acceptance by the general public of disseminating a genetically modified virus into the wild should not be assumed. Ultimately, societal and political support are essential components of successful wildlife vaccination programs and should not be ignored.

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Brucellosis in North American Wildlife

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Brucellosis: An Introduction

Brucellosis is a group of diseases caused by bacterial species in the genus *Brucella*. *Brucella* spp. organisms are small, nonspore-forming gram-negative coccobacilli that live and replicate inside the host's cells, especially phagocytic cells and placental trophoblasts. Classically, each species has been subdivided into biovars dependent on the presence of certain antigens as determined by reacting the organisms with different antisera. Since genomic typing has developed, however, the relationship between genotypes and biovars is being reevaluated.

Most, but not all, *Brucella* spp. cause zoonotic disease. Brucellosis in humans is known as undulant fever and is characterized by persistent and recurrent bouts of fever, chills, night sweats, headache, arthralgia, arthritis, anorexia, nausea, weight loss, malaise, and dementia. *Brucella* infections in humans usually respond to antibiotic therapy, although recrudescence is not uncommon and polymerase chain reaction (PCR) indicates that brucellar DNA may be demonstrated in patients years after antibiotic treatment.[1]

The causative organism of a severe, sometimes fatal, disease in soldiers in Malta in 1887 was identified by David Bruce and later given the name *Brucella melitensis*. A similar organism, *B. abortus*, was identified a decade later by Bernard Bang as causing "infectious abortion" in cattle. In the following decades, *B. suis* was identified in domestic swine, *B. canis* in dogs, *B. ovis* in sheep, and *B. neotomae* in desert woodrats *(Neotoma tepida)*. Until recently, those organisms comprised the genus *Brucella*. However, since 1994, several new species of *Brucella* have been identified. In marine mammals, two new species have been reported worldwide (currently named *B. pennipedialis* and *B. ceti*, in seals and cetaceans respectively), and are reviewed by Nymo et al., 2011.[2] *Brucella inopinata* has been identified in numerous frog species (reviewed by Mühldorfer et al., 2016).[3] *Brucella microti* was first isolated common voles *(Microtus arvalis)* in the Czech Republic[4] and subsequently has been found to be present in the soil from the same area,[5] and in addition, was later isolated in red foxes *(Vulpes vulpes)* in Austria and a wild boar *(Sus scrofa)* in Hungary.[6,7]

The most recent discoveries of new *Brucella* species were *B. papionis* in captive baboons (*Papio* spp.),[8] *B. vulpis* in red foxes in Austria,[9] strains closely related to *B. vulpis* in rodents (*Myodes glareolus*; *Microtis* spp.) and shrews (*Sorex* spp.) in Germany,[10] and as yet unnamed strains found in Australian rodents (*Rattus assimilis*; *Melomys* spp.).[11]

Cooperative State-Federal Brucellosis Eradication Program

To understand the importance of the presence of brucellosis in wildlife reservoirs in North America, familiarity with the 80-year eradication effort in the United States is helpful. In the early 1900s, brucellosis was considered the most economically devastating disease of livestock, and in 1934, the Cooperative State-Federal Brucellosis Eradication Program was begun. The program has used quarantine, test and slaughter, calfhood vaccination, and, more recently, adult vaccination to accomplish its goal. In 1934, nearly 50% of the cattle herds tested had seropositive or suspect animals and by the 1990s less than 200 affected herds remained. For over a decade cattle and captive bison *(Bison bison)* herds, nationwide, have been negative for brucellosis with the exception of herds in the Greater Yellowstone Area (GYA) that acquire the infection from free-ranging elk *(Cervus canadensis)*.[12] National and state efforts to control swine brucellosis were begun in the late 1950s, at which time the disease was prevalent in domestic herds. Currently, swine brucellosis has been eradicated from the commercial swine industry.

Brucellosis in Wildlife

There are four general ecosystem infections of brucellosis in North American wildlife: (1) *B. abortus* in bison and elk in the GYA and in bison in and around Wood Buffalo National Park (WBNP), Canada; (2) *B. suis* in invasive wild swine; (3) *B. suis* biovar 4 in caribou *(Rangifer tarandus)* and other wildlife in the Arctic; and (4) *B. ceti* and *B. pinnipedialis* in marine mammals. The successful eradication

of *B. abortus* from cattle, ranched bison, and most public bison herds in the United States and Canada leaves bison and elk in the GYA and bison in WBNP as the remaining reservoirs of the infection. *Brucella suis* remains present in many wild swine populations and occurs occasionally in backyard or "transitional" domestic swine operations having contact with wild swine.

There are many published surveys of wildlife for brucellosis, although most are limited to examination of serum for antibodies to *Brucella* spp. The serologic tests used are usually those developed for cattle to detect antibodies to *B. abortus*. When using these tests to detect antibodies to other species of *Brucella*, one is dependent on cross-reacting antibodies developed against shared antigens in the organism's cell wall. Factors to take into account when interpreting the results of these studies include: undetermined or variable specificities and sensitivities of tests when used in nonbovine species; detection of antibodies indicates possible exposure to the agent, not necessarily active infection; and false positive results may occur due to cross-reactive antibodies developed on exposure to several non-*Brucella* bacterial species, such as *Yersinia enterocolitica* strain 09. In the case of marine mammals, considerable variation on the same serum sample between standard *B. abortus* tests has been observed and numerous assays have been developed specifically for detection of antibodies to brucellosis in pinnipeds and cetaceans.

Brucellosis in Bison and Elk in the Greater Yellowstone Area

Brucellosis was first serologically diagnosed in wild bison in Yellowstone National Park in 1917, in elk in Yellowstone in 1931, and on the National Elk Refuge, Jackson, Wyoming in 1930. Populations of bison and elk in the GYA are approximately 5000 and 100,000 respectively. *Brucella* seroprevalence in GYA bison is approximately 50%–60% and is highly variable in elk, ranging from negligible to 40% depending on the year and location.[13] Recent surveys in elk show an average of 21.9% positive on feeding grounds and 3.7% positive on land in proximity to feeding grounds.[13] Until about 2006, it was thought that the 22 state and federal elk feeding grounds in Wyoming and intermittent winter feeding grounds in Idaho were necessary for maintenance and transmission of infection in elk. However, it has been observed that seroprevalence in some elk herds remote from feeding grounds has markedly increased,[13] indicating expanding infection among elk populations. This has resulted in many cases of transmission to cattle and captive bison herds in the GYA.[12] Factors that may have influenced the apparent switch from elk as a spillover host to a maintenance host include increasing populations of elk, land use changes resulting in larger congregations of elk on private property during winter and spring,[14] and the reintroduction of wolves to the GYA which has influenced elk behavior.[15]

Brucellosis in Wood Buffalo National Park Bison

Canada's sole known wildlife reservoir of brucellosis consists of several subpopulations of bison within and adjacent to WBNP in Alberta and the Northwest Territories. Currently, bison populations within the greater WBNP region are estimated at 4500 animals and have generally increased in recent years in spite of a coinfection with bovine tuberculosis and predation by wolves.[16] In a survey conducted in the mid-1980s, 25% of 72 WBNP bison examined had evidence (culture and/or serology) of *B. abortus* infection.[17]

The Disease in Bison

In bison, *B. abortus* is transmitted primarily through ingestion or mucosal contact with organisms shed in the placenta, fetus, and birth fluids at abortion or calving. It is also shed in the milk; however, it appears that calves often clear any infection and become seronegative by weaning. In fact, 5-month-old calves may be seronegative, though suckling milk from which *B. abortus* can be isolated.[18] Post-weaning seronegative calves, regardless of prior serostatus due to maternal antibody, are susceptible to infection and abortion. Seroconversion to positive rates (indicating at least exposure to *B. abortus*) in the GYA are 20% per year for calves up to 3 years of age and then decrease to 10% per year.[18]

Latent infection, also known as the "heifer syndrome," is a phenomenon which is rare but has been documented in cattle. Calves born to infected dams become latently infected due to in utero or neonatal exposure. They become seronegative after weaning and show no evidence of infection until their first parturition in which they seroconvert and may abort or shed organisms. Understandably, this phenomenon may be problematic for eradication strategies. Latent infection has not been documented in bison; however, it is too early to conclude that it does not occur due to the limited number of observations made thus far.

Among female bison exposed to *B. abortus*, some become infected and do not abort or shed organisms; others abort one or more times. Some infected cows may shed at parturition, have one or more normal calves without shedding, and then have an abortion with marked shedding of brucellae. Additionally, the infection causes metritis and mastitis in cows, with reduced birth rates in seropositives compared to seronegatives.[19] Infection of the mammary gland often occurs but not as frequently as in cattle.[20] Retained placenta may accompany abortion or calving and likely contributes considerably to transmission among herd-mates, especially juvenile male bison who are still present with the nursery groups and very interested in the hanging placenta.

In bulls *B. abortus* causes seminal vesiculitis, epididymitis, ampullitis, and occasionally orchitis, which may affect fertility. It is thought that venereal transmission is not a significant factor in the epidemiology of the disease

in bison. This is extrapolated from our knowledge of the disease in cattle and is supported by the findings of a single limited study.[21] In using several seronegative bulls to breed positive cows, we have not seen seroconversion in the bulls. In a study collecting semen from wild bison bulls in the spring (prebreeding season), 1–8 CFU *B. abortus*/mL of semen could be recovered.[22] In recently infected bulls, we have isolated up to 2000 CFU *B. abortus* per mL of semen. After instilling 10×10^7 CFU *B. abortus* strain 19 into the vagina of cows at breeding, we observed transient seroconversion but were unable to isolate the organism from tissues 6 months later.[23] Nonreproductive tract lesions in bison are occasionally seen and include arthritis, abscesses, bursitis, and hygroma formation. Following infection, bison develop antibodies to *B. abortus*, detectable on several tests currently used for cattle. The success in obtaining a *B. abortus* isolate from a seropositive bison varies with the stage of infection, the harvest of appropriate tissues, and the rigor of the isolation process. In recently infected animals, a large percentage of seropositives will be culture positive.[24] In a small sample of random seropositive bison from the GYA, we isolated the organism from 47% of seropositives.[20] Antibody levels to *B. abortus* may slowly decrease over time but are surprisingly persistent with only a rare animal changing from seropositive to seronegative over several years.[18]

The Disease in Elk

The pathogenesis and clinical disease in elk are similar to what is seen in bison, although reports vary regarding percentage of abortions in infected elk. In male elk, epididymides, seminal vesicles, and ampullae may be infected with *B. abortus.* Also in elk, bursa, joint, and tendon sheath infections due to *B. abortus* are not uncommon. Infection of the mammary gland is not as prevalent in elk as in cattle. There appear to be differences in intraspecies transmissibility of brucellosis in elk and bison with elk being less efficient transmitters. This is likely, at least in part, due to behavioral differences. Calving events among bison, especially early in the calving season, attract other cows and calves that intimately interact with the birthing process through sniffing and licking. In contrast, elk generally calve away from the herd and maintain some isolation for several days, even in confinement. Of course, the presence of a feeding ground changes this isolation dynamic considerably as the infected cow elk may abort or dribble infectious discharge on the feedline, exposing others to the agent.

Brucellosis in Moose

Natural and experimental *B. abortus* infection has been reported in the moose *(Alces alces)*.[25,26] Reported lesions include pericarditis, myocarditis, pleuritis, peritonitis, arteritis, lymphadenitis, orchitis, hepatitis, and arthritis. In experimentally infected moose, antibody responses developed within 15 days and remained high along with persistent bacteremia. The disease is thought to be generally fatal in moose, and this species is considered a likely dead-end host.

In many areas of Alaska and Canada, moose share habitat with caribou and reindeer. *Brucella suis* biovar 4 infects caribou and reindeer (see section "Brucellosis in Caribou") and has been isolated from bone lesions from a wild debilitated moose killed in the Northwest Territories.[27] An experimental infection of a moose with biovar 4 produced severe clinical illness, and infection of blood, lymph nodes, liver, and spleen.[28] It was the authors' opinion the infection would have been fatal in the wild.

Brucellosis in Bighorn Sheep

Brucella abortus infection has been observed in bighorn sheep *(Ovis canadensis)* in a research facility and was attributed to fence-line contact with aborting infected elk.[29] The infection produced abortion, placentitis, orchitis, epididymitis, and lymphadenitis in the bighorns and apparently resulted in the death of two rams. The organism has not been isolated from bighorns in the wild.

In North America, serological titers to *B. ovis* have been detected in wild bighorn sheep. An experimental infection of bighorns demonstrated seroconversion, abortion, epididymitis, ampullitis, seminal vesiculitis, prostatitis, and necrotizing and pyogranulomatous orchitis (Fig. 45.1).[30] Compared with domestic sheep, bighorns developed more frequently detectable bacteremia and equivalent or more severe lesions.

Brucellosis in Wild Pigs

Brucella suis infection was first isolated in an invasive wild pig in North America in 1974 in an animal collected in South Carolina, USA.[31] In the following four decades, *B. suis* has been isolated from wild pigs in eight additional United States including Hawaii. *Brucella suis* biovar 1 predominates in North America; however, biovar 3 has been identified in a Hawaiian wild pig.[32] In another five states there is evidence of *Brucella* exposure in wild pigs in the form of antibody detection. As serologic tests are not very sensitive in detecting brucellosis in swine, it is possible that the disease may go underreported in some areas.[32,33] At least 38 states in the United States and 3 provinces of Canada are known to harbor invasive wild pigs with numbers likely exceeding 5 million.[34,35] The presence of *B. suis* in North American wild pig populations that have great potential for continued range expansion poses a constant threat to the domestic swine industry (especially transitional swine herds) and other domestic animals, as well as to human health. However, it is difficult to predict how the prevalence and range of brucellosis in wild pigs may change with changing wild pig populations, as there is little information regarding the epidemiology of brucellosis in these animals in North America. Much more research must be done regarding the epidemiology of this disease in

• **Figure 45.1** Bighorn sheep ewe *(Ovis canadensis)* experimentally infected with *Brucella ovis* aborting fetus and ram inspecting the fetus indicating possible route of transmission. (Photos courtesy of Matt McCollum.)

wild pigs in order to be able to effectively understand and manage it in these free-ranging populations.

Brucella suis infection in swine may produce the following clinical signs: weak or stillborn piglets, abortion (although less common than in cattle infected with *B. abortus*), orchitis, and lameness and posterior paralysis due to arthritis. Associated lesions may include placentitis, seminal vesiculitis, epididymitis, arthritis, and abscessation of various tissues. Transmission in swine generally occurs through the venereal route as well as by exposure to aborted fetuses or other products of parturition. *Brucella suis* infection in humans and other species may occur through aerosol and oral routes, as well as via breaks in the epidermis, and may be severe. There are several reports of *B. suis* being contracted by slaughterhouse workers and hunters while butchering or field dressing infected pigs.[36,37]

Brucellosis in Caribou

The northern portions of North America are home to hundreds of thousands of free-ranging caribou occupying boreal, montane, and arctic environments. Additionally, there are approximately 20 herds of reindeer and domesticated caribou, often also free-ranging and located primarily in the Seward Peninsula and Aleutian Islands of Alaska. Reindeer and their herders comprise a small but growing commercial livestock industry whereas hunting of caribou provides meat and other animal products for a large number of indigenous peoples in proximity to those animals.

Brucellosis was serologically first detected in humans in Alaska in 1939. Subsequently, an organism resembling *B. suis* was isolated from human blood and bone marrow, and epidemiologic studies suggested caribou as a source of infection. In 1963, the organism was isolated from caribou,[38] and later studies demonstrated the human and reindeer isolates from Alaska, Canada, and Siberia were the same organism that was classified as *B. suis* biovar 4.[39] A survey conducted in the 1960s found that in communities where caribou were eaten, 5%–20% of the residents had positive titers to *Brucella*. The infection is pan-Arctic and whereas *R. tarandus* is the natural host, other species are susceptible including muskox, foxes, moose, dogs, wolves, bears, and some rodents.[28,40–42] At present, the seroprevalence of *B. suis* biovar 4 infection is estimated to be less than 5% in Alaskan reindeer (R. Gerlach, personal communication, December 12, 2016) and highly variable in caribou, occasionally exceeding 40% in Canada.[43] Brucellosis has not been diagnosed in imported reindeer in the lower 48 states of the United States or in the small number of woodland caribou that range in southern British Columbia, northwest Montana, and northern Idaho.

Transmission of the disease is by ingestion of or contact with infected products of parturition. Whether venereal transmission or vertical transmission through the milk occurs is unknown. In *R. tarandus*, *B. suis* biovar 4 produces abortion, weak calves, retained placentas, lameness, sterility, orchitis, epididymitis, seminal vesiculitis, metritis, mastitis, nephritis, and bursitis.[44] An alternate pathway of transmission was tested by Vashkevich (1978),[45] who demonstrated transovarian transmission of brucellosis from infected to naïve reindeer by the warble fly *(Oedemagena tarandi)*. Disease management tools for reindeer consist of vaccination (adjuvented, killed *B. suis* biovar 4) and test and cull of seropositives. Currently no specific disease management is practiced in caribou.

Brucellosis in Wild Carnivores

Several serologic surveys have indicated exposure of North American wild carnivores to *Brucella* spp., summarized by Davis (1990).[46] These species include wolves, arctic foxes, red foxes, coyotes, and black, grizzly, and polar bears[42];

however, naturally occurring disease has not been reported in these carnivores. Experimental infections have suggested that carnivores usually develop detectable antibodies and transient infection of lymph nodes and organs but do not usually shed significant numbers of brucellae.[47] Transmission of *B. abortus* from coyotes to cattle has been observed under experimental, close-confinement conditions,[48] but wild carnivores are generally considered "spillover" hosts and are not thought to be significant transmitters of infection in nature.[46] In selected areas such as the GYA, wild canids could serve as effective sentinels for the presence of brucellosis in free-ranging ungulates.

Brucellosis in Marine Mammals

Brucella spp. were first isolated in 1994 from harbor seals *(Phoca vitulina)*, harbor porpoises *(Phocoena phocoena)*, and a common dolphin *(Delphinus delphis)* found dead on the Scottish coast.[49] Also in 1994, a similar organism was isolated from an aborted fetus of a captive bottlenose dolphin *(Tursiops truncatus)* in California.[50,51] *Brucella* spp. infections have since been detected by serology and/or culture in numerous species of cetaceans and pinnipeds, with nearly global distribution.[2,52] Additionally, a recent report describes the isolation of a unique *Brucella* strain from an osteolytic lesion of a southern sea otter *(Enhydra lutris nereis)* on the coast of California.[53] Molecular and biochemical data suggest the organism represents a novel lineage distinct from *B. ceti* and *B. pinnipedialis* though more closely related to pinniped strains. Reports of evidence of *Brucella* spp. infection or exposure in North American marine mammals are summarized in Table 45.1.

Brucella ceti has been associated with a variety of lesions in several cetacean species (see Table 45.1) including abortions

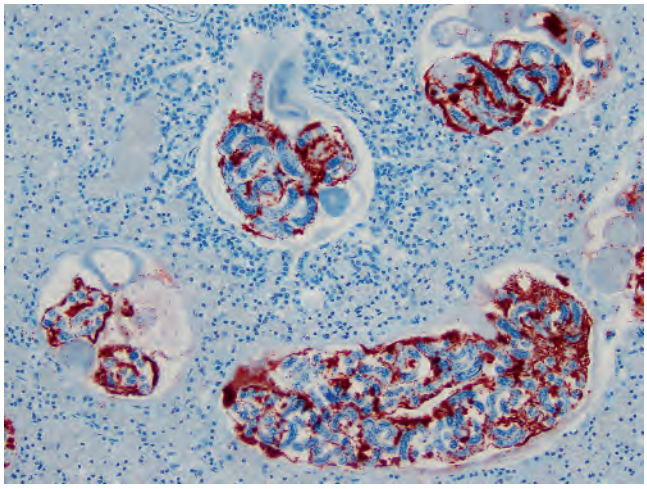
TABLE 45.1 Summary of Publications Reporting Evidence of *Brucella* spp. Infection or Exposure in Marine Mammals by Species

Common Name (Species)	Location	Pathology	Type of Tests Performed	Reference
Common bottlenose dolphin *(Tursiops truncatus)*	Northern Gulf of Mexico, USA	Pneumonia	Serology, Polymerase chain reaction (PCR), Histopathology, Culture	Colegrove et al. (2016)[55]
	Captive facility, San Diego, CA, USA	Pulmonary abscess	Culture, PCR	Cassle et al. (2013)[63]
	Gulf of Mexico, Texas, USA	Vertebral osteomyelitis	Culture, Histopathology	Goertz et al. (2011)[64]
	Captive facility, San Diego, CA, USA	Abortion, placentitis; lung granuloma	Serology, PCR, Histopathology, Immunoperoxidase technique	Miller et al. (1999)[50]
	Captive facility, San Diego, CA, USA	Abortion	Culture	Ewalt et al. (1994)[51]
Harbor porpoise *(Phocoena phocoena)*	Bay of Fundy, Canada	Orchitis	Serology, PCR, Histopathology	Niemanis et al. (2008)[65]
Sperm whale *(Physeter microcephalus)*	Hawaii, USA	Lymphoid depletion, chronic meningitis, pneumonia	Culture, PCR, Histopathology	West et al. (2015)[66]
Beluga *(Delphinapterus leucas)*	St. Lawrence Estuary, Canada; MacKenzie Delta, Canada; Nunavuk, Canada; Hudson Bay, Canada; Baffin Island, Canada; Grise Fjord, Canada	N/A	Serology	Nielsen et al. (2001)[67]
Narwal *(Monodon monoceros)*	Baffin Island, Nunavut, Canada	N/A	Serology	Nielsen et al. (2001)[67]
Harbor seal *(Phoca vitulina)*	Alaska, USA	N/A	Serology	Hueffer et al. (2013)[68]
	Puget Sound, WA, USA	N/O	Serology, PCR, Histopathology, Immunohistochemistry	Lambourn et al. (2013)[69]
	Alaska, USA	N/A	Serology	Zarnke et al. (2006)[70]

TABLE 45.1 Summary of Publications Reporting Evidence of *Brucella* spp. Infection or Exposure in Marine Mammals by Species—cont'd

Common Name (Species)	Location	Pathology	Type of Tests Performed	Reference
	New England, USA	N/O	Serology, Culture, Histopathology, Immunohistochemistry	Maratea et al. (2003)[58]
	Atlantic Coast, USA; St. Lawrence Estuary, Canada; Nova Scotia, Canada; Vancouver Island, Canada	N/A	Serology	Nielsen et al. (2001)[67]
	Puget Sound, WA, USA	Pulmonary abscess	Serology, Culture, Histopathology, Immunohistochemistry	Lambourn et al. (1998)[71]
Hooded seal *(Cystophora cristata)*	St. Lawrence Estuary, Canada; Gulf of St. Lawrence, Canada; Nova Scotia, Canada; Newfoundland, Canada	N/A	Serology	Nielsen et al. (2001)[67]
Gray seal *(Halichoerus grypus)*	Gulf of St. Lawrence, Canada; Nova Scotia, Canada	N/A	Serology	Nielsen et al. (2001)[67]
Harp seal *(P. groenlandica)*	Magdalen Islands, Quebec, Canada	N/O	Serology, Culture	Forbes et al. (2000)[72]
	New England, USA	N/O	Serology, Culture, Histopathology, Immunohistochemistry	Maratea et al. (2003)[58]
	Gulf of St. Lawrence, Canada; Newfoundland, Canada	N/A	Serology	Nielsen et al. (2001)[67]
Ringed seals *(Pusa hispida)*	Newfoundland, Canada; Nunavut, Canada	N/A	Serology	Nielsen et al. (2001)[67]
	Baffin Island, Nunavut, Canada	N/O	Serology, Culture	Forbes et al. (2000)[72]
	Nunavut, Canada; Holman Island, Northwest Territories, Canada	N/A	Serology	Nielsen et al. (1996)[73]
Northern fur seal *(Callorhinus ursinus)*	Alaska, USA	Placentitis	Serology, PCR, Histopathology, Immunohistochemistry	Duncan et al. (2014)[56]
Hawaiian monk seal *(Monachus schauinslandi)*	Hawaii, USA	N/A	Serology	Nielsen et al. (2005)[74]
California sea lion *(Zalophus californianus)*	San Miguel Island, CA, USA	N/O	Culture, Histopathology	Goldstein et al. (2011)[75]
Polar bears *(Ursus arctos)*	Alaska, USA	N/A	Serology	O'Hara et al. (2010)[42]
Atlantic walrus *(Odobenus rosmarus rosmarus)*	Nunavut, Canada	N/A	Serology	Nielsen et al. (2001)[67]
	Nunavut, Canada	N/A	Serology	Nielsen et al. (1996)[73]
Southern sea otter *(Enhydra lutris)*	California, USA	Osteolytic lesions, myelitis, the myocardial mottling, hepatosplenomegaly	Culture, Histopathology, Immunohistochemistry	Miller et al. (2017)[53]

N/A, Not applicable-serologic study only; *N/O*, none observed.



• **Figure 45.2** Photomicrograph of lung tissue from harbor seal *(Phoca vitulina)* containing cross sections of *Parafilaroides* sp. nematodes. Note immunohistochemistry positive *(red)* staining brucellae in uterine lining and surrounding larvae of nematodes; also colonizing lumen of gastrointestinal tract in upper right quadrant of upper right nematode. (Specimen courtesy of M. Garner and D. Lambourn.)

and placentitis, epididymitis and orchitis, meningoencephalitis also referred to as neurobrucellosis, subcutaneous abscesses, bone and joint lesions, and heart lesions, recently reviewed.[54] *Brucella*-associated in utero pneumonia was also reported in perinatal bottlenose dolphins associated with the northern Gulf of Mexico unusual mortality event of 2010–2014.[55]

Although *Brucella* isolates have been obtained from numerous organs from many species of pinnipeds, reported pathologic lesions associated with the *B. pinnipedialis* are lacking, with a couple of exceptions. A necropurulent placentitis with associated brucellae demonstrated by immunohistochemistry (IHC) was reported in a northern fur seal *(Callorhinus ursinus)* from the Pribilof Islands of Alaska.[56] Secondly, multifocal pneumonia has been observed in lung tissue from Pacific harbor seals *(P. vitulina richardii)* with *Brucella*-infected *Parafilaroides* sp. lungworms (Fig. 45.2).[57] It is thought the focal pneumonic lesions are due to the death or parturition of the infected lungworms. Interestingly, *Brucella*-infected lungworms have also been observed in a harp seal *(Phoca groenlandica)*[58] and a harbor porpoise.[59]

Mechanisms of transmission of marine mammal brucellosis are largely unknown. The occurrence of placentitis and abortions in some cetaceans suggests contact with infected products of parturition, as in terrestrial mammals, may be of importance. Additionally, venereal transmission, vertical transmission from mother to fetus or newborn, and transmission by ingestion of infected fish or nematodes have been suggested.[54] In humans, one case of laboratory-acquired marine brucellosis[60] and three naturally acquired cases have been reported.[61,62] No disease management strategies are currently being practiced for brucellosis control in wild marine mammals.

Future Directions and Conclusions

Management of brucellosis in animals and humans is extremely challenging at both the individual and population levels. Although we have known about and dealt with this disease in ruminants for over a century, efforts continue to develop better ways to manage and eradicate it from animal populations. Now we have the emergence/recognition of *Brucella* infections in several other species, and a dearth of knowledge about each of these. Therefore future directions for research should include a good deal of pathogenesis and epidemiology work for the new species, and development of improved mitigation strategies for the infections in wild ungulates and marine mammals. Such research topics should include strategies to minimize transmission, and methods of remote detection and vaccination (see Chapter 44). As brucellosis has been largely eradicated from commercial livestock industries, its continued presence in selected wildlife populations has emerged as an important and politically charged issue. With the continuing emergence of these diseases in wildlife species, solutions may only be found based on good science, public education and involvement, and innovative collaborative work among research scientists, regulatory and wildlife veterinarians, and wildlife biologists and managers.

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Update on Melioidosis in Zoo and Wild Animals

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Melioidosis is a severe, often fatal, systemic disease of humans and other animals caused by a gram-negative bacillus, *Burkholderia pseudomallei*. A wide range of animal species affected by melioidosis differs in their susceptibility to the infection and to the disease. Rising awareness of the disease resulted in an increase in melioidosis case reports from more than 20 countries. The list of species at risk continues to expand.[1,2] Literature reports of melioidosis in zoo species represent only a fraction of the information exchanged between zoo veterinarians and managers. Imported animals in apparent good health have spread the disease to areas previously not affected, where they have been responsible for sporadic cases outside endemic areas. An outbreak of melioidosis at the Jardin des Plantes in Paris in the 1970s was traced to either imported horses from Iran or a giant panda *(Ailuropoda melanoleuca)* from China. The disease spread widely outside the zoo, infecting animals and humans.[3,4] This chapter will provide an update on progress made at the Hong Kong Ocean Park on diagnosis and treatment of infection in marine mammals and birds and summarize the status of melioidosis in other zoo species.

Etiology and Epidemiology

B. pseudomallei is a gram-negative, bipolar staining, aerobic, motile, rod-shaped bacterium. It is an environmental saprophyte commonly isolated from contaminated soil, surface water, and heavy rain water. *B. pseudomallei* is remarkably resistant in the environment. The bacterium was still cultivable after 16 years in distilled water,[5] 70 days in an acid environment at pH 4.5, and 35 days in saltwater at 32 ppt.[6,7] *B. pseudomallei* may also live within amoeba (*Acanthamoeba* sp), fungi (*Gigaspora* sp), and plants.[8–10] *B. pseudomallei* is sensitive to exposure to ultraviolet (UV) light and is killed in less than 5 minutes in pools with free chlorine concentrations higher than 0.3 ppm or oxidation-reduction potential (ORP) readings higher than 500 mV (Martelli & Hui, unpublished).[10a]

Recommended methods for environmental sampling are published.[11] A standard operating procedure on a simplified method for isolation of *B. pseudomallei* from soil may be downloaded from www.melioidosis.info. Studies comparing culture and polymerase chain reaction (PCR) to screen for *B. pseudomallei* in a soil sample found a higher positivity rate by PCR than by culture.[12,13] PCR detects DNA signal from both live and dead bacteria. DNA from dead cells may be excluded by using selective nucleic acid intercalating dyes, ethidium monoazide (EMA), or propidium monoazide (PMA).[14]

Strain typing of *B. pseudomallei* by multilocus sequence typing (MLST) requires pure bacterial isolates.[15,16] An updated library of *B. pseudomallei* MLST may be found at http://bpseudomallei.mlst.net. MLST analysis of 146 isolates from Ocean Park between 1999 and 2010, including 111 environmental and 35 clinical samples, demonstrated that five of the eight environmental strains have caused disease in animals at Ocean Park: ST70, ST32, ST37, ST660, and ST684. The most prevalent strain in both environmental and clinical samples is ST70.[17]

Infection typically occurs after exposure to soil or water contaminated with *B. pseudomallei.* Modes of infection include inhalation, ingestion, or through open wounds, although a definite route of infection is seldom identified. Transplacental infection was reported in goats and pigs.[1,18] Other routes such as biting insects, sexual transmission, or suckling have been suggested but are considered very rare.[19–23]

Cases of melioidosis at Ocean Park correlate with meteorologic conditions, with increased incidence associated with heavy rain and strong winds. Studies in Taiwan, Hong Kong, and Singapore have also linked increased rates of infection to high rainfall.[24–26] In Hong Kong Ocean Park between the months of May and October, 10%–45% of rainwater samples collected during heavy rainfall are positive for *B. pseudomallei*. Other local conditions, including soil composition and wind speed, also play a major role in the incidence of the disease.

In humans, factors predisposing to melioidosis include debilitating conditions such as diabetes, thalassemia, renal disease, alcoholism, and prolonged steroid therapy.[27] In

animals, decreased host immunity from illness, chronic stress, steroid therapy, and the nature of the environmental exposure are believed to be predisposing factors.[1,27]

Diagnosis

Positive bacterial culture of *B. pseudomallei* confirms the diagnosis. Culture is 100% specific, but sensitivity may be as low as 60%, depending on the method of sample collection, media used, and expertise of the microbiologist.[28]

Ashdown medium is the most reliable selective culture medium for the specific isolation of *B. pseudomallei*, although *B. pseudomallei* does grow in commercially available blood culture media. Colonies of motile, oxidase positive, gram-negative bacilli, resistant to gentamicin and colistin, sensitive to amoxicillin-clavulanic acid, grown under aerobic conditions, with a characteristic colonial morphology of metallic sheen and progression to dry and wrinkled colonies should be presumptively identified as *B. pseudomallei*.[29] It is important to collect blood for culture before antibiotic therapy begins because blood cultures from confirmed cases are usually negative after antibiotic treatment has begun. The API 20NE & Vitek systems (bioMerieux, France) are widely used to confirm the isolates biochemically. Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) improves accuracy and speed.[30–32]

Monoclonal antibody against exopolysaccharides on the cell surface of *B. pseudomallei,* such as latex agglutination (LA) and immunofluorescence assay (IFA), are highly specific and sensitive and may be used on cultures to provide an early diagnosis.[27,33,34] Lateral flow immunoassay (LFI) for detection of capsular polysaccharide (CPS) antigen may also be applied to blood, pus, sputum, urine, and bacterial colonies.[35,36]

A variety of PCR detection methods for *B. pseudomallei* have been developed. Targeted genes include type III secretion system, 16S rRNA, 23S rRNA, flagellin C, ribosomal protein subunit, groEL, Tat-domain protein, etc.[29,37] PCR has been shown to be specific and sensitive when applied to bacterial isolates. However, PCR may have low specificity and sensitivity when applied directly to clinical specimens.

Serology is an essential tool in the diagnosis of individual infections and for mass surveillance. Indirect hemagglutination assay (IHA) is the earliest described serologic test for melioidosis and is routinely performed in endemic areas.[38] An updated standard operating procedure for IHA may be downloaded from www.melioidosis.info.

A cetacean-specific enzyme-linked immunosorbent assay (ELISA) was described by Chow (2004).[39] Despite its high specificity and sensitivity, the usefulness of the cetacean ELISA is limited due to the long interval between infection and seroconversion that may take up to 18 days.[27]

For other species, including birds, Ocean Park Hong Kong uses a microagglutination test (MAT) developed in-house from multiple strains (Chan, unpublished).

Clinical Presentation, Pathology, Treatment, and Management

Cetaceans

The clinical and pathologic presentations of melioidosis in cetaceans at Ocean Park are detailed in Kinoshita.[27] In the decade since that report, there have been three additional cases of cetacean melioidosis, including two confirmed by blood culture and another by serology. All cases occurred between May and October and only days after a typhoon or heavy rain. All were successfully treated. Initial clinical presentation was invariably acute to peracute. Early clinical signs are nonspecific, such as pyrexia, lethargy, inappetence, and lack of response to the trainers. Pyrexia is a reliable early indicator of infection. Rectal temperature is measured at a depth of 15–20 cm. During the times of higher risk (wet season, typhoon season), it is advisable to take rectal temperature twice daily (BID). A temperature increase of half a degree or higher is reported immediately and warrants investigating further. Blood must be immediately collected for analysis and culture when the previously mentioned nonspecific symptoms are detected in a cetacean during the wet season or shortly after a heavy rainfall in an area with a high incidence of melioidosis. When melioidosis is suspected at presentation, ceftazidime at 30 mg/kg is administered intravenously immediately after blood collection. All confirmed cases of melioidosis had initial blood pictures showing leukocytosis, neutrophilia, elevated fibrinogen, prolonged erythrocyte sedimentation rate (ESR), and very low iron. Pronounced hypoferemia with values lower than 10 µmol/L and as low as 2 µmol/L are the result of *B. pseudomallei's* secretion of malleobactin, a molecule with a superior ability to sequester iron from circulating blood.[40,41] Other biochemistry changes reflect multiple organ insult due to disseminated septicemia. Fibrinogen, measured on citrated blood, is a very responsive and early marker of inflammation in cetaceans. Later in the disease, hyperferremia may manifest as a result of mixed iatrogenic and bacterial hepatic insult.

The current treatment of the initial acute phase is intensive and differs from the previously reported treatment only in that quinolones are no longer used.[27] The initial therapy consists of intravenous (IV) injections of ceftazidime at 30 mg/kg 3 times daily (TID) for a minimum of 3 weeks, or longer if episodes of pyrexia, leukocytosis, or hypoferremia persist. Seroconversion of IgG may require up to 18 days in dolphins; hence the 3-week duration of the initial therapy. Should ceftazidime be poorly tolerated or contraindicated, meropenem at 20 mg/kg IV TID for 3 weeks could be an alternative. However, this remains theoretical and to date we have not encountered bacterial resistance or dolphin intolerance to ceftazidime. Phlebitis is very likely to occur after administration of IV meropenem.[27]

This initial intensive therapy is followed by 20 weeks of eradication therapy consisting of TID oral administrations (PO) of amoxicillin-clavulanic acid (Clavulox) at 16 mg/kg,

corresponding to 12.8 mg/kg of amoxicillin and 3.2 mg/kg of clavulanic acid. A recent long-acting antibiotic cefovecin (Convenia) was found to be effective in vitro against all the strains of *B. pseudomallei*, clinical or environmental, isolated in Hong Kong Ocean Park (Martelli & Hui, unpublished). Dosing interval for cefovecin at 8 mg/kg subcutaneous (SC) is 17 days.[42] Currently cefovecin is not being considered to treat the acute phase, but SC injections every 17 days offer a tempting alternative to TID PO amoxicillin-clavulanic acid for the eradication phase. More studies are needed (see also pinnipeds later).

Positive response to the treatment is characterized clinically by a rapid return to normal body temperature and a gradual improvement of appetite and demeanor. In cetaceans, daily blood samples to monitor hematology, biochemistry, and fibrinogen are used to assess efficacy of the treatment. Serology using ELISA is performed once a week on the daily serum collected that week to capture the moment of seroconversion. Serology will eventually confirm or refute whether an animal with negative blood cultures had contracted melioidosis. Animals that do not seroconvert after 3 weeks and remain unwell require further work-up.

In two cases the initial leukocytosis was followed by a severe leukopenia and neutropenia. A single intramuscular (IM) injection of granulocyte colony-stimulating factor (G-CSF, Neupogen) at 2 μg/kg led to a normalization of the leukogram.

Supportive treatment includes 2–4 L of water per 100 kg of body weight PO daily and ad hoc nutritional support. Liver supplements including silymarin (Legalon), s-adenosylmethionine (SAM-e), and multivitamins are given for the entire duration of the treatment including the eradication phase.

Three strains of *B. pseudomallei,* ST32, ST37, and ST70, were confirmed to have infected the dolphins in Hong Kong. Infections by ST37 have a better prognosis, with up to 60% survival compared with zero survival for ST32 and ST70 (Martelli & Hui, unpublished).

We believe the next milestone in the treatment of cetacean melioidosis will be the development of a permanent venous access port to avoid repeat injections in the small tail vessels.

Pinnipeds

Clinical presentations of melioidosis in pinnipeds are mostly acute or peracute. Clinical signs are anorexia, lethargy and prostration, lack of response to trainers, and pyrexia. In facilities with high endemicity, unresponsive pinnipeds should be restrained for clinical examination that includes rectal temperature and blood sampling for hematology, biochemistry, and bacterial culture. As a precaution, a suitable antibiotic such as meropenem at 18–20 mg/kg may be administered SQ during the clinical examination. In confirmed cases of melioidosis, blood picture shows leukocytosis, neutrophilia, marked hypoferremia, and increased haptoglobin. In pinnipeds, haptoglobin levels are a more reliable indicator of early inflammation than fibrinogen.[43] Since Kinoshita's previous report,[27] an additional five cases of melioidosis were managed in three California sea lions *(Zalophus californiae)* and two harbor seals *(Phoca vitulina).* Both harbor seals and two Californian sea lions survived. The Californian sea lion that did not survive died within hours of presentation, shortly after blood sampling and the first injection of meropenem. The animal was extremely depressed and pyrexic on presentation. *B. pseudomallei* was detected by IFA on the blood sample and cultured from all organs at necropsy. This was an extraordinarily aggressive form of melioidosis.

The treatment previously described in pinnipeds consisted of SQ injections of meropenem 18.5 mg/kg TID for 2–3 weeks followed by an eradication therapy comprising of four antibiotics.[27] This treatment protocol, although successful in seven of 12 cases, caused many iatrogenic complications such as necrotizing dermatitis, iatrogenic myositis, SQ cellulitis, hematuria, trauma due to restraint, and loss of appetite. Access to a pool had to be restricted to allow repeat captures, something much disliked by the animal and their handlers. Relapses during eradication treatment were not rare and proved fatal in a grey seal *(Halichoerus grypus).* The current revised protocol's most significant addition is the placement under general anesthesia of a central venous access catheter immediately after the decision to treat for melioidosis is made. Through a central line, IV medications, including rehydration fluids, may be easily administered painlessly under voluntary behavior or with only cage restraint. The animal does not need to be confined on land for the duration of treatment, which markedly reduces stress and favors recovery in aquatic species. A central line allows ad libitum blood draws in species for which blood sampling is challenging. This in turn allows a better assessment of the efficacy of the treatment. Central venous catheters may be left in place for months and require minimum care.[44] With a central venous access, the antibiotic of choice is ceftazidime at 30 mg/kg IV TID for 2–4 weeks. The catheter is left in place during the first months of the eradication therapy, allowing blood draws or supplemental rehydration and immediate IV access in case of relapse.

For the eradication therapy the four-drug protocol mentioned previously was abandoned and replaced successfully with PO amoxicillin-clavulanic acid at 16 mg/kg TID for 20 weeks. Alternatively, SQ injections of cefovecin at 4 mg/kg once monthly for 5 months have also achieved successful eradication.[44]

In cases for which placing a central line is not possible, meropenem SQ at 18–20 mg/kg TID for 2–4 weeks is followed by the eradication therapy described previously.

Contrast computed tomography is advised near the end of the treatment or in recalcitrant cases to detect residual abscesses.

Atypical clinical presentations in pinnipeds include a mammary infection that progressed to a fatal septicemia in a California sea lion[27] and a pustular alopecia in a harbor seal.

That adult male seal presented with oscillating pyrexia, capricious appetite, extensive pustular dermatitis, and alopecia. It was diagnosed with bacterial dermatitis related to molting. Treatment with two different courses of antibiotics and topical agents was unsuccessful. Melioidosis was diagnosed 3 weeks after initial presentation based on rising antibodies (ELISA). This seal was treated with SQ cefovecin at 4 mg/kg once a month for 6 months. All symptoms regressed, and recovery was complete. This was an uncharacteristic presentation of melioidosis in a marine mammal.

In harbor seals, 4/4 infections with ST37 resulted in death. Also in harbor seals, 3/3 infections with ST70 survived, whereas 2/2 sea lions infected with ST70 died. Pinnipeds have also become infected with ST660, a strain never isolated from cetaceans kept at the same park. The data on strains are presently too scarce for analysis.

Primates

Melioidosis is virtually undefinable in humans.[40] Symptoms in humans range from benign skin infections to fulminant septicemia, wasting disease, and quiescent infections, although it is commonly thought of as an acute respiratory infection.[40,45] Symptoms of melioidosis in nonhuman primates range from septicemia, to wasting or chronic wounds. Multiple organ infections with disseminated abscesses are the common necropsy findings.[1,2] Differential diagnosis must include tuberculosis and chromobacteriosis.

Great and lesser apes and New and Old World monkeys have contracted melioidosis.[2,46,47] Four of four Western lowland gorillas *(Gorilla gorilla gorilla)* succumbed to melioidosis shortly after their arrival in Singapore in 1983.[48] Two other gorillas were imported to Singapore in 1992. One died of melioidosis 6 months after arrival, and the remaining animal was sent to a European zoo. Respiratory distress was the more evident clinical sign. *B. pseudomallei* was cultured from multiple organs on postmortem. It is interesting to note that gorillas kept in Jakarta (Indonesia) since 2002 have to date not contracted melioidosis (Oh, personal communication). This emphasizes the role of local conditions in the occurrence of melioidosis. Strains reported in primates include ST296, ST612, ST132, ST36, ST 131, and ST109.[47] The human treatments for melioidosis are suitable for primates. An initial IV intensive phase of 2 weeks or longer with ceftazidime 30 mg/kg IV TID or IV meropenem 18.5 mg/kg TID followed by an eradication phase of 12–20 weeks with PO trimethoprim/sulfamethozazole at 25 mg/kg BID or PO amoxicillin-clavulanic acid at 16 mg/kg TID.[49,50] Long-term sedation may assist with the IV treatment phase. Discouraging access to the contaminated soil or water through husbandry and enclosure design helps to minimize contact with the pathogen.

Carnivores

Melioidosis in carnivores may present as septicemia or as localized infections of the skin, limbs, or epididymis or even ocular disease.[51] Multiple small and large creamy abscesses are disseminated throughout the body at necropsy. Felidae, canidae, ursidae, and herpestidae are susceptible.[1,2] Meerkats are reportedly very susceptible to melioidosis in Thailand and in Northern Australia[47,52] yet have never become infected in Singapore (Oh, personal communication) because of different environmental conditions. Treatment is adopted from primates or pinnipeds protocols but with species-specific challenges. Prolonged treatments with cefovecin at 8 mg/kg twice monthly SQ and or trimethoprim/sulfamethozazole 25 mg/kg PO BID or amoxicillin-clavulanic 16 mg/kg PO BID may not treat septicemic states but could be attempted in chronic cases.

Herbivores

Herbivores are regularly affected by melioidosis in endemic countries, presumably because their feeding habits favor soil ingestion and because of the frequency of hoof and interdigital disease in this taxon. The list of published species at risk is long and incomplete.[1,2,47] It is prudent to assume that all herbivores and all marsupials are susceptible to melioidosis. Epidemics have occurred in ruminants and manifest as herds with widespread lameness, limb swelling, wasting disease, and lack of stamina.[1] On necropsy, lung, liver, and spleen abscesses and bacterial cultures will confirm the diagnosis. Equidae may be infected and seroconvert but are overall more resistant, although melioidosis may be fatal.[1]

Treatment of individual herbivores is rarely attempted and could follow any of the treatments previously mentioned. The pasture or exhibit is generally the reservoir of the pathogen and ideally would be abandoned or converted. Application of calcium oxide,[53] good foot care, elevated or hanging food troughs, sound social composition, and good soil drainage may lower exposure to *B. pseudomallei* and reduce the incidence of the disease.

Birds

The list of avian species affected by *B. pseudomallei* is growing and includes macaroni penguins *(Eudyptes chrysolophus)*,[54] Galliformes, ratites, raptors and many psittacines, and Columbiformes.[55] Pathology includes splenic and hepatic abscess, pneumonia, and generalized congestion. The clinical presentation is variable, and birds are considered quite resistant to the disease.[1,55] In endemic areas, differential diagnosis of nonspecific clinical findings such as choanal congestion, radiographic evidence of pulmonary congestion, splenomegaly, and hepatomegaly must include melioidosis. Avian patients may present with only minor or vague complaints such as lameness or ill-doing. Twelve cases of avian melioidosis were diagnosed in Ocean Park between 1998 and 2007. Of those, 10 were presented dead (83%), one died under treatment, and only one recovered fully (8%). Psittacines represented 10 of the 12 cases, indicating either a higher susceptibility of psittacines to the disease

or, more likely, a more attentive husbandry staff for that group of birds.

In 2008 we undertook efforts to improve detection and treatment of avian melioidosis. Between 2008 and 2016, nine cases of avian melioidosis were diagnosed. Three were presented dead (33%); two failed to respond and were euthanized. Four birds recovered fully (44%), a net improvement over the previous decade. The hemogram of avian melioidosis shows pronounced leukocytosis and heterophilia often more than 70,000 or even 90,000 white blood cells (WBCs)/μL and 70% or higher respectively. Affected psittacines demonstrate elevated aspartate transaminase (AST) and creatine kinase (CK) accompanied by initial leukocytosis, heterophilia, and positive serology. These enzymes decrease during treatment in spite of repeated muscle trauma from ceftazidime IM injections. This suggests, but in no way affirms, that elevated WBCs, heterophils, CK, and AST support a suspicion of infection by *B. pseudomallei* in psittacines. Paired serology using MAT confirms an active infection and guides the treatment plan.

The current treatment for systemic avian melioidosis consists of an intensive therapy of 2–4 weeks of IM ceftazidime at 100–150 mg/kg TID. The eradication phase consists of 170 mg/kg of amoxycillin-clavulanic acid PO BID for 20 weeks. Concurrently 20 mg/kg of terbinafine PO once daily (SID) are administered on alternating weeks for 20 weeks to prevent secondary fungal disease from prolonged use of antibiotics.

A localized infection occurred in a Harris hawk presenting with a necrotizing inguinal dermatitis. A pure culture of *B. pseudomallei* (ST70) was grown from the lesion. The animal was successfully treated with IM marbofloxacin 10 mg/kg BID for 2 weeks followed by PO cefuroxime 100 mg/kg SID for 20 weeks. There is clearly still room for more options and efficacy in the diagnosis and treatment of avian melioidosis.

Studies show that wild birds present a low but real risk of dispersing melioidosis.[55]

Other Species

Other species, including reptiles and fish, may become infected or carry the disease.[1,2] Pet iguanas *(Iguana iguana)* were implicated in importing *B. pseudomallei* in California and in the Czech Republic.[56,57]

Conclusion

In conclusion, global increase in awareness has multiplied the number of reports and expanded the known distribution and impact of melioidosis in humans and animals. Management of zoological collections in endemic areas requires dedicated veterinary and husbandry practices. Preventative measures against melioidosis such as species selection, enclosure design, sanitation practices, and specific staff training are important to minimize the impact of melioidosis. There are no effective vaccines against melioidosis to date. Zoo managers in endemic areas must seek access to excellent veterinary resources and build awareness of melioidosis throughout their company and the public.

In Ocean Park Hong Kong, across-the-board institutional awareness resulted in investments that reduced contact with the pathogen and increased diagnostic and therapeutic capabilities. Reducing the interval between case presentation, diagnosis, and treatment of melioidosis markedly improved prognosis and survivability. Previously melioidosis accounted for a third of all marine mammal deaths at Ocean Park,[25] but there has been zero mortality due to melioidosis in cetaceans since 2006. More intensive management, combining behavioral training and advanced vascular access, contributed to achieving zero mortality in pinnipeds since 2008. There has also been progress in avian melioidosis diagnosis and treatments.

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SECTION 10

Aquatic

47

Techniques for Addressing Parasites in Saltwater Aquariums

CLAIRE ERLACHER-REID

Parasitic infections of fish are common and are often secondary opportunistic infections; therefore it is recommended to establish a thorough quarantine protocol (Box 47.1) in efforts to reduce the risks of introducing parasitic infections to an established aquarium system.[1–4] Occasionally, breakthrough parasitic infestations still occur, despite an intensive quarantine protocol, due to parasite life cycle, anatomic location of parasite on affected animal, or treatment failure.[5] Implementing a thorough routine preventative medicine protocol (Box 47.2) may facilitate diagnosis and guide treatment and/or management decisions for parasitic infections of established fish collections (see also Chapter 49).

Diagnostics

Diagnostics should first consist of evaluation of the environment, water quality, and visual observation of the fish. Clinical signs of parasitic infections may be nonspecific and may include the following: abnormal/erratic swimming, flashing (i.e., rubbing body across surfaces), clamped fins (i.e., holding fins close to body), increased respiratory rate and/or piping (i.e., gulping at surface), color changes to skin and/or gills, excessive mucus on skin and/or gills, raised nodules, ulcerative and/or erosive lesions on the skin, loss of scales and/or fins, exophthalmia and/or cloudy eyes, coelomic distension, emaciation, or gross visual observation of large external parasites. The majority of fish diseases can be diagnosed by evaluation of water quality and wet mount examination of tissues with light microscopy.[6] Therefore skin scrapes, fin clips, and gill biopsy wet mount examination with light microscopy should be considered the minimum diagnostic database for most species of fish when presented for a physical examination. To avoid potential parasitic detachment that could decrease the sensitivity of your diagnostic test, when possible, it is best to acquire these samples prior to sedation, while ensuring human and animal safety are still prioritized.[6] When there is high morbidity and mortality in a collection of fish and ante mortem diagnostics have not revealed a definitive diagnosis, humane euthanasia and necropsy on a subset of fish, including wet mount examination of internal organs and histopathology, may be considered a reasonable diagnostic tool. Other diagnostics that may be pursued to evaluate for the presence of parasites in some saltwater fish species include fecal examination, freshwater dip, endoscopy, and coelomic saline flush. Identification of the parasite to species or genus is not always required to determine an initial therapeutic course of action.[6]

Treatment

The sensitivity of skin, fin, and gill wet mount diagnostic examinations may be low in subclinical or minimally affected fish because the acquired sample represents only a small portion of the actual tissue; therefore a negative test result does not ensure that the population of fish examined are truly parasite free.[7] For this reason, prophylactic treatments may be used as part of a quarantine protocol to prevent the introduction of parasites into an established system.[3,4]

Numerous factors must be taken into consideration when deciding on a treatment plan besides the target parasite. Environmental factors (e.g., temperature, salinity, dissolved oxygen, alkalinity) may influence treatment and monitoring. It is important to know, for example, that for each 5 mg/L of formalin added to a system, it depletes 1 mg/L of oxygen.[1] Thus, increasing aeration and closely monitoring dissolved oxygen are essential when treating with formalin, especially at high temperatures. The decision to treat individual fish or an entire aquarium system/population often guides the veterinarian's medication selection and desired route of administration. When oral medications are chosen, the animal's current appetite and the food preference of the species typically guides the formulation selected (e.g., bioencapsulation in brine shrimp, medicated flakes or gel, gruel). The life cycle of the target parasite influences treatment frequency and duration. Oviparous parasitic species usually require multiple repeated treatments and a longer duration of treatment when compared with viviparous parasitic species. Furthermore, it is important to know

• BOX 47.1 Quarantine Considerations for Fish Maintained in Public Display Aquaria

All new incoming fish species should be placed in quarantine for a minimum of 30 days (45–60 days usually recommended for cold water species).

Entrance and Exit Examinations

Entrance and exit examinations on a subset of the population (~2%–5%) should occur within 1 week of quarantine arrival and 1 week prior to quarantine departure. The following criteria should be met when possible during both of those examinations:

- Visual inspection
- Body weight
- Skin scrape (+/– fin clip) wet mount examination
- Gill clip wet mount examination when feasible
- Blood sampling for hematology and plasma biochemistry, especially in moray eel and elasmobranch species (not recommended in fish <8 cm total length)
- Thorough necropsy of all deceased fish when possible including postmortem wet mount samples of skin scrapes, fin clips, gill biopsies, and all internal organs

Treatments

Treatment protocols should be designated by veterinary staff. This may include prophylactic treatment of quarantined fish to prevent parasitic introduction to established systems (e.g., praziquantel, formalin, fenbendazole, copper) and/or specific treatment based on diagnostic examinations.

Medical Records

Written or computerized records should be maintained for each system documenting the following criteria daily to monitor trends during the quarantine period:

- Water quality parameters
- Number of mortalities
- Treatments administered (e.g., dose, duration/frequency, and route)
- Estimated percentage of food intake for the population

Biosecurity

- Fish should ideally be quarantined in an isolated facility located away from collection animals
- Teleost fish, elasmobranchs, and invertebrate species should be quarantined in separate systems by taxa when possible
- There should be designated staff members assigned to work only in the quarantine facility. Alternatively, if that is not possible, collection fish should be handled first prior to employees entering the quarantine facility for the day
- Foot baths should be maintained at all quarantine entryways and exits, and if possible, between systems
- Designated nets and equipment should be assigned to each system. Alternatively, if that is not possible, gloves, hand washing stations, and net disinfection stations should be readily available and easy to access

This information was compiled and summarized from previous published literature on fish quarantine protocols; therefore the reader is encouraged to refer to these references for complete details.[3,4]

• BOX 47.2 Routine Preventative Medicine Considerations for Fish Maintained in Public Display Aquaria

Elasmobranchs

Routine Health Evaluations

At minimum all elasmobranchs should be visually examined by a staff veterinarian annually. When feasible, handling an elasmobranch for a brief physical examination once a year is advised and should be discussed among staff veterinarians and aquarium leadership. The decision to handle should be based on species, human and animal safety, enclosure and ability to catch, available equipment, and available space. If handled for a physical examination, the following sampling criteria should be met when possible:

- Appropriate restraint for select species to ensure both animal and human safety (e.g., tonic clonic immobilization, oxygen narcosis, behavioral or manual restraint in a net or sling, or chemical)
- Body weight and morphometric measurements
- Blood sampling for hematology, plasma chemistries, and point-of-care analyzer (e.g., blood gasses and lactate)
- Skin scrape and gill biopsy wet mount examination when possible
- Ultrasound examination, especially females
- Coelomic wash for species considered susceptible to *Eimeria southwelli* (e.g., cownose rays)
- Radiographs for select species (e.g., sand tiger sharks with suspected spinal deformity)

Teleost Fish

Routine Health Evaluations

At minimum all teleost fish should be visually examined by a staff veterinarian annually. If a fish is handled for any cause, the following minimal physical examination criteria should be performed when feasible:

- Appropriate restraint for select species to ensure both animal and human safety (e.g., manual restraint in a net/sling or chemical)
- Weight and appropriate morphometric measurements
- Skin scrape (+/– fin clip) wet mount examination
- Gill clip wet mount examination when feasible
- Blood sampling for hematology and plasma biochemistry when possible (not recommended in fish <8 cm total length)

the consequences the selected treatment may have on the filtration, microbe community, and plants in the exhibit and species-specific sensitivities to the medication prior to treating. If this knowledge is not known, it is advised to consult with experienced colleagues, treat a smaller number of fish, or use surrogate species and monitor them closely prior to treating an entire system or population to avoid unexpected adverse results.[1]

Treatment for parasitic infections may consist of environmental manipulation (e.g., temperature or salinity changes), the addition of biological control (e.g., cleaner fish), manual removal of parasites, and chemical medications. It is usually recommended to treat the entire aquarium environment, when possible and when applicable, for the best chance of successful therapy.[1] However, once parasites are introduced to an established system, treatment may become problematic, especially when involving large mixed-species aquariums

with complex life support systems. When that does occur, efforts are usually focused on parasitic management by maintaining animal health and appropriate temperature, water quality, filtration, substrate, and stocking density for the housed aquarium species to prevent parasitic flare-ups and subsequent morbidity and mortality. Indefinite hyposalinity (Table 47.1) has been a valuable tool for the successful management of *Neobenedinia* sp. in large mixed-species exhibits containing elasmobranchs and teleost fish. There has also been evidence to suggest that short-term use of hyposalinity, along with temperature increases, may aid in resolution of *Cryptocaryon irritans* infections in large tropical marine exhibits as well (K. Heym, personal communication, March 16, 2017). In cases of parasitic flare-ups in which a specific species is markedly compromised and the entire system cannot be treated, it may become necessary to remove those fish from the environment and treat individually. Manual removal of parasites, short-term dips and baths, medications administered via gastric lavage, and parenteral treatment are often reserved for treating individual fish or anorectic fish. Because parasitic infections are often secondary and opportunistic, it is important to complete a thorough physical examination on heavily parasitized fish to determine if other medications (e.g., antimicrobials) and supportive care are needed in addition to antiparasitic agents.

Challenges and Novel Treatment Considerations

Degradation of Praziquantel and Formalin in a Recirculating System

Research has discovered significant degradation of both praziquantel and formalin treatments in saltwater aquariums when using standard published doses and frequencies.[8,9] Failure to maintain therapeutic concentrations in a system could lead to decreased efficacy, recurrence of pathogen, and possibly the development of resistance.[10] The microbial population within the aquarium system is thought to be the primary contributing factor for the degradation of these treatments. Degradation rates appear to increase with subsequent dosing of these medications, possibly related to increased bacterial activity or bacterial growth that may use these medications as an energy source after the first exposure.

Investigations have demonstrated that an initial dose of praziquantel at 2 mg/L and formalin at 25 mg/L degraded to less than detectable limits in naïve recirculating saltwater systems in approximately 9 days and 14 hours, respectively. The rate of removal for each of those medications continued to increase with subsequent treatments at those same doses.[8,9] Similar degradation has been observed in clinical settings, with praziquantel reaching nondetectable levels in as little as 8 hours in a freshwater aquarium (M. Hyatt, personal communication, February 27, 2017) and in as little as 4–6 hours in a saltwater aquarium (S. Boylan, personal communication, February 27, 2017). These investigations emphasize the significance of monitoring drug concentrations throughout treatment to determine appropriate dosage frequencies and to ensure therapeutic levels are maintained. Removing fish from an experienced system into a naïve system or enclosure for each treatment may help to minimize rapid degradation.

Capsalid Management in a Large Mixed-Species Saltwater Aquarium

Capsaloidea or capsalids, including the genera *Neobenedenia* and *Benedenia*, are large oviparous monopisthocotylean flatworms (approximately 3–10 mm) commonly found in tropical saltwater aquariums.[6] They may infest the skin, gills, and eyes of teleost fish and elasmobranchs, causing flashing, erratic swimming, scale loss, erosions of the skin, and ulcerations of the skin and eyes, and subsequently may lead to death. Diagnosis may be made with gross observation of the parasites, wet mount examination of the skin and gills, or histopathology. The parasites may also easily be recovered from the bottom of a bucket or tank following a freshwater dip, and this method is often used to reduce parasitic loads in heavily parasitized fish prior to further treatments. Capsalids produce many triangular-shaped eggs (more than 80 in a day in some species) that may take 4–21+ days to hatch depending on environmental temperature.[6] These eggs also contain long sticky threads that are used for attachment to fish, substrate, nets, and other objects. For these reasons, they are extremely difficult to eliminate from a system once established, and efforts are usually focused on management to reduce outbreaks.

Chemical treatments are often difficult in large aquarium systems due to cost, volume, differences in species sensitivities, difficulty maintaining therapeutic levels, and bacterial degradation of the medication. Chronic exposure to reduced salinity is thought to reduce egg viability and may be a useful tool in the management of capsalids in large aquarium systems. Indefinite hyposalinity (see Table 47.1) has proven successful at preventing recurrent outbreaks of external monogeneans (*Neobenedenia* sp.) in a 500,000-gallon mixed-species exhibit housing sea turtles, sand tiger sharks *(Carcharias taurus),* nurse sharks *(Ginglymostoma cirratum),* southern stingrays *(Dasyatis americana),* spiny lobsters *(Panulirus argus),* and teleost fish (K. Heym, personal communication, March 16, 2017). Hyposalinity has been maintained in this exhibit since 2011, with no obvious adverse effects observed in the collection species. Only when attempts were made to increase the salinity did outbreaks and resultant morbidity and mortality reoccur. Biological control with cleaner fish may also be a viable solution for reducing parasitic load in large aquarium exhibits.[6]

Leech Infestation in a Large Mixed-Species Saltwater Aquarium

Inadvertent introduction of *Branchellion torpedinis* leeches into closed saltwater aquariums has reported. These marine

Text continued on p. 331

TABLE 47.1 Treatment Protocols for Commonly Diagnosed Parasites in Saltwater Public Aquaria

Type	Examples	Diagnosis	Treatment Options	Comments
Protozoa: Motile Ciliates	*Cryptocaryon irritans*	Wet mount of skin or gills; histopathology	Copper (Cu^{2+}) 0.15–0.2 mg/L of free Cu^{2+} for 3–6 weeks. Tx concentration should be attained slowly over 3 days[6] Formalin: • 25 mg/L (0.025 mL/L) EOD up to 4 weeks[27] • 125–250 mg/L (0.125–0.25 mL/L) for 60 min q 3 days[6,28] Chloroquine diphosphate: • 10 mg/L prolonged bath for 2–3 weeks[27] • 10 mg/L q 5 days for four doses[6] Hyposalinity: • 14–18 g/L for 21–30 days. Salinity should be reduced slowly by 5–10 g/L a day. • 15–18 g/L for 42–56 days along with temperature increases in large aquariums housing elasmobranchs, teleosts, and sea turtles may aid in resolution of infection (K. Heym, personal communication, March 16, 2017) • 10 g/L for 3 h q 3 days for 4 txs[6,27] Transfer fish to clean aquarium between chemical txs or transfer to a clean aquarium q 3 days[6] Temperature manipulations: Peak reproduction occurs between 20°C and 30°C requiring frequent and repeated medication dosing. Reproduction stops at 19°C[6]	Tx typically multimodal requiring addition of hyposalinity and/or transfer to clean aquarium and/or temperature manipulations in conjunction with chemical txs Copper, in the form of copper sulfate, is typically the tx of choice
	Uronemia spp. Scuticociliatosis	Wet mount of skin, gills, or internal organs; histopathology	Metronidazole: • Bath at 50 mg/L daily for 10 days reportedly was successful in treating an outbreak of scuticociliatosis *(Philasterides dicentrarchi)* in Australian Pot-bellied Seahorses[24] • 50 mg/kg PO SID for 7 days followed 30 days later with ponazuril at 10 mg/kg PO SID for 3 days, and repeated in 2 weeks may have been helpful in reducing parasitic load in a zebra shark with subcutaneous scuticociliatosis (S. DiRocco, personal communication, March 2, 2017) Jenoclean (Atacama extract 97% [Zeolites] + citric acid 3%) at 50 mg/L for 30 min to treat scuticociliatosis *(Philasterides dicentrarchi)* in Olive Flounder *(Paralichthys olivaceus)*[29] Hydrogen Peroxide at 50 mg/L for 30 min to treat scuticociliatosis *(Philasterides dicentrarchi)* in Olive Flounder[29] Formalin: • 200 mg/L (0.2 mL/L) formalin bath for 2 h SID for 6 days used in Japanese flounder[6] • 125–250 mg/L (0.125–0.25 mL/L) for 1 h may be helpful to clear early superficial skin infestation[6,28] • 25 mg/L (0.025 mL/L) 24 h bath repeated EOD for 2–3 txs may be helpful to clear superficial skin infestation. May be used as an adjunct to a freshwater bath[6,28] Other: Improve husbandry	When restricted to external surface of fish, organisms are easily treated. When organisms invade deep muscles, internal organs, and blood vessels, there is a poor prognosis[6] *Philasterides dicentrarchi* and potentially *Uronema* spp. reportedly pathogenic in sea dragons and seahorses[23–25]

	Trichodina spp.	Wet mount of skin or gills; histopathology	Improve water quality and husbandry Formalin: • 25 mg/L (0.025 mL/L) 24 h bath repeated EOD for 2–3 txs if needed[6,28] • 125–250 mg/L (0.125–0.25 mL/L) 1-hour bath repeated SID for 2–3 days if needed[6,28] Cu^{2+} at 0.15–0.2 mg/L of free Cu^{2+} until effect[6,28] Others: Freshwater dip/bath	Often an indicator of poor sanitation or overcrowding[30]
	Brooklynella spp.	Wet mount of skin or gills; histopathology	Formalin: • 125–250 mg/L (0.125–0.25 mL/L) 1 h bath repeated SID for 2–3 days if needed[6] • 25 mg/L (0.025 mL/L) 24 h bath repeated EOD for 2–3 txs if needed[6]	Often not susceptible to Cu^{2+}[6]
Protozoa: External Dinoflagellate	*Amyloodinium ocellatum*	Wet mount of skin or gills; histopathology	Cu^{2+} at 0.15–0.2 mg/L prolonged immersion for 2–3 weeks[31] Chloroquine diphosphate at 10 mg/L prolonged immersion once or redose in 7–8 days for 2–4 txs if needed[6] Hydrogen Peroxide at 75 mg/L (0.21 mL of 35% H_2O_2/L) for 30 min, retreat after 6 days and immediately move fish to an uncontaminated system[6] Others: Temperature manipulation, hyposalinity, freshwater baths/dips	Chloroquine recently tx of choice but Cu^{2+} still most widely used Can infest both elasmobranchs and teleosts[6] Clownfish seem to be particularly susceptible to this parasite[30]
Protozoa: External Flagellate	*Ichthyobodo* spp.	Wet mount of skin or gills; histopathology	Formalin: • 25 mg/L (0.025 mL/L) 24 h bath repeated EOD if needed[6,28] • 125–250 mg/L (0.125–0.25 mL/L) 1 h bath repeated SID if needed[6,28] Metronidazole at 40 g/kg of feed at 2% BW per day for 10 days[6] Secnidazole at 20 g/kg of feed per day at 2% BW for at least 2 days[6] Triclabendazole at 40 g/kg of feed per day at 2% BW for at least 5 days[6] Other: Improve husbandry/sanitation	Parasite has a direct life cycle; therefore a single tx is usually effective
Protozoa: Coccidia	*Eimeria southwelli*	Wet mount of coelomic saline flush; histopathology	Cu^{2+} wire particles (50 mg, Copasure) at a dose of 36.7 mg/kg administered PO once appears to be the current tx of choice[16] Toltrazuril at 10 mg/kg/day PO for 5 days may help to control but does not eliminate infection[30]	Associated with morbidity and mortality in cownose rays[14]
Metazoan: Monogeneans	Monopisthocotyleans Gyrodactylidae (viviparous) Dactylogyridae (oviparous) Capsalidae (oviparous) Monocotylidae (oviparous) Polyopisthocotylea (oviparous)	Wet mount of skin or gills; gross visual observation of large monogeneans; histopathology	Praziquantel: • 2–10 mg/L immersion for 3–6 h bath, repeat in 7 days[6,28] • 2–3 mg/L weekly for 4–5 txs used in conjunction with Cu^{2+} tx for oviparous monogeneans (R. George, personal communication, January 27, 2017) • 8 mg/L prolonged immersion for 20 days, redose every fourth day • 20 mg/L immersion for 1.5 h[6] • 100 mg/L bath for 4 min or 20 g/kg of feed q 48 h at 1% BW were both effective in reducing polyopisthocotylea in rockfish[32] • 5 mg/L immersion for 1,800 min (30 h), 10 mg/L immersion for 120 min, or 20 mg/L for 45–90 min reportedly reduces monogenean loads on eagle rays but does not eliminate[12,13] (R. George, personal communication, February 20, 2017)	Oviparous species require multiple repeated txs (≥3) at appropriate intervals and a longer duration of tx when compared with viviparous species Reproductive rate is controlled by temperature (typically faster at warmer temperatures)[6]

Continued

TABLE 47.1 Treatment Protocols for Commonly Diagnosed Parasites in Saltwater Public Aquaria—cont'd

Type	Examples	Diagnosis	Treatment Options	Comments
			• 5 mg/L praziquantel prolonged immersion for 1,800 min (30 h) in a naïve treatment tank, moving animals to a second naïve treatment tank, then treating with 3 mg/L praziquantel q 5 days for 4 to 5 months in conjunction with 10 mg/L chloroquine has been utilized successfully by at least one facility to treat monogeneans in eagle rays such that all life stages ceased to be detected after 2 months of treatment. Chloroquine concentration should begin at 3 mg/L and slowly increase by 2–3 mg/L EOD until a final concentration of 10 mg/L is achieved. No obvious adverse effects were noted in eagle rays or Atlantic stingrays at this facility; however, cownose rays demonstrated changes in swimming behavior during the treatment and were removed. It is also recommended to remove ozone from the treatment tanks because it may react with the chloroquine and cause inappetance or other adverse effects in elasmobranchs (R. George, personal communication, February 20, 2017). • 100 mg/kg BW via oral gavage reportedly reduces monogenean loads in eagle rays but does not eliminate. More research is currently underway[5] • 100 mg/kg BW split into four doses SID for 3 days[6] Cu^{2+} 0.15 mg/L of free Cu^{2+} for 3–4 months to treat oviparous monogeneans. Treatment concentration should be attained slowly over 3 days. Often used as an adjunct to praziquantel (3 mg/L weekly for 4–5 txs). Has been used without adverse effects in cownose rays but has caused inappetence in southern and Atlantic stingrays (R. George, personal communication, January 27, 2017) Hyposalinity: • <20 g/L may reduce egg viability in oviparous species[6] • Indefinite hyposalinity (20–24 ppt) has proven successful at preventing recurrent outbreaks of external monogenean (*Neobenedenia* sp.) parasites in a large mixed species exhibit (500,000 gallons) housing teleosts, elasmobranchs, and sea turtles (K. Heym, personal communication, March 16, 2017) Formalin: • 150–250 mg/L (0.15–0.25 mL/L) bath for 60 min[6,28] • 15–25 mg/L (0.015–0.025 mL/L) prolonged immersion[6,28] Trichlorfon (Dylox): • 2–5 mg/L bath for 60 min[6] • 0.25–1 mg/L prolonged immersion for 2 tx at 3-day intervals for dactylogyrid species.[6] Has been used as an adjunct treatment prior to praziquantel use. Mebendazole: • 100 mg/L bath for 10 min[6] • 1 mg/L prolonged immersion for 24 h[6] Biological control: French angel fish, neon gobies, cleaning gobies, and blue-lined cleaner wrasse pick monogeneans off other fish[6,33] Other: Freshwater dip/bath to reduce parasite load, chloramine T	Praziquantel is generally the tx of choice; however, it is strongly advised to measure drug levels in the water to confirm therapeutic levels are being maintained. This knowledge may then be used to decide the frequency of repeated or redosed txs Capsalids have an affinity for ocular tissue Dacylogyrids and Polyopisthocotylea have an affinity for gill tissue. Gill monogeneans are often more resistant to treatment than skin parasites[6] Dactylogyroidea are most commonly found in freshwater fish

Metazoan: Digeneans	Wet mounts of gill, skeletal muscle, intestinal contents, or other organs; histopathology	Prevention: exclude birds and mammals from contact with fish, disinfect and quarantine, intermediate host usually mollusk Praziquantel: • 1 mg/L immersion for 90 h[6] • 2–10 mg/L for 24 h prolonged immersion[6] • 10 mg/L bath for 1 h[6] • 25 mg/kg BW IM once[6] • 50 mg/kg BW PO once[6] • 330 mg/kg BW PO once[6] Other: Cu^{2+}, Slaked lime, and Bayluscide molluscicides	Most common in freshwater wild fish. Uncommon in aquaria due to the complex life cycle involving a variety of host animals Self-limiting and does not progress in aquariums without exposure to hosts
Metazoan: Cestodes	Wet mount of intestinal content or other internal tissues/ organs; histopathology	Praziquantel: • 5 mg/kg BW PO q 7 days up to 3 txs[28] • 50 mg/kg BW PO once[6] • 2 mg/L bath for 1–3 h, repeat in 1 week if needed[6] Other: Exclude intermediate host contact with fish and water supply	Tx targets adults not larvae Do not feed live foods that might transmit larval cestodes
Metazoan: Nematodes	Wet mount of feces, intestinal contents, or other internal tissues/ organs; histopathology	Fenbendazole: • 2 mg/L once a week for 3 weeks[6,28] • 2.5 mg/g of food daily for 3 days, repeat in 2–3 weeks[28] • 25 mg/kg BW PO for 3 days[6] • 50 mg/kg BW PO once a week for 2 txs[6] Levamisole: • 4 g/kg of food once a week for 3 weeks[28] • 2.5–10 mg/kg BW PO SID for 7 days[28] • 10 mg/kg BW PO once a week for 3 weeks[28] • 10 mg/kg BW PO once, then repeated in 14 days resolved *Huffmanela* sp. lesions in sandbar sharks[34] • 10 mg/kg BW IM once, repeated at 14 and 28 days. Cleared *Huffmanela* sp. egg tracks from a sandbar shark *(Carcharhinus plumbeus)*[34] • 1–2 mg/L immersion for 24 h, repeat in 2–3 weeks[28] • 10 mg/L bath for 3 days[28,30] Other: Piperazine, trichlorphon, and mebendazole. Avoid feeding organisms to fish that may harbor the larvae (e.g., live copepods are a common source)	Tx targets adults, not larvae. Encysted nematodes are difficult to treat Fish may be definitive hosts for adult nematodes or act as an intermediate host or transport for larval nematodes Most nematodes are oviparous, but there are some that are viviparous and some with direct life cycles Ivermectin has been used but is not recommended due to low therapeutic index[30]
Metazoan: Leeches (Annelids)	Gross visual observation or wet mount of skin, gills, and oral cavity samples; histopathology	Trichlorfon (Dylox) at 0.25–0.4 mg/L for a 5–6 h bath was used successfully to treat *Branchellion torpedinis* leeches in elasmobranchs. Repeated tx in 30 days may be needed (A. McDermott, personal communication, January 26, 2017). It is important to remember when calculating the dose that commercial preparations of organophosphates vary in percentage of active ingredients[6]	Premedication with atropine at 0.04–0.08 mg/kg IM is advised (A. McDermott, personal communication, January 26, 2017) Spotted eagle rays anecdotally appear more sensitive to this treatment; therefore, pre-medication with ≥0.06 mg/kg of atropine is highly advised Organophosphates are typically the tx of choice

Continued

TABLE 47.1 Treatment Protocols for Commonly Diagnosed Parasites in Saltwater Public Aquaria—cont'd

Type	Examples	Diagnosis	Treatment Options	Comments
Metazoans: Crustaceans	Copepods Branchiurans Isopods	Gross visual observation or wet mount of gills, skin, or oral cavity samples; histopathology	Diflubenzuron: • 0.01 mg/L immersion for 48 h q 6 days for 3 txs[28] • 0.03 mg/L immersion for *Lernaea* spp.[6] • 75 mg/kg BW PO for 14 days for sea lice[6] Enamectin: • 50 µg/kg BW PO for 7 days for sea lice[6,28] • 2 mg per 100 g of dry gel food, add water, and mix to create a medicated gel. Feed approximately 2% BW for an estimated fish intake of 400 µg/kg/day for 10 days for treatment of copepods (L. Adams, personal communication, March 14, 2017) Trichlorfon: • 15–300 mg/L for 15–60 min at 3–18°C for sea lice[6] • 2–5 mg/L for 60 min for isopods[6] • 0.25–1 mg/L immersion[28] Dichlorvos: • 0.5–2 mg/L for 30–60 min for sea lice[6] • 15 mg/L for 1 min for sea lice[6] Hydrogen Peroxide at 1250 mg/L (1.25 mL of 50% H_2O_2/L) for up to 30 min bath[6] Formalin at 150–250 mg/L (0.15–0.25 mL/L) immersion for 60 min[6,28] Carbaryl (1-napthyl N-methylcarbamate), available as a suspension (Sevin 225 mg/mL) has been used as an alternative to trichlorfon for successful treatment of isopods, branchurians, and copepods in temperate marine fish (<18°C) and freshwater eels: • 0.25–0.35 mg/L for 4–5 days • 0.25–0.35 mg/L for 2 txs at 48 h intervals with water changes in between (L. Adams, personal communication, March 14, 2017) Milbemycin oxime (Interceptor) alone or with spinosad (Triflexis) has been used for treatment of copepods: • 0.5–1 mg/kg PO once • 4 mg/100 g of gel food (0.004%) fed one day (L. Adams, personal communication, January 27, 2017) Other: Manual removal, biological control, and freshwater dip/bath	Diflubenzuron is considered the most effective tx for crustacean parasites[30] Enamectin has been associated with rare neurotoxicity and death when a fish ingests a greater amount of food than anticipated. Reduce the dose for ravenous fish (L. Adams, personal communication, March 14, 2017) It is important to remember when calculating trichlorfon doses that commercial preparations often vary in percentage of active ingredients[6] Carbaryl treatment may cause anorexia and at a dose of 0.5 mg/L has resulted in muscle tetany or spasms in fish but has resolved 24 h following removal of the medication (L. Adams, personal communication, March 14, 2017)

The listed doses have been acquired from both published and anecdotal references and may not be appropriate for all species in all water conditions. Many of the listed treatments are not yet supported with pharmacokinetic data; therefore the reader is encouraged to use caution. If adverse reactions are observed, fish should be removed from the treatment immediately. *BW*, Body weight; *EOD*, every other day; *h*, hour(s); *IM*, intramuscular; *min*, minutes; *PO*, orally; *q*, every; *SID*, once daily; *tx(s)*, treatment(s).

leeches exclusively parasitize elasmobranchs, resulting in ulcerations at attachment sites, lethargy, anorexia, anemia, and potential death in as little as 5 days (A. McDermott, personal communication, January 26, 2017).[6,11] Leeches may also serve as vectors for infectious diseases. Affected species have included sawfish *(Pristis pristis),* guitarfish *(Rhina ancylostoma),* zebra sharks *(Stegostoma fasciatum),* spotted eagle rays *(Aetobatus narinari),* manta rays *(Manta birostris),* southern stingrays, and experimentally yellow stingrays *(Urobatis jamaicensis)* (A. McDermott, personal communication, January 26, 2017).[11] Leeches are most commonly recovered from the claspers, pectoral fins, eyes, oral cavity, and cephalic lobes and appear to remain permanently attached to the host if not removed. Manual removal when leeches are easily accessible is a treatment option, but the process is time consuming. Trichlorfon (Dylox) for a 5- to 6-hour bath has also been used with success; however, it is strongly recommended to premedicate with atropine approximately 45–60 minutes prior to trichlorfon treatment (see Table 47.1). It is also important to remember when calculating the dose that commercial preparations of organophosphates vary in percentage of active ingredients.[6] Leech cocoons will hatch in approximately 30 days; therefore repeated treatment may become necessary at that time.[11] Topical or systemic antibiotic administration and blood transfusions have also been helpful in the supportive care and recovery of the affected host when deemed necessary by the veterinarian. Future studies to establish pharmacokinetic effects and safety for trichlorfon in various species of elasmobranchs are warranted.

Monogeneans in Spotted Eagle Rays *(Aetobatus narinari)*

Decacotyle floridana and *Clemacotyle australis* are monocotylid monogenean parasites that have been associated with morbidity and mortality in spotted eagle rays.[5,12,13] These parasites have been recovered from the skin and gills of spotted eagle rays, and clinical signs have included abnormal swimming postures, bottom resting, and rubbing on the walls of the enclosure.[12] Praziquantel immersion (see Table 47.1) reportedly reduces parasite loads and is often used as a management tool; however, this treatment does not successfully eradicate the parasite, requires repeated therapy and frequent handling, and is further complicated by bacterial degradation of the medication in the water.[12,13] A multistep treatment protocol involving a prolonged immersion of praziquantel in a naïve treatment tank for 30 hours followed later by immersion with chloroquine in combination with praziquantel for 4–5 months in a second naïve treatment tank (see Table 47.1) was utilized successfully by at least one facility to treat monogeneans in eagle rays such that all life stages ceased to be detected. No obvious adverse effects were noted in eagle rays or Atlantic stingrays with this treatment protocol at this facility; however, cownose rays demonstrated changes in swimming behavior during this treatment and were removed. It is also recommended to remove ozone before treatment because it is believed to react with chloroquine and cause inappetance or other adverse effects in elasmobranchs (R. George, personal communication, February 20, 2017). Praziquantel administered via gastric gavage to anesthetized spotted eagle rays has also resulted in dramatic decreases in parasite loads but did not eliminate (see Table 47.1).[5] Research investigating pharmacokinetic data for this oral treatment regimen and various other chemical immersions along with additional management options are currently ongoing.

Eimeria southwelli in Cownose Rays *(Rhinoptera bonasus)*

Eimeria southwelli are apicomplexa coccidia parasites that may be associated with morbidity and mortality in cownose rays at high numbers.[14] In small numbers and in the absence of clinical signs, this parasite might be considered normal flora that may clear over time without treatment.[15] Clinical signs have included discoloration of the skin, emaciation, and death. Diagnosis includes microscopic identification of organisms on a wet mount obtained from a coelomic saline flush. A coelomic flush may be obtained in one of two ways: (1) gently passing a lubricated sterile red rubber catheter (3–5 Fr) attached to a syringe through one of the coelomic pores found on either side of the cloaca or (2) inserting a winged infusion set attached to a syringe (21-gauge, 19-mm needle) into the right ventral paramedian body wall cranial to the pelvic girdle. Sterile 0.9% saline may be infused into the coelomic cavity at 1% of the animal's body weight and aspirated for microscopic examination.[14] The current most successful treatment when clinical signs are apparent appears to be copper wire particles (Copasure) administered orally once (see Table 47.1).[16] No obvious negative effects have been documented to date, and the animals do not appear to excrete copper to detectable levels in their enclosure following treatment (E. Clarke, personal communication, January 26, 2017). Further studies to establish pharmacokinetic effects and safety for copper wire particles in elasmobranchs are needed. Toltrazuril, ponazuril, clindamycin, and sulfadimethoxine treatments administered at various doses, frequencies, and routes have been attempted with inconsistent or inconclusive results. A preliminary study evaluating the use of ponazuril in cownose rays did not achieve blood concentrations considered to be therapeutic in other species, but the parasite load did decrease and there were no negative side effects observed (S. Cassle, personal communication, January 24, 2017). Cloacal prolapse may be a complication of the parasitic infection and/or treatment attempts associated with the infection.[17]

Copper Immersion in Cownose Rays *(Rhinoptera bonasus)*

The use of copper immersion treatment has typically been avoided in elasmobranchs due to intolerance and mortalities

associated with respiratory, osmoregulatory, and ionoregulatory distress.[3,15,18–21] However, it has been used safely as a prolonged immersion in cownose rays for 3–4 months in conjunction with praziquantel prolonged immersion weekly for 4–5 treatments (see Table 47.1) to treat capsalid parasite infestations (R. George, personal communication, January 27, 2017). Closely monitoring copper and praziquantel concentrations in the water is extremely important throughout the course of treatment. Although no adverse effects have been observed in cownose rays, it has been associated with inappetence in southern and Atlantic stingrays *(Dasyatis sabina)* (R. George, personal communication, January 27, 2017). Pharmacokinetic studies to evaluate the effects and safety of copper immersion at various concentrations in elasmobranchs are warranted.

Scuticociliatosis in Sygnathid and Elasmobranch Species

Philasterides dicentrarchi are ciliated protozoa in the subclass Scuticociliatia that have been identified as the cause of disease outbreaks in a range of marine teleost fish in aquarium and aquaculture settings.[22] Severe outbreaks of this parasite have recently been reported in aquarium-maintained Australian pot-bellied seahorses *(Hippocampus abdominalis),* weedy sea dragons *(Phyllopteryx taeniolatus),* and leafy sea dragons *(Phycodurus eques)* and may be associated with stressful environmental conditions (e.g., temperature fluctuations, poor water quality, transport) resulting in compromise of the immune system. Clinically these animals have presented with nodular or ulcerative epidermal lesions, hyperemia or depigmentation, anorexia, lethargy, irregular respirations, abnormal swimming, and/or death. Histopathologic examination revealed ciliate invasion into the gills, dermis, subdermal connective tissues, vasculature, skeletal muscle, ovary, kidney, intestines, thyroid, and/or brain.[23–25] Treatment with a prolonged immersion of metronidazole (see Table 47.1) for 10 days appeared to be successful in treating systemic disease in Australian pot-bellied seahorses; however, knowledge of additional treatment options for sygnathids is lacking in the literature, and further pharmacokinetic research is warranted.[24]

Philasterides dicentrarchi has also been the cause of a rapidly lethal systemic infection in aquarium-maintained zebra sharks, Port Jackson sharks *(Heterodontus portusjacksoni),* and Japanese horn sharks *(Heterodontus japonicus)* characterized by necrotizing hepatitis, meningoencephalitis, and/or thrombosing branchitis. Clinical signs prior to death were brief and included lethargy, anorexia, and/or behavioral abnormalities.[22] Ciliate infections resembling scuticociliates have also been identified in swell sharks *(Cephaloscyllium ventriosum),* dusky smooth-hounds *(Mustelus canis),* southern stingrays, and California bat rays *(Myliobatis californica)* (C. Erlacher-Reid, unpublished, 2016).[22,26] These infections have varied in severity, ranging from external infections of the gills and skin, along with bacterial, flagellate, or viral infections, to deep invasive lesions involving the kidney, brain, and/or liver. It is thought that this disease may have been underreported or unrecognized in elasmobranchs until now or is an emerging disease.[22] Systemic invasion has been associated with a poor prognosis. To date, there are no known effective treatment protocols published in elasmobranchs. However, treatment with oral metronidazole followed a month later with oral ponazuril may have been helpful in clearing or reducing parasitic load in a zebra shark with subcutaneous scuticociliatosis (see Table 47.1). Follow-up on the case to monitor for recurrence is currently ongoing (S. DiRocco, personal communication, March 2, 2017).

Summary

Prevention and early diagnosis of parasitic infections are recommended by establishing a thorough quarantine and preventative medicine protocol whenever possible. There are a multitude of published and unpublished protocols that have been used for the treatment and management of parasitic infections in fish with variable success; therefore the reader is strongly encouraged to use caution and perform a thorough literature search and discuss treatment options with experienced colleagues to select the treatment protocol that is best suited for each specific situation (see Table 47.1). There are numerous considerations that should be addressed prior to and during treatment, including potential species-specific sensitivities, life cycle of the parasite, and the relationship between and among water quality parameters, parasites, fish, and treatments. The reader is encouraged to monitor drug concentrations throughout treatment to determine appropriate dosage frequencies and ensure therapeutic levels are maintained. Further pharmacokinetic research evaluating the effects and safety of antiparasitic medications in various species of fish are greatly needed in the available peer-reviewed literature. The World Association for the Advancement of Veterinary Parasitology (WAAVP) has created guidelines for testing the efficacy of ectoparasiticides in finfish, and this may serve as a useful resource for designing meaningful studies.[35]

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Touch-Pools: The Other Side of the Hand

CATHERINE HADFIELD AND KATHRYN A. TUXBURY

Introduction

Zoos and aquariums are striving to provide immersive guest experiences to foster excitement about animals and conservation. Touch-pools give guests unique opportunities to interact with fish and aquatic invertebrates through close observation and touch. Compared with display-only habitats, touch-pools provide greater public engagement, educational opportunities, and physiologic benefits.[1,2] For animals to thrive, it is important that touch-pools are designed to enhance animal welfare and mitigate potential stressors (e.g., handling, noise, visual stimuli, and water contamination). Balancing guest experiences and animal needs can be optimized through providing animals with excellent environmental conditions and ample habitat choices and ensuring that guest experiences are moderated by well-trained staff. The focus of this chapter is on planning and managing new touch-pools and how those processes differ from display-only habitats. Many of these ideas can be applied to established touch-pools.

Setting Goals

Specific goals are necessary for any exhibit but are particularly important for touch-pools. They should be determined before design starts because they help to guide species selection and habitat plans. Animal health and welfare, education, and conservation goals should be included, with specific metrics that can be regularly assessed. At the National Aquarium and the New England Aquarium, the following animal health and welfare goals have been used:

- meet or exceed animal care standards as described by the Association of Zoos and Aquariums (AZA);
- track morbidity and mortality to ensure both are equal to those of nontouch conspecifics;
- have a long-term population plan that is sustainable.

Other potential goals may include:

- guest learning about the species displayed;
- more direct guest interactions with staff;
- increased guest discussion about conservation;
- changing guest behaviors to support conservation goals.

Species Selection

The species and number of animals should be determined early in the design process to optimize the habitat for the species. Factors should be considered within the framework of the animal health and welfare, education, and conservation goals. Some questions that may be used to help guide planning for species and animal numbers are as follows.

- How will the environmental needs be met for all species in the habitat?
- Are the species compatible at the planned stocking density and sex ratios?
- Can animals be individually identified to improve monitoring?
- What breeding activity is likely in the habitat?
- What morbidity and mortality are likely and how will this be mitigated?
- What is the anticipated time on exhibit for each species?
- What is the expected life span?
- Will the species outgrow the habitat and, if so, what is the subsequent management plan?
- Where will the animals be obtained from and how often? Is the sourcing responsible and sustainable?
- Can quarantine needs be met?
- Can holding needs be met to allow for rotation or for medical or breeding management?
- Are the animals likely to show the desired behaviors to meet the education and conservation goals?

Overall, species determined to have stable populations with little turnover are more appropriate for reaching a wide variety of animal health and welfare, education, and conservation goals.

Multiple elasmobranch species are displayed in touch-pools and appear to do well based on anecdotal information, although some of these species will outgrow touch-pools. Species include cownose rays *(Rhinoptera bonasus)*, Atlantic stingrays *(Dasyatis sabina)*, southern stingrays *(Dasyatis americana)*, round rays *(Urobatis halleri)*, yellow stingrays *(Urobatis jamaicensis)*, blue-spotted ribbontail rays *(Taeniura lymma)*, blue-spotted stingrays *(Neotrygon kuhlii)*, little skates *(Leucoraja erinacea)*, juvenile zebra sharks *(Stegostoma*

fasciatus), coral catsharks *(Atelomycterus marmoratus)*, epaulette sharks *(Hemiscyllium ocellatum)*, white-spotted bamboo sharks *(Chiloscyllium plagiosum)*, and brown-banded bamboo sharks *(Chiloscyllium punctatum)*. Teleosts are less common and often limited to sturgeon *(Acipenseridae)* or fish that will not be touched but add visual interest. Invertebrates are common in tide-pool touch habitats. Invertebrates that appear to do well based on anecdotal information include whelks (e.g., *Busycotypus canaliculatus*, *Busycon carica*, *Buccinum undatum*), hermit crabs (e.g., *Pagarus* spp.), Atlantic horseshoe crabs *(Limulus polyphemus)*, limpets (e.g., *Lottia* spp.), and snails (e.g., *Littorina littorea*).

Training and enrichment plans for the species selected should be determined early on because they have a significant impact on animal welfare.[3–5] Valuable training goals for touch-pools include desensitization to touch and positive reinforcement training for touching, target-feeding, and routine husbandry behaviors. Feeding cues and target-feeding out of reach of guests help to reduce feeding-related aggression.[4]

Planning

In the authors' experience, the design, construction, and commissioning of touch-pools often takes longer than display habitats. All groups that are involved in building and managing the habitat should be involved in the planning process, including education and media representatives. Additional time for animal training prior to moving to the habitat, as well as once in the habitat, is important in meeting goals. This can be accomplished by slowly increasing guest numbers in a controlled manner. A soft opening provides a gradual acclimation and time for environmental or management changes. Staff and volunteer training for touch-pools is also time-consuming but is essential for animal care, guest management, and education goals.

The space required by a touch-pool is greater than that required by a display-only habitat because of the habitat shape, additional life-support equipment needs, and guest routing based on peak attendance and anticipated stay-times.

The shape of the habitat must provide refugia, sometimes known as retreat spaces; this is a fundamental welfare requirement.[6] These are spaces that are out of reach of guests and potentially out of sight. They provide the opportunity for each animal to control its interactions with humans. The ideal refugia would be part of the habitat that extends into a space that is separated from the guest space. More commonly, refugia are still in sight of guests but out of direct touch, for example, wide or deep sections (>18 inches or 50 cm) that guests cannot reach, or caves or tunnels (Fig. 48.1). For touch-pools where guests have access on all sides, the habitat should be large enough to provide ample refugia in the center. To maximize the impact of refugia, staff should not catch animals from those areas.

For good animal care and guest interactions, the habitat shape should allow interpreters to have access to the animals and a view of the guests. This may involve bays

• **Figure 48.1** Touch-pools demonstrating wide areas for refugia and bays for staff access (A) and larger system with mangrove décor for additional refugia (B). (A Courtesy National Aquarium; B Courtesy New England Aquarium.)

that extend into the habitat or a central dry space. The habitat shape may create narrow sections; it is important that those sections are wide enough to allow animals to pass through freely. In large touch-pools, interpreters may enter the habitat in waders or wetsuits, although this may be a more stressful scenario for the animals and should always be preceded by a cue.[7] Accessibility and visibility to the public are important factors. Concrete tanks with acrylic windows are preferred because they provide better sound-dampening.[8] Large windows may increase the need for refugia that animals can choose to use.

The design of the interior of the habitat is also important for animal health. Substrate and décor that is suitable for the target species must be included to meet the behavioral needs of the animals. This may include sand, rocks, small coral structures, mangrove roots, and/or sea grasses. Substrate may also reduce noise exposure for resident animals.[8]

The life-support system of a touch-pool often needs to be larger or more complex than a display habitat of a similar volume to manage possible contaminants (e.g., skin oils, lotions, sunscreen, insect repellant, perfume, and human hair). Water turnover must be high (more than once per hour) with excellent surface skimming. The biological

filtration needs to accommodate the maximum animal bioload as well as the possible contaminants, based on peak attendance. In saltwater systems, good foam fractionation is essential. Both ultraviolet light and ozone disinfection are strongly recommended. Where elasmobranchs are in a system using ozone, the levels of nitrate, iodate, and iodide should be monitored to avoid goiters.[9]

It is essential for welfare reasons that animals are provided with a regular, suitable period of full darkness. This may conflict with late night events, custodial needs, and safety lighting requirements, and needs should be discussed prior to construction. To help maintain appropriate temperature, the heating, ventilation, and air-conditioning (HVAC) system must be able to cope with regular changes in guest numbers as well as seasonal temperature and humidity fluctuations.

As with any exhibit, the authors have found that animal health and welfare are improved with the provision of suitable off-exhibit holding space for all the species. However, this may be even more important for a touch-pool where animals may benefit from rotations off-exhibit. Holding space should be in a quiet area close to the touch-pool to minimize transport time. Having the holding space on the same water system as the exhibit allows animals to be transferred without an acclimation, but a water quality issue on exhibit will then affect the holding space. The best option may be a combination of holding space that is on the same water system and holding space that is on a separate water system nearby.

Hand-washing and drying stations at the touch-pool entrance could reduce contamination of the system, but compliance is likely to be low and it is probably more effective to rely on the life-support system. Best practices dictate that hand-washing and drying stations are provided after touch-pools to reduce the risk of zoonotic disease transmission. The zoonotic risk is low, particularly compared with terrestrial animal interactive experiences such as petting zoos.[10] However, with young guests and an increasing proportion of immunosuppressed people, services should always be provided to reduce the risks. Hand-cleaning stations should be easily visible, always available, on an exit route, and accessible at different heights. Signage should be clear and readily visible. It may help to have digital screens to attract guests to the stations. Running water, foaming hand wash, and hot-air or jet-air drying are usually recommended, although hand-sanitizers are associated with greater compliance.[11] Compliance is still poor (typically 30%–60% at petting zoo hand-washing stations) but is improved by verbal reminders by staff at the exit who are actively dispensing hand sanitizer.[11,12]

Contingency planning is essential with any new exhibit. Some scenarios are specific to touch-pools:

- contamination by human bodily fluids (e.g., blood or vomit);
- human injury (e.g., from a stingray barb, an urchin spine, a bite, or a fall);
- a zoonotic question or complaint.

Other scenarios should be considered with any exhibit:

- a catastrophic life-support failure (e.g., pump failure decreasing dissolved oxygen);
- significant morbidity or mortality;
- a visible animal abnormality, which may represent a genuine health concern or a cosmetic issue that guests may notice;
- welfare concerns.

It is extremely helpful to be able to close off a touch-pool area and stop guest access if needed for animal care.

Monitoring of Touch-Pools

Monitoring of touch-pool habitats and animals should be similar to display-only habitats, but closer tracking may be required to ensure that health and welfare standards are met.

Water quality parameters must be assessed regularly. This should include temperature, dissolved oxygen, pH, salinity, nitrogenous wastes, calcium, alkalinity, and hardness. Touch-pools may show fluctuations in dissolved oxygen, temperature, and ammonia based on guest numbers, particularly in shallow areas and after peak guest attendance. In-line monitoring of dissolved oxygen and temperature are helpful to monitor trends, particularly around animal introductions, peak guest attendance, and any changes to the life-support system. Water quality monitoring may include bacterial counts. These are typically run based on indicator bacteria tests (e.g., total and fecal coliform bacteria).[13] These may not represent changes in pathogenic bacteria, but after a baseline is established for a specific system, they can be used to determine the level of disinfection required to maintain the baseline.

Feeding and behavior records should be reviewed regularly, particularly because touch-pool animals are often managed by a wider range of staff than display habitats of a similar size. Feed records, weights, and body condition scores should be used to evaluate feeding needs on a routine basis. Behaviors that should be tracked include bite wounds, mating, or stereotypies. If animals are exhibiting undesirable behaviors, environmental and behavioral modification plans should be instituted quickly. For example, aggression may be reduced by providing increased foraging opportunities. However, individuals or species that are not thriving in a touch-pool and not responding to the changes provided should be transferred to different habitats where they can thrive.

Routine examinations are often indicated to assess health and welfare criteria. This may consist of visual or hands-on examinations with appropriate diagnostic tests. Routine trimming of the venomous barb in stingrays is common in touch-pools. These events can provide unique educational opportunities.

Animal health and welfare criteria should be assessed continuously and reviewed on a routine basis. All touch-pool animals, vertebrate and invertebrate, should do as well as conspecifics in display-only habitats. It is likely that it will take 2–3 years to find the optimal balance of

animal, life-support, and guest dynamics, at which point the population and management should be well established.

Staff and Guest Considerations

Touch-pools are typically maintained by a combination of animal husbandry and guest experience/education staff, some of whom are often volunteers. All staff should have extensive training on the natural history of the various species, conservation and education goals, and common questions that arise. Unusual facts and "juicy questions" encourage guest interaction and may be more effective than scripted talks.[14] Staff must also have a solid understanding of animal touch rules. Instructions are often that animals may be gently touched with two fingers but not moved or picked up. Written signs may help, but their wording is important and signs are not always read.[15,16] Although guests have good intentions, they may adversely affect the animals through lack of direction, lack of experience, and enthusiasm (e.g., moving, manipulating, or grabbing animals). Staff should be trained in effective guest control, with a focus on positive reinforcement of preferred behavior.

Evaluation of the guest metrics is important to determine if the touch-pool goals are being met. This may include guest numbers, time spent at touch-pools, number of interpreter interactions, and guest evaluations.[1,14,17]

Conclusion

The immersive experiences provided by touch-pools can foster excitement about animals and conservation. With good planning and management, touch-pools can meet animal health and welfare, education, and conservation goals. Examples of goals may include meeting or exceeding AZA animal care standards, morbidity and mortality rates equal to nontouch conspecifics, and a sustainable management plan. Animals can thrive and be ambassadors for change.

The authors would like to encourage staff at zoos and aquariums to objectively assess touch-pool habitats and to contribute data to the literature. Studies exist on guest effects on mammals and birds, as well as effects of fish and aquatic invertebrate touch-pools on families.[1,18–20] There are abundant opportunities to study guest effects on health and behavior of fish and aquatic invertebrates, as well as water quality, morbidity and mortality comparisons, and how touch-pools affect us all.

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Sharks and Medicine

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Sharks are common and popular display animals in zoos and aquaria. There are more than 440 known species of shark, with variations in lifestyle and environment. Most species are marine, with few exceptions that are not typically maintained in aquaria.

Among the most common sharks maintained in human care are nurse sharks *(Ginglymostoma cirratum),* sand tiger sharks *(Carcharias taurus),* sandbar sharks *(Carcharhinus plumbeus),* blacktip reef sharks *(Carcharhinus melanopterus),* bonnethead sharks *(Sphyrna tiburo),* leopard sharks *(Triakis semifasciata),* zebra sharks *(Stegostoma fasciatum),* and whitespotted bamboo sharks *(Chiloscyllium plagiosum).* Larger species, such as whale sharks *(Rhincodon typus)* and hammerhead sharks (*Sphyrnidae* spp.), are less frequently maintained but are found at some institutions.[1]

Sharks are in the same class as rays, both of which are characterized by their cartilaginous skeletons. However, there are differences in the approach to medical management between these elasmobranchs. This chapter will focus on the management of shark species commonly housed in aquatic and zoological institutions.

Biology, Anatomy, and Physiology

The basic body plan and anatomy are similar among all shark species. Their skeleton is composed of cartilage instead of bone, and they have small placoid scales covering their bodies. Sharks have a two-chambered heart consisting of an atrium and a ventricle. Blood is carried from the heart to the capillaries in the gills, where oxygen exchange occurs. The branchial arteries distribute the blood throughout the body, and deoxygenated blood returns to the heart.[2]

Most species have five pairs of gill slits. Ventilation is dependent on the lifestyle of the shark. Open water pelagic sharks primarily use ram ventilation in which water is forced over their gills while constantly swimming. Few obligate ram ventilators are kept in aquaria due to the challenge of providing adequate space for continuous swimming in a captive setting. In contrast, benthic species can move water over their gills through buccal pumping. This allows them to rest comfortably on the bottom and tolerate hypoxic environments. Many species are intermediate ventilators and use some combination of ram ventilation and buccal pumping.[2]

Sharks have large livers that comprise up to 25% of an individual's body weight. They have a simple stomach and a short small intestinal tract that empties into the spiral colon (also known as the spiral valve). The large surface area of the spiral colon compensates for the overall shortness of the digestive tract and allows for more efficient nutrient absorption. Sharks have a rectal gland that is located proximal to the cloacal opening and is thought to play a role in sodium balance.[2]

Sharks lack bone marrow and lymph nodes and use alternative hematopoietic organs such as the thymus, spleen, epigonal organ, and Leydig cells. The ability of the epigonal organ and Leydig organ to produce lymphomyeloid tissues is unique to elasmobranchs.[2]

Husbandry and Management

As with all aquatic animals, water quality is of paramount importance to maintaining good health. Parameters such as temperature, pH, salinity, nitrates, nitrite, ammonia, alkalinity, and heavy metal levels may vary between shark taxa and should be thoroughly researched prior to bringing a new species to an institution.

Exhibits need to be designed with both animal and human safety in mind. Ideally an exhibit should be set up so that the animal is accessible and may safely be manipulated or restrained. This allows for management of the individual in case of illness or injury. Mixed species exhibits are possible when size and demeanor of the species to be housed together is taken into consideration.

Restraint

Manual Restraint

Many small- to medium-sized sharks can be manually restrained with adequate facilities and experienced handlers. The goal of any restraint is to minimize stress and ensure the safety of both the animal and the handlers. The handler should wear gloves to protect from the abrasive nature of

their skin. Many sharks respond well to operant conditioning and may be trained to target and voluntarily move into a sling. Small sharks can be held safely. Many sharks go into tonic immobility when placed in dorsal recumbency. This reflex, documented in multiple species, including blacktip reef sharks and leopard sharks, causes the individual to become immobile and allows for safer handling.[3] Although this restraint technique may be useful for nonpainful procedures, it has not been documented to have analgesic effects and in some instances has been linked to hyperesthesia, so additional analgesia should be used when performing painful or invasive procedures.[4]

Because this method of restraint requires that the shark must be physically turned upside down, it is applicable only to individuals and species that are small enough to do so safely.

Larger sharks may be restrained using a shark box by using a sling to remove them from the water and place them in the box. The restrainers then apply firm pressure along the body with one person holding the jaw shut. The box is small enough to restrict movement and allow safe sample collection. It is important for all restraint that there is not a prolonged pursuit because this may lead to elevated lactate and acidemia.[5]

Chemical Restraint

There are many species of shark that are too large or dangerous to manually restrain, and sedation or anesthesia are required. Anesthetics may be administered through immersion bath, injection, or orally, and numerous protocols have been described.[6,7] Equipment may vary depending on species and environment, but a list of commonly used materials is presented in Box 49.1. There is wide variability in species-specific physiology. As a result, the efficacy of any anesthetic may depend on a number of factors, including temperature, metabolism, drug receptors and distribution, and hepatic transformation.[7] A complete review of shark anesthesia is beyond the scope of this chapter, and the reader should consult additional source material for specific protocols.[7]

• BOX 49.1 Common Equipment Needed for Shark Anesthesia

Appropriately sized container: tub, shark box
Stretcher
Tank water for anesthesia and recovery
Methods of ventilation: red rubber tubes, air pumps, catheter tip syringes
Thermometer
pH meter
Dissolved oxygen meter
Air stone
Protective gloves

Physical Examination

A physical examination on a shark should begin from afar by obtaining a thorough history and observing how the animal interacts with its environment and conspecifics. Appetite, attitude, swimming pattern, and evaluation of any external lesions may all be assessed without needing to restrain the individual.

When performing a physical examination, the handler should evaluate the shark from nose to tail. The integument should be assessed for any lesions, cuts, or abrasions. Overall body condition should be evaluated, and if possible a weight should be obtained. Ocular examination may be performed with the use of a light source, slit lamp, or ophthalmoscope. A safe evaluation of the oral cavity may be done by inserting a polyvinyl chloride (PVC) pipe into the oral cavity and using a light source to evaluate the mucosa and teeth. Respiratory rate and effort should be noted by monitoring opercular movement. Auscultation has little utility for monitoring of cardiac parameters, but a Doppler, electrocardiogram (ECG), or ultrasound may be used to obtain heart rate and rhythm. Because ECG clips may be difficult to place on the skin of sharks, leads should be connected to needles and placed in the appropriate locations. Evaluation of the cloaca for any swelling, lesions, discharge, or discoloration should be included in the examination. Measurements are often taken to track growth and condition.

Diagnostics

Blood may be collected from several locations in a shark, depending on the species and the method of restraint. The dorsal sinus and the ventral coccygeal vein are the two most commonly used sites. The ventral coccygeal vein is preferred because blood in the dorsal sinus pools, whereas the blood in the ventral tail vein is actively circulating.[8] When accessing the ventral coccygeal vein, it is important to ensure that the needle is inserted at a 90-degree angle on midline (Fig. 49.1). In many cases the needle needs to be advanced through cartilage prior to reaching the vessel. In larger species a spinal needle may be used. When obtaining blood from the dorsal sinus the needle should be inserted on midline caudal to the dorsal fin at a 45-degree angle (Fig. 49.2).

Blood should always be placed immediately into anticoagulant tubes. In sharks, dry heparin or ethylenediaminetetraacetic acid (EDTA) can both be used; however, EDTA can cause rapid hemolysis in stingray species, so if both species are maintained in a collection it is preferable to use heparin tubes in all cases. If possible, a hemocytometer count should be performed immediately after collection to prevent thrombocyte and white blood cell aggregation. If this is not possible, an aliquot can be preserved in 10% formalin for later evaluation; this maintains cell morphology and prevents thrombocyte aggregation.[9]

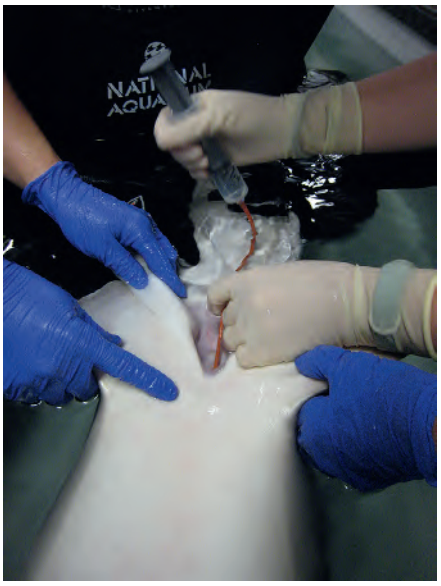
The unique physiologic characteristics of sharks must be taken into account when interpreting blood work results.

• **Figure 49.1** Image of blood collection from the ventral coccygeal vein in a blacktip reef shark *(Carcharhinus melanopterus).*

• **Figure 49.2** Image of blood collection from the dorsal sinus in a sandbar shark *(Carcharhinus plumbeus).*

Marine elasmobranchs retain and reabsorb urea and other solutes, thereby ensuring that plasma remains hyperosmotic to their saline environment.[10] Normal blood urea nitrogen (BUN) should range from 1000 to 1300 mg/dL, and low values may indicate loss from renal disease or decreased production due to hepatic disease. Marine elasmobranchs have efficient salt excretion mechanisms in the kidney and specialized rectal gland that compensate for the influx of sodium (Na) and chloride (Cl) from the environment. However, serum concentrations of these electrolytes are higher than seen in mammals. Differential counts vary by species, but generally there should be 50%–75% lymphocytes, 10%–30% heterophils, 0%–10% eosinophils, 0%–1% basophils, and 0%–3% monocytes.[11] Basophils are rare or absent in many shark species studied. The use of acute phase proteins as markers of inflammation is being explored but has only been investigated in limited species.[12] Reference ranges have not been established in many sharks but Tables 49.1 and 49.2 provide values for some commonly kept species.

Skin scrapes and biopsies may be taken if lesions are detected on examination. It may be difficult to obtain a fecal sample from sharks, but a cloacal wash can be done to obtain a sample. This procedure involves gentle insertion of a lubricated red rubber tube cranially into the cloaca, saline infusion, and gentle suction (Fig. 49.3). This allows for parasite evaluation, as well as provides material to culture if enteritis is suspected.



• **Figure 49.3** Image of cloacal wash being performed to obtain a fecal sample on a blacktip reef shark *(Carcharhinus melanopterus).*

Imaging

The shark skeletal system is composed of calcified cartilage, resulting in excellent radiographic detail, making radiographs a useful tool in evaluating skeletal structures.[11] However, it is often difficult to evaluate soft tissue and organs.[11] When possible, both a dorsoventral and a lateral image should be obtained. The use of contrast medium may be useful in evaluating the gastrointestinal tract but often requires multiple images.[11] Because obtaining radiographs requires removing the shark from the water, there may be increased risk to the animal as well as potential damage to the equipment.

Ultrasound is very useful in assessing organ shape, location, and consistency and is a complement to radiology.[13] It may also be used to monitor gilling and heart rates as well as diagnose pregnancy. It is important to note that sharks have a large, lipid-filled liver, which will appear more hyperechoic compared with mammals.

Advanced imaging, such as computed tomography (CT) or magnetic resonance imaging (MRI), is not typically used in sharks due to their limited availability and complicated logistics. Studies on preserved specimens have revealed that it may be a useful modality to characterize internal structures.[14] Due to logistic challenges, advanced imaging is more often used to evaluate specific lesions of interest and is not generally performed as part of routine scanning.

TABLE 49.1 Hematologic Reference Ranges for Select Shark Species

Parameters	Smooth Dogfish *(Mustelus canis)**	Spiny Dogfish *(Squalus acanthias)*†*	Atlantic Sharpnose *(Rhizoprionodon terraenovae)*†*	Bonnethead *(Sphyrna tiburo)*†*
PCV (%)	20.1–32.1*	21.4–55.9	18.9–30.8	22–35
WBC (per µL)	11,438–25,580	21,400–55,900	34,600–119,600	35,300–83,100
Neutrophils (%)	11.2–21.2	5.17–39.2	0–2.3	1.5–16
Neutrophils (per µL)	2,488–5,035	1,300–18,200	0–2,600	6,700–8,500
Lymphocytes (%)	16.2–39	20–49	20.2–57.1	22.7–55
Lymphocytes (per µL)	3,268–11,162	7,600–23,600	10,400–47,400	10,400–37,500
Monocytes (%)	2.4–8.4	1–8.3	0–8.3	1–7
Monocytes (per µL)	550–1,794	410–3,300	0–6,500	470–4,600
Fine eosinophilic granulocytes (%)	8.1–31.7	5.45–26.8	13.5–38.3	9.75–40.5
Fine eosinophilic granulocytes (per µL)	1,190–7,544	2,000–11,200	5,700–26,800	4,700–19,100
Coarse eosinophilic granulocytes (%)	1.6–5.7	4–24.3	4–26.2	0.75–17
Coarse eosinophilic granulocytes (per µL)	334–1,287	1,100–11,400	2,200–22,700	340–12,100
Granulated thrombocytes (%)	19–36.6	10.7–26	6.5–36	7–39
Granulated thrombocytes (per µL)	3,814–8,836	3,500–12,600	3,700–29,000	3,400–27,500

*Range (took low mean—SD and high mean + SD).
†Persky ME, Williams J, Burks RE, et al: Hematologic, plasma biochemistry, and select nutrient values in captive smooth dogfish *(Mustelus canis)*. *J Zoo Wildl Med* 43:842–851, 2016.
§Haman K, Norton T, Thomas A, et al: Baseline health parameters and species comparisons among free-ranging Atlantic sharpnose *(Rhizoprionodon terraenovae)*, bonnethead *(Sphyrna tiburo)*, and spiny dogfish *(Squalus acanthias)* sharks in Georgia, Florida, and Washington, USA. *J Wildl Dis* 48:295–306, 2012.

Diseases

A variety of bacterial pathogens have been reported in shark species. The bacteria *Tenacibaculum maritimum* has been documented to cause necrotic skin lesions in sand tiger sharks.[15] A Chlamydia-like bacterium was isolated in association with epitheliocystis lesions in a leopard shark.[11] Vibrio, specifically *Vibrio carchariae* and *V. damsela*, have been associated with disease and mortality in multiple shark species, although some, like the lemon shark *(Negaprion brevirostris),* appear to have resistance.[16,17] *Aeromonas salmonicida* has caused hemorrhagic septicemia in blacktip reef sharks.[11] *Flavobacterium* sp. have been isolated from bonnethead sharks in association with neurologic disease.[11] A pyogranulomatous meningoencephalitis of presumed bacterial etiology was found in a basking shark *(Cetorhinus maximus),* but the exact organism was not identified.[11]

Viral diseases are less commonly reported in sharks. Viral erythrocytic necrosis has been noted in dusky smooth-hound *(Mustelus canis)* and leopard sharks. The causative agent is an iridovirus that affects the red blood cells, leading to hemolysis and potentially death. Young animals with no immunity to the virus are most often affected. A viral skin disease, associated with a herpesvirus, has also been reported in dusky smooth-hounds and is characterized by white to gray discoloration of the skin. This often occurs after the animal has undergone a stressful event, but there is no associated systemic disease, and lesions often resolve without intervention.[11]

There are several reported incidences of pathogenic fungal disease in sharks. *Paecilomyces lilacinus* fungal infection was seen in a hammerhead shark, leading to systemic and terminal disease.[18] *Fusarium solani* has been documented in several captive hammerhead sharks, causing mycotic dermatitis and affecting the head and lateral line system, and has also caused fatal disease in juvenile bonnethead sharks.[19,20] Coinfection with *Exophiala pisciphila* and *Mucor circinelloides* in a zebra shark also caused fatal systemic fungal infection.[18] *Exophiala* sp. was detected in a swell shark *(Cephaloscyllium ventriosum)* that displayed abnormal circular swimming patterns and mineralization of the cartilage of the skull and cervical vertebrae.[21]

Numerous parasites have been reported in shark species. The monogenean parasite *Dermophthirius nigrellii* has been found in wild lemon sharks.[22] Another monogenean, *Dionchus penneri,* can cause chronic skin lesions in blacktip reef sharks (see also Chapter 47).[23]

Several cestode species have been documented, including: *Paraorygmatobothrium* spp. tapeworm in lemon sharks, *Pedibothrium* spp. in nurse sharks, *Crossobothrium* spp. in

TABLE 49.2 Biochemical Reference Ranges for Select Shark Species

Parameters	Smooth Dogfish *(Mustelus canis)**	Spiny Dogfish *(Squalus acanthias)*†*	Atlantic Sharpnose *(Rhizoprionodon terraenovae)*†*	Bonnethead Sharks *(Sphyrna tiburo)*†*
Glucose (mg/dL)	91.6–117	28.2–58.0	129–222	165–191
Urea nitrogen (mg/dL)	968–1017			986–1028
Phosphorus (mg/dL)	4.1–6.3	2.8–5.7	5.5–9.6	7.5–10
Calcium (mg/dL)	16.2–17.5	9.3–15.6	15.9–21.7	16.2–17.2
Total protein (g/dL)	2.1–3.4			2.7–3.4
Albumin (g/dL)	0.4–0.6			0.4
Globulin (g/dL)	1.7–2.9			2.3–3
Albumin/globulin ratio	0.2–0.4			0.13–0.17
Aspartate aminotransferase (IU/L)	3.8–17.5	1.7–19	8.2–51.6	33–66
Creatinine kinase (IU/L)	2.2–8.5	BDL	107–626	47–233
Sodium (nmol/L)	251.5–257.4			279–285
Potassium (mmol/L)	3.5–4.8	3.2–4.8	4.9–7.6	6.4–7.9
Chloride (mmol/L)	249.5–260.7			285–296
Bicarbonate (mmol/L)	5.4–13.6			2–4
Anion gap (mmol/L))	0–1.2			–7.6–0.1
Osmolality (mOsm/kg)	833–855.8	699–1,210		1,078–1,111
Creatinine (mg/dL)	<2*	0.1–0.13	0.2–1.1	0.1–0.7
Lactate dehydrogenase (IU/L)	<10*			<5
ALT (U/L)		5.9–21.6	3.5–16.0	BDL
Amylase (U/L)		BDL	584–2,030	812–1,800
Lipase (U/L)		2.7–152	1.5–19.7	8.0–68.3
Cholesterol (mg/dL)		56.5–145	6.8–165.2	75–149
Triglycerides (mg/dL)		20–82.3	BDL	20.2–45.3

*Values too low to measure; out of range. *BDL*, Below detectable range.
†Persky ME, Williams J, Burks RE, et al: Hematologic, plasma biochemistry, and select nutrient values in captive smooth dogfish *(Mustelus canis)*. *J Zoo Wildl Med* 43:842–851, 2016.
§Haman K, Norton T, Thomas A, et al: Baseline health parameters and species comparisons among free-ranging Atlantic sharpnose *(Rhizoprionodon terraenovae)*, bonnethead *(Sphyrna tiburo)*, and spiny dogfish *(Squalus acanthias)* sharks in Georgia, Florida, and Washington, USA. *J Wildl Dis* 48:295–306, 2012.

wild seven gill sharks *(Notorynchus cepedianus), Tetraphyllidean* spp. in the spadenose shark *(Scoliodon laticaudus),* and *Calliobothrium schneiderae* in smooth-hound sharks *(Mustelus lenticulatus)*.[24–27] Nematodes have been shown to cause a parasitic meningoencephalitis in nurse sharks.[28]

Noninfectious diseases of sharks include trauma, foreign body ingestion, neoplasia, and deleterious environmental impacts. Sharks may potentially ingest foreign objects that end up in their enclosures. This is a particular consideration in aquaria where the public has access to the tank. Documentation of neoplastic disease is uncommon in sharks, but oral fibropapillomas, hepatic adenomas, intrahepatic cholangiocarcinoma, testicular mesothelioma, melanoma, and lymphosarcomas have been reported.[11]

Spinal deformities are commonly reported in captive sharks. It has been postulated that some of these deformities could be capture related, particularly when pound nets are used.[29] There have also been correlations with nutritional deficiencies, particularly potassium, zinc, and vitamin C, which play a critical role in cartilage development.[29] In addition, congenital abnormalities of the vertebrae and skeletal system have been documented in several species.[30]

Goiter in association with the addition of ozone to a system has been reported in multiple species. When

aquarium water is ozonated, it reduces the amount of environmental iodine available, which is critical for thyroid hormone synthesis.[31] Urogenital sinus calculi have been reported in a sand tiger shark.[32]

Treatment and Therapies

Pharmacokinetic and pharmacodynamic studies of therapeutics in sharks are limited. Treatment dosages are typically extrapolated from other species. Cefovecin, a third-generation cephalosporin, maintains therapeutic serum concentrations for 4 days in white-spotted bamboo sharks *(Chiloscyllium plagiosum)* at a dose of 8 mg/kg subcutaneously.[33] A single 40 mg/kg intramuscular dose of florfenicol in this species was shown to maintain therapeutic concentrations for 120 hours in serum and 72 hours in cerebrospinal fluid.[34]

In cases of acute trauma and/or severe blood loss, blood transfusions may be performed. Cross-matching should be done prior to any transfusion to ensure that the donor is compatible.[35]

Nutritional support is important in anorectic animals, and patients may be assist fed. In some animals this is done by inserting whole fish into their oral cavity using tongs to encourage eating. If the shark does not respond to this, a fish gruel using their diet and vitamins may be made and tube fed. Gruel is administered by inserting a small piece of PVC into the oral cavity to pass a tube, then advancing the tube past the esophageal sphincter into the stomach. A total of 2%–5% of body weight should be given.

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Decompression Medicine in Aquatic Species (Fish and Sea Turtle Focus)

DANIEL GARCÍA-PÁRRAGA AND JOSÉ LUIS CRESPO-PICAZO

Introduction

Dysbarism refers to any pathologic condition resulting from variation in ambient pressure. In aquatic species, these pressure changes are associated with rapid vertical movements through the water column. There are recognized complications associated with pressure changes in air-breathing vertebrates: barotrauma, decompression sickness (DCS), nitrogen narcosis, and high-pressure nervous syndrome. Of these four, only DCS and barotrauma have been described in aquatic species.

Pressure alters gas-filled spaces through Boyle's law, which states that within a closed, but compressible system, the pressure and volume are inversely proportional.[1] Barotrauma refers to injury caused by the effect that Boyle's law has on gas-filled spaces, causing mechanical distortion and tissue trauma during ascent (through gas expansion) or descent (through gas compression). DCS (also called diver's disease, caisson workers' disease, or the bends) is a collection of clinical symptoms caused by gas bubbles that develop from supersaturated gas in tissues and blood following decompression.[2] Henry's law states that the amount of dissolved gas in a liquid is proportional to the partial pressure of the gas.[1] During descent, the increasing pressure results in increased solubility of gases within the body, and the blood and tissues can absorb greater concentrations of any compressed gas. During ascent, when the blood or tissue gas tension exceeds ambient pressure (supersaturation), gas may come out of solution and form bubbles. Direct effects of intravascular and extravascular bubbles include vascular obstructions and tissue distortion. Secondary effects include endothelial damage, platelet aggregation, activation of the coagulation cascade (leading to a disseminated intravascular coagulation), activation of the complement and immune system, capillary leakage, plasma extravasation, and hemoconcentration.[2–4] In terrestrial mammal models, the risk of DCS correlates with dive depth (pressure), time at depth (dive duration), ascent rate (pressure reduction), temperature, and allometric variation in cardiac output.[4–7]

Conceptually, it is critical to differentiate gas embolism (GE) and DCS; the former involves the presence of any gas bubbles in the tissue or vasculature, whereas DCS by definition is symptomatic GE associated with a decompression event that resolves by recompression therapy. The former has been reported in a range of species, but to our knowledge there is only one report of DCS for marine vertebrates, the loggerhead turtle.[8]

In the present chapter, we will discuss the current knowledge on how to diagnose and treat problems associated with dysbarism in aquatic species, particularly in two vertebrate groups: fish (as gill-breathing representatives) and sea turtles (as breath-hold diving representatives)—most commonly affected as a consequence of interaction with fisheries. Their distinctive anatomy and physiology will alter the potential outcome after a change in pressure.

Decompression Medicine in Sea Turtles

General

DCS in any air-breathing marine vertebrate was first described in the loggerhead sea turtle *(Caretta caretta)* in the Valencian Region (Spain).[8] More recently, GE has also been described in other countries, including Brazil and Italy, and in other sea turtle species, including one leatherback *(Dermochelys coriacea)* accidentally captured in a trawl, one green turtle *(Chelonia mydas)* bycaught in a gillnet, and one Olive Ridley sea turtle *(Lepidochelys olivacea)* in a trawler (Crespo-Picazo, and Parga, personal observation). These findings highlight the potential global effect of decompression disease on all sea turtle species worldwide.

Although the pathophysiologic mechanism of GE in sea turtles is still not completely understood, one hypothesis suggests that fishing net entanglement could interfere with a physiologic mechanism that causes a complete pulmonary shunt preventing gas exchange during diving. Because sea turtles are breath-hold divers, they cannot eliminate the excess solubilized nitrogen through respiration under water.

Recent studies reveal that capture depth and animal size could have a significant impact on the likelihood of DCS.[9] It was further hypothesized that variation in water temperature, the ascent rate, the duration of entrapment, and the type of fishery could all be important to alter the risk of GE.[3,4] However, further research is needed to determine which of these factors are relevant for sea turtles.

From the medical point of view, forced submergence may have many other significant effects on the physiology of sea turtles other than GE and DCS, such as hypoxia, acidosis, electrolyte unbalance, and exertional myopathy, among others.[10–12]

Diagnosis

Diagnosis of DCS includes the animal's history (type of fishing gear, duration the gear was in the water, fishing depth, initial condition and progression through time), external clinical signs, and complementary diagnostic tests. Diagnostic imaging, including ultrasound, radiographs, or computed tomography (CT), is the most practical and reliable resource to accurately diagnose GE (see Chapters 31 and 32).

Clinical History and Symptoms

Turtles suffering from DCS show a narrow range of clinical signs that can be easily overlooked. Valuable information can be obtained from fishermen involved, because progression of animal condition over time after surfacing seems to be especially relevant.

In our experience, bycaught turtles exhibit three main behaviors right after surfacing: (1) apparently normal; (2) hyperexcited with or without neurologic signs that may develop over time (including extended neck and opened mouth, flipper retraction, limb paresis, breathing difficulties, loss of regional nociception or superficial sensitivity, and even full paralysis, depending on localization and amount of gas bubbles); or (3) comatose. GE in various degrees of severity has been diagnosed following all three initial presentations, generally inducing a progression from active to comatose in moderate to severe cases if no specific treatment is applied. Positive buoyancy and erratic swimming have been observed some hours after surfacing in some individuals if returned directly to the water.

Diagnostic Imaging

Combining ultrasound and radiographic examination results in the easiest, quickest, and most complete basic approach for a tentative diagnosis of GE and presumptive DCS in sea turtles. Radiography is the simplest semiquantitative diagnostic tool and the most practical to categorize the severity of GE (Figs. 50.1A–C, 50.2A–C, and 50.3A–C). On the radiographs, dorsal-ventral (DV) and lateral-lateral (LL) projections are recommended to allow a general view of the amount and distribution of gas. Intravascular gas is observed as radiolucency (negative contrast) within or distending the vessels, or even in severe cases the cardiac chambers. Whole body DV projection generally provides sufficient information to classify degree of GE, depending on the amount and distribution of gas. Images representative of each category are summarized in Table 50.1. In mild cases the LL projection of the kidney region is the most sensitive, allowing detection of relatively small amounts of gas. Radiographs from dead bycaught specimens are useful to aid with interpretation of lesions prior to necropsy.

During ultrasound examination, common findings are the presence of gas bubbles in echocardiography and/or renal image and in severe cases, increased free fluid inside the coelomic cavity. Ultrasound is the most sensitive method to determine the presence of gas bubbles because even a small accumulation of gas can be seen in renal vessels as hyperechoic spots generating typical comet tail artifacts (see Figs. 50.1D, 50.2D, and 50.3D). However, ultrasound is less effective than radiography to estimate total volume of pathologic gas and global organic distribution.

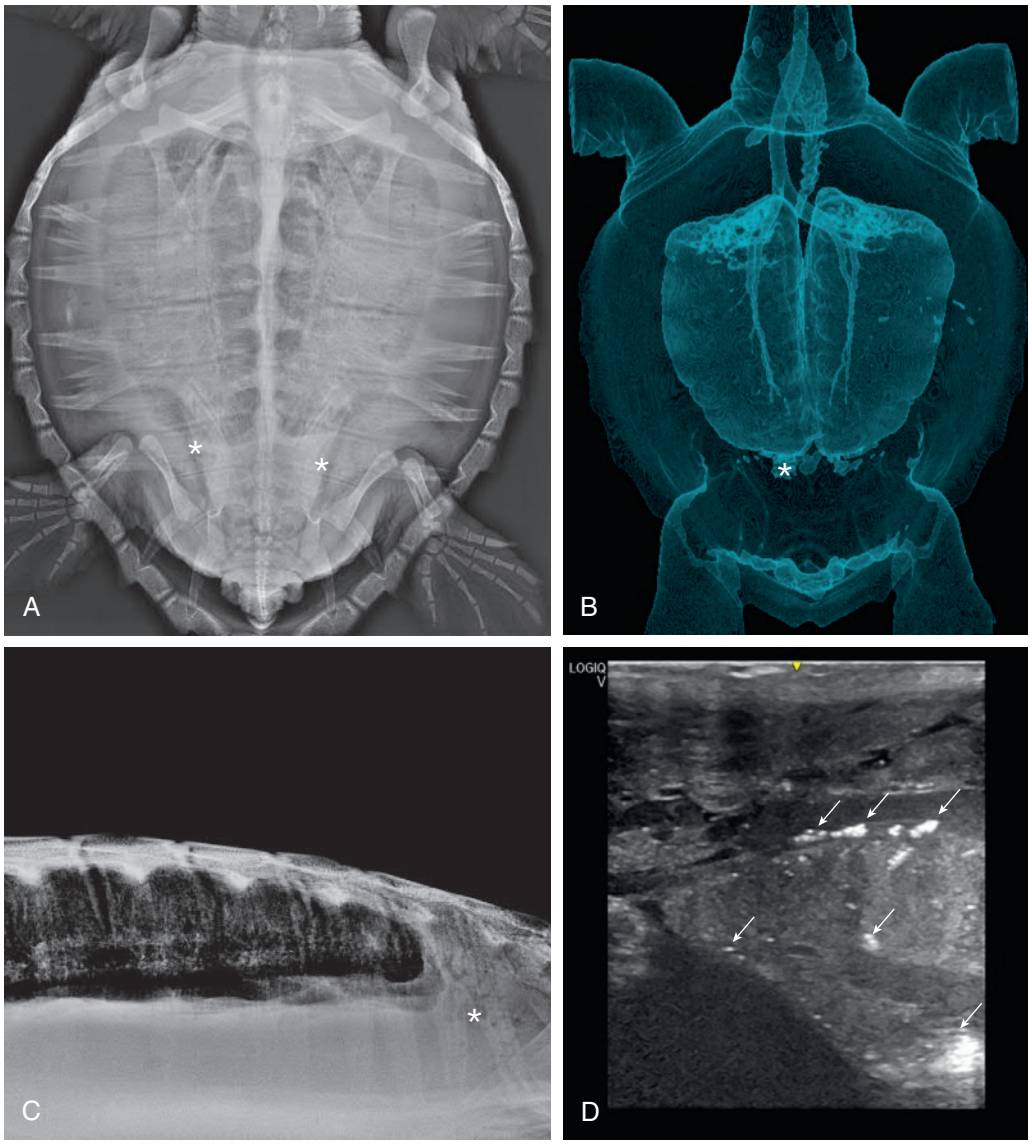
CT scan is the preferred method to quantify and discriminate the presence of gas bubbles in different tissues, but it is often not readily available and generally requires anesthesia or at least sedation in active individuals. The whole body of the turtle should be scanned under maximum resolution and thinnest slice thickness to increase sensitivity and detect minor bubbles. CT images provide additional information about the presence of gas in areas where ultrasound cannot penetrate, such as the parenchyma of the central nervus system (CNS) and inside the vertebral canal. Bubbles in the spinal cord region are frequently observed, even in mildly affected individuals (Fig. 50.4A).

Image datasets of individuals with mild, moderate, and severe decompression are included (see Figs. 50.1, 50.2, and 50.3).

Magnetic resonance imaging (MRI) offers additional prognostic information to help determine tissue damage and presence of sequelae in turtles that survive. Anatomic alterations, lack of tissue circulation/perfusion, or even metabolic activity can provide information about the extent of damage.

Laboratory Profile

The primary acute biochemistry changes detectable in affected individuals with large presence of gas include a moderate to severe increase in uric acid (UA) concentration and a notable electrolyte imbalance (mainly hyponatremia and hyperkalemia) at presentation. Severe elevation in plasma creatine kinase (CK) and moderate elevation in lactate dehydrogenase (LDH) enzyme activities are typically observed over the course of days following treatment. In addition, a left-shifted inflammatory response post treatment is frequently seen. However, it is difficult to discern which alterations correspond to the presence of GE versus the other pathologic conditions that overlap with gas bubble formation during forced submergence.

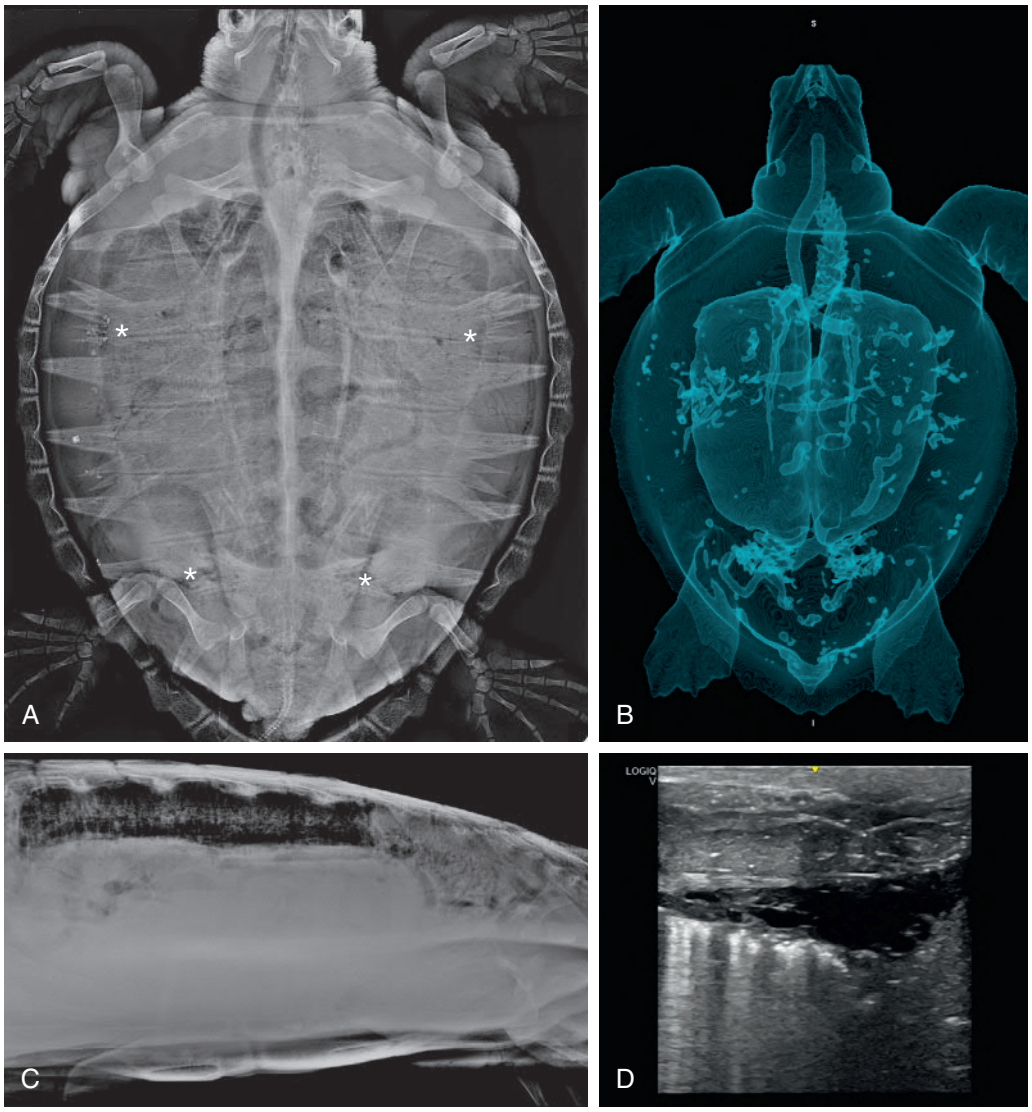


• **Figure 50.1** Imaging findings on admission in loggerhead sea turtles *(Caretta caretta)* with mild gas embolism. (A) Dorsal-ventral radiographic image showing presence of gas as radiolucent angiograms *(black)* on the renal veins *(asterisks)*. (B) Computed tomography dorsal view of three-dimensional air volume–rendering of gas spaces. Small amount of abnormal gas is clearly visualized caudal to pulmonary silhouette. (C) Lateral-lateral radiographic image revealing intravascular gas caudal to lungs. (D) Renal ultrasound showing gas bubbles *(white arrows)* evidenced as hyperechoic spots artifacts dispersed inside kidney parenchyma and renal vessels.

Postmortem Studies

Ideally necropsies should be performed in the first hours postmortem to avoid gas bubble formation from putrefaction. Special attention should be made to minimize gas infiltration artifacts under traction of tissues and section of blood vessels (especially when removing the plastron). In mild cases, gas may be impossible to detect on necropsy but is usually noticeable in moderately and severely affected patients. In these cases, gas often can be grossly visible in the median abdominal, mesenteric, gastric, pancreatic, hepatic, and renal veins, as well as in the cardiac chambers (especially the right atrium), the sinus venosus, the post cava, and other major vessels. In severe cases the spleen can be emphysematous. Externally, the kidneys often exhibit multifocal extensive red areas consistent with marked congestion and acute renal infarcts. Intestinal mucosa often reveals segmental congestive transversal bands from 1–3 cm wide, along different intermediate sections. The lungs of some animals present cranial pulmonary emphysema. Other gross findings include coelomic transudate in severe cases.

Gas sampling and analysis to identify gas bubble composition can be performed according to previously described methods.[13] Gas composition of the bubbles should be consistent with air embolism or gases from decompression, ruling out putrefaction or gas infiltration.[14]



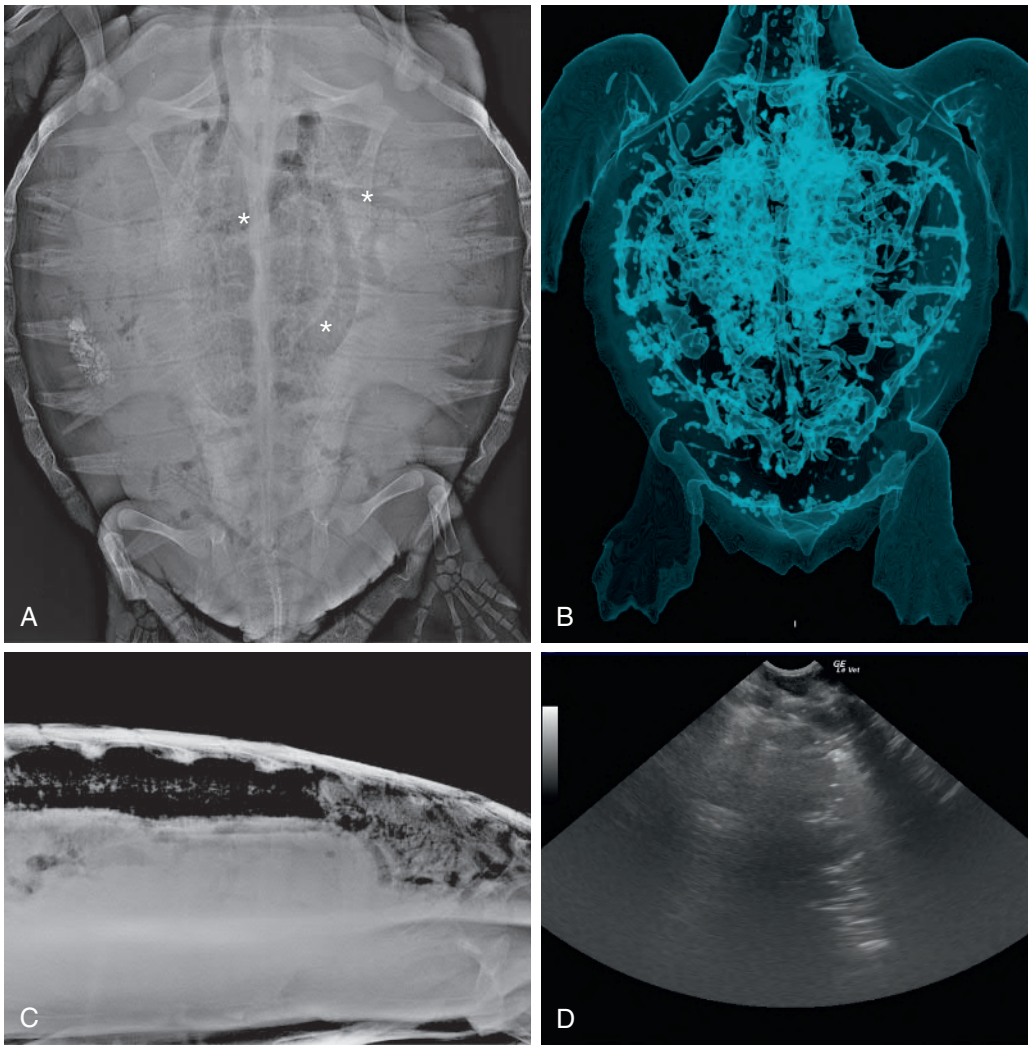
• **Figure 50.2** Imaging findings on admission in loggerhead sea turtles *(Caretta caretta)* with moderate gas embolism. (A) Dorsal-ventral radiographic image clearly showing presence of gas on renal and hepatic veins *(asterisks)*. (B) Computed tomography dorsal view of three-dimensional air volume–rendering of gas spaces. Gas amount is noticeably larger than in mild cases and widely distributed over the kidney and liver parenchyma. (C) Lateral-lateral radiographic image revealing significant intravascular gas caudal to lungs. Lung field extension is limited due to liver gas expansion. Evident gas accumulation is present in the caudal coelom and partially visible ventral to lungs. (D) Renal ultrasound revealing intraluminal gas in renal parenchyma and major vessels evidenced as hyperechoic spots and comet tail artifacts. Note the loss of detail in parenchyma due to gas bubble accumulation and comet tail artifacts in the renal portal vein.

Histopathology typically reveals moderate to severe multisystemic congestion with the presence of intravascular gas bubbles in organs such as the lung, liver, kidney, and heart. In addition, perivascular edema and hemorrhages, varying in extent and severity among the evaluated animals, are also detected in different tissues. Acute, multifocal, myocardial necrosis with vacuolar degeneration of myocytes, alveolar edema, diffuse microvacuolar hepatocellular degeneration, sinusoidal edema, and intrahepatocyte hyaline globules are frequently evident.

Treatment

For moderate to severe cases, hyperbaric oxygen treatment (HBOT) has been used to successfully treat patients (see Fig. 50.4C).

In case of suspect GE, normobaric oxygen should be administered as soon as possible through an endotracheal tube (in unconscious individuals), facemask, or in a closed chamber to mitigate effects of hypoxia and facilitate nitrogen elimination until HBOT is available. Basic



• **Figure 50.3** Imaging findings on admission in loggerhead sea turtles *(Caretta caretta)* with severe gas embolism. (A) Dorsal-ventral radiographic image clearly showing large presence of intravascular gas in cardiovascular system. Large vessels are filled with gas; i.e., postcava, hepatic veins, *(asterisks)*. Lung shape contrast is usually partially reduced or absent. (B) Computed tomography dorsal view of three-dimensional air volume–rendering of gas spaces. Note pulmonary physiologic gas has almost disappeared and gas is distributed following the main cardiovascular circuit. (C) Lateral-lateral radiographic image revealing marked lung compression against the carapace due to the large presence of gas in intracoelomic organs. (D) Renal ultrasound is impeded due to massive presence of intravascular and tissue gas.

conventional emergency treatment[8,15] will help to promote blood circulation and tissue perfusion and to reestablish voluntary respiration. Stimulation of acupuncture point GV26 (nasal philthrum) with a hypodermic needle also helps with resuscitation.[16]

Due to the simple design of some custom-made pressure chambers, animals cannot be artificially ventilated when inside, so it is important to verify that sea turtles are spontaneously breathing before HBOT. If respiration is interrupted, nitrogen elimination will be impeded, possibly resulting in the death of the patient inside the chamber. Nonbreathing severely embolized turtles have undergone brief recompression cycles while intubated. This allows the gas to compress, helping the nitrogen to go back into solution, reestablishing blood flow, and accelerating oxygen diffusion through lungs, leading to successful treatment in some critical cases. Intubated animals should be closely monitored and extubated as soon as they regain consciousness and start breathing voluntarily.

Animals that have been through recompression therapy should be reevaluated using radiography and ultrasound immediately after treatment, to evaluate the outcome of the therapy. In all cases the recompression therapy can be repeated as necessary if some gas is still present.

Human-based recompression protocols were adapted to turtles, based on differences in anatomy and physiology. The time at elevated pressure was prolonged (up to 14–16 hours) using pure oxygen during the entire procedure. At present, an initial pressure of 2.8 ATA (1.8 ATM) is maintained for the first 2 hours. The pressure is then

TABLE 50.1 Criteria for Categorization of Gas Embolism in Live and Recently Dead Animals Based on the Amount and Distribution of Gas Observed on Diagnostic Imaging

Severity of GE	Symptoms	Ultrasound	Simple Radiography	Computed Tomography	Prognosis/ Treatment
Mild	Most commonly asymptomatic. Some signs of pain including flipper retraction or specific neurologic signs could appear with time in some individuals.	Small amount of gas observed in kidney region and sporadic circulating bubbles in cardiac chambers, mainly right atrium	Small amount of gas detectable mainly on LL radiographs, poorly evident in DV projection	Limited amount of gas in kidney region and discrete gas bubble dispersion around the body including spinal cord region	Good. High survival rates but with potential aftereffects. No treatment needed but most probably benefits from oxygen therapy.
Moderate	Frequently hyperexcited at early stages progressing to neurologic impairment or comatose status with time.	Ultrasound images reveal considerable amounts of gas bubbles in kidney vasculature and parenchyma as well as within cardiac chambers, especially the right atrium.	Gas visible in kidneys at LL projection but also disperse on liver and peripheral vessels on DV radiographic projections.	Gas is evident throughout all body, especially accumulating in renal vasculature, liver veins, great vessels (pre and post cava) and cardiac chambers.	Reserved. Poor prognosis without treatment. Ideally HBOT (good success rate if early applied). If hyperbaric chamber is not available, normobaric oxygen should be applied for 24 h.
Severe	Typically comatose cases at admission due to fast progression of symptoms, leading to death in the first hours if untreated. Occasionally, gas bubbles can be observed externally in the anterior segment of the eye.	Ultrasonography is usually impeded by the large amount of gas present in tissues and vessels. If accessible, abundant bubbles and accumulated gas are observed in almost any detectable blood vessel or cardiac chambers.	Gas is very evident in kidney, liver, and major systemic vasculature, and even cardiac chambers can be delineated in DV radiographs being filled with gas. Lung fields are mostly faded out in the DV projection due to partial lung collapse due to liver and major vessels gas expansion.	Lung section is significantly reduced to the top of the carapace, whereas liver veins, spleen, coelomic major vessels and cardiac chambers are almost completely filled with gas (not just bubbles but up to several hundreds of ml of gas). Renal vasculature is also all filled with gas.	Poor. Survival options only if HBOT applied in the first few hours.

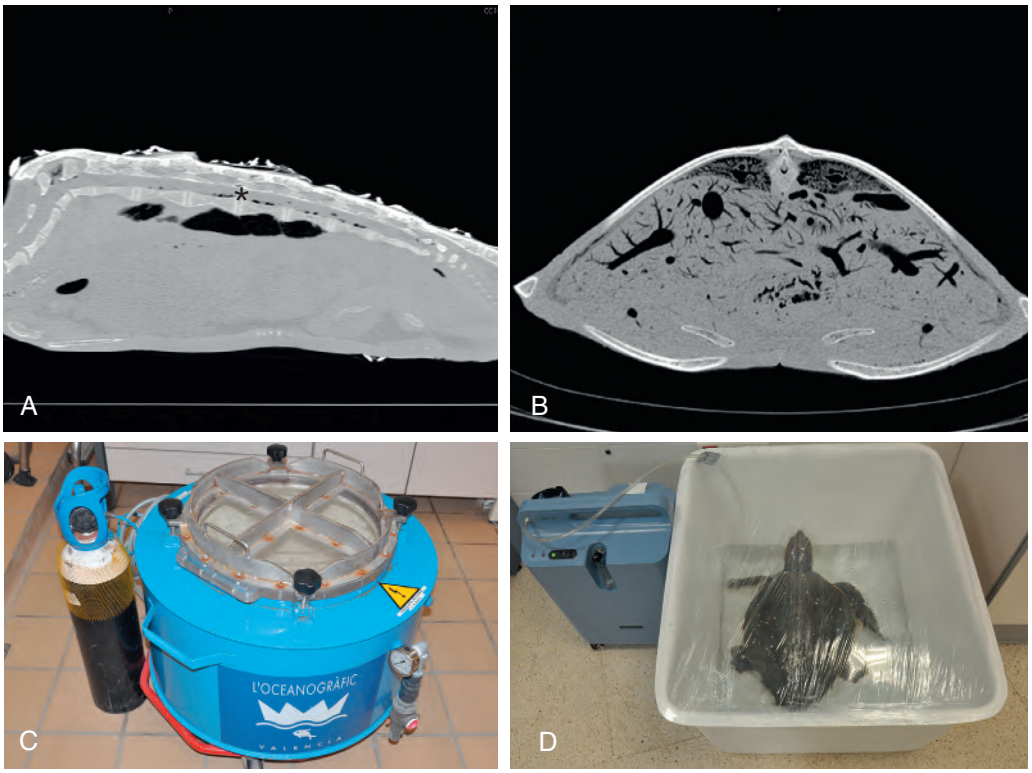
DV, Dorsal-ventral; *GE*, gas embolism; *HBOT*, hyperbaric oxygen treatment; *LL*, lateral-lateral.

gradually decreased over a period of 4 hours to a final pressure of 1.9 ATA, where the pressure is held constant for the next 6 hours. Then the pressure is slowly further reduced back to atmospheric pressure (1 ATA) over a period of 2 hours. It is essential to continuously observe breathing during the entire procedure, especially during periods when the pressure decreases, because breath holding during this time can lead to barotrauma. The current protocol is still experimental and can be modified according to different technical requirements. To maximize safety, it is important to remember that oxygen is flammable and explosive.

If there is not access to a hyperbaric chamber, the patient will benefit from breathing normobaric oxygen. Turtles can be placed in a plastic film–sealed tub connected to an oxygen concentrator partially filled with water up to the level of the nares of the turtle to help improve breathing (see Fig. 50.4D).

In the authors' experience with more than 130 bycaught loggerheads, treatment success mostly depends on severity of clinical signs at arrival, total amount and distribution of intravascular gas, time to HBOT, and evidence of pulmonary water aspiration.

Sublethal effects cannot be ruled out in affected surviving individuals. In fact, cardiac, renal, and CNS damage have been evident on histopathology in some individuals that survived the initial insult.



• **Figure 50.4** (A) Computed tomography (CT) midsagittal image from a loggerhead sea turtle *(Caretta caretta)* suffering from mild gas embolism. Notice that even in mild cases, abnormal gas is detected in spinal cord region *(asterisk)*. (B) CT transverse image of middle-coelom from severe gas embolism. Image reveals severe presence of intravascular gas throughout hepatic parenchyma and enlarged liver volume. Lungs appear dorsally compressed by liver expansion. (C) Custom-made hyperbaric chamber for sea turtle hyperbaric oxygen therapy. (D) Container for alternative treatment with normobaric oxygen by means of a hermetic plastic film sealed basin connected to an oxygen concentrator.

Decompression Medicine in Fish

General

Fish that are hauled to the surface by conventional fishing techniques may suffer from barotrauma and/or GE. The severity depends on depth, speed of ascent, water temperature, swim bladder type, and total gas volume at original depth. The response and outcome of decompression is highly variable between and within species, with physoclists species (which lack a connection between swim bladder and digestive tract) being more severely affected. Hauling fish to the surface at slow or controlled rates does not necessarily reduce the effects of barotrauma, because the bladder can require several hours or even days to adjust (depending on the species).

The authors have even detected the presence of gas bubble formation in tissues and vessels of deep sea sharks (little sleeper sharks *[Somniosus rostratus]*) accidentally bycaught by trawling at 900 m. Gas bubbles in sharks have been noted on CT scan, and associated lesions were also present on necropsy and confirmed on histopathology (García-Parraga, unpublished observation). These animals lack any air cavities and had not been exposed to any supersaturated water, so GE could be formed only through the gas dissolved in tissues coming out of solution during and after ascension. Although we speculate the bubbles are most likely composed of carbon dioxide, the actual composition remains unknown.

The physiopathology of the GE component causing most lesions and external signs associated to a decompressive event in teleost fish is somehow uncertain. However, according to most recent studies, it seems that main lesions, including those involving internal gas bubble formation, are mainly associated to swim bladder hyperinflation and gas leakage through tissues following anatomic less resistance paths instead of a real exolution of dissolved blood/tissue gases as a consequence of supersaturation in association with a pressure drop.[1,17]

Although it seems to be an infrequent finding, GE due to decompression has also been reported in teleost fish transported by airlines.[18]

Although with some similarities in the pathology and therapeutics of dysbaric processes, water supersaturation can also lead to gas bubble formation in fish without being exposed to any environmental pressure changes but generally through increased gas absorption across the gills from supersaturated waters.[19] Supersaturation occurs when the total pressure of gases dissolved in water is higher than the ambient atmospheric pressure. Excess of gases are absorbed

coming out of solution in the bloodstream and forming emboli in different tissues, and in fish most commonly around the eyes, subcutaneous spaces under skin and flippers, or in the gill filaments. This condition is most commonly known as gas bubble disease (GBD) syndrome in aquarium fishes, with cold-water fishes being especially sensitive. Nitrogen is the main problematic gas because oxygen is assimilated metabolically and thus is less likely to form persistent bubbles.[20] Regardless of gas bubble origin (decompression or supersaturation), treatment approach for excess of entrapped gas and/or gas bubble formation can overlap for both processes, with focus on eliminating retained pathologic gas/bubbles.

Diagnosis

Observable signs of barotrauma in fish may include stomach eversion due to swim bladder overexpansion or even rupture, exophthalmia caused by retrobulbar gas emboli, intraocular gas bubble formation potentially visible in the cornea and/or anterior chamber of the eye, and emphysema in dermis especially visible in the fins (Fig. 50.5A, B, and E).[1] Patients are generally positively buoyant and in most cases unable to swim back to depth without intervention. Depending on severity, GE can be detected through diagnostic imaging techniques, mostly ultrasound (see Fig. 50.5D), radiography (see Fig. 50.5C), and CT; or bubbles can be seen in blood vessels of virtually any organ, including skin (see Fig. 50.5B), gills, eyes (see Fig. 50.5A), viscera, and peritoneal cavity during necropsy. Common findings include vascular and cardiac air, swim bladder overdistension or rupture, hematomas, hemorrhages, and potentially internal organ torsion associated with displacement.[21,22]

Treatment

Although prevention is always a better approach than treatment when dealing with decompressed fish, affected patients once diagnosed should be recompressed as soon as possible to minimize tissue damage. Treatment has traditionally been addressed by two means: venting or recompression.

Venting involves the insertion of a hypodermic needle through the body wall to deflate a fish's swim bladder or any air cavity. Major complications of barotrauma can be quickly corrected with relative ease through the use of venting, but the outcome varies depending on the application, fish species, and severity of trauma.[23]

This technique has been widely promoted because of its ease of use for mitigation of acute barotrauma. However, the efficacy has been under intense debate and there are contradictory results from different studies. Some studies indicate that venting the swim bladder at the surface with a syringe and repositioning the stomach when everted, results in only approximately 40%–50% survival of the fish at the surface and an unknown mortality following release. Increased survival has been reported by placing scuba divers at mid depth to vent air bladders of fish coming up a line from depth.[24,25] Some authors point out that there is little evidence that venting increases survival, being equally ineffective for freshwater and marine fishes. In other studies the effects of venting varied with capture depth, with the efficacy decreasing with depth.[26] The available evidence suggests that venting is generally discouraged in favor of recompression therapy. However, some studies still consider venting as an alternative option for barotrauma treatment in some fish species, reaching very high post reintroduction survivorship comparable to recompression therapy if applied rapidly enough.[27] Following venting, an injured swim bladder may not be capable of regulating volume properly, or at best it may require a prolonged recovery period; so although venting can be practical under certain circumstances and conditions where recompression is not feasible, the recommendation at present would be to apply full recompression instead of venting, whenever possible.

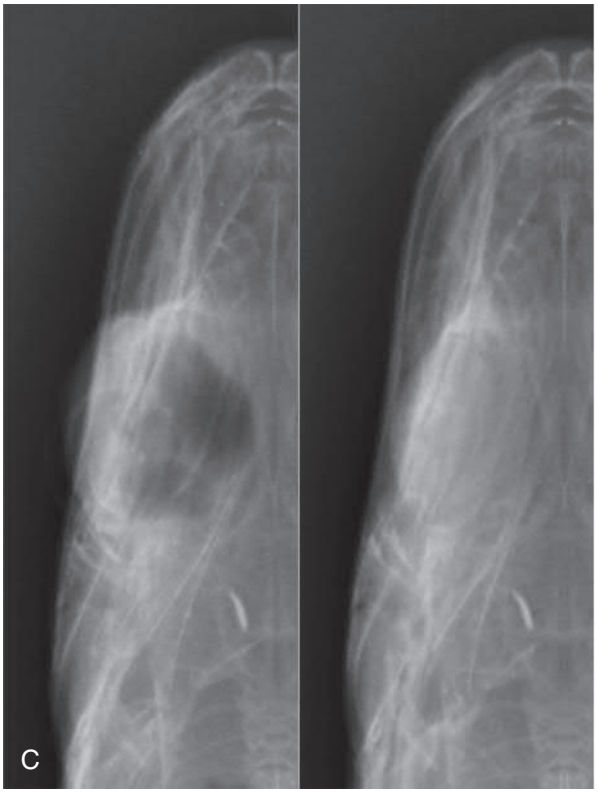
For gas exophthalmia, mechanical removal of the gas from the retrobulbar space or even from inside the eye can be achieved using the appropriate syringe and needle. The needle should be the smallest gauge possible and long enough to reach the gas pocket, which is easily identifiable through radiography or guided by ultrasound (see Fig. 50.5C and D). This intervention should be repeated as required throughout the course of the disease. Ultrasound would typically aid in verifying the gas location within the eye as well as potential damaged eye structures before and after treatment. If gas bubbles in the eye are left untreated, the consequences for the fish could include buphthalmos, the rupture of the globe, atrophy (phthisis bulbi), infection, and even death.

Hyperbaric treatment is another treatment option. Different studies reference that rockfishes showing severe barotrauma can recover if quickly returned back to depth[21,28] or with recompression in hyperbaric chambers.[23,29] Time to recompression seems to be critical, and in some cases, animals can develop temporary or even permanent lesions associated with original barotrauma and GE.

• **Figure 50.5** (A) John Dory fish *(Zeus faver)* showing intraocular bubbles. (B) Subcutaneous bubbles in dorsal fin from John Dory fish. (C) Head dorsal-ventral radiographic comparison from juvenile piper gurnard fish *(Trigla lyra)* with retrobulbar gas compared with normal. Note radiolucent anomaly behind the eye and the exophthalmia on the right x-ray. (D) Ocular ultrasound from two Ballan wrasse *(Labrus bergylta).* Upper image shows normal anatomy with posterior echo reinforcement. Bottom study revealed marked exophthalmia and comet tail artifact behind the eye due to presence of gas. (E) Rosy rockfish *(Sebastes rosaceus)* showing bilateral exophthalmia and corneal gas bubbles, as well as tight body (by swim bladder distension) due to decompression event after being caught around 100 m deep. (Courtesy Bonnie Rogers, California State University). (F) PVC double hyperbaric chamber used by Monterey Bay Aquarium for fish recompression treatment (Courtesy Joe Welsh, Monterey Bay Aquarium).


A


B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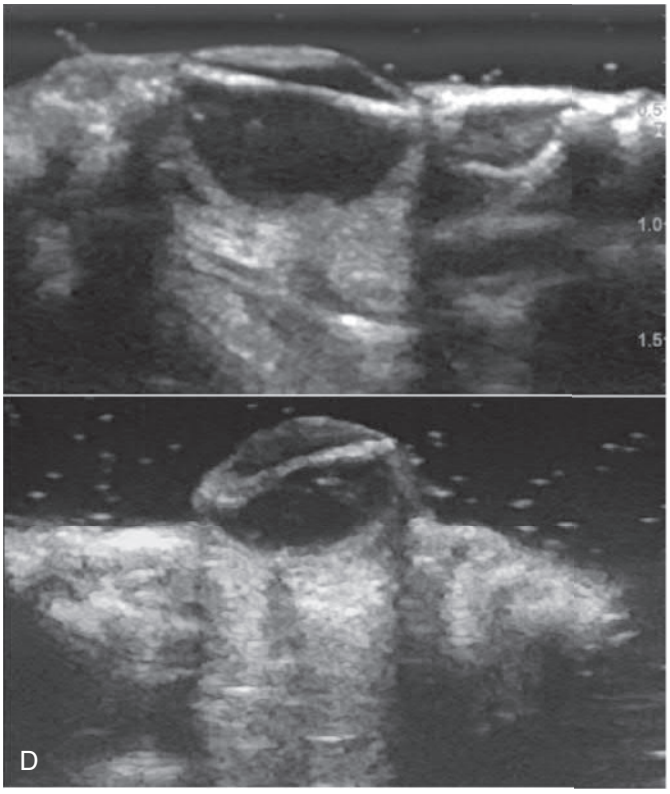
1.0
1.5
D


STAINLESS
HARDENED
E


F

Recompression treatment can be done by two different means: resubmersion of the fish to depth or the use of a hyperbaric chamber.

Resubmersion of fish to depth with remote sinking release devices or adapted cages: bottom cage/trap in the tank/ocean

One study showed that rockfish of the genus *Sebastes sp*. caught at depths near 100 m, and then decompressed but rapidly returned to depth and released, survived several months developing active healthy reproductive organs.[30]

The California Department of Fish and Game, in collaboration with California and Oregon Sea Grant, has published a brochure called "Bring That Rockfish Down" (2008), in which they promote and suggest methods for fishers to sink unwanted or prohibited species back to depth. This brochure also recommends against the venting or gas aspiration technique. The National Oceanic and Atmospheric Administration has held barotrauma workshops and has opened a website (www.fishsmart.org) also encouraging the use of sinking release devices. These devices range from the simple and homemade to commercially available remotely operated or pressure-activated units.[23]

In aquariums, it is not uncommon to put positively buoyant or gas bubble–affected individuals on a cage at the bottom of a deep tank to facilitate gas reabsorption. The trap should be lined with fine mesh synthetic netting to cushion the interior and avoid abrasion. This system of the cage and a weight can also be used in the ocean to send back to depth fished dysbaric individuals, increasing survivorship.

Hyperbaric chambers have been used successfully to recuperate fish suffering from decompression injury when collected at depth, as well as to treat fish suffering with GBD, including gas exophthalmia or gas bubble pockets under skin.

In addition, the Monterey Bay Aquarium (MBA) is one of the most experienced institutions using two types of hyperbaric chambers for treatment of fish:

1. Single Chamber: a single chamber made of stainless steel filled to approximately two-thirds capacity with seawater and then pressurized with oxygen while placed into a refrigerated chamber or on ice to help reduce the water temperature and increase the gas solubility of the water. Depending on the number of fish in the chamber, the chamber can remain pressurized up to 8 hours before gas/water renovation is needed. Hyperbaric oxygen toxicity, although reported for some vertebrate species with pressures over 2.8–3.0 ATA,[31] does not seem to be affecting fish treated in these pressure chambers. The authors have used pressurized oxygen at 4 ATA without any evident deleterious effect in fish, whereas the MBA reports that their standard oxygen pressurization is 7 ATA for 4–8 hours, and they never observed any problem associated to it.
2. Double chamber: a custom-made chamber of polyvinyl chloride (PVC) pipe that includes a staging chamber, being pressurized with a water pump. This double-chambered system contains bypass valves to allow upper chamber pressurize/depressurize cycles, water source changes, or other flow adjustments without disturbing a steady pressure in the main chamber. Because this second model is filled with pressurized water instead of gas, the risk for major accidents is less. For safety reasons, these chambers made of PVC piping should not be used with pressurized gases and especially oxygen.

Both single and double hyperbaric chambers are portable, inexpensive, and work well for fish up to 40 cm.[23]

Pressure in hyperbaric chambers can be adjusted to suit the patient, depending on severity of signs, symptoms, and the kind of chamber available. The animals should be rapidly pressurized until normal buoyancy is restored or they sink due to swim bladder gas compression. Fish are generally initially recompressed at 5 ATM (6 ATA) for approximately 24 hours and then a controlled decompression (0.2–0.7 ATM) every few hours (typically in periods over 8–10 hours). The whole process can take from 3–4 days to more than a week.[23] Fish buoyancy should be controlled during the whole process. In case uncontrolled positive buoyancy is observed, recompression should be reapplied and pressure reduction should be more gradual. If fish are still slightly overbuoyant when taken out of the chamber, a cage can be used to retain the fish for a few more hours at the bottom of the tank until reaching neutral buoyancy.

Hyperbaric treatment is reported to be faster and with decreased probability of relapse than when using the gas aspiration technique or venting. In addition, the prognosis is generally better.

Supportive Medical Treatment

Combination of injectable antibiotics, antiinflammatories, and a carbonic anhydrase inhibitor (acetazolamide) are commonly used as required (there are frequent relapses) on GE/GBD-affected fishes to facilitate recovery until no pathologic gas pockets are present.

Present studies indicate physical injuries, and behavioral impairment associated with dysbarism may compromise fish in the hours and weeks following onset, even with recompression. Potential visual aftereffects in fish that had experienced exophthalmia, buphthalmia, and/or intraocular gas are of concern. There is evidence of optic nerve stretching and visual impairment associated with dysbaric exophthalmia.[32,33] In case of gas exophthalmia, prognosis is better if the gas is located in the retrobulbar space compared with gas bubbles located inside the globe; as in the latter, bubbles can cause significant damage to eye structures, including retinal detachment and complete vision loss.

Acknowledgments

We are grateful to the many excellent colleagues, animal care specialists, technical personnel from local authorities, and students who have enabled us to complete part of the studies mentioned in this chapter. Many thanks to all

professionals who have inspired us and supported our work along these years. Our special gratitude to two main referees of this work, Dr. Andreas Fahlman and Dr. Sally Nofs, who provided constructive criticism to the content of this chapter, improving it significantly. Finally our thanks to Mr. Joe Welsh from MBA, who provided additional comments and references for discussion, as well as a great number of images to be included in this chapter.

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SECTION 11

Amphibians and Reptiles

51

Euthanasia of Ectotherms

GREGORY A. LEWBART

Introduction

Over the past 5 years there have been significant advances in the area of humane treatment and euthanasia of ectothermic animals. This chapter addresses and summarizes these advances and includes four tables with American Veterinary Medical Association (AVMA)-approved and other generally accepted methods of euthanasia.

Some definitions are warranted at this point. Ectotherms are animals that have little internal control of their body temperature and generally are at or close to the ambient environmental temperature. Poikilothermic animals may be ectotherms, and vice versa, but the terms are not synonymous. Poikilotherms are animals with highly variable body temperatures. Many ectotherms are not poikilothermic. For example, tropical reef fish are ectothermic, but because their environmental and body temperatures are fairly constant, they are not poikilotherms. In contrast, marine iguanas (*Amblyrhynchus cristatus*) are both ectothermic and poikilothermic because their daily body temperature may vary widely depending on whether they are feeding in 20°C water or basking on a hot equatorial piece of dark lava rock.

For this chapter the emphasis will be on animals kept as pets, for display, education, and research. Many species of ectotherms are consumed by humans or fed to other animals; however, humane slaughter and pest control are peripheral to this chapter, and details related to these topics are contained in the 2013 AVMA Guidelines for the Euthanasia of Animals.[1]

Invertebrates

Invertebrates (an artificial taxon, in which millions of species share only one negative trait, the lack of a developed vertebral column) comprise more than 95% of the animal kingdom's species. Although there is ongoing debate about an invertebrate animal's ability to "feel pain," and/or detect noxious stimuli, we all should agree that taking a conservative and humane approach to the care of any creature is warranted and expected by society. Recent publications related to invertebrate animal welfare include articles on individual species like the land snail, *Succinea putris*,[2] and thoughtful review articles on the importance and timeliness of humane treatment of invertebrates.[3–12] Several of these articles focus on cephalopods, the most sentient of the molluscs and arguably of all the invertebrates.[10–12]

It is beyond the scope of this chapter to review every published method of euthanasia for invertebrates. Table 51.1 contains most of the commonly used methods, with taxa-specific details and guidance, and references where appropriate.

Fishes

The group of animals commonly referred to as "fish" is a diverse group of approximately 33,500 species.[13] Approximately 96% of these species fall into the division called Teleostei (the bony fishes), but this taxon does not include bony finned fishes such as bowfin, gar, paddlefish, and sturgeon. There are also other distinct groups, including the chimeras, hagfish, lamprey, and of course the elasmobranchs (rays, sharks, and skates). Although we lump these amazingly diverse and somewhat unrelated species into a single artificial taxon (fishes), it is important to keep in mind their diversity, especially when it comes to husbandry, medicine, and euthanasia.

In recent years there has been considerable debate on what constitutes fish welfare and, in fact, whether or not fish can "feel." The "pro" studies and reviews[14–16] outnumber the "con" publications.[17,18] Like most of my colleagues, I believe that fish do feel pain, should be treated humanely, and deserve a humane death when that decision has been made.

There have been a number of articles addressing captive fish welfare and euthanasia that accept the fact that we need to treat fish humanely and with compassion.[19–25] Table 51.2 summarizes the commonly used methods, both pharmaceutical and physical, with comments and references where appropriate. Fig. 51.1 demonstrates the most effective means of delivering a fatal injection to a fish.

Amphibians

The class Amphibia is a diverse group of vertebrates that occupy a variety of habitats and geographic areas. There

TABLE 51.1 Euthanasia Methods for Invertebrates

Invertebrate	Compound/Method	Dosage/ Concentration	Comments	References
Sponges (Porifera)				
	Magnesium chloride	7.5%	This is a two-step euthanasia*	34, 35
Coelenterates				
Jellyfish	Eugenol	120 mg/L	Moon jellies *(Aurelia aurita)*. Two-step euthanasia*	36
	MS-222 (tricaine methanesulfonate); buffered	300–600 mg/L	Moon jellies *(Aurelia aurita)*. Two-step euthanasia*	37, 38
Gastropods				
Terrestrial Snails	Ethanol	5%	Two-step euthanasia*	2
" "	Decarbonated beer	4.74%	Two-step euthanasia*	2
Aquatic snails	Ethanol/menthol (Listerine)	10% in saline	Two-step euthanasia*	39
	Magnesium salts (magnesium chloride or magnesium sulfate)	20%–30%	Two-step euthanasia*	34, 40
Aplysia sp. (sea hares)	Magnesium chloride or magnesium sulfate	7.5% intracoelomic injection	Two-step euthanasia*	41
	Sodium pentobarbital	0.4 mg/mL H_2O	Two-step euthanasia*	42
Abalone	2-phenoxyethanol	0.5–3 mL/L	Two-step euthanasia*	43
	Benzocaine	100 mg/L	Two-step euthanasia*	44
	Magnesium sulfate	4–22 g/100 mL water	Two-step euthanasia*	43
	Sodium pentobarbital	1 mL/L (400 mg/L)	Two-step euthanasia*	42, 45, 46
	2-phenoxyethanol	0.5–3.0 mL/L	*	41
Cephalopods				
	Ethanol	3%–10% in seawater	Two-step euthanasia*	11, 47
	Magnesium chloride	7%–10% in seawater	Two-step euthanasia*	11, 47
Bivalves				
Oysters				
	Magnesium chloride (3.5%)		Variable effects with long induction. Two-step euthanasia*	48–50
	Propylene phenoxetol (1% solution)	1–3 mL/L	Dosage is species dependent. Has been used in *Tridacna* clams. Two-step euthanasia*	49, 50
Scallops				
	Chloral hydrate	4 g/L	Variable effects, temperature dependent*	51
	Magnesium chloride	30–50 g/L immersion	Two-step euthanasia*	51
Annelida				
Leeches	Benzocaine	400 mg/L	*Hirudo medicinalis*. May be suitable for other aquatic annelids*	52
	Ethanol	5%	*Hirudo medicinalis*. As an immersion*	53, 54
Earthworms	Ethanol	5%	*Lumbricus terrestris*. As an immersion*	55

TABLE 51.1 Euthanasia Methods for Invertebrates—cont'd

Invertebrate	Compound/Method	Dosage/ Concentration	Comments	References
Arachnida				
Tarantulas	Alfaxalone	200 mg/kg intracardiac	Combine with: 20 mg/kg ketamine, 5.0 mg/kg morphine, or 20 mg/kg xylazine*	56
Spiders and Scorpions	Carbon dioxide **Isoflurane/Sevoflurane**	3%–5% 3%–5%/4%–6%	Induction chamber system* Induction chamber system*	35 53, 57–59
Crustaceans				
Small crabs; Lobsters	Eugenol (clove oil)	0.125 mL/L 75–100 ppm	*	60, 61
Hermit crabs	Isoflurane			59
Marine crabs	Ketamine	20 mg/kg IV	Reliable and deep anesthesia Add xylazine (20 mg/kg) for greater effects*	62
Crayfish	Ketamine Lidocaine	40–90 mg/kg IM 400–1000 mg/kg IM	* *	63 63
American lobsters	Potassium chloride	1 g/kg (330 mg/mL solution) IV	Inject at base of second walking leg	64
Crabs	Procaine Xylazine	25 mg/kg IV 16–22 mg/kg IV	 *	65 60
Echinoderms				
Sea stars	Magnesium chloride MS-222 (tricaine methanesulfonate); buffered	7.5%–8% 1–10 g/L	* *	66, 67 68
Sea urchins	MS-222; buffered	0.4–0.8 g/L	*	69

*A two-step euthanasia is defined as euthanasia that requires a second method to humanely kill the animal. In most cases the animal has been rendered unconscious by the first step before the second step, which may include a fixative such as 10% neutral buffered formalin or 70% ethanol, is applied. Freezing or physical destruction may also constitute second-step applications.

are currently more than 7600 described species, almost 90% of which are anurans (frogs and toads).[26] The other two orders, Caudata (salamanders) and Gymnophiona (caecilians), contain 9% and 3% of the species, respectively. Most species have a two-stage life cycle and can respire through their skin. This presents different challenges when euthanasia is being considered. For example, euthanasia for a larval form (tadpole) might require a different protocol than a metamorphosed individual (frog or salamander).

Lillywhite et al. provide a detailed review of anesthesia and euthanasia in amphibians.[27] Other recent articles that address these topics include Atwood[28] and Shine et al.[29]

Table 51.3 summarizes the commonly utilized methods, both pharmaceutical and physical, with comments and references where appropriate.

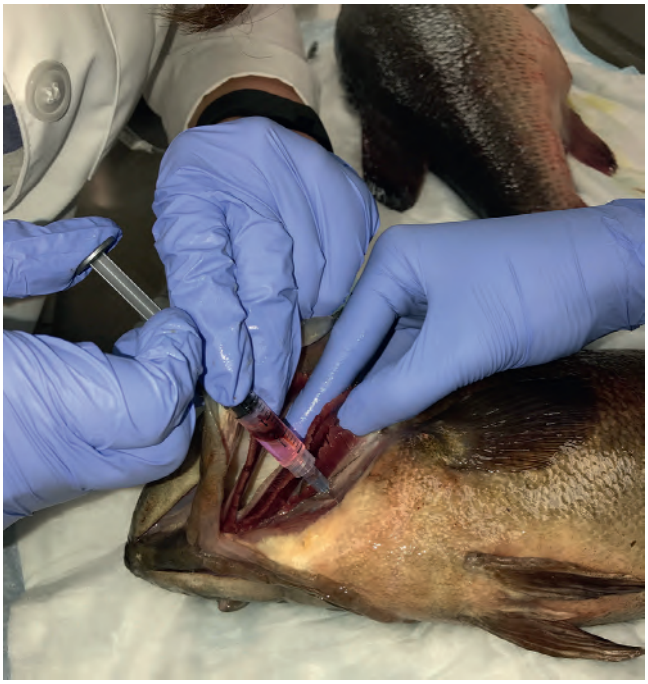
Reptiles

The class Reptilia is broken into 4 orders and 48 families containing 10,450 species, as of August 2016.[30] Taxonomy and nomenclature are dynamic disciplines, so these numbers change frequently and may not be universally accepted by herpetologists. The snakes and the lizards belong in the same order (Squamata) and the turtles, crocodilians, and the

TABLE 51.2 Euthanasia Methods for Fishes

Compound/Method	Dosage/Concentration	Comments	References
Benzocaine	20–250 mg/L immersion until respiration stops.	Higher doses may be used. The solution should be buffered.	1, 19, 20
Carbon dioxide	Bubble into water until respiration stops.	Fish may become hyperactive before losing consciousness. Use in a well-ventilated area*	1, 70
Dexmedetomidine	0.05–0.10 mg/kg IM	Combined with ketamine at a dose of 1–13 mg/kg*	1, 19
Ethanol	10–30 mL of 95%/L as an immersion to effect	*	1
Eugenol (clove oil)	50–150 mg/L to effect	A stock solution is generally made by diluting eugenol 1:9 with 95% ethanol*	1, 19
Ice slurry (rapid cooling)	Rapid immersion in an ice slurry	Not recommended for fish longer than 3.8 cm. Research supports use for juvenile zebrafish	1, 20, 24, 71
Isoflurane	5–20 mL/L	Volatility makes this a risk to humans. Ventilation precautions should be taken*	1
Isoeugenol	60–120 mg/L	Dosing may vary among species*	19
Ketamine	12–88 mg/kg IM	Dosing may vary among species*	1, 19
Pentobarbital	60–100 mg/kg IV, ICe, intracardiac		1
2-phenoxyethanol	200–600 mg/L	Dosing may vary among species.*	1, 19
Propofol	2.5–6.5 mg/kg IV or 5–10 mg/L immersion	*	1, 19

*A two-step euthanasia is defined as euthanasia that requires a second method to humanely kill the animal. In most cases the animal has been rendered unconscious by the first step before the second step, which may include double pithing, decapitation, or injectable pentobarbital.



• **Figure 51.1** The most effective means of delivering a fatal injection to a fish is via the intracardiac route. In this case a largemouth bass *(Micropterus salmoides)* is receiving an injection of pentobarbital after being rendered unconscious with MS-222.

single species of Tuatara each have their own order. With so many taxa, universal methods of euthanasia do not apply. However, many methods do apply to more than one taxonomic group, and Table 51.4 lists the various compounds and methods that are generally accepted or approved in the veterinary medical community.

In addition to the 2013 AVMA Guidelines for the Euthanasia of Animals,[1] some recent articles deal with specific taxa and/or methods. Nevarez et al.[31] performed a detailed study on euthanasia of American alligators (*Alligator mississippiensis*), and Lillywhite et al.[27] report on the merits of hypothermia for small reptile euthanasia. Mader[32] and Music and Strunk[33] emphasize the importance of a two-step euthanasia, especially when an owner or curator is present, and suggest heavy sedation with ketamine, dexmedetomidine, and midazolam; or butorphanol, tiletamine-zolazepam, and dexmedetomidine.

TABLE 51.3 Euthanasia Methods for Amphibians

Compound/Method	Dosage/Concentration	Comments	References
Benzocaine	182 mg/kg	Apply topically to *Xenopus laevis* in a 2 × 1 cm area with 20% benzocaine.	72
Carbon dioxide	Inhalant in a chamber	This is not a recommended method but may be used if followed by a secondary procedure*	1, 28
Hypothermia	Cooling and then freezing	Despite not being a recommended AVMA method, Lillywhite et al. (2017) make a strong case for hypothermia and freezing in some amphibian species	27, 29
Isoflurane	5% in a chamber	Volatility makes this a risk to humans. Ventilation precautions should be taken*	73
Pentobarbital (sodium)	60–100 mg/kg IV, ICe, or in the lymph sacs. 1100 mg/kg with 141 mg/kg sodium phenytoin ICe.	Intracoelomic is the preferred route. This regimen is less preferred because cardiac arrest may take up to 3 h	1, 72, 73
Propofol	60–100 mg/kg ICe		74
Tricaine methanesulfonate (MS-222)	5–10 g/L	Buffering and a secondary method are required*	1, 28, 72, 73

*A two-step euthanasia is defined as euthanasia that requires a second method to humanely kill the animal. In most cases the animal has been rendered unconscious by the first step before the second step, which may include double pithing, decapitation, or injectable pentobarbital.

TABLE 51.4 Euthanasia Methods for Reptiles

Compound/Method	Dosage/Concentration	Comments	References
Captive bolt		Penetrating and nonpenetrating are both effective in alligators	31
Carbon dioxide	Inhalant in a chamber	This is not a recommended method but may be used if followed by a secondary procedure*	1
Hypothermia	Cooling and then freezing	Despite not being a recommended AVMA method, Lillywhite et al. (2017) make a strong case for hypothermia and freezing in some reptile species	27, 29
Isoflurane	5% in a chamber	Volatility makes this a risk to humans. Ventilation precautions should be taken*	73
Pentobarbital (sodium)	60–100 mg/kg IV, ICe, or intracardiac	Sedation with an appropriate injectable (e.g., ketamine/dexmedetomidine; alfaxalone; propofol) or inhalant anesthetic (isoflurane; sevoflurane) is suggested	1, 32, 33, 73
Propofol	60–100 mg/kg ICe		74
Spinal cord severance		This should be followed immediately by pithing of the brain	31
Tricaine methanesulfonate (MS-222)	250–500 mg/L ICe followed by 0.1–1.0 mL 50% MS-222 solution ICe	Buffering is recommended	75

*A two-step euthanasia is defined as euthanasia that requires a second method to humanely kill the animal. In most cases the animal has been rendered unconscious by the first step before the second step, which may include double pithing, decapitation, or injectable pentobarbital.

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52

Ranaviral Disease in Reptiles and Amphibians

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Wildlife diseases have been on the rise across the world.[1] Although some of these diseases receive a great deal of attention, such as chytridiomycosis in worldwide amphibian declines, white-nose syndrome in North American bats (see also Chapter 72), West Nile virus in birds, and chronic wasting disease in cervids,[1–3] others are poorly understood and require further study (see also Chapter 39). Disease events may have a dramatic impact on local populations, becoming more critical as population size decreases.[1] One such fatal disease in amphibians and reptiles results from viruses in the family Iridoviridae. Infections and death have been reported in numerous species, contributing to population declines.

The family Iridoviridae consists of two subfamilies and five genera (Table 52.1).[4] They are large, icosahedral, DNA viruses that may be found in an enveloped or nonenveloped form. Diseases caused by viruses in the genus *Ranavirus* are a growing concern in free-ranging and captive amphibian and reptile populations.[5] These viruses are found worldwide, cause significant morbidity and mortality in affected species,[5] and were placed on the World Organisation for Animal Health (OIE) list of reportable diseases for amphibians.[6] Detailed information on taxonomy, molecular characteristics, surveillance techniques, and ecology is available elsewhere.[7]

Geographic and Host Distribution

The discovery of frog virus 3 (FV3) was first reported in leopard frogs *(Rana pipiens)* being studied for Lucke renal adenocarcinomas in the 1960s.[8] Since that time, numerous outbreaks have occurred in both free-ranging and captive herpetofauna across the world.[5,9] Outbreaks have occurred throughout the world, including North America (Arizona, Colorado, Florida, Georgia, Idaho, Kentucky, Maine, Massachusetts, Minnesota, New Hampshire, New York, North Carolina, North Dakota, Pennsylvania, Tennessee, Texas, Utah, Wyoming, Saskatchewan, Ontario), South America (Brazil, Uruguay, Venezuela), Europe (Croatia, Denmark, Germany, Netherlands, Spain, United Kingdom), Asia (China, Japan), and Australia.[5]

Various ranaviruses account for epizootics, including FV3, *Ambystoma tigrinum* virus (ATV), soft-shelled turtle iridovirus (STIV), and Bohle iridovirus (BIV). FV3 is the only iridovirus identified in turtles in North America and is also the most commonly reported iridovirus for anurans. ATV affects salamanders in the western United States, whereas BIV is the main ranavirus identified in Australia.[10,11] Many manuscripts describe the detection of these ranaviruses using only polymerase chain reaction (PCR) of the major capsid protein (MCP); thus terminology such as FV3-like virus indicates that the sequence was homologous to the type species but further characterization was not performed. This has meant very little in a clinical setting because disease syndrome for FV3 and FV3-like diseases are assumed to be synonymous; thus all mention of FV3-like is considered to be FV3 in this chapter.

Ranaviruses have a wide species distribution, with significant differences in species susceptibility even within the same isolate.[12,13] There are several species that appear uniquely sensitive to these viruses, including tiger salamanders *(Ambystoma tigrinum)* to ATV,[11] wood frogs *(Lithobates sylvaticus)* to FV3,[12,14,15] and eastern box turtles *(Terrapene carolina carolina)* to FV3.[16] However, all anurans, caudates, and chelonians should be considered susceptible.

Age class often aids in diagnosis in amphibian diseases. Many reports indicate that larval amphibians in North America are more susceptible to mortality than adults, whereas adult mortality is more commonly reported in European amphibians.[9,17,18] Adult chelonians have been more commonly reported to develop FV3-like infections than juveniles.[16] However, there are fewer overall reports in reptiles with FV3, leading to the likelihood that adult reptiles are more likely sampled and diagnosed than juveniles. In Australia, juveniles of two species of tortoise (Krefft river turtle *[Emydura krefftii]* and saw-shelled tortoise *[Elseya latisternum]*) were susceptible to BIV, whereas adult tortoises, juvenile crocodiles, and three species of snakes were not affected after experimental transmission.[18] All age classes of reptiles and amphibians should be considered susceptible to this disease; however, juvenile/larval age classes may be at increased risk in some species.

TABLE 52.1 Taxonomy of Iridoviruses and Species Affected

Subfamily	Genus	Species	Taxonomic Groups Affected
Alphairidovirinae	Lymphocystivirus	Lymphocystis disease virus 1	Teleost fish
	Megalocystivirus	Infectious spleen and kidney necrosis virus	Teleost fish
	Ranavirus	Frog virus 3*	Teleost fish, amphibians, reptiles
		Ambystoma tigrinum virus	Salamanders
		Bohle iridovirus	Teleost fish, frogs, reptiles
		Epizooitic hematopoietic necrosis virus	Teleost fish
		European catfish virus	Teleost fish
		Santee-Cooper ranavirus	Teleost fish
		Singapore grouper iridovirus	Teleost fish
Betairidovirinae	Chloriridovirus	Invertebrate iridescent virus 3	Invertebrates
	Iridovirus	Invertebrate iridescent virus 6*	Invertebrates, lizards
		Invertebrate iridescent virus 1	Invertebrates

*Indicates type species.

Pathogenesis

Many different factors have been shown to affect the pathogenesis of ranaviruses in amphibians and reptiles, including species susceptibility, environmental factors, and host immune response.[13] However, there remains much we do not know about the pathogenesis of this group of viruses in these animals.

Transmission in amphibians may occur through contact with infected individuals, water, or fomites.[17] Natural ranaviral infections in Burmese star tortoises *(Geochelone platynota)* were speculated to occur via ingestion of infected amphibians.[19] Transmission studies of FV3 in red-eared slider turtles *(Trachemys scripta elegans)* using an oral inoculum of FV3 was unsuccessful; however, direct intramuscular injection of the virus was found to be effective at generating an infection model in this species.[20,21] Chelonians cohoused with infected amphibians or exposed to shared water with an infected fish induced disease in 20% and 30% of individuals, respectively.[22] Recently, FV3-like DNA was detected in mosquitoes during an eastern box turtle mortality event and may serve as another route of transmission.[23]

New cases of ranavirus may occur through a live animal reservoir host or persistence in the environment.[17] Attempts to definitely identify the vertebrate and invertebrate reservoir hosts for FV3 have been lacking. Much of this is because there have been limited field studies attempting to define the potential range of hosts. In amphibian populations, proposed reservoir species include those that develop over 1 year (American bullfrog *[Lithobates catesbienus]*), have neotenic development (tiger salamanders), or have aquatic adult life stages (red-spotted newt *[Notophthalmus viridescens]*; black-bellied salamander *[Desmognathus quadramaculatus]*).[24] A PCR survey in Ontario, Canada demonstrated that caudate salamanders are likely both the reservoir and the hosts of FV3 infections based on prevalence and viral load.[25] Isolation of BIV was successful in an adult brown tree snake *(Boiga irregularis)* and indicates a permissible reservoir host.[18]

Eastern box turtles that recovered from a ranavirus infection experimentally reinoculated a year later demonstrated less than a 20% mortality rate.[26] However, surviving animals all shed up to millions of viral copies without showing clinical signs, thus supporting their role as a potential reservoir.[26]

Clinical Signs and Pathology

Amphibians and reptiles infected with ranaviruses share several common clinical and pathologic characteristics, including systemic disease with epithelial necrosis. Although many reported infections lead to death prior to any premonitory signs,[9,24] there are examples of antemortem clinical signs. Skin ulcerations are also commonly seen in amphibians,[9,27] although amphibians may also demonstrate systemic hemorrhages in the absence of skin lesions (Fig. 52.1A).[28] Adult salamanders may develop abnormal swimming patterns and subcutaneous edema, whereas loss of pigmentation, lordosis, epithelial sloughing, and petechiation are reported in frogs.[9,29]

In larval amphibians, erythema at the base of the tail, ventrum, and legs, as well as swelling of multiple areas of the body, have been observed (see Fig. 52.1B).[24] Ecchymosis and petechiation of the skin are common.[9] The gallbladder may also be enlarged, but this is attributed to anorexia.[9]

Chelonians are commonly reported to develop nasal, ocular, and oral discharge, in addition to oral plaques (see Fig. 52.1C).[21,30] Epithelial lesions are also observed in reptiles but with less consistency than fish or amphibians.[9,31] In a survey of natural exposure to FV3 in eastern box turtles, only diarrhea and fractures were associated with FV3-like virus prevalence.[32] In an experimental challenge of FV3-like virus in eight red-eared sliders, lethargy (100%) (see Fig. 52.1D) and skin abscesses (66%) were seen at trials conducted at both 22°C and 28°C, whereas nasal discharge (100%), oral plaque (100%), and ocular discharge (100%)

• **Figure 52.1** (A) Clinical signs of a larval wood frog *(Lithobates sylvaticus)* demonstrating hemorrhages of the tail base; (B), Larval silvery salamander *(Ambystoma platineum)* with disseminated hemorrhages; (C), An eastern box turtle *(Terrapene carolina carolina)* with dehydration and lethargy; (D), An eastern box turtle with lethargy, ocular, and nasal discharge. (Photos A and B Courtesy Kelsey Low; C and D Courtesy Matthew Allender.)

were seen only at 22°C, but not at 28°C; control turtles at both temperatures demonstrated only rare lethargy (12.5%) or leg swelling (25%).[21]

Pathologic findings in susceptible individuals are similar between adults and juveniles of both amphibians and reptiles.[9,33] Infections lead to systemic organ failure due to cellular necrosis in the spleen, liver, kidney, and intestines.[9,33] Hemorrhagic syndromes are common in anurans in the United Kingdom[34] and in salamanders in western North America[35] and were observed in one experimentally inoculated red-eared slider.[20] Tiger salamanders have been observed with polypoid lesions early in the course of the disease that progress to cover most of the body.[36] There are also nonspecific changes noted with ranavirus infections, such as lymphocytosis, lymphoid depletion, and vacuolation of hepatocytes and renal tubular cells.[33] Basophilic viral inclusions have been inconsistently observed in affected tissues.[20,24] When present, inclusions have been identified in erythrocytes, leukocytes, epithelial cells, meninges, gills, neuroepithelium, nasal tissues, adipose, trachea, muscle, and osteoclasts.[9,21,24,29,30,36]

Diagnosis

Several methods have been proposed for the diagnosis of ranaviruses, including histopathology, PCR, virus isolation, enzyme-linked immunosorbent assay (ELISA), electron microscopy, restriction fragment length polymorphism (RFLP), and cytology.[9,24,37]

Conventional PCR targeting the MCP is commonly used to identify infections but is limited by the fact that it allows detection of only a 530-bp segment (or less) and does not confirm active infection. Diagnosis is most successful on fresh or frozen tissues, but a method for successful recovery from formalin-fixed tissues exists.[38] Evaluation of antemortem (toe clip) versus postmortem (liver) samples in anurans was evaluated for detection of FV3.[39] The study found that 88% of samples that were positive in liver

samples were also positive in toe clips, leading the authors to conclude that both methods are effective for detection.[39] The specificity and sensitivity of PCR were also evaluated comparing postmortem (necropsy) and antemortum (tail clips) in salamanders.[40] The study demonstrated that tail clips underestimate prevalence, although its agreement increases as time after exposure increases.[40] In reptiles, necropsy tissues are most commonly tested. Agreement of necropsy tissues were compared with antemortem sampling and concluded whole blood and oral swabs were 100% specific and 100% sensitive, whereas cloacal swabs were 100% specific and 83% sensitive if comparing with any sample taken during the 30-day trial.[21] However, experimental transmission in eastern box turtles demonstrated whole blood detection spiked at 13 days and oral swabs not until 16 days.[26] In the same study, whole blood detection ended by day 27, but oral swab detection persisted until day 55.[26] Therefore it is recommended that a dual testing strategy of both whole blood and oral swabs is needed for greatest testing success.

Quantitative PCR (qPCR) has been developed to detect fewer viral copies than conventional PCR. Primers target a segment of the ranavirus DNA polymerase gene.[41] This assay was validated in cell culture for several ranaviruses and was found to be effective in detecting virus in fish tissues experimentally infected with epizootic hematopoietic necrosis virus (EHNV)[41] but has difficulty in differentiating ranavirus species. A similar assay was developed based on a 56-bp segment of the MCP of FV3 and validated in *Terrapene* heart cell culture, eastern box turtle whole blood extracts, and plasmid-spiked cell cultures.[42]

Immunohistochemistry is useful in quickly confirming the presence of a pathogen in formalin-fixed tissue. An immunohistochemical (IHC) method has been developed using rabbit antiserum against purified virus to definitively demonstrate systemic ranavirus infection in several tissues from fish and amphibians.[43,44] Immunohistochemistry also has been developed in the United Kingdom that is capable of detecting ranavirus in lymphocytes, fibrocytes, and melanomacrophages, among other tissues from amphibians.[45] IHC may be an invaluable diagnostic tool when working on an outbreak and has been used in Australia for this purpose.[46] However, IHC may be applied only to biopsy or necropsy tissues, which are an invasive method that is not a preferred method for antemortem diagnosis.

Serologic assays have been developed to detect antibodies against iridoviruses in amphibians and reptiles.[18,47] Antibodies in cane toads *(Bufo marinus)* were detected using an ELISA against purified BIV and EHNV.[48,49] In chelonians, FV3 antigen and an anti–desert tortoise IgY were used in an indirect ELISA.[47] This assay demonstrated a high coefficient of variation (>15% in some replicates) but was used for evaluation of gopher tortoises and box turtles with low prevalence (<1.5%)[47]; however, the high coefficient of variation may have led to false positives.

Although the previous diagnostic tests are specific to characterizing ranaviruses, additional diagnostic methods that are less specific should also be considered when attempting to determine the presence or absence of these viruses. Clinical pathology is commonly used to characterize the health status of an animal. Although not specific, it may be used with other diagnostic methods in a parallel testing method to potentially increase the sensitivities of the assays. Intracytoplasmic inclusions were identified in an eastern box turtle with natural infection[30] but rarely in red-eared sliders following experimental infection.[21] This same experimental challenge found a significant decrease in total protein over time.[50]

Treatment and Prevention

Therapeutic interventions in free-ranging settings are often impractical for combating disease outbreaks. However, in captive animals or in those situations in which an endangered group of free-ranging animals is brought into captivity, an appropriate treatment protocol needs to be established.

Acyclovir is a guanine analog antiviral drug.[51] It is active against herpesviruses due to the presence of the thymidine kinase (TK) enzyme, which rapidly activates acyclovir to the monophosphate form.[52] Several isolates of iridoviruses have been shown to have TK genes or functional TK enzymes.[53,54] In vitro studies against an iridovirus using acyclovir indicated only a dose-dependent partial inhibition at 25 µg/mL.[16] The half-life of acyclovir after a single oral valcyclovir dose in eastern box turtles is 14.1 hours, and that of marginated tortoises *(Testudo marginata)* given oral acyclovir is 8.8 hours.[55,56] These results are slower than homeotherms, which could allow a reduction in dosing intervals. Famciclovir at three doses was used during a ranavirus outbreak in eastern box turtles in a zoo.[57] There were no significant differences in survival based on dose, but other supportive care measures were used and may have contributed to survival.

Besides pharmacologic treatment, other mechanisms to control FV3 include temperature alteration and disinfection. The effect of temperature on the pathogenesis of iridoviruses has been shown to be important. Tiger salamanders experimentally inoculated with ATV showed high mortality at 10°C and 18°C, whereas lower mortalities were noted at 26°C.[58] Furthermore, viral loads were highest in the animals kept at 10°C and lowest in the animals kept at 26°C.[58] In adult red-eared sliders, there was 100% mortality in turtles exposed to FV3 at 22°C and only 50% at 28°C.[21] Although FV3 growth is inhibited at temperatures greater than 32°C, the effects of other ranaviruses in other species clearly indicate either virus or species-specific differences in pathogenesis, and further studies are needed in several species to better characterize the role of temperature in development of disease.

Chlorhexidine at 0.75%, sodium hypochlorite at 3%, and potassium peroxymonosulfate at 1% were all effective at inactivating ranavirus after a 1-minute exposure,[59] whereas potassium permanganate (100% concentration) after a 60-minute exposure, chlorhexidine at 0.25%, and sodium hypochlorite less than 3% were found to be ineffective.[59]

Mortality Events

Disease events in amphibians are often clustered into local epizootics, with significant impact on local populations.[9] These epizootics have been scattered across numerous habitats and landscapes in the United States and, in some cases, have occurred on an annual basis.[11,60] Outbreaks in amphibians have accounted for between 1 and 600 deaths during each outbreak, accounting for up to 90% mortality of a single population.[9] Animals in higher-density populations had higher mortality rates and died more quickly; however, it was also observed that extinction events were unlikely because, as mortality reduced density below a critical threshold, remaining animals were able to recover.[61]

Mortality events in turtles are less well described, likely due to differences in natural history and surveillance methods. An outbreak in a zoological institution affecting eastern box turtles demonstrated greater than 40% mortality.[57] It was hypothesized that a native turtle entered the enclosure and initiated the outbreak.[57] Disease in this outbreak was unique in the detection of copathogens, in which turtles codetected with herpesvirus and *Mycoplasma* had a nonsignificantly lower mortality than individuals only detected with ranavirus.[57] The role of copathogens in susceptibility to ranavirus outbreaks needs further investigation.

Several reports exist in which surveys of reptile and amphibian populations failed to demonstrate molecular evidence or exposure to ranavirus. Nonclinical painted turtles *(Chrysemys picta)* and Blanding turtles *(Emydoidea blandingii)* did not show any PCR evidence of FV3-like virus.[62] In addition, several rehabilitation centers have zero or no prevalence in surveys across many states and locales.[32,42] In cases in which captive animals are affected, clinical course of disease may be short with minimal signs. Affected free-ranging animals are likely lost to the disease before being detected, reinforcing that this virus is associated with an acute disease process. The low prevalences observed in so many locations does not correspond to protection during outbreaks, in fact in a multiyear survey of ponds that experience recurrent ATV outbreaks in tiger salamanders, prevalence ranged from 0% in 1 year to 57% in other years.[11] Unfortunately, disease predictability outside these annual occurrences has not been successful.

Conservation

The global trade in amphibians is a significant factor in disease epidemiology and threatens conservation; the concern for this has grown so large that it has received listing status by the OIE to ensure the safety of international trade.[63,64] It was suggested that human transport of bait salamanders from the Midwest United States to Arizona and Colorado was responsible for the introduction of ATV to these otherwise naïve environments.[65,66] The authors concluded that because of the limited genetic variation seen in ranaviruses in North American salamanders, human movement is a plausible mechanism for its spread.[65,66] Furthermore, in a survey of fisherman in the western United States, bait salamanders were commonly released into local waters, which the authors concluded could serve as a significant threat to releasing ranavirus into native populations.[66] More importantly, these salamander isolates are able to infect but not kill fish in the environment, potentially leading to viral persistence and the spread of infections in the face of amphibian outbreaks.[67] In a cross-sectional study evaluating green frogs *(Rana clamitans),* the prevalence of ranavirus was influenced by industrial activity, degree of human influence, and distance to human habitation.[68] It is clear from these studies that humans have both directly and indirectly increased ranaviral disease in amphibians, and therefore it is reasonable to consider that these or other anthropogenic factors will occur in reptiles.

In conclusion, ranavirus has been identified as a threat to individual and population health in both free-ranging and captive amphibian and reptiles. This disease has a short incubation period with high mortality; thus if these characteristics are observed, ranavirus should be a leading differential. There are numerous diagnostic and treatment options, but qPCR is the most sensitive method to detect viral DNA in clinical animals and may detect subclinical carriers. Many aspects of disease occurrence are unknown, and future research should continue to improve our ability to determine the environmental or husbandry conditions that lead to a mortality event.

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Anuran Reproduction

ELLEN BRONSON AND CARRIE K. VANCE

There are more than 6700 known species of Anura (frogs and toads) in the world, of which at least 2000 are currently considered threatened or endangered,[1] more than any other vertebrate taxa. A number of complex factors have led to amphibian declines around the world, including habitat loss, disease, climate change, pesticide use, and pollution.[2–5] As a result of these biotic and abiotic factors, many amphibian populations are now extinct or have become critically endangered in their native habitats.[5] Because amphibians have developmental stages that use both aquatic and terrestrial environments, they are often considered sentinels for ecosystem health. However, amphibians are also threatened by specific pathogenic microorganisms, including ranaviruses, the trematode *Ribeiroia ondatrae*, and the fungus *Batrachochytrium dendrobatidis* (Bd).[6,7] Bd has spread across several continents at an alarming rate, causing extinctions of numerous amphibian species.[8,9] As a hedge against further amphibian extinctions, zoological parks, aquaria, and other institutions have established assurance colonies with the goal of maintaining viable populations in captivity for species sustainability and future reintroductions.[10,11] In some cases the captive assurance colonies consist of a significant proportion, if not all, of the remaining individuals of the rescued species.[4,11]

To sustain these assurance colonies and maintain genetic diversity for long-term species survival, successful reproduction of founders and descendants is required. Despite the best efforts of the zoological community, many amphibian species fail to naturally reproduce in captivity.[5] Consequently, amphibian biologists, veterinarians, and researchers around the world have partnered to develop hormone therapies and assisted reproductive technologies (ARTs) for enhanced reproductive output and management of genetic diversity.[12–15] Amphibian reproduction is a complex sequence of events that couples environmental, social, and physical cues leading to hormonal cascades associated with the hypothalamic-pituitary-gonadal (HPG) axis, eventually resulting in gamete development and release.[14,16–18] Amphibian ART spans a wide range of approaches (e.g., hormone therapy, egg expression, in vitro fertilization), which are used to either facilitate or circumvent specific steps in the natural cycle when reproductive failure becomes apparent. An important aspect in implementing ART is identifying which stages of the reproductive cycle might be compromised if there is reproductive failure. This is not a trivial step because the environmental cues and breeding behaviors are often species specific, thus requiring a solid understanding of the reproductive cycle for a given species.[19]

Anuran Reproductive Physiology

With few exceptions, anurans (frogs and toads) exhibit external fertilization, in which the female deposits an egg mass (termed oviposition or spawning) while the male fertilizes it.[10,19] Egg maturation, or oogenesis, is signaled by progesterone to resume meiotic division and is typically triggered by a combination of factors such as photoperiod and temperature. The egg grows in size due to the accumulation of vitellogenin, produced by the liver in response to estrogen. After the eggs are mature and the environmental conditions are right, the female will seek out a male for breeding. A cascade of hormones through the HPG axis leads to ovulation of the eggs from the ovary into the oviduct, and the egg mass becomes surrounded by egg jelly.[10,18,20] Spawning follows (release of eggs from the cloaca), such that the eggs are laid as a loosely bound string. Typically the male amplexes the female to fertilize the eggs, embracing the female tightly with the front legs around the cranial coelom, and fertilization by the male occurs as the eggs are expressed. Most temperate anurans lay eggs in water; however, the various species display a wide array of reproductive strategies. Most anurans leave the fertilized egg mass following reproduction, although there are a few species that participate in protecting the embryos until hatching of the larval (tadpole) stage.

Elucidating the behavioral cues that each species is responsive to can take many years and be very difficult to identify and replicate in the captive setting. Many species require specific levels or changes in humidity, temperature, barometric pressure, water depth, and light length and intensity.[4,19,21] Other factors may include tactile or acoustic characteristics such as male calling or spray and vibration effects of a replicated waterfall. Temperate species often do best after a period of low temperature inducing brumation or hibernation followed by slow warming to induce egg production, egg maturation, and breeding behaviors.[22]

Many tropical species breed at the onset of, or during, the rainy season, so misting systems or rain chambers, as well as temperature changes, may replicate the natural environment and spur reproduction.

Health Factors Affecting Reproduction

Poor health of amphibians may impair the normal hormone cascade and result in cessation of oogenesis and spermatogenesis. For example, an animal in poor health or poor nutrition will likely abort the process of vitellogenesis or egg maturation if fat stores are insufficient, and instead the body will begin to resorb the eggs. Principal energy reserves must be redirected for survival and metabolism and will be directed away from nonessential activities, such as reproduction, which is energy demanding. Another important health aspect is egg retention in female anurans, which is similar to dystocia in other vertebrates. These retained eggs remain either attached to the ovary or are ovulated into the oviduct but not deposited, and this state may lead to bacterial infection and sepsis.[23] Occasionally, reproductive hormones are needed to stimulate the release of these eggs from the ovary or oviduct to preserve the health of the female.[10,24] For example, there has been a clinical need for the administration of exogenous hormones to release egg masses from Panamanian golden frogs *(Atelopus zeteki),* Wyoming toads *(Anaxyrus baxteri),* boreal toads *(Anaxyrus boreas),* and tomato frogs (*Dyscophus* spp.).[4,10,23,25] Panamanian golden frogs tend to develop very large egg masses and have long periods of amplexus (weeks to months); thus the reproductive season is metabolically challenging for both sexes, and high mortality is seen due to decimation of fat supplies and decreased food intake. Frequently, females do not lay eggs even when amplexed, so the energy demands may be even higher for months while the eggs resorb.[4] For these reasons the administration of exogenous hormones has been used to stimulate egg release and decrease mortality.

Hormonal Regulation of Reproduction

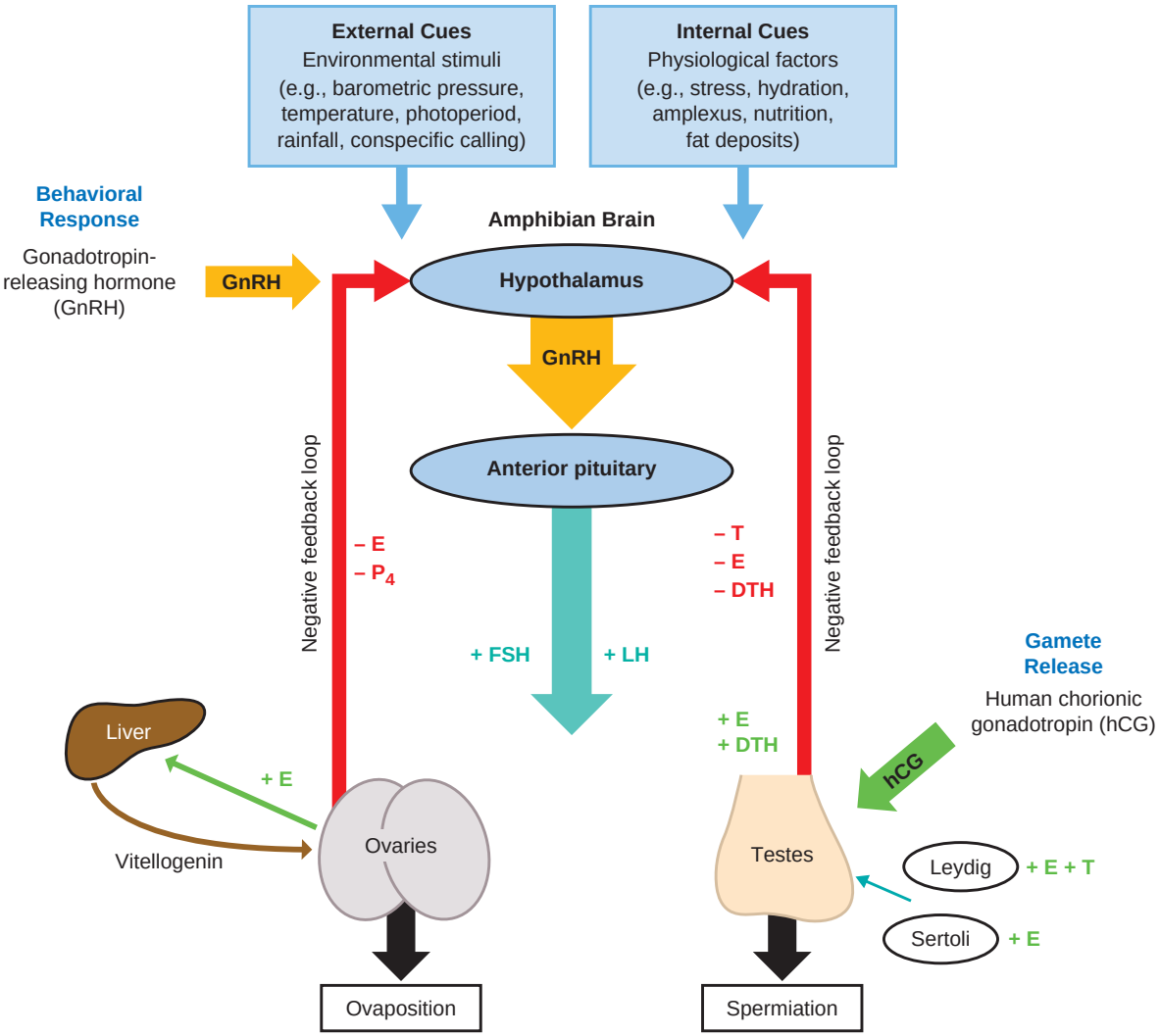
Amphibians share many of the main reproductive neuropeptides, pituitary hormones, and gonadal steroids with other vertebrates. Gonadotropin-releasing hormone (GnRH) is produced in the neurons of the amphibian hypothalamus, which stimulates the release of luteinizing hormone (LH) and follicle-stimulating hormone (FSH) from the anterior pituitary gland (Fig. 53.1).[18] LH and FSH have a stimulatory effect on steroidogenesis in the gonads. In males, they lead to the release of testosterone, causing spermatogenesis and sperm maturation and release, and they also play a role in calling and amplexus.[22] In females, these gonadotropins lead to ovulation, egg transport through the oviduct, production and secretion of egg jelly, and spawning (see Fig. 53.1).

Exogenous Hormone Use in Anurans

Despite the best efforts of curators, keepers, and veterinarians to replicate the natural habitats and environmental cues to stimulate breeding, some amphibian species do not reproduce well in captivity without the use of external hormones. For example, the Puerto Rican crested toad *(Peltophryne lemur),* Mississippi gopher frog *(Lithobates sevosus),* Houston toad *(Anaxyrus houstonensis),* and Wyoming toad have rarely bred in captivity without the use of exogenous hormones.[22,24,26] The hormone cascade involved in both spermiation and ovipositioning is well-preserved among species, although in the development of protocols, some species seem to have better success with one or the other of the hormones that are commercially available. Human chorionic gonadotropin (hCG) has LH-like activity on the ovary or testes. Because it is a mammal-derived protein, much higher doses are required in amphibians to stimulate a response compared with when the hormone is given to mammals (approximately 2000 times higher per kg). The most commonly used hCG product is Sigma-Aldrich C-1063 (CAS Number 9002-61-3). LH and FSH specific to amphibians have not been developed, and synthetic commercial forms of these products have proven to be ineffectual in amphibians.[23] The most frequently used hormone analog in amphibians is gonadotropin-releasing hormone agonist (GnRH-A), commonly and hereafter referred to as luteinizing hormone–releasing hormone analog (LHRHa), available as the commercial product Des-Gly 10, D-Ala 6-LHRH ethylamide acetate hydrate (Sigma-Aldrich, L4513; CAS Number 79561-22-1). Purchased as a lyophilized powder, it is reconstituted in sterile saline or phosphate-buffered saline prior to use.[21] The reconstituted liquid may be frozen but is stable only for 24 hours once thawed. Another group of drugs that have been investigated more recently in anurans with promising but mixed results are the dopamine antagonists combined with LHRHa.[27,28] Dopamine inhibits GnRH synthesis and secretion from the hypothalamus, thereby reducing or eliminating the secretion of LH and FSH. By administering a dopamine antagonist, the inhibition of GnRH is removed and the effect of the exogenous hormone(s) can theoretically be enhanced.[17,21,28,29] In anurans a combination of LHRHa and the dopamine antagonist metoclopramide has been termed "Amphiplex" by Trudeau and has been tested in a number of amphibian studies.[27–29] However, the efficacy of such combinations is known to be variable in fish species, and some mortality has been reported in amphibians, so future studies will need to delineate the efficacy and safety in the various anuran species. Administration of exogenous hormones is considered most effective if given intracoelomically with a small-gauge needle (Fig. 53.2). Other administration routes, such as absorbance through the skin, injections into the dorsal lymph sac, subcutaneously, or intramuscularly, have been used but typically with lower success.[21,30,31]

Exogenous Hormone Induction of Spermiation in Male Frogs

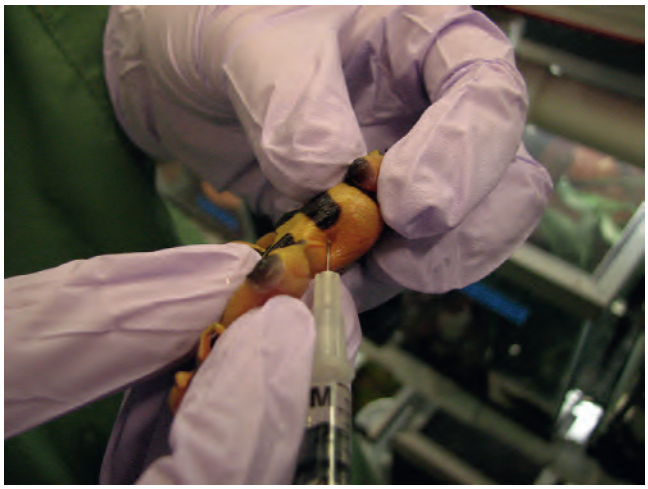
Following exogenous hormone administration, sperm are released into the cloaca and are mixed with urine, forming



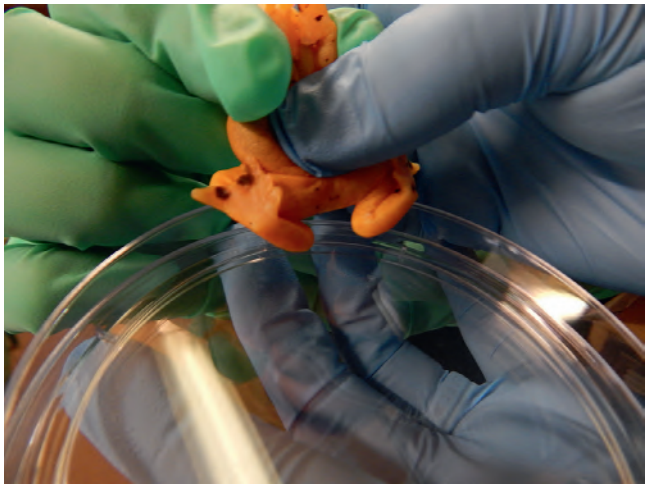
• **Figure 53.1** Diagram of the hormone cascade through the hypothalamic-pituitary-gonadal (HPG) axis. Gonadotropin-releasing hormone (GnRH) is released by the hypothalamus in response to environmental or endogenous cues and binds receptors at the anterior pituitary. The gonadotropin hormones—luteinizing hormone (LH) and follicle-stimulating hormone (FSH)—are released and act at the level of the gonads to stimulate steroidogenesis in the testes, and follicular growth and ovulation in the ovaries and yolk formation (vitellogenesis) in the liver. LH is the regulator of the Leydig cells, which produce testosterone (T) and estrogens (E). Testosterone is transported to Sertoli cells and converted to dihydrotestosterone (DHT) and estrogen which may regulate positive and negative feedback loops in anuran species.

spermic urine. Spontaneous expression of spermic urine occurs when the animal is picked up and held over a Petri dish and is a result of the natural defense mechanism seen upon handling, especially in toads. Spermic urine may also be obtained by gentle palpation with light pressure to the ventral coelom (Fig. 53.3). For frogs a small-gauge catheter inserted into the cloaca and urinary bladder may be necessary to obtain urine.[21,31] In most toad species, the highest concentration of sperm can be found 3–9 hours after hormone administration, but in frog species the response is earlier, with sperm being released in the urine 30–90 minutes after hormone treatment.[14,30,32–34] If hormones are used to induce sperm production for natural breeding or artificial fertilization (AF), it is imperative to know this expected time frame to coordinate with the window of availability of eggs from the female for a particular species.[13,22,34] Moreover, males need sufficient recovery time (approximately 2 weeks) between hormone injections or spermiation can temporarily cease.[21,35]

Select species-specific protocols for hormone-induced spermiation are presented in Table 53.1. LHRHa is the hormone of choice for inducing natural mating in most zoos and aquaria because it elicits a stronger amplexus response than does hCG.[14,36] For instance, LHRHa initiates a good amplexus response in American toads *(Anaxyrus americanus)* and Fowler toads *(Anaxyrus fowleri)* with lower sperm production, whereas hCG tends to generate better sperm production but a lower behavioral response.[13,34] Hence, hCG is excellent for collection of sperm for AF and cryopreservation. When hCG is combined with LHRHa, it may produce an even stronger effect than with LHRHa alone.[18,34] LHRHa is used for induction of spermiation and



• **Figure 53.2** Injection site and method of intracoelomic hormone administration in a Panamanian golden frog *(Atelopus zeteki).* The small needle (28 gauge) should be placed in an area free of visible organs into the caudal coelomic cavity. The frog's head is to the upper right. (Courtesy Ellen Bronson, Maryland Zoo.)



• **Figure 53.3** Positioning of a Panamanian golden frog *(Atelopus zeteki)* for release of spermic urine into a Petri dish. Many toad species will urinate as a defense mechanism when handled. Light digital pressure to the lower abdomen may be applied to release spermic urine. (Courtesy Ellen Bronson, Maryland Zoo.)

stimulation of natural reproduction in many frog species, such as the Günther's toadlet *(Pseudophryne guentheri),* European common frog *(Rana temporaria),* Argentine horned frog *(Ceratophrys ornata),* and African bullfrog *(Pyxicephalus adspersus).*[32,33,37] LHRHa combined with the dopamine antagonist metoclopramide has been successfully used to collect spermic urine in the Panamanian golden frog and Northern leopard frog *(Lithobates pipiens).*[27,28]

Anuran sperm exhibit a unique osmotic tolerance regarding motility and osmotic damage compared with mammalian sperm.[14,22,38] Anuran sperm are inactive in the isotonic environment of the testes (~280 mOsmol/kg) and then become briefly stimulated by the lower osmolality of urine (~50 mOsmol/kg) and will remain active for short periods of time (typically 10–15 minutes) when deposited in environmental water (<20 mOsmol/kg) for external fertilization.[32,38] Sperm will remain active for an extended time period (hours) if left in spermic urine. If the spermic urine is kept chilled, the sperm can remain active for up to 2 weeks in some cases.[10,18,39] Although motility will decrease over time, cooled sperm has been used successfully for AF in a few cases.[10,26,39] In the instance of deceased animals, sperm collected from testes macerates and subsequently stored at 0°C–4°C for several days has been successfully used in AF.[10,32,39,40] Shishova et al. demonstrated that whole carcasses of frogs could be frozen and the sperm retrieved following thawing to produce fertilized eggs.[41] Cryopreservation is another amphibian ART that is showing great promise in contributing to sustainability of amphibian species. Drs. Vance and Kouba at Mississippi State University, USA are leading the effort to create a National Amphibian Genome Bank for storing frozen sperm in a metabolically arrested state to assist the Association of Zoos and Aquariums in genetically managing captive amphibian populations.[13,21]

Monitoring of Female Reproductive Status via Ultrasound

To date, ultrasonography has been used in amphibians mainly for medical diagnostic purposes[42–44]; however, this technique also has potential for analysis of female reproductive status.[45–47] In nongravid females, the reproductive tract is difficult to discern via ultrasonography,[44] but well-developed oocytes are relatively easy to visualize during imaging, which may be useful for determining whether the female is ready to lay eggs or if priming doses will be needed to promote egg maturity. Egg development may be assessed using a 0–3 scale,[47] where oocytes are discernible based on the characteristic pattern of hyperechoic and anechoic (light and dark) areas throughout the abdomen during imaging (Fig. 53.4). In the scale developed by Dr. Ruth Marcec, a female exhibiting a grade 0 development would not be treated with exogenous hormones but might begin a long-term priming regimen to promote egg development, followed with ultrasound monitoring for several weeks until the eggs advance to a stage 1 or 2. Typically, females that are grade 2 need a priming dose followed by an ovulatory dose, but females with mature eggs (grade 3) can lay without a priming dose, although there are species-specific variations to these assessments. Ultrasound can also be used to determine if a female resorbs eggs or is experiencing dystocia in cases where there are no externally visible indicators.[4,47]

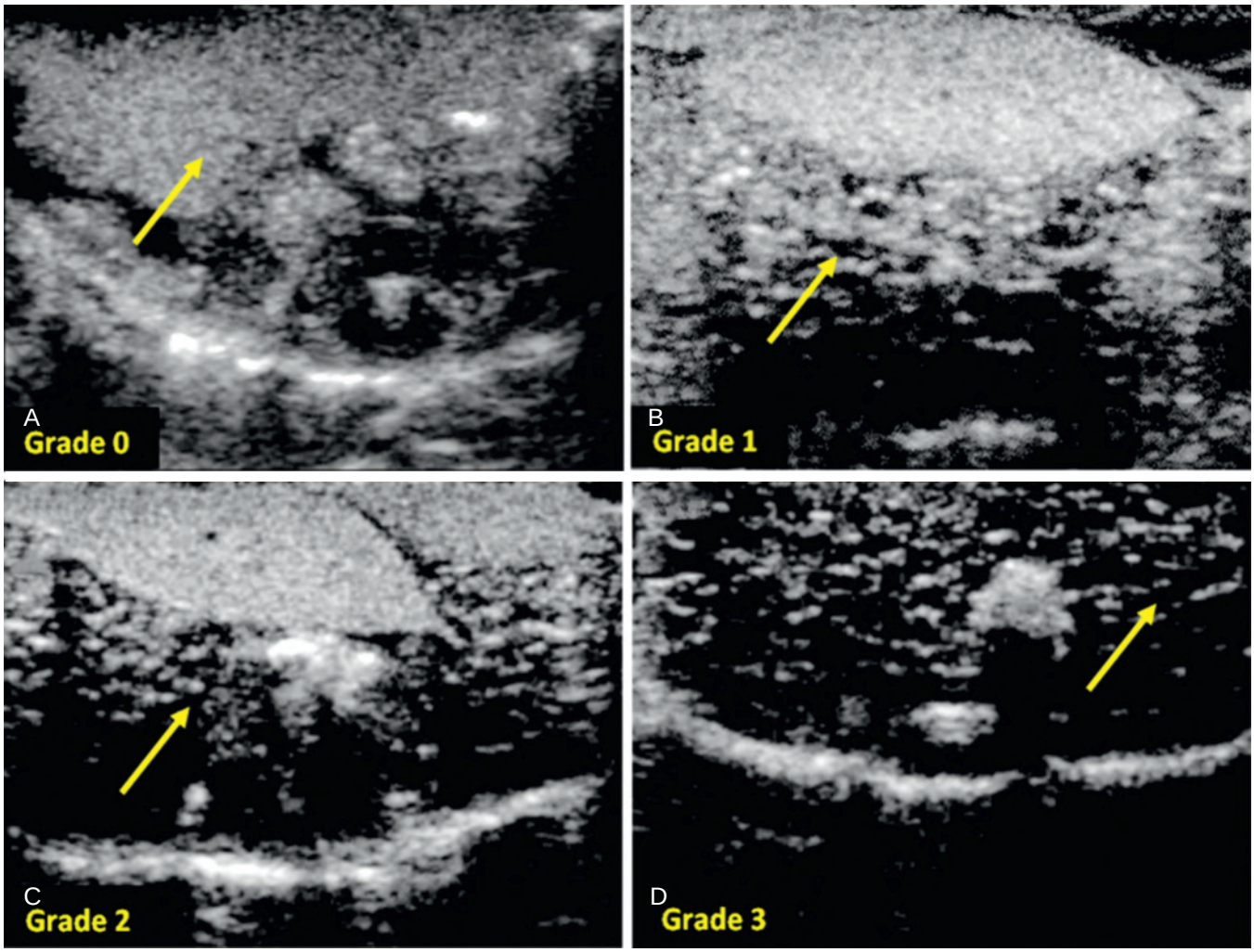
Exogenous Hormone Induction of Spawning in Female Frogs

Both GnRHs, hCG and LHRHa, have been applied to females of different anuran species to facilitate egg development and oviposition (Table 53.2). If the goal is to aid in natural laying, the timing of the hormone treatment for the

TABLE 53.1 Hormones and Dosages Used to Induce Spermiation in Select Male Anuran Species

Scientific Name	Species	Exogenous Hormone	Dosage	Reference
Anaxyrus americanus	American toad	hCG	300 IU	Kouba 2012
Anaxyrus boreas	Boreal toad	LHRHa	300 IU	Roth 2010
Anaxyrus fowleri	Fowler's toad	hCG	300 IU	Kouba 2009
Atelopus zeteki	Panamanian golden frog	LHRHa	4 µg/g	Della Togna 2017
Atelopus zeteki	Panamanian golden frog	Amphiplex	LHRHa 0.4 µg/g + MCP 10 µg/g	Della Togna 2017
Atelopus zeteki	Panamanian golden frog	hCG	10 IU/g	Della Togna 2017
Bufo baxteri	Wyoming toad	hCG	300 IU	Browne 2006
Bufo baxteri	Wyoming toad	LHRHa	4 µg	Obringer 2000
Ceratophrys cranwelli	Chacoan horned frog	LHRHa	0.1–0.5 mg/kg	Waggener 1998
Ceratophrys ornata	Argentina horned frog	LHRHa	0.1–0.5 mg/kg	Waggener 1998
Lepidobatrachus laevis	Budgett's frog	LHRHa	0.375 mg (0.29–0.58 µg/kg)	Waggener 1998
Lepidobatrachus llanensis	Llanos frog	LHRHa	0.6 mg/kg	Waggener 1998
Lithobates pipiens	Northern leopard frog	hCG/LHRHa	500 IU LHRH + 10 µg LHRHa	Kouba 2009
Lithobates pipiens	Northern leopard frog	LHRHa	0.1–0.4 µg/kg	Waggener 1998
Lithobates pipiens	Northern leopard frog	Amphiplex	LHRHa 0.4 µg/g + MCP 10 µg/g	Trudeau 2010
Litoria raniformis	Southern bell frog	LHRHaLucrin	20 µg	Mann 2010
Peltophryne lemur	Puerto Rican crested toad	hCG	4 IU/g SC	Crawshaw 2008
Peltophryne lemur	Puerto Rican crested toad	LHRHa	0.1 µg/g SC	Crawshaw 2008
Pseudophryne corroboree	Corroboree frog	hCG	20 IU/g in DLS	Byrne 2010
Pseudophryne corroboree	Corroboree frog	LHRHa	5 µg/g in DLS	Byrne 2010
Pseudophryne guentheri	Günther's toadlet	LHRHa	2 µg/g	Silla 2011
Pyxicephalus adspersus	African bullfrog	LHRHa	0.1–0.5 mg/kg	Waggener 1998
Rana temporaria	European common frog	LHRHa	50 µg	Shishova 2011
Xenopus laevis	African clawed frog	LHRHa	0.1–0.5 mg/kg	Waggener 1998
Xenopus tropicalis	Tropical clawed frog	hCG	100 IU	Browne 2007

male needs to be synchronized with the expected time of egg laying, and the pair is typically encouraged to amplex naturally.[21,34] When amplexus does not occur naturally, the female can be administered hormone and allowed to either spontaneously oviposit in the absence of a male, or the eggs can be expressed from the cloaca through gentle pressure. Once collected, the eggs may be fertilized with stored sperm by conducting AF. Spawning may be induced using a single ovulatory dose of hCG or LHRHa, or it may require one or more lower concentration priming doses to advance egg maturation prior to oviposition. Some species, such as the American toad, Puerto Rican crested toad, or the common coquí *(Eleutherodactylus coqui),* have been found to reliably lay eggs after receiving only an ovulatory dose of exogenous hormones.[24,48,49] However, in other cases a priming dose may be required approximately 24–96 hours prior to the ovulatory dose if the cues that stimulate egg recruitment are not available, such as in the off season or when hibernation is needed.[10,19] For example, a single low-dose priming injection of LHRHa followed by an ovulatory dose of LHRHa is sufficient to induce female Günther's toadlets to spawn.[37] Fowler's toads typically receive two low-dose priming injections of hCG 72 hours apart, followed by a higher ovulatory dose of hCG + LHRHa 48 hours later to trigger egg laying.[21] Spawning without hibernation has been achieved in female Wyoming toads using either one or two priming doses and an ovulatory dose of hCG and LHRHa.[22,25] In Northern leopard frogs, both sexes were treated with LHRHa plus metoclopramide to induce amplexus and egg laying within 48–96 hours.[28] However, LHRHa alone was sufficient to achieve the same outcomes in female Panamanian golden frogs.[4]



• **Figure 53.4** Ultrasound images of various egg development stages in the Mississippi gopher frog *(Lithobates sevosus). Arrows* indicate ovarian tissue at each grade of oocyte development. Fluid is indicated by hypoechoic areas and tissue or eggs by hyperechoic areas. (A) Grade 0, no or minimal egg development, ovarian tissue is uniform and undeveloped *(arrow).* (B) Grade 1, minimal egg development as eggs begin to form in small pockets in the ovaries *(arrow).* (C) Grade 2, increasing egg development where eggs begin to be distinguishable from tissue *(arrow).* (D) Grade 3, fully developed individual eggs are seen as hyperechoic specks surrounded by fluid. Imaging was performed with a Sonosite MicroMaxx ultrasound machine equipped with a 38-mm broadband linear array transducer (range 6–13 MHz). A scan depth of 2.7 cm was used. (Image credit: Allison Julien, Mississippi State University.)

If the female anuran has a mature cohort of eggs to ovulate, the final ovulatory dose of hCG/LHRHa typically stimulates egg laying 12–48 hours post administration of hormone. In many of the ranid species, the females may be stripped of eggs by carefully stroking from cranial to caudal to push eggs from the oviducts into the cloaca and then externally for collection.[31,37] In other species, such as the Panamanian golden frog, the eggs enter the oviducts just hours before laying, and these frogs cannot be stripped easily or at all.[4] Female anurans that do not spawn will eventually resorb the egg mass partially or entirely before the next season, but this condition may lead to additional health problems due to the increased metabolic demands on the female.

Artificial Fertilization

AF is the human-made mixing of sperm and eggs together within a Petri dish and is the term typically used to describe in vitro fertilization for an animal that displays external fertilization. This process is theoretically simple and achievable in a zoo setting because it may be done without complex equipment, media, or technical expertise. However, timing is essential because the gametes, especially the oocytes, will be viable only for a short time once removed from the cloaca (about 20 min). Typically, the egg mass is removed from the water as it is being laid or is slowly removed from the female's cloaca gently with a pair of forceps. Once removed, a process of dry fertilization is performed, whereby sperm is added

TABLE 53.2 Hormones and Dosages Used to Induce Oviposition in Select Female Anuran Species

Scientific Name	Species	Hormone	Priming Dose(s)	Ovulatory Dose	Reference
Anaxyrus americanus	American toad	hCG, hCG/LHRHa	100 IU (twice)	500 IU/20 µg	Kouba 2009
Anaxyrus baxteri	Wyoming toad	hCG/LHRHa	500 IU/4 µg; 100 IU/ 0.8 µg	500 IU/4 µg	Browne 2006
Anaxyrus boreas	Boreal toad	LHRH		0.1 µg/g	Roth 2010
Anaxyrus fowleri	Fowler's toad	hCG, hCG/LHRHa	100 IU (twice)	500 IU/20 µg	Kouba 2009
Anaxyrus fowleri	Fowler's toad	hCG/LHRHa		500 IU/4 µg	Browne 2006
Anaxyrus fowleri	Fowler's toad	LHRHa		20 µg	Browne 2006
Anaxyrus fowleri	Fowler's toad	LHRHa/ Progesterone		20–60 µg/5 mg	Browne, Li 2006
Atelopus zeteki	Panamanian golden frog	LHRHa	4 µg	4 µg	Bronson 2015
Eleutherodactylus coqui	Common coqui	LHRHa		20 µg	Michael 2004
Lithobates pipiens	Northern leopard frog	LHRHa/ Metoclopramide		0.4 µg/g + 10 µg/g	Trudeau 2010
Mixophyes fasciolatus	Great barred frog	hCG	100 IU	100 IU	Clulow 2012
Peltophryne lemur	Puerto Rican crested toad	LHRHa		0.1 µg/g	Crawshaw 2008
Pseudophryne corroboree	Corroboree frog	LHRHa	1 µg/g	5 µg/g	Byrne 2010
Pseudophryne guentheri	Günther's toadlet	LHRHa	0.4 µg/g	2 µg/g	Silla 2011
Xenopus laeves	African clawed frog	hCG	10 IU	100–200 IU	Browne 2007
Xenopus tropicalis	Tropical clawed frog	hCG		200 IU	Browne 2007

directly onto the egg jelly and allowed to sit for 5 minutes before flooding the dish with aged tap water. The cleavage rate of the fertilized embryos may be observed through a dissecting microscope 4–6 hours following insemination of the eggs. AF has been performed successfully in several amphibian species to date.[10,32,33,40] Wyoming toad tadpoles produced from AF have been released into the wild.[22]

Role of Zoo Veterinarians in Anuran Reproduction

Zoo and wildlife veterinarians play an important role in both the development of protocols for the medical management of reproductive disorders, as well as in the support of reproductive specialists in the development of ARTs. As amphibian species become increasingly imperiled in our changing world and assurance colonies of endangered amphibians become one of our only hopes in maintaining these species and returning them to their native habitats in the future, the further development of ARTs will continue to increase in importance. Having appropriate reproductive protocols at the ready may both increase our success in maintaining and expanding healthy populations in captivity as well as saving genetic material for the future.

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54

Minimally Invasive Surgery of Amphibians

NORIN CHAI

More than 7645 species of amphibians exist of which 6745 belong to the Anura (frogs and toads), 695 to the Caudata (newts and salamanders), and 205 to the Gymnophiona (caecilians).[1] Only the commonly maintained companion animal species are discussed here; therefore caecilians have been excluded. The term minimally invasive surgery (MIS) is generally used to refer to any procedure that is less invasive than open surgery used for the same purpose. It should be safe and associated with a lower postoperative patient morbidity compared with a conventional approach for the same operation. In our case it will mostly refer to rigid endosurgical procedures, mainly laparoscopy. There have been sporadic reports of amphibian endoscopy since the 1980s. Most previous reports describe the use of endoscopy to examine the gender or retrieve foreign bodies.[2–5] In contrast to reptile medicine, where laparoscopy has been well developed, literature is still scarce in amphibians. The greatest limiting factor is probably equipment compatibility due to the small size of most amphibians. Still, with appropriate equipment, many species of newts, salamanders, frogs, and toads may be internally examined, with minimal trauma and discomfort. Laparoscopy may be a useful complementary tool to radiography and ultrasonography. However, it is sometimes difficult to interpret images in such small animals. Misdiagnosis is not uncommon. In the author's experience, laparoscopy in amphibians permits visualization of almost all the coelomic organs from a single point of entry. In Fowler's *Zoo and Wild Animal Medicine*, Volume 8, an overview of current techniques and applications of MIS in wildlife has been provided, including a discussion on MIS-specific risks and disadvantages and on recent developments in human and domestic animal surgery that have implications for wildlife surgery.[6] The major concepts described may be appropriate here as well. Amphibian surgery is a very small specialized field; MIS in amphibians is even smaller. Updated information on endoscopy in amphibians has been recently described.[7] This chapter will provide a brief overview of current techniques of MIS in amphibians and an example of their application in fundamental research.

General Considerations

In the author's experience, the main indications of MIS in amphibians are listed in Box 54.1. Depending on the equipment, the small size of the animal would be the most common contraindication. As usual, sick animals with high anesthetic risks should not undergo laparoscopy. Despite the variation in size and the nature of the procedures that may be performed, the basic materials needed for MIS are listed in Box 54.2. The amphibian patient should be handled with care, and wrapping with wet paper towel is a good technique to restrain an animal for a quick examination or for medication administration. Wearing moistened, powder-free gloves prevents the transfer of microorganisms or chemicals from the handler as well as protection against secreted toxins. Manual restraint is used for short and nonpainful procedures. For a better visualization, it is advisable to fast large frogs and toads for 24–48 hours prior to anesthesia. General anesthesia with analgesia is required for MIS. Updated information on anesthesia, analgesia, and basic and advanced surgical procedures has been recently described (see also Chapter 60).[8] The benefits of MIS are numerous, but most useful is the ability to collect samples for a definitive diagnosis, accurate prognosis, and to direct therapy. In general, complications resulting from a properly performed laparoscopy procedure itself are rare. However, iatrogenic endoscope trauma may adversely affect organs and biopsy results, as well as result in hemorrhage. The most dramatic complication occurs when the ventral abdominal vein is damaged. This may happen on very small animals, even from a paramedian approach. The problem is that there is no way to control the hemorrhage. If complications occur, the procedure is immediately stopped. After a warm, anesthetic-free bath or shower, the animal is soaked in the balanced electrolyte solutions, the amphibian Ringer solution (6.6 g NaCl, 0.15 g CaCl2, 0.15 g KCl, and 0.2 g $NaHCO_3$ per liter of dechlorinated water), until the recovering of its biological functions.[9] A major concern is the risk-benefit ratio of proceeding with a MIS in an amphibian. Cognitive bias has been previously discussed

• BOX 54.1 Indications of Minimally Invasive Surgery

- Gender identification
- Retrieval of foreign bodies from the upper gastrointestinal tract
- Rapid, magnified, ante- or postmortem assessment of intracoelomic lesions in cases of high mortality in a colony
- Valuable diagnostic tool when confronted by non-pathognomonic clinical signs, such as anorexia, weight loss, or loss of pigmentation
- Biopsies of tissues and coelomic organs
- Reproductive endosurgery

• BOX 54.2 Standard Equipment

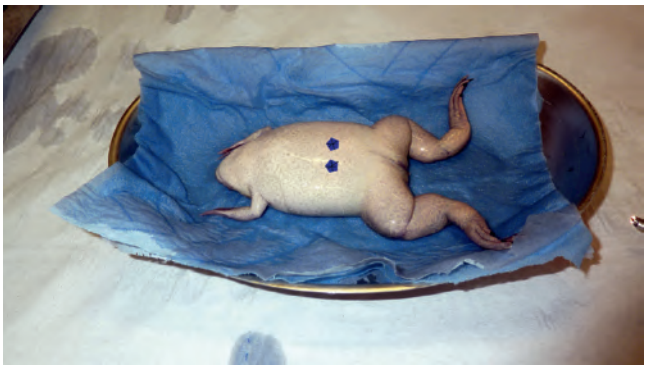
- 1.9-mm integrated telescope
- 2.7-mm diameter, 18-cm length, 30° oblique rigid telescope with a 4.8-mm operating sheath
- Endovideo camera and monitor
- Xenon light source and light cable
- 1-mm or 1.7-mm endoscopic biopsy forceps and grasping forceps. Endoscopic needle is optional.
- Carbon dioxide (CO_2) insufflator with silicone tubing. Care must be taken when using CO_2 insufflation, because it may quickly dry out the organs and the mucosa. In the author's experience, the endoscopic procedure should not last more than 5 min. A simple syringe for air or saline infusion is also practical.

in wildlife MIS.[6] This bias is also true in amphibians. The author believes that amphibian MIS should be performed by a practitioner already experienced at least with reptile MIS. Practical recommendations should follow the edict "first do no harm."

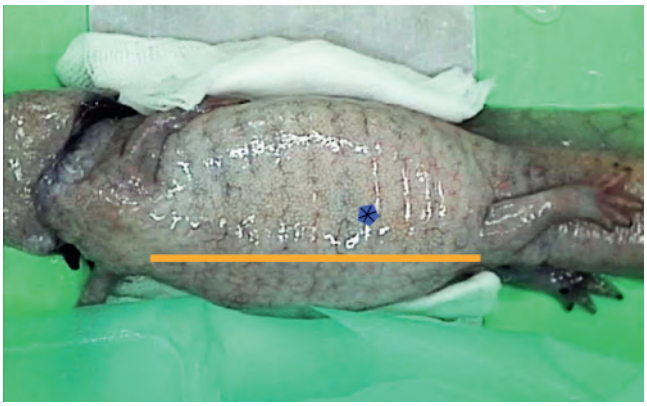
Clinical Applications of Minimally Invasive Surgery in Amphibians

Laparoscopic Examination and Endoscopic Biopsy

Laparoscopy may be defined as the exploration of the coelomic cavity using a rigid endoscope, indicated for many diagnostic and surgical procedures. Despite the diversity in body shape, and the visceral anatomy and its distribution in amphibians, the process is similar for all species. The author uses dorsal recumbency for all anurans. In more laterally compressed amphibians (e.g., newts), lateral recumbency is used. By convention (or habit acquired with endoscopy in birds), the animal is placed on its right side. Insufflation is useful, but lower flow rates are used and maintained for intracoelomic pressures compared with those reported in small animals. Increased intracoelomic pressure compresses the lungs because there is no true diaphragm. Furthermore, assisted ventilation is rarely possible during MIS in amphibians. With the animal positioned in dorsal or lateral recumbency (depending on the body shape), the surgical



• **Figure 54.1** On dorsal recumbency, the 3-mm paramedian skin incision site is localized by these *two blue marks* on this adult African clawed frog *(Xenopus laevis)*. (Copyright Norin Chai.)



• **Figure 54.2** This axolotl *(Ambystoma mexicanum)* is on right lateral recumbency, the left pelvic limb simply placed against its tail base. A *line* drawn between the shoulder and the hind limbs is divided into three equal parts, and the skin incision site is localized by the *blue mark*. (Copyright Norin Chai.)

field is aseptically prepared by gently wiping the surgical site with sterile gauze soaked in 0.75% chlorhexidine solution and left on the surgical site for at least 10 minutes before surgery. In dorsal recumbency, a 3-mm paramedian skin incision is made in the mid-coelom (between the shoulders and the cloaca), either left or right (Fig. 54.1). Care must be taken not to damage the macroscopic glands, lymph hearts, and blood vessels, especially the mid-ventral vein. On right lateral recumbency, the left pelvic limb is simply placed against the tail base. A line drawn between the shoulder and the hind limbs is divided into three equal parts (Fig. 54.2). The incision site is located on the boundary between the second and third parts. In all cases the underlying muscle is grasped and elevated away from the coelomic viscera, and small hemostats are gently forced through the coelomic musculature and into the coelomic cavity. The hemostats are removed and replaced by the telescope within its sheath and obturator (with insufflation line attached to one of the ports) (Fig. 54.3). By making a small skin incision and breach in the muscle, the sheath will be tight-fitting and insufflation gas leakage will be minimal. Typically, CO_2 insufflation pressures of 0.5–2 mm Hg with a flow rate not exceeding 0.5 L/min are used. In some situations, saline infusion may be preferred over gas.

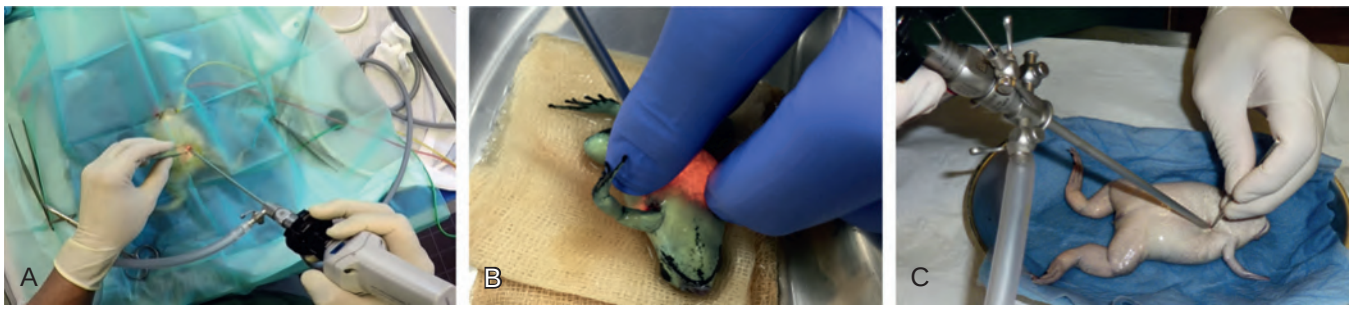
Amphibians have an undivided pleuroperitoneal cavity. The only separated compartment is the pericardial sac. This common coelom permits the visualization of liver, gallbladder, heart, lungs, digestive tract, gonads, kidneys, bladder, and fat body from only a single entry point.

In general, the liver of anurans consists of two completely separated lobes (Fig. 54.4A–G).[10] The liver of caudates, such as the axolotl *(Ambystoma mexicanum)*, is a single elongated organ that may be partially subdivided. One must pay attention to the ventral vein at this point (see Fig. 54.4H and I). A large gallbladder lies on the midline in the interlobular connective tissue of the liver (see Fig. 54.4J). It is important to look for the spleen (see Fig. 54.4K). The stomach is situated dorsally within the left side of the coelom. The intestines fill the contralateral right side (see Fig. 54.4L–N). The intestine may be subdivided into the narrow, coiled small intestine followed by a short, wide large intestine that leads to the cloaca. The lungs lie dorsal to each lobe of the liver and the heart is further cranial, between the shoulders. Each gonad (described later) is associated with a conspicuous fat body which is subdivided into numerous digitiform lobes, pressed up against the pleuroperitoneal cavity. The size of the fat bodies varies greatly with the stage of reproductive cycle. The ovaries vary in size, depending on stage of the reproductive cycle, and may be massive, occupying a large part of the pleuroperitoneal cavity. The small, ovoid testes are less apparent, located in a dorsal position and thus covered by other viscera (Fig. 54.5A–E). The kidneys, paired, dark, flattened, with ovoid structures, are also located dorsocaudally (see Fig. 54.5F). The urinary bladder is large and thin-walled in anurans. Endoscopic biopsy of parenchymatous organs or intracoelomic masses may be performed. Hepatic or renal samplings submitted for culture and histology are used for diagnosis antemortem and postmortem. Collecting liver biopsy samples using laparoscopic surgery is technically easy to perform (see Fig. 54.4O). Laparoscopy provides adequately sized and lesion-specific tissue samples for histopathologic analysis and other tests (e.g., culture and heavy metal analysis).

After examination, the scope is removed and the animal deflates immediately. The coelomic membrane and the skin are closed in one layer with one or two interrupted sutures (Fig. 54.6). Monofilament nylon seems to be the most appropriate suture in amphibian skin.[11] Then the animal is transferred to a warm, anesthetic-free bath and is rinsed copiously with fresh, well-oxygenated water.

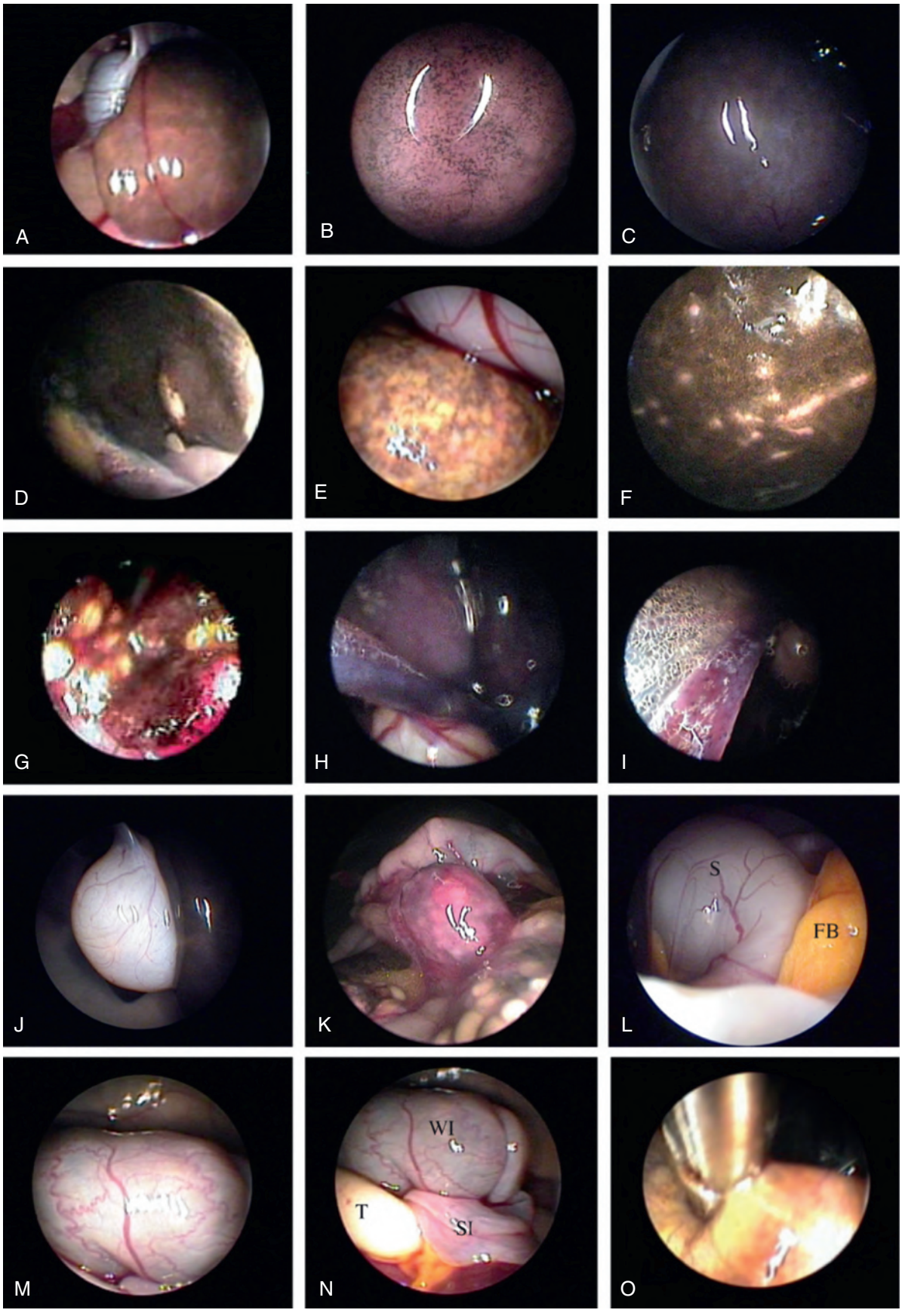
Endoscopic Orchiectomy

Endoscopic orchiectomy in a bullfrog *(Lithobates catesbeianus)* is used here as an example of MIS. The animal is placed in dorsal recumbency. Prior to orchiectomy, a laparoscopic examination is conducted and the gonads identified as described previously (Fig. 54.7A). A second entry is achieved under direct endoscopic visualization and guided by transillumination of the body wall, thereby avoiding



• **Figure 54.3** (A) Laparoscopic examination of an African clawed frog *(Xenopus laevis)*. A 3-lead system electrocardiogram is used to assess the heart rate. The insufflation line is visible, attached to one of the ports of the telescope's sheath. (B) Laparoscopic examination of a Golden poison frog *(Phyllobates terribilis)*. Due to the size of the animal, the monitoring is difficult, thus minimal. (C) A small skin incision allows the sheath to be tight-fitting. Please also note that the incision in this African clawed frog is more cranial: it was for a biopsy of the myocardium. (Copyright Norin Chai.)

• **Figure 54.4** (A) The liver of anurans consists of two completely separated lobes, here in an African clawed frog *(Xenopus laevis)*. (B and C) In the African clawed frog, the normal hepatic gross appearance varies from pale gray, pink brown, to black. Note the normal dark pigmentation due to the presence of melanomacrophages. This is common in most amphibians. (D) Hepatic cystic lesion in an African clawed frog caused by *Contracaecum* sp. (E) Hepatic lipidosis in a wide-mouth frog *(Lepidobatrachus laevis)*. (F) Focal hepatic discoloration in an African clawed frog caused by *Mycobacterium liflandii* infection. (G) Several hepatic abscesses in a western clawed frog *(Xenopus tropicalis)* caused by *Mycobacterium szulgai* infection. (H and I) The ventral vein travels cranially between the lobes of the liver to further divide with a branch entering each lobe. (J) The large gallbladder often appears yellowish (normal). (K) Spleen with multiple abscesses in an African clawed frog caused by *Mycobacterium gordonae* infection. (L) The stomach *(S)* may be found caudal to the liver varying in size and shape depending on the species and nature of the prey. Note the normal fat bodies *(FB)*. (M and N) Coelomic organs with the small intestine *(SI)*, the wide large intestine *(WI)*, and testis *(T)*. (O) Biopsy of the liver. (Copyright Norin Chai.)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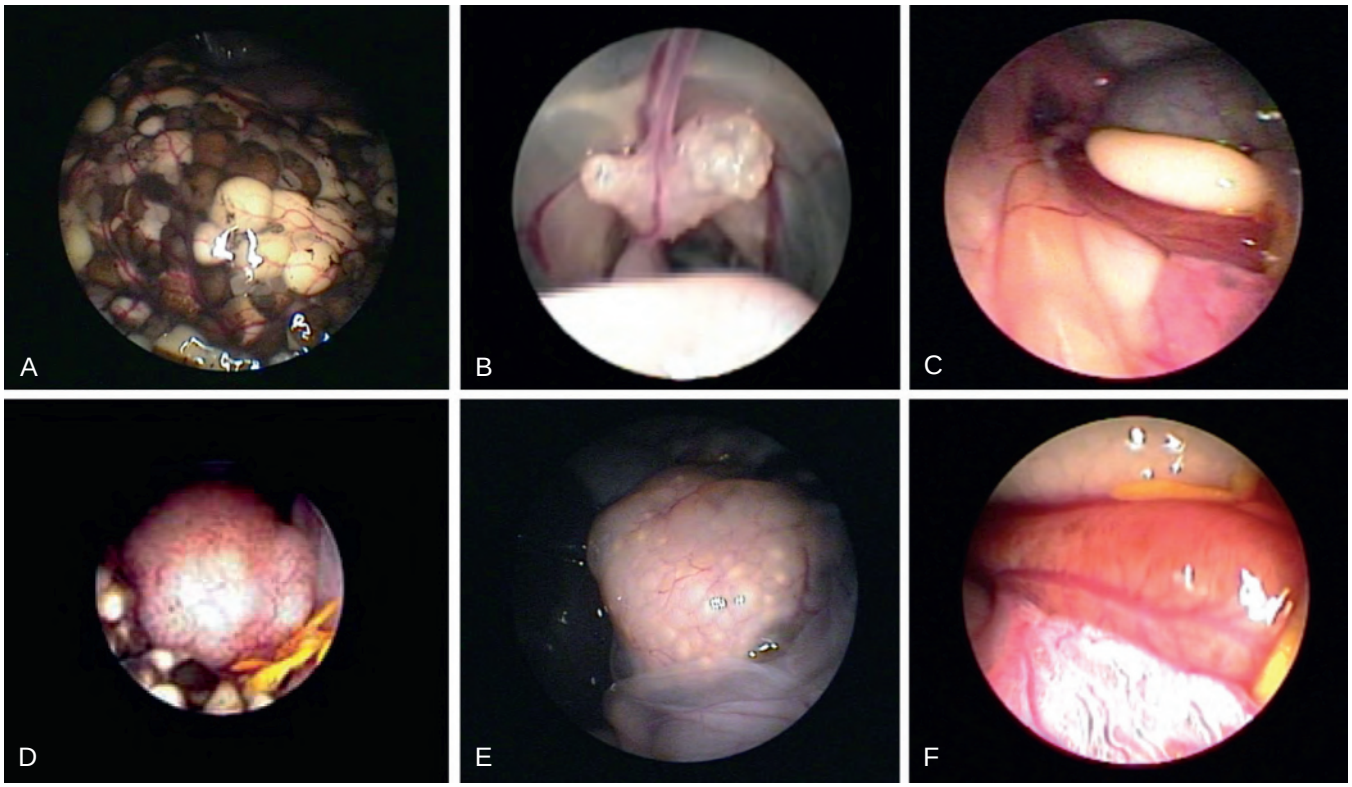


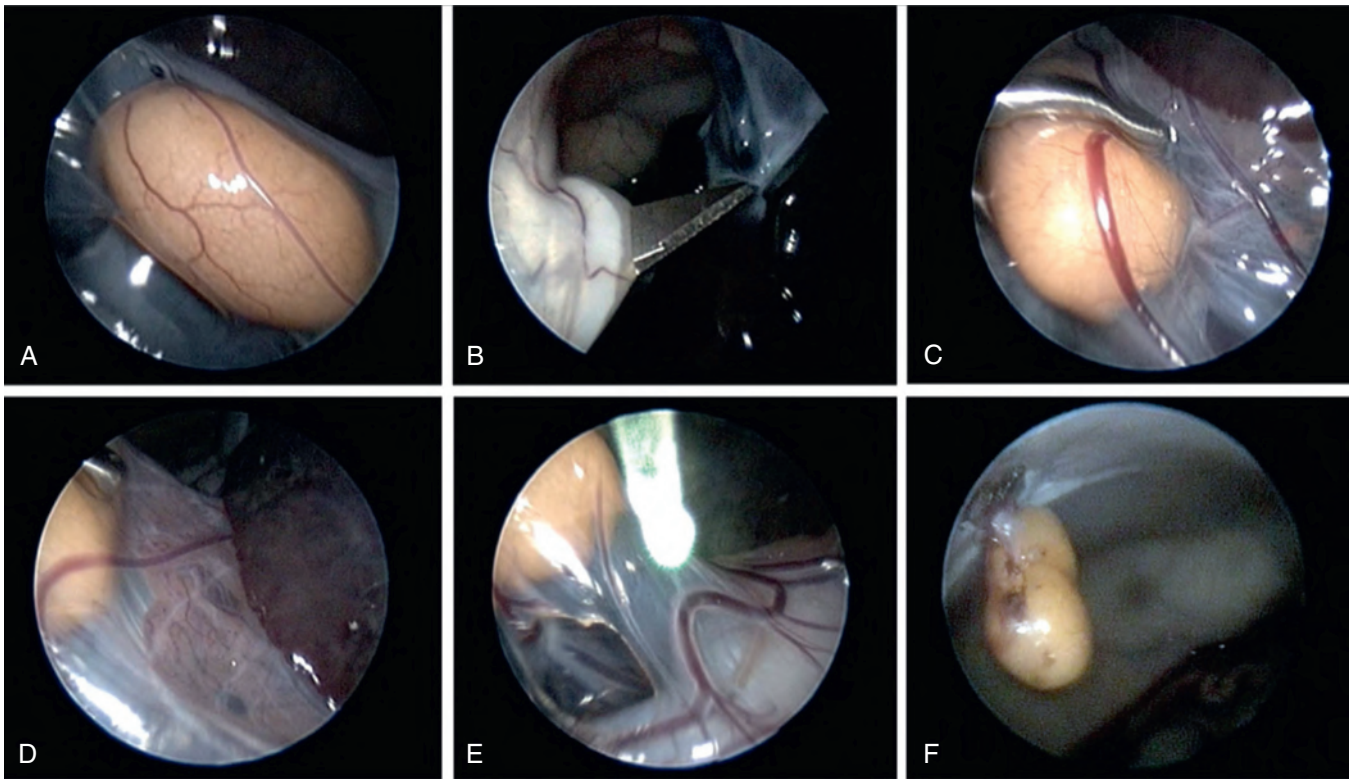
• **Figure 54.5** (A) In mature females, the ova extend cranially, cover the parietal surface, and may occupy large parts of the coelom as showed here in an African clawed frog *(Xenopus laevis)*. (B) Gender identification in immature great crested newt *(Triturus cristatus)*. Note the immature ovaries that are dorsally located. (C) Testis of an adult two-colored leaf frog *(Phyllomedusa bicolor)*. (D) Ovarian abscess in a western clawed frog *(Xenopus tropicalis)* caused by *Mycobacterium szulgai*. (E) Ovarian neoplasia in a two-colored leaf frog. (F) Kidney of a two-colored leaf frog. The kidneys are dark red cigar-shaped cylinders located caudal, lateral to the spine. (Copyright Norin Chai.)

• **Figure 54.6** Closing the coelomic membrane and the skin in one layer with 1 interrupted suture in a Golden poison frog *(Phyllobates terribilis)*. (Copyright Norin Chai.)

large blood vessels (see Fig. 54.7B). A simple incision with a size 11 scalpel blade is sufficient to allow the insertion of an atraumatic 5 mm rigid grasping forceps. The gonad is held with the forceps (see Fig. 54.7C and D), while the surrounding blood vessels and the epididymis are cauterized with diode laser (see Fig. 54.7E). After cauterization, the testicle is removed from the coelom (see Fig. 54.7F). Once the endoscope has been removed, the animal deflates immediately. The coelomic membrane and the skin are closed in one layer with one or two interrupted sutures. Then the animal is transferred to a warm, anesthetic-free bath and is rinsed copiously with fresh, well-oxygenated water.

Application of Minimally Invasive Surgery in Fundamental Research in Amphibians

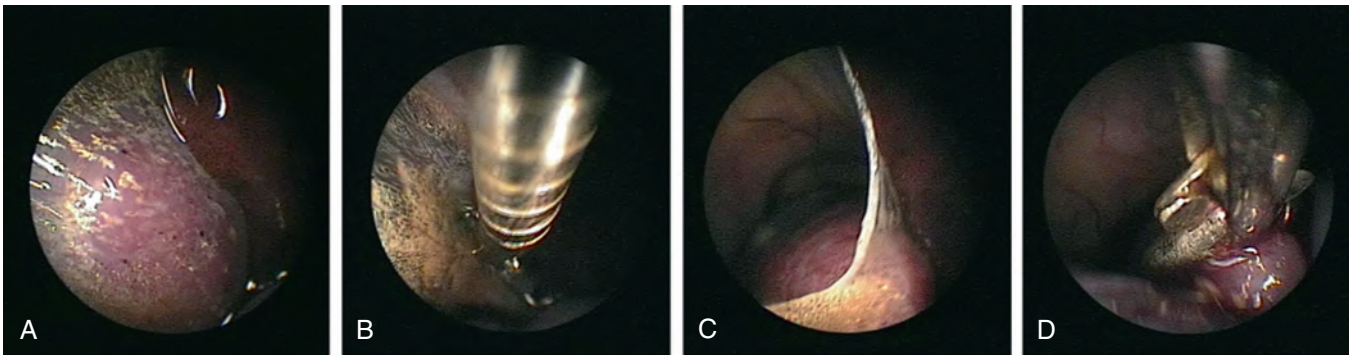
Ischemic heart disease is the leading cause of mortality worldwide. Myocardial infarction results in the loss of cardiac myocytes.[12] In adult humans, these cardiac myocytes cannot be replaced. Fundamental research using model organisms focuses increasingly on determining mechanisms and factors of repair and scarring, with the hope of unlocking the ability to restore damaged cardiac tissue in humans.[13] Two



• **Figure 54.7** Orchiectomy by minimal invasive surgery in a bullfrog *(Lithobates catesbeianus)*. (A) The abdominal cavity is evaluated and the testicles identified. (B) A second entry is achieved with a 11 scalpel blade under direct endoscopic visualization. (C and D) The gonad is held with atraumatic 5 mm rigid grasping forceps. The surrounding blood vessels (E) are cauterized with a diode laser. (F) After cauterization, the testicle is removed from the celom. (Copyright Norin Chai.)

main model organisms used in cardiac regenerative studies are zebrafish *(Danio rerio)* and mice.[13] Both show marked differences, adult zebrafish having a highly efficient cardiac regenerative capacity, while adult mice lack this ability.[12] Amphibians are evolutionarily placed between these two organisms and have been historical models for heart development and cardiovascular physiology.[14] Urodeles such as the neotenic axolotl and adult eastern newt can regenerate their heart ventricle.[15] Although *Xenopus* sp. has been considered as a powerful model organism for regeneration research over the past decades, only limited information is available on the outcome of cardiac injury in this species and other frogs. The author has developed an endoscopy-based resection method to explore the consequences of cardiac injury in adult African clawed frog *(Xenopus laevis)*. This method allowed in situ live heart observation, standardized tissue biopsy size, and reproducibility. The procedures were performed with the same list of material described in Box 54.2. Frogs were fasted 24 hours prior to anesthesia. Presurgical preparations included hydration of the animal in a shallow dechlorinated water bath. Analgesia was performed with an initial dose of butorphanol (1 mg/kg) followed by meloxicam (0.4 mg/kg), both injected into the lymph sacs. After 10 minutes, anesthesia was performed by transferring the animals to a bath of buffered 1% tricaine methanesulfonate (MS-222) for 8 minutes. Loss of righting reflex suggested a light stage of anesthesia. The surgical plane was indicated by the loss of withdrawal reflexes. For this procedure, the 3-mm paramedian skin incision was made just beneath the sternum. Care was taken to not damage the mid-ventral vein. Following skin incision, the abdominal membrane was elevated, incised, and carefully dissected. The telescope-sheath system was inserted into the pleuroperitoneal cavity, which was insufflated with CO_2. After endoscope insertion, the heart was located behind the falciform ligament (Fig. 54.8A); then using the endoscopic biopsy forceps, the falciform ligament was opened and the single ventricle chamber was immediately apparent (see Fig. 54.8B and C). The pericardial sac was carefully pinched open; then during the ventricle diastole, a biopsy was performed toward the heart apex (see Fig. 54.8D). Once the scope was removed, the animal deflated immediately, naturally removing the CO_2. The coelomic membrane and the skin were closed in one layer using interrupted sutures with monofilament nylon. The animal was then transferred to an anesthetic-free bath and rinsed copiously with fresh, well-oxygenated dechlorinated water. The minimal invasive surgery itself (from incision to skin suture) took an average of five minutes.

Finally, using this endoscopy-guided cardiac injury procedure, it has been shown that the adult *Xenopus* sp. heart was unable to regenerate.[12] However, adult *Xenopus* sp. may complement adult mammalian models to study the consequences of heart injury after tissue removal.



• **Figure 54.8** Biopsy of the myocardium in an African Clawed Frog *(Xenopus laevis)*. (A) Note the heart behind the falciform ligament. (B and C) With an endoscopic biopsy forceps, the falciform ligament is opened and the single ventricle chamber is immediately apparent. (D) The pericardial sac was carefully pinched open; then during the ventricle diastole, a biopsy was performed toward the heart apex. (Copyright Norin Chai.)

Furthermore, adapting this minimally invasive method of cardiac injury to other species could help in translating the collected information into therapies aimed at salvaging and repairing failing human hearts.

Recovery and Postoperative Management

Recovery from water-bath–based anesthesia (MS-222) is accomplished by thoroughly rinsing the animal with anesthetic-free dechlorinated water. The author finds that a bath well oxygenated decreases the recovery time for aquatic species. Aquatic animals should have their head out of water during recovery. As the animal begins its recovery, the withdrawal reflex and gular respirations are the first to return followed by the righting reflex. The amphibian should be considered recovered when all of the reflexes have returned and heart and respiration rates have returned to preanesthetic values. The primary factors to consider postoperatively are analgesia, and continued vigilance concerning hydration, nutrition, and hygiene. The continuation of preemptive analgesia using opioids and/or nonsteroidal antiinflammatory agents should be a routine part of postoperative care.

An important adjunct to maintain hydration and restore the health of ill amphibians is to soak the animals in balanced electrolyte solutions. A simple formulated solution for use in amphibian patients consists of one part of saline (0.9% NaCl) mixed with two parts of 5% dextrose.[9] Alternatively, a solution may be formulated with seven parts of saline mixed with one part of sterile water. An appropriate dose of either solution is 25 mL/kg of body weight.[9] Two other classic formulations are amphibian Ringer's solution and Holtfreter's solution (3.46 g NaCl, 0.1 g $CaCl_2$, 0.05 g KCl, and 0.2 g $NaHCO_3$ per liter of dechlorinated water). It may be necessary to assist the amphibian patient with feeding for a brief period after any surgical procedure. A/D Prescription Diet (Hill's Pet Nutrition) or highly palatable energy supplement for cats and dogs like Nutrigel (Virbac) are suitable choices for nutritional support in amphibians that are not self-feeding (Fig. 54.9). Antibiotic therapy is not routinely recommended after MIS and will depend on the context. In all cases, it should be in conjunction with microbial investigation.

• **Figure 54.9** A two-colored leaf frog *(Phyllomedusa bicolor)* is assist-fed with a highly palatable energy supplement for cats and dogs (Nutrigel, Virbac). (Copyright Norin Chai.)

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55

Medical Aspects of the Hungarian Meadow Viper Reintroduction

ENDRE SÓS AND BÁLINT HALPERN

Introduction and History of the Project

The Hungarian meadow viper *(Vipera ursinii rakosiensis;* [HMV]*)* is a small and elusive venomous snake which was first described by the famous Hungarian zoologist, Lajos Méhely in 1893.[1] This taxon is considered a subspecies within the intensively studied *Vipera ursinii* species complex (Orsini viper), which includes lowland and mountain members (Fig. 55.1). Aspects of the life history of the HMV have only recently been understood (Table 55.1).

Formerly, this species inhabited many suitable habitats and its distribution covered areas in Romania and Austria as well. Until 2004 it was thought to be found only within the boundaries of Hungary, but four small populations were recently rediscovered in Transylvania, Romania. Despite this finding, the current distribution area is much more restricted than the historical range.[2] There are only two remaining fragmented populations in the Hanság and Kiskunság areas of Hungary, whereas in all the other former habitats the species has become extinct. There are two subpopulations found in the Hanság and nine in the Kiskunság areas. The total population size is estimated to be between 500 and 1000 individuals. As with many reptile taxa, an accurate census is difficult and population trends are based on observations in the wild. Viper collectors from the 1970s reported that 50 vipers could be found in 1 day at some sites; however, in field studies at the end of the 1990s, only one viper was found in 50 days spent searching.

The main cause of population declines is the continuous loss of habitat due to the intensification of agriculture and use of grasslands after World War II. Because this subspecies is the inhabitant of steppe remnants, draining of marshy meadows and plowing of grasslands greatly reduced and fragmented the suitable natural habitats, and mechanical mowing techniques physically threatened the survival of snakes and changed the structure of remaining habitats. The illegal pet trade and the overall persecution of venomous animals further reduced the number of the vipers and enhanced this negative trend. Current viper populations occur on diverse grasslands, where high prey abundance and several different microclimates still provide an ideal habitat for the species. Despite its name, areas with the lowest elevation and unpredictable water levels are dangerous for HMVs and are therefore not preferred.

The HMV has been protected in Hungary since 1982, strictly protected since 1986, and was raised to the highest conservation category in 1992. This viper is the most endangered vertebrate species in the country and became directly threatened with extinction at the end of the 20th century. Its critical situation was also recognized internationally; therefore it is included in the Bern Convention Appendix II,[5] is listed in Appendix I of the Convention on International Trade in Endangered Species of Wild Flora and Fauna,[10] and categorized as "Endangered" on the International Union for Conservation of Nature and Natural Resources (IUCN) Red List.[9] The Bern Convention approved a European conservation action plan for the meadow viper in 2005.[8] In Hungary an appropriate species conservation plan exists for the protection of this species, and a complex conservation project was initiated, cofunded by European Commission's LIFE and later LIFE+ funds.

In April 1993, at the start of these complex conservation efforts, the total HMV population was estimated at less than 500 individuals. At that time, conservation experts initiated a long-term conservation program and agreed on a number of principles, which included basic research, learning about the life history of the species, and identifying other possible threat factors and reasons for the severe and continuous population decline.

Conservation Actions and Results

In 2001 a 2-day Population and Habitat Viability Assessment (PHVA) meeting was organized at Budapest Zoo,

• **Figure 55.1** The endangered Hungarian meadow viper *(Vipera ursinii rakosiensis)* is one of the lowland subspecies of the Orsini viper *(Vipera ursinii)* species complex.

TABLE 55.1 Life History Traits of the Hungarian Meadow Viper *(Vipera ursinii rakosiensis)*

Biological Characteristics	Value/Fact
Length (adult)	45–60 cm
Length (newborn)	14 cm
Weight (adult)	50–70 g (gravid female may reach 150 g)
Weight (newborn)	2.4 g
Feeding	Orthopterans, lizards, rarely birds and rodents
Sexual maturity	3–4 years
Breeding season timing	Mating in April, parturition from late July until early September
Gestation period	116–135 days
Way of reproduction	Ovoviviparous
Number of offspring	5–20 (observed maximum 27)
Venom	Weak, fatality is not described

facilitated by the International Union for Conservation of Nature Conservation Breeding Specialist Group (IUCN CBSG). The various stakeholders of the event produced the following general conclusions: Wide-scale mapping and data collection are crucial; minimal viable population size needs to be determined; the exact reasons of continuous population size decline need to be identified; communication among stakeholders needs to be improved; consensus is needed regarding the best type of habitat management among the nature conservation bodies; "viper-friendly" habitat management at all remaining habitats and sites needs to be achieved; decisions affecting the level of the water table need to take into account the presence or absence of vipers; the creation of a captive breeding center at Kiskunság is needed; a reintroduction strategy needs to be devised; and the creation of a zoo assurance population is needed.[6] The last three points contain medical aspects already under consideration at that time.

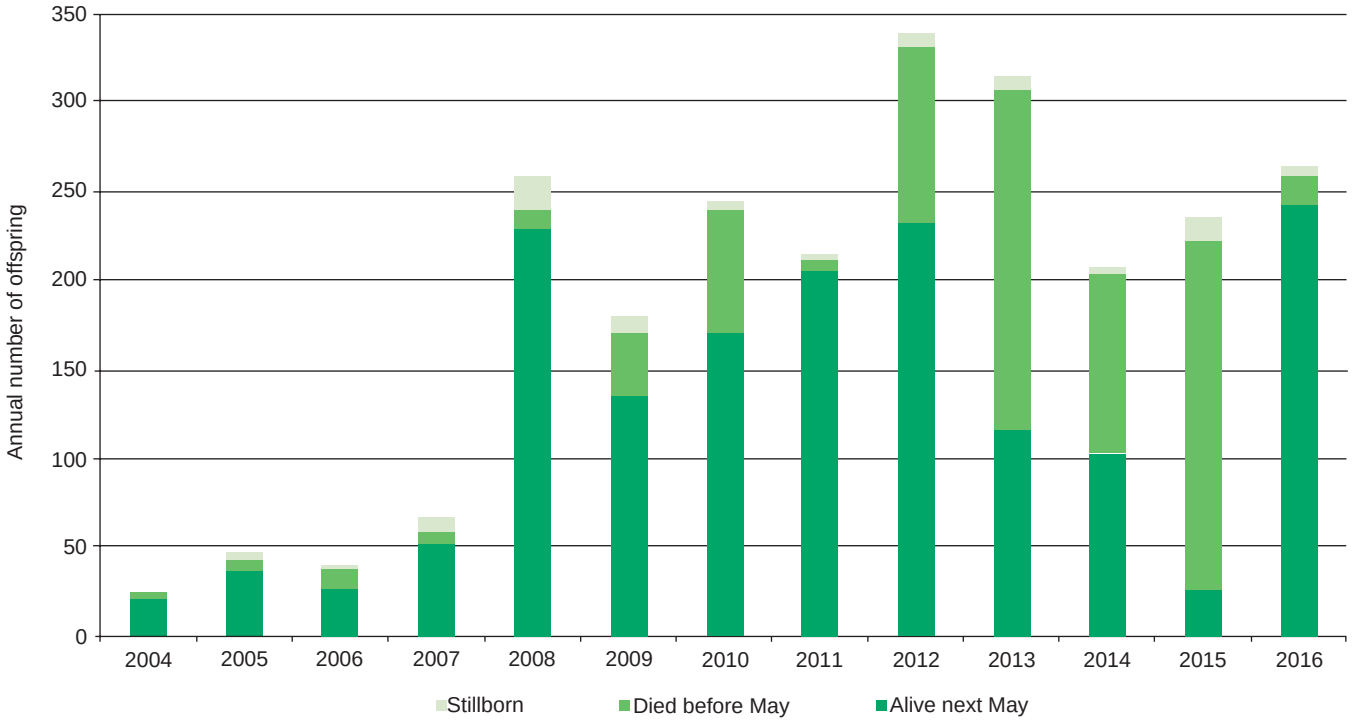
In 2004 BirdLife Hungary, several national parks in the country, and Budapest Zoo initiated a complex, European Union LIFE-Nature fund endorsed conservation program.

The Hungarian Meadow Viper Conservation Centre (HMVCC) was established as part of this project, with 16 adult individuals collected from six different wild populations because there were no legal animals kept in captivity. The main goal of the operation of the HMVCC was to breed the vipers collected from the endangered populations and establish a sufficient sized, stable, and genetically diverse captive population that would serve as a solid base for future reintroductions. This original plan was achieved by the end of 2016, when the number of vipers bred at the HMVCC reached approximately 2350 vital individuals (stillborn vipers or individuals that died within a very short period of time were excluded) (Fig. 55.2). The first reintroductions took place in March 2010, when 30 adult snakes were released into a reconstructed habitat in Kiskunság National Park. By the end of 2016, a total of 464 animals have been released at six locations (three in the Kiskunság and three in the Hanság). Snakes were released by relocating the animals in the artificial burrows that are used in the seminatural terraria at the HMVCC. At the release sites, vipers are continuously monitored and recorded after release, and individuals either are identified according to their head scale patterns or are radio-tagged.

Medical Considerations and Management

One of the first actions in 2001 at the Budapest Zoo was the creation of a breeding center for the closely related Steppe viper *(Vipera renardi),*[4] which served as a model species to gather scientific information (husbandry and medical aspects) for the HMV conservation breeding program. This activity proved to be essential because visceral gout was found in a number of Steppe vipers before the native species program was begun. During the early part of the modeling phase, the relative humidity of the holding facilities was kept at only 30%–40%, which most likely led to the development of this lethal condition. Due to this experience, humidity was raised to 70%, which prevented further cases of gout in the Steppe vipers and established the basis of proper husbandry of the HMV.

The HMVCC was officially opened in June 2004 in the area of the Kiskunság National Park, adjacent to currently known viper habitats. The HMVCC has two subdivisions: one for the adult animals housed in outdoor terraria, and another for the youngsters housed in indoor terraria. Outside, seminatural terraria were created, providing conditions as close to natural as possible, where vipers from geographically isolated populations had the chance to breed among appropriate and annually changing climatic conditions while eliminating problems, such as inbreeding,



• **Figure 55.2** Breeding results of the Hungarian Meadow Viper Conservation Centre.

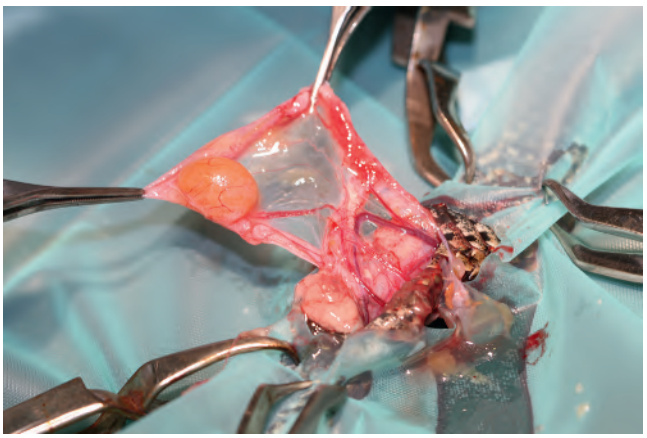
arising from small size of recent populations.[7] Similar to many other species, juvenile mortality is the highest in the first year of life of the animals. Therefore young vipers born at the HMVCC reached adulthood in higher percentage than those in natural populations, due to prey abundance, lack of predators and separate keeping in terraria during their first winter. At the beginning of the program (between 2004 and 2008), juveniles were housed indoors in a heated facility for their first winters and were released into outdoor, seminatural enclosures late in the following spring. This practice helped to establish a relatively large and strong population within a short time frame. After the rapid growth of the captive population, this practice was neither possible logistically, nor important for the benefit of the program; thus all vipers (including the youngsters) spent their first winter in artificial wintering burrows in their seminatural terraria. These circumstances closely mimic the natural conditions but allow only limited control of the animals. An annual mortality of approximately 10% of juveniles was observed in the cohorts of 2004–2008, but since 2009 a higher first-year mortality was recorded, although this level in most of the years is considered still lower than in the wild, where predation must also be factored in for this age group.

The breeding element of the conservation program was a crucial contribution factor for the survival of the HMV. However, it had several medical implications, including the wild source populations, the captive conservation breeding populations, and the intended animals for release. Screening, quarantine, and other preventive medicine tools needed to be implemented by the program overall (including incoming founder animals, translocated individuals within the HMVCC, and assigned individuals for release). Individual treatment was also possible, but it was mainly restricted to captive or rescued animals at the HMVCC, or snakes exhibited in two zoos (Budapest and Szeged) in Hungary. Because the conservation breeding program was lacking even basic information, it was essential to collect microbiologic samples from seemingly healthy individuals from the beginning, when establishing a solid captive population for breeding purposes. Each May between 2005 and 2008, during the release process into seminatural terraria, cloacal swabs, fecal samples, and skin impressions were collected to gather data about the health status of the young animals, as well as to obtain a clear picture of the normal enteral flora and the parasite status of the snakes (these samples were used for bacteriologic, parasitologic, and virologic studies). This step was of utmost importance, even for the latter stages of the project, because releases could pose a potential threat to native populations through the transmission of diseases. We found that cloacal swabs and fecal material were easily collected even from 9- to 10-month-old youngsters. The results did not yield the presence of pathogenic organisms, but similarly to many other reptile species, *Salmonella* sp. was found to be part of the normal enteral flora of HMVs, and all obtained samples were free of ophidian paramyxovirus (OPMV). Regular parasitologic, bacteriologic, and virologic sampling of HMVs continues to be part of the medical protocol of the HMVCC.

Diagnostics and Special Medical Conditions

Due to the small size and anatomy of the HMV, clinical examination has its limitations. The primary site for blood

• **Figure 55.3** The delicate ventral aspect of the caudal vein is the preferred site for blood collection in the Hungarian meadow viper *(Vipera ursinii rakosiensis).*



• **Figure 55.4** Ovariohysterectomy in the Hungarian meadow viper *(Vipera ursinii rakosiensis).*

collection is the ventral aspect of the caudal vein (Fig. 55.3). Although blood can be drawn from the right palatine vein as well, it is not advisable, due to the close proximity of the fangs and the collected blood is often mixed with snake venom. Because only a small amount may be taken at a time (maximum 1% of body weight), the samples were mainly used for genetic analyses. Evaluation of the health status was difficult because the collection of the proper volume may be compromised; therefore sometimes the most relevant and informative values were measured exclusively. Due to the small size of the species, blood collection from the heart is contraindicated.

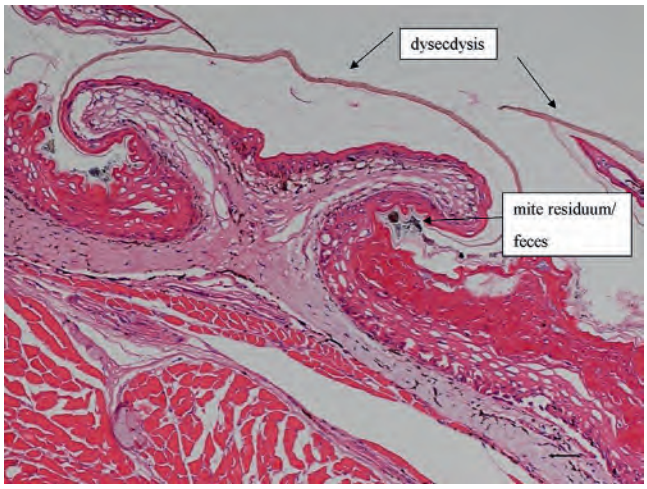
Apart from the physical examination (including sampling) and blood work, diagnostic imaging was generally very useful due to the small size of the species. Due to the fact that the main goal of the program was conservation breeding, we used radiography and ultrasonography to assess the reproductive tracts. We may conclude that, for the detection of late pregnancy, both methods are suitable, but because of the potential teratogen radiologic effect of radiology, we strongly advise use of ultrasound equipment (with 8–11 MHz frequency probes).

As a rare reproductive medical condition, dystocia was observed several times and caesarean sections were performed in these instances. The parturition process may take some time in vipers, and a relaxed environment is of utmost importance. According to our protocol, dystocia is present if labor is longer than 24 hours. The first such case took place in August 2005, when parturition appeared to have ended in an adult female after seven juveniles were born and despite conservative medical treatment (oxytocin and calcium), surgery was needed to remove five more young, of which two were still alive. The surgical technique was performed similarly to other snake species by way of a paramedian laparotomy, visualization and elevation of the uterus, removal of the fetuses, and suturing the wall of the uterus and the coelomic wall. One must consider that the proper closure of the wound edges is quite challenging due to the thin muscle layer. The contributing factors of dystocia in HMVs are not fully understood. However, based on our experience, although these females could theoretically produce offspring in the subsequent season, the likelihood of a repeated dystocia is very high; therefore we recommend ovariohysterectomy in these individuals (Fig. 55.4).

Healthy newborn vipers usually complete their first shedding within 10 minutes of birth; however, we do encounter problems with shedding relatively frequently. The affected animals are emaciated, refuse to eat, lose weight rapidly, and are unable to go through the normal sloughing process. Usually 2–4 layers of dry, parchment-like skin may be found on their bodies, which is strongly attached, and it is impossible to simply remove it. Despite force feeding, topical hydration, warm baths, and other supportive treatment, only approximately 30% of these affected animals may be saved.

During the late autumn of 2006, we lost 17 young vipers born that same year out of 39 individuals. The clinical symptoms were rather complex, including respiratory, integumentary, and neurologic signs. One key element of the differential diagnosis was to exclude the possible involvement of OPMV infection because it could have been detrimental to the future success of the whole program. Disease was detected only among the young individuals in the inside part of the HMVCC, and spread was presumed to be from neighboring individuals in terraria in one row. The final conclusion of this massive mortality was a reptile mite *(Ophionyssus natricis)* and secondary bacterial infection (Fig. 55.5).

Apart from the problems with juveniles, in a few cases mechanical trauma had to be treated in rescued animals. Injuries were caused by lawn mowers and attack by predators. For such cases, devising a safe anesthesia protocol was mandatory. In our experience, for premedication, we recommend the combination of ketamine hydrochloride (20–40 mg/kg) and midazolam (1–2 mg/kg) be given intravenously or into the coelomic cavity. Intravenous administration may greatly reduce induction times and dosage, but due to the small size of the species, it is often not possible, and larger

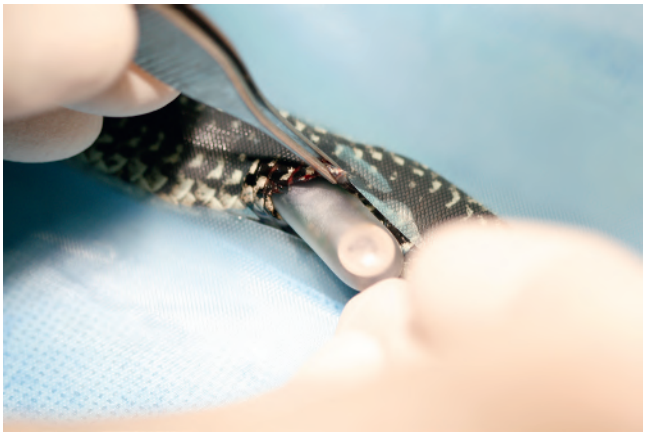


• **Figure 55.5** Reptile mite *(Ophionyssus natricis)* infection in the Hungarian meadow viper *(Vipera ursinii rakosiensis).* Dysecdysis (hyperkeratosis and spongiosis) and mite residuum and feces are visible under the scales. (Courtesy Nadia Robert.)

• **Figure 55.6** Radiograph of a radiotelemetry surgically implant in a Hungarian meadow viper *(Vipera ursinii rakosiensis).*

volumes may cause local tissue circulation disorders in the tail. Following premedication, maintenance with isoflurane or sevoflurane is straightforward. Vipers are usually agitated enough not to withhold their breath; therefore masking them after premedication is possible in the majority of cases. Although intubation is straightforward, we preferred the use of masks when using gas anesthesia for safety reasons because we did not want to manipulate close to the fangs during procedures. However, it was sometimes necessary (e.g., when the surgical site was on the head of the animal). Nevertheless, because the depth of the anesthesia is difficult to assess in reptiles, we strongly recommend an experienced person to physically restrain the viper for safety reasons during the entire procedure.

As the program reached the release phase, postrelease monitoring of individuals and the continuous evaluation of the project were important elements with medical linkages. Former experience in the 1990s was controversial with radiotelemetry in the species[3]; therefore the experts of the Research Institute of Wildlife Ecology, Vienna, Austria (FIWI) were involved in the design and production of tailor-made tagging devices. To achieve this task, preprogrammed, very-high-frequency radio-tags, with a detection range of 200–300 m, were surgically implanted into various, larger and subsequently similar-sized snake species initially and finally into the coelomic cavities of 23 HMVs. These tags also operated as temperature loggers, recording data every 5 minutes for 1 year. Before releasing tagged vipers, we carried out tests in the outdoor terraria of the HMVCC in 2011 by implanting 10 vipers (2 with radio-tags and 8 with temperature loggers, which were the same size, but without an antenna) (Fig. 55.6). These individuals were monitored closely to determine whether or not the implant affected their general behavior or quality of life. We concluded that all snakes showed normal behavior: feeding, defecating, shedding, and some even producing offspring ($n = 3$), and



• **Figure 55.7** Surgical implantation of a temperature logger into a Hungarian meadow viper *(Vipera ursinii rakosiensis).*

all of them grew significantly during the tagged period. The tags were implanted under anesthesia, and the vipers generally required a month for healing before sutures were removed (Fig. 55.7). We also concluded that these tags operated reliably (frequency of operation and life span).

During all surgeries related with tag implantation or removal, there was only one casualty out of 63 procedures. This female overwintered with her radio tag and had surgery almost immediately after emerging from hibernation. Postmortem evaluation did not reveal complications linked to the surgical procedure. We concluded that this incident might be explained by the inability of the snake to metabolize the anesthetic drugs normally, due to reduced post-winter metabolism because hibernation was significantly prolonged by unusual snow cover that year (March 2013). We also concluded that in similar cases, surgical interventions and anesthesia should be postponed to a later date, when individuals are showing signs of normal metabolism, including feeding and digesting.

We strongly feel that the medical elements were very important bricks in the structure of the HMV project. A proper medical program may ensure not only the health of the individuals of the conservation breeding program but provide a One Health concept for the whole population, including released and genuinely wild individuals.

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56 Ophidiomycosis

JEAN A. PARÉ

Background

Fungal isolates recovered from cutaneous lesions in a ball python *(Python regius)* in the United Kingdom in 1985, a captive corn snake *(Pantherophis [Elaphe] guttatus)* in western New York in 1986, a garter snake (*Thamnophis* sp.) in Germany in 1999, captive brown tree snakes *(Boiga irregularis)* in Maryland in 1999, a captive Pueblan milk snake *(Lampropeltis triangulum campbelli)* in Wisconsin in 2001, a file snake (*Acrochordus* sp.) in Australia in 2003, salt marsh snakes *(Nerodia clarkii)* in Central Florida in 2006, a captive Eastern diamondback rattlesnake *(Crotalus adamanteus)* in Tennessee in 2006, a captive green anaconda *(Eunectes murinus)* in California in 2008, and a captive broad-headed snake *(Hoplocephalus bungaroides)* in Australia in 2010 were all, under light microscopy, morphologically identical to each other and to the *Chrysosporium* anamorph of *Nannizziopsis vriesii* (CANV).[1] These fungi were therefore classified as CANV-like or CANV complex isolates until recent molecular studies demonstrated that they differed significantly and formed their own separate clade.[1] The monotypic genus and species *Ophidiomyces ophiodiicola (Oo)* were erected to accommodate these former CANV-like snake isolates.[1] Over the last 10 years, fungi initially identified as *Chrysosporium ophiodiicola* were recovered from a black rat snake *(Pantherophis [Elaphe] obsoleta)*, free-ranging eastern massasaugas *(Sistrurus catenatus catenatus)*, and timber rattlesnakes *(Crotalus horridus)* with dermatomycosis.[2–4] These isolates were later found to be molecularly identical to *Oo*. The disease was referred to as snake disfiguration syndrome due to massive distortion of facial features in several infected wild massasaugas, but lesions were not as severe in other free-ranging crotalids and sympatric colubrid snakes; therefore the disease was renamed snake fungal disease (SFD). This term should be discouraged and the disease more accurately referred to as ophidiomycosis.[5]

Etiology

Ophidiomyces ophiodiicola, the causal agent of ophidiomycosis, is consistently isolated from snakes with SFD.[5] Corn snakes and cottonmouths *(Agkistrodon piscivorus)* experimentally challenged with *Oo* conidia developed skin lesions typical of SFD, providing further evidence to support a causative role.[6,7] *Ophidiomyces ophiodiicola* is an onygenalean ascomycetous fungus in the family Onygenaceae.[1] It is an anamorphic fungus for which a teleomorph has not yet been identified in spite of repeated in vitro mating attempts. It grows as a white, velvety to powdery colony with a whitish or slightly yellow reverse. Growth is relatively slow and is enhanced on selective agars that contain cycloheximide, an inhibitor of ubiquitous saprophyte fungi that would otherwise overgrow the medium and impede or mask *Oo* growth. Chloramphenicol or another antibiotic in the agar will prevent bacterial growth that could further inhibit fungal proliferation. Vegetative *Oo* hyphae are unpigmented, narrow and parallel-walled, septate, and branched. Aleurioconidia are formed but the fungus mainly arthroconidiates, often heavily at the surface of infected integument. Arthroconidia are a result of segmentation of hyphal cells at the septa and are readily identifiable in histologic sections or cytologic preparations as rectangular conidia in more or less linear arrangements. An enzymatic arsenal allows *Oo* to degrade keratin, proteins, chitin, lipids, and a variety of other substrates so that it may well survive on various environmental substrates or items in the absence of a host.[8] Maximal growth occurs at 25°C, although it will not grow or grow only very slowly at 35°C. It can, however, grow at temperatures as low as 14°C. It is tolerant of pH ranging from 5 to 11, although a pH of 9 allows for optimal growth.[1,8]

Distribution and Host Range

Ophidiomycosis has been reported in captive snakes from North America, Europe, and Australia.[1,9] Only in North America has the infection been documented in free-ranging snakes, chiefly in the Midwest, New England, and down the Atlantic coast. Ophidiomycosis has also been diagnosed in eastern fox snakes *(Pantherophis gloydi)* in southern Ontario, Canada. Host range is strictly limited to snakes and includes crotalids (timber rattlesnake, eastern diamondback rattlesnake, eastern massasauga, pygmy rattlesnake *[Sistrurus miliarus]*, copperhead *[Agkistrodon contortrix]*, cottonmouth, colubrids (corn snake, garter snake, ribbon

snake *[Thamnophis sauritus]*, water snakes [*Nerodia* spp.], black rat snake, eastern fox snake, black racer *[Coluber constrictor]*, northern pine snake *[Pituophis m. melanoleucus]*, mud snake *[Farancia abacura]*, rainbow snake *[F. erytrogramma]*, ring-necked snake *[Diadophis punctatus]*, eastern milk snake *[Lampropeltis t. triangulum]*), acrochordids (file snake, *Acrochordus* sp.), pythonids (ball python), boids (green anaconda), and elapids (broad-headed snake). Only recently has ophidiomycosis been diagnosed outside of North America, in wild European snakes, specifically grass snakes *(Natrix natrix)* in the British Islands and a dice snake *(N. tessalata)* in the Czech Republic.[9] It is likely that all snakes are susceptible.[10]

Epidemiology

Available data indicate that infection of captive snakes with *Oo* antedates that of wild snakes by two decades or more.[1,9] The two first known *Oo* isolates were collected a year apart, in 1985 and 1986, from geographically distant sources: a captive ball python in the United Kingdom and a corn snake in western New York State. In Australia in 1976, three wild-caught carpet pythons *(Morelia spilotes)*, within a few months in captivity, died of fungal dermatitis in which *Oo*-like arthroconidial tufts were histologically disclosed at the skin surface.[11] *Geotrichum candidum*, recovered from these pythons, was likely a misidentification, as *Oo* had not yet been described. In fact, in the past, *Oo* and other former CANV-like fungi have been consistently mistaken in the lab for other arthroconidiating fungi, such as *Trichophyton* spp., *Trichosporon* spp., *Geotrichum candidum*, and *Malbranchea* sp. A case of severe, deep granulomatous facial mycosis in a captive western massasauga *(Sistrurus c. tergeminus)* in a Central Florida Zoo in 1979 was likely a case of ophidiomycosis misdiagnosed as phycomycosis (zygomycosis).[12] *Ophidiomyces ophiodiicola* was likely present on several continents as early as in the 1980s. The global trade in pet reptiles might well have facilitated movement of this pathogen.

Oo is rarely recovered from the skin of healthy captive snakes. It was isolated from only 1 of 91 healthy snakes sampled in one study, in contrast with aspergilli, penicillia, and other ubiquitous saprophytes.[13] This one isolate, from an African rock python (*Python sebae*) in a southwestern zoo, remains the only *Oo* isolate not to have been cultured from an actual skin lesion.[13] Attempts to identify *Oo* by means of polymerase chain reaction (PCR) from the skin of 31 wild massasaugas that were all free of lesions were unsuccessful,[14] further underlining a strong association between the presence of this fungus on the skin of snakes and the presence of lesions/disease. Many case reports of ophidiomycosis in captive snakes involve recently captured animals, suggesting that the fungus was carried by these animals and that the stress of captivity may have resulted in disease. Ophidiomycosis is contagious and can spread among captive snakes. Propagation is likely by means of arthroconidia on the surface of infected or shed skin. Arthroconidia are hardy, persist in the environment, and can survive freezing.

When, where, and how ophidiomycosis (SFD) appeared in wild snakes in North America remains speculative. Mild skin lesions typical of hibernation sores/blisters in timber rattlesnakes at emergence were confirmed as ophidiomycosis, raising the possibility that this disease has been around for some time and that climate change and other factors may have resulted in the increased expression and severity of infection. Spillover of infection from captive to free-ranging snakes appears much less likely.

Wild snakes may become infected with *Oo* through contact with sick snakes or with exuvia left behind by sick snakes, with contaminated soil, or with fomites. Recent use of newer, more sensitive molecular assays did disclose the presence of *Oo* at very low levels on the skin of some normal-looking snakes[15,16]; therefore the risk exists that healthy carriers may introduce disease in a collection or population. Koch's postulates for *Oo* have been fulfilled in experimental challenges of corn snakes and cottonmouths.[6,7] The severity of lesions in infected animals appears to vary between snake species, ranging from disfiguring in many massasaugas to very mild in northern pine snakes; it also varies across individuals within the same species.

Pathogenesis, Clinical Signs, and Disease Outcome

Conidia come in contact with intact or abraded/breached snake skin; they germinate and colonize the epidermal corneum with proliferating vegetative hyphae that release keratinases and other proteases. This results in epidermal necrosis and the recruitment of inflammatory cells, initially heterophils. Eventually hyphae move down into the deeper epidermal layers and then through to the dermis. Hyphal proliferation may extend to underlying muscles and bones, especially in massasaugas and other crotalids. Clinically, focal or multifocal and coalescing scale necrosis occurs. The head and face seem particularly affected, maybe because the head is the part of the body that first contacts a potential source of the fungus and is also most likely to present abrasions or other wounds. Scales and scutes are discolored, swollen, with subsequent epidermal brown crusting (Fig. 56.1). Ulceration occurs, as sometimes does hyperkeratosis with thickened crusts or eschars. Granulomas in the subcutis may cause swelling, and infection may extend to underlying muscles and bone and result in disfiguration. Very rarely does terminal fungemia or dissemination of the fungus to internal viscera occur. Snakes typically remain alert until disease has progressed. Lesions on the head and often around the nares and the nasolabial pits in crotalids progressively impede hunting and feeding, so that animals lose condition and die.

Infected snakes often undergo more frequent ecdysis, as if to better rid themselves of skin fungal loads. Infection also leads to behavioral changes and aberrations that may affect

• **Figure 56.1** Free-ranging timber rattlesnake, *Crotalus horridus*, with multifocal active scale necrosis and extensive scarring over the head due to *Ophidiomyces ophiodiicola* infection. The spectacle is pleated and deformed.

• **Figure 56.2** Multiple swellings due to subcutaneous *Ophidiomyces ophiodiicola* mycetomas over the body of a black rat snake, *Pantherophis obsoletus*.

survival, such as seeking open areas or exiting hibernacula in the winter months.

A peculiar presentation occurs in some colubrids, mainly rat snakes (*Pantherophis* spp.), in which multiple subcutaneous mycetomas result in the presence of swellings along the body (Fig. 56.2). The reasons for this atypical form of ophidiomycosis in pantherophine snakes are unclear, but clinicians need to consider ophidiomycosis in rat snakes with multiple cutaneous lumps.

• **Figure 56.3** (A) Right lateral view of the head of a free-ranging corn snake, *Pantherophis guttatus*, with ophidiomycosis. (B) Right lateral view of the head of the same corn snake, *P. guttatus*, postecdysis. The hyphema resolved and the snake was released. ([A] Photo courtesy of Michael Bisignano; [B] Photo courtesy of Julie Larsen Maher, WCS.)

Many snakes that exit hibernacula with mild lesions in the spring seem to improve at each subsequent shed throughout the summer. This is likely a result of increased ambient temperature, leading to a more efficient immune response in infected snakes. *Oo* displays limited thermotolerance and restricted growth at warmer temperatures (32–35°C) and solar ultraviolet radiation may exert some antifungal effect. Shedding may sometimes result in drastic improvement (Figs. 56.3A and B). Although snakes with mild lesions at egress may improve over the summer, lesions on snakes at ingress may be much more problematic, as dropping temperatures in hibernacula will favor fungal growth in increasingly torpid animals. The fate of sick snakes entering hibernation with ophidiomycosis remains unknown, but the finding of snakes at spring emergence with scars and old or chronic lesions of ophidiomycosis suggests that at least some of them survive.

Diagnosis

Ophidiomycosis is the most common fungal disease of captive and free-ranging snakes and should figure prominently on the list of differential diagnoses for any snake with cutaneous lesions. Crusts, scabs, swellings, or ulcers on the face and body are all suggestive of ophidiomycosis. The

presence of arthroconidia at the surfaces of lesions, whether on cytology of impression smears from lesions or in tissue sections, is highly suggestive of ophidiomycosis. A firm diagnosis relies on culture or PCR from clinical or necropsy material and demonstration of morphologically compatible fungal elements in tissue sections. Conventional PCR assays are available, and newer and more sensitive qPCR and TaqMan PCR assays have recently been developed.[15,16] Ideally, in situ hybridization for *Oo* in tissue sections would best demonstrate causation.

Treatment

Wild snakes with mild lesions at emergence may improve through successive shedding cycles over the summer and may therefore not require treatment. Hospitalization may, however, be required for snakes with more severe lesions and captive snakes with ophidiomycosis. Strict isolation and proper biosecurity measures should be instituted to curtail the risk of contagion. A number of disinfectants—such as bleach, ethanol, quaternary ammonium, and others—have demonstrated activity against *Oo*.[17] The same compounds should be used in the field to disinfect boots and gear between sites.

Treatment consists of general supportive measures along with systemic and topical antifungals. Sick snakes should be provided with fluid, thermal, and nutritional support as needed. This may be enough to achieve improvement with each ecdysis cycle when lesions are mild. Surgical debridement of lesions is indicated when shedding fails to yield improvement, to remove crusts and eschars in which conidia would otherwise remain sequestered. Topical antiseptics may be useful on wounds following debridement. Itraconazole, voriconazole, and terbinafine were all active in vitro against *Oo* isolates,[18] but delivery of these oral compounds is problematic in venomous snakes. Osmotic pumps and implants for parenteral delivery of voriconazole and terbinafine, respectively, are being investigated. Broad-spectrum antibiotics may help to prevent or treat potential secondary bacterial infections. Duration of treatment should extend beyond resolution of skin lesions and may require overwintering sick wild snakes in a captive environment. Snakes with lesions should not be allowed, whenever possible, to hibernate.

Conclusion

Ophidiomycosis is a progressive and contagious dermatomycosis of captive and free-ranging snakes for which the outcome is often fatal. This disease should figure prominently on the list of differential diagnoses for snakes with skin lesions.

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Fibropapillomatosis in Marine Turtles

ANNIE PAGE-KARJIAN

Fibropapillomatosis (FP) in marine turtles is a debilitating, infectious disease characterized by single or multiple tumors that may develop anywhere on an afflicted turtle's body (Fig. 57.1). FP mainly affects green turtles *(Chelonia mydas)* but has been reported in all marine turtle species.[1–7] There is evidence that FP does not negatively impact green turtle population recovery, survival probability, or somatic growth; however, FP disease may have severe negative effects on the health of an individually afflicted turtle.[8–11] FP tumors are histologically benign; however—depending on their location, size, and degree of invasiveness—they can be fatal in some cases.[1] For example, turtles with periocular or corneal tumors (Fig. 57.2) may have difficulty acquiring food and/or avoiding boats; turtles with oropharyngeal masses may have difficulty feeding and/or breathing; turtles with flipper tumors may have reduced swimming ability and/or be more likely to become entangled in fishing gear; and turtles with internal tumors may experience organ dysfunction and/or physiologic imbalances.[12,13] In free-ranging marine turtles, FP is most frequently observed in juveniles, and the presence of FP-afflicted turtles has been associated with shallow/inshore waters, especially habitats affected by anthropogenic impacts such as agricultural, urban, and industrial development.[1,14–17] FP is a major concern for caretakers of captive or rehabilitating turtles because extensive quarantine measures are necessary for marine turtles with FP, and prognoses for turtles with severe FP are complicated by poor nutritional condition, poor general health on admission, and secondary or opportunistic infections.[18–21]

Etiology

Most evidence points to a herpesviral etiology for FP, with the majority of molecular data suggesting that chelonid herpesvirus 5 (ChHV5) is the main causative agent of FP. A series of transmission experiments demonstrated three of the four Koch's postulates, and in vitro replication of the virus was demonstrated when de novo formation of ChHV5-positive intranuclear inclusions were observed in three-dimensional cultures of green turtle skin cells.[1,21–23] A consistently strong statistical association of ChHV5 with FP tumors has been confirmed by many subsequent studies using molecular technologies such as polymerase chain reaction (PCR) and in situ hybridization.[25,26] The virus has been visualized in tumors via transmission electron microscopy (TEM), and immunohistochemistry and reverse transcriptase PCR have further demonstrated that ChHV5 is transcriptionally active in epithelial cells of FP tumors.[21,26–31] FP is a complex disease wherein multiple factors likely play a role in tumor development and progression, including ChHV5 infection as well as environmental, microbial, and/or immune-related cofactors (see also Chapter 39).

Epidemiology

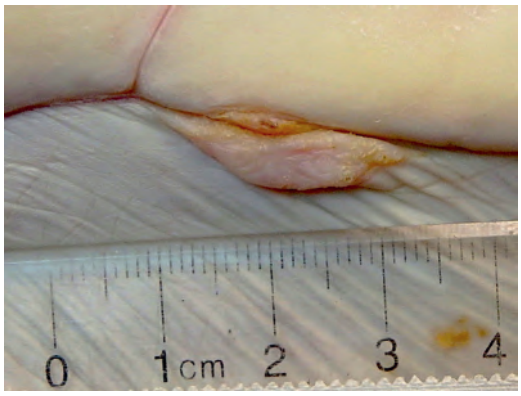
FP disease occurs worldwide but is mainly reported in warmer waters in and around the tropics.[1,32] The prevalence of FP disease has reached epizootic proportions in several green turtle populations.[1,33] FP seems to be primarily a disease of juvenile green turtles following their migration to near-shore habitats.[34] Although FP was once identified as a major cause of green turtle strandings in Hawaii, more recent studies show that prevalence of the disease in Hawaiian green turtles is now in decline.[8,35] Two plausible explanations for this include the development of herd immunity and the removal of a tumor-promoting environmental insult in the near-shore turtle foraging habitats.[8,36,37] FP prevalence seems to be more stable in green turtles in the southeastern United States, with very high prevalence in some areas of Florida, where FP is considered a major cause of mortality.[11] In other locations, FP prevalence is increasing, and it has recently been reported from numerous new localities in the Atlantic and Pacific Oceans.[16,38–40] In many studies, ChHV5 DNA has been detected via molecular techniques in tissue samples collected from marine turtles with FP and from turtle populations in which FP prevalence is high.[29,41–43] ChHV5 prevalence independent of FP tumor prevalence has also been reported; for example, ChHV5 has been detected in normal skin biopsies from nontumored turtles in populations where FP is common.[43,44]

FP tumors are a proven source of infectious ChHV5 particles, and direct, horizontal transmission of ChHV5 was demonstrated in a series of infectivity trials.[20–22,28,36,45] The high prevalence of active ChHV5 infection in early tumors suggests that virus production and shedding predominantly

• **Figure 57.1** Severe multifocal fibropapillomatosis of a juvenile green turtle *(Chelonia mydas)* from the Florida Keys. Large verrucous tumors arise from and infiltrate the skin of the axial regions, ventral neck, front flippers, inguinal regions, cloaca, tail, and hind flippers as well as the posterior portion of the plastron. (Photo credit The Turtle Hospital.)

• **Figure 57.2** Fibropapilloma lesions on the left eye of a juvenile green turtle *(Chelonia mydas)* from the Florida Keys. Multilobulated verrucous tumors cover the epithelial surfaces of the conjunctiva, palpebrae, sclera, and cornea, partially obscuring the turtle's vision. Cutaneous tumors may also be seen on the left and right front flippers. (Photo credit The Turtle Hospital.)

occur early in the progression of FP disease.[26,28] It has also been postulated that ChHV5 transmission within a population may largely depend on a few highly infectious individuals with small tumors (<20 cm^2 surface area) permissive to viral production.[26] In areas where FP is endemic, viral shedding into the surrounding environment via sloughing of virally infected epidermal cells from FP tumors represents a key source of infection; in areas with high FP prevalence, epizootic transmission cycles are likely perpetuated in this way.[34] ChHV5 transmission may be magnified by mechanical vectors such as marine leeches *(Ozobranchus* spp.*)*.[30] Tissues other than cutaneous tumors may also be involved in ChHV5 replication and transmission cycles, as suggested



• **Figure 57.3** Flat, plaque-like cutaneous fibropapilloma lesion on the shell-skin interface of a juvenile green turtle *(Chelonia mydas)* from the Florida Keys. The tumor is smooth and sessile, with a broad base (2.0 cm × 0.6 cm). (Photo credit The Turtle Hospital.)

by a high prevalence of ChHV5 antibodies detected in areas of Florida with 0% FP prevalence.[46] In several studies, ChHV5 DNA has been identified via PCR in epidermal samples taken from nontumored turtles, suggesting that some turtles without gross evidence of FP disease are ChHV5-infected and may serve as a potential source of viral transmission.[43,44] To date, however, this assumption is unsupported by demonstration of infectious viral particles from apparently normal tissues. Because the full suite of potential FP transmission routes remains unknown, biosecurity is of utmost importance when handling marine turtles with FP. Thus measures of quarantine and prevention of transmission via fomites or personnel should be strictly implemented.[19,47]

Clinical Signs

FP tumors are typically proliferative masses that can occur anywhere on or within the turtle's body (see Fig. 57.1).[1,45,48] Morphologically, FP tumors may have a wide variety of gross appearances: flat plaques (Fig. 57.3), pedunculated, sessile, verrucous, smooth, or polypoid nodules, or a combination of multiple types. The number, color, and size of FP masses may vary widely, depending on tumor location and severity of disease. Secondary invaders such as bacteria and/or fungi readily infect ulcerated FP lesions.[27] The typical histologic description of cutaneous FP tumors includes papillary epidermal hyperplasia supported by broad fibrovascular stalks with a varying ratio of epidermal to dermal proliferation.[1,28] Lymphocytes and macrophages may be found at tumor margins and infiltrating tumors in moderate to marked numbers. Histologic evidence of clinical regression is observed in some tumors.[19] Visceral tumors are perceived as more chronic lesions that develop following cutaneous tumor proliferation.[1,13,28] Histologic descriptions of visceral tumors include fibromas, myxofibromas, and fibrosarcomas.[1,18,28,49]

Stranded and free-ranging sea turtles with FP are often debilitated and/or cachectic. Severe FP has been associated

with various abnormalities in clinical pathology data, including anemia, leukopenia, lymphopenia, eosinopenia, and heterophilia.[32,48,50] Hypoproteinemia, hypocalcemia, hypoalbuminemia, and hyperglobulinemia may also be observed in green turtles with FP.[32,48–53] Suggestive of anemia of chronic disease and antigenic stimulation, these changes are compatible with the clinical presentation of FP. Turtles afflicted with severe FP often present with a number of associated comorbidities, including bacterial, fungal, and/or parasitic coinfections, ileus, buoyancy issues, and boat-strike trauma.[19]

Diagnosis

Although cutaneous FP may easily be recognized on gross examination, definitive diagnosis requires histopathology findings compatible with FP. Follow-up diagnosis of ChHV5 DNA using molecular techniques is also recommended. If possible, all rehabilitating turtles with FP should be imaged to rule out visceral tumors. Widely available imaging techniques include radiography and ultrasonography; however, small soft tissue masses may evade diagnosis with these techniques. Observation of suspicious internal lesions on imaging may be followed by laparoscopy and biopsy.[19] Endoscopic examination also has limitations, however: dorsal lung and extraparenchymal lesions may be missed, and endoscopic procedures are not recommended in severely debilitated turtles.[13,19] If available, computed tomography (CT) or magnetic resonance imaging (MRI) may be preferred, as these techniques do not require anesthesia and tend to be more accurate in identifying small internal tumors.[54,55]

ChHV5 infection may be inferred via identification of viral DNA in biological swabs using molecular diagnostic techniques. For example, ChHV5 DNA has been demonstrated in cloacal, oral, and ocular swabs taken from turtles with cutaneous FP lesions, although these sample types are not as sensitive for ChHV5 DNA as tumor and/or skin biopsies.[25,56] Suspected cases of ChHV5 infection must be confirmed via direct visualization of areas of herpesvirus morphogenesis using histopathology and/or TEM in combination with molecular diagnostics such as PCR, in situ hybridization, or immunohistochemistry. There are several validated and published PCR assays targeting different ChHV5 genes, although assays offered by commercial diagnostic laboratories may be less specific than those used in research.[14,25,29,30,34,41,42,57] Confirmatory sequencing of PCR amplicons is required for proper diagnosis of ChHV5 infection. A serologic immunoassay based on recombinant antigen has been validated for detecting antibodies to ChHV5 glycoprotein H but is not currently commercially available.[46]

Treatment

Supportive care is essential for the successful treatment of FP, as the overall health of the turtle may strongly affect the course of FP disease and host immunosuppression, stress, and comorbid conditions can lead to viral reactivation.[58] Supportive care of turtles with FP should include water of suitable quality and temperature, adequate and species-appropriate nutrition, fluid therapy, pain management, and treatment of secondary infections.[59] Antiviral therapeutics (e.g., L-lysine, acyclovir) may be used to supplement supportive care; however, to date no controlled studies have been performed on the efficacy of these treatments against FP lesions.[19]

Although evidence suggests that some marine turtles with less severe FP will undergo spontaneous FP lesion regression, this should not be expected in most cases.[13,19,60,61] Surgical excision is currently the most effective way to treat cutaneous, oral, and ocular FP lesions. Local or general anesthesia can be used, depending on the size, number, and degree of invasiveness of the tumors. Multiple tumor excisions tend to require general anesthesia. The technique of choice is carbon dioxide (CO_2) laser–mediated tumor removal; other options include sharp excision, cryosurgery, radioscalpel, electrochemotherapy, and electrocautery.[13,19,62] The CO_2 laser helps minimize hemorrhage to the tumor removal site as it simultaneously cauterizes and seals the excision site or sites while it cuts tissue.[13] Laser power, pulse rate, and handpiece size may be varied according to surface area extent and depth of the tumor or tumors. Plaque-like or broad-based FP lesions may be ablated using a lower power, whereas pedunculated or narrow-based tumors may be excised using a higher-power technique. Extreme care should be exercised in removing ocular lesions, including low power and low pulse rate, avoiding corneal tissue by ablating ocular tumors at an angle. Sutures are usually not needed, and tumor excision sites may be left open to heal by secondary intention; however, sutures may be required in the case of removal of a very deep tumor. Perioperative analgesics and antibiotics should be administered. Careful postoperative monitoring should be implemented after tumor removal, including dry-docking patients for up to 24-hours postsurgery. Cutaneous lesions may heal completely in as little as 12 weeks. It is acceptable to perform multiple tumor removal surgeries, and this may be preferred in turtles with large tumor burdens.[13] The number of tumor removal surgeries was not significantly related to prognosis in one study.[19] A 4- to 6-week period of healing should be allowed between surgeries.[13] An important caveat of tumor removal surgery is the possibility of tumor regrowth: one study found that 38.5% of green turtles that underwent tumor removal surgery experienced FP regrowth within an average of 36 days postsurgery.[19] Although regrown tumors can be surgically removed, it is best to avoid repeated cycles of tumor removal and regrowth. To help prevent tumor regrowth, a wide margin of apparently normal tissue should be included in tumor excision whenever possible, as normal skin surrounding tumors may serve as a source of ChHV5-infected cells.[41] The likelihood of tumor regrowth may also be reduced by lowering tank water temperatures by 2°C–5°C after tumor removal surgery to help prevent viral reactivation.[19]

Prognostic Indicators and Release Considerations

Tumor number, anatomic distribution, morphologic appearance, and progression, as well as overall body condition and severity of comorbid conditions should dictate triage criteria for FP-afflicted marine turtles. If left untreated (and in some cases regardless of treatment), certain tumor locations and morphologies are associated with poor case outcomes; these include visceral or intraocular tumors, tumors that have eroded thorough bony structures (e.g., carapace, plastron), and aggressive recurrent tumors.[13,19] Turtles with these lesions may be considered outright euthanasia candidates. Other tumor types are more easily treated and should be considered on a case-by-case basis, taking into account comorbid conditions, available resources and treatment options, and quarantine capabilities. Although in one study, green turtles with ocular tumors were significantly less likely to survive rehabilitation than turtles with tumors but without ocular tumors, turtles with less severe ocular tumors may be candidates for treatment if materials and trained personnel are available. In the same study, turtles with only flat, plaque-like FP lesions had a significantly better prognosis, including spontaneous lesion regression in more than 50% of cases, as compared with turtles with more verrucous-types of FP lesions.[19] In general, FP is considered more of an incidental finding in loggerhead turtles *(Caretta caretta)*, and rehabilitation efforts focused on loggerheads with FP may have a more favorable outcome than those focused on green turtles with FP.[20] Based on clinical findings, medical opinion, and permitting conditions, the attending veterinarian should determine intake criteria, euthanasia candidacy, and release criteria for turtles with FP.

To be eligible for release, a turtle must be deemed capable of survival in its current condition. As long as the turtle is clinically stable and the overall assessment of survival following release is favorable, the presence of FP lesions should not prevent the release of rehabilitated turtles. It is recommended that green turtles be rehabilitated and released as quickly as possible, because the stress of captivity may contribute to FP tumor development de novo or exacerbate cycles of tumor removal and regrowth in ChHV5-infected turtles.[19] Thus reduction of tumor burden and rehabilitation to a clinically stable condition, including treatment of any comorbid conditions, are acceptable goals in preparing a turtle for release. The risk of introducing ChHV5 into naive wild turtle aggregations via clinical or subclinical carriers may be reduced by releasing rehabilitated turtles within the same geographic area from which they were recovered. Marine turtle rehabilitation programs should take into account FP prevalence within local wild marine turtle populations when evaluating resources and developing rehabilitation goals.

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Rehabilitation Medicine of Confiscated Turtles

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Introduction

Chelonians are among the world's most preyed upon animals. They have been a source of sustenance and traditional medicine for humans for as long as there has been a historical record. The rate of human predation on turtles over the millennia is unknown, but even if it has been constant, it is currently unsustainable, as the human population has increased globally. There are many factors affecting increases in predation including human and accompanying domestic animals, encroachment on habitat, increasing wealth in regions where consumption of turtles is a sign of prosperity and the use of turtle products for traditional medicine is still highly valued, globalization and modernization of trade methods (internet) and routes, and the popularity of live turtles as pets and status symbols.[1,2] Much of the demand for turtles is centered in Asia, although the Western world has fueled the demand for turtles as pets. As awareness of the scope of the turtle trade became known worldwide, the phrase "Asian turtle crisis" took hold, and organizations such as the International Union for Conservation of Nature and Natural Resources (IUCN), Turtle Survival Alliance (TSA), and the Wildlife Conservation Society (WCS),[3] among others, started to both track the level of trade and also assist countries where such trade was taking place to begin to regulate it through legislation. This had the effect of helping local law enforcement to intervene/interrupt the trade in their jurisdictions and, in turn, has resulted in the confiscation of turtles and the associated conundrum of what to do with them in both the short and long term.

Confiscations may range in numbers from a few very valuable/rare animals that have been well treated to thousands of specimens of multiple species that have undergone mass transport piled upon each other in truck beds or crates (Fig. 58.1) with no thought of environmental factors.[3] Time in captivity and transit may range from a few days to months. The journey starts with collection from the wild by local people who hold the animals for variable times until they are turned over to overland transporters as part of a larger network that often mixes species from different habitats and localities. Sometimes the animals are transported between continents on ships or airplanes until they finally end up in live markets, where they are sold for food or as pets. Rarely is food or water provided along the way. When confiscations occur, there is usually scant information about the origins of the turtles, time in transit, and death rates. With very few exceptions, the animals have been severely stressed. Death rates of confiscations vary widely, depending on all the previous factors and the type of care that may be provided. The following is an attempt to provide guidance and act as a reference for dealing with confiscated turtles and the medical problems associated with them. Information has been gathered from colleagues, direct and indirect experience, and publications.[4]

Be Prepared

Prior to becoming involved with turtle confiscations, it is a good idea to have established some aids and protocols. These should include checklists of medicines and equipment items to be gathered and/or packed (Box 58.1); easy references for drug and anesthesia protocols (Table 58.1); names and contact information of veterinarians, veterinary technicians, and support personnel that should/could be contacted; and triage and treatment protocols (Table 58.2). When word of a confiscation is received, those protocols can be reviewed and individualized for the situation and species involved. Confiscations can be overwhelming in terms of both number of individuals and number of different species. Numbers of live animals have been known to exceed 5000 animals of multiple species in a single confiscation.[5,6] If the site of the confiscation and treatment of animals is in an area that is relatively easy to reach and has access to resources, it is not essential to pack more than enough to get through the first week of the event. However, if resource availability is unknown or limited, supplies may be needed to last for 3–6 weeks. In that case, packing more supplies and arranging for additional supplies to be brought in by other personnel over time become imperative. Occasionally animals may be transported, immediately after appropriate

• **Figure 58.1** Conditions of transport of Chelonians. (A) Truck used for transport of approximately 8000 Philippine forest turtles *(Siebenrockiella leytensis)*. (B) Close view of turtles with broken shells in the bottom of the truck. (C) Truckload of confiscated Philippine forest turtles. (All photos courtesy Katala Foundation.)

repacking, to alternate triage centers so that personnel and supplies do not have to be sent to one site. Where only one species is involved, the equipment and supplies can be tailored to the size and type of animal. Gavage tubes of appropriate size, specific tube-feeding formulas, needle and syringe sizes, amounts of hydration fluids, total amounts and dilutions of drugs, and mouth specula are only some of the size and taxa-dependent adjustments that should be made. For drugs that are most commonly used, it is very useful to have spreadsheets that calculate the milligrams and volumes based on weights so that they do not have to be generated in the field. Hard copies of the protocols, waterproofed, are always necessary in the field, so these should be easily available for packing.

In terms of necessary personnel, for most large confiscations, one or two logistics people are essential. They will be involved with transportation logistics, communication with and among all the personnel, food and lodging, data recording and compilation, ordering supplies, organizing teams, final reports, and liaising between veterinary teams and other support staff. At least one veterinarian—well versed in chelonian medicine, treatment, and surgery—is necessary; in some instances up to seven veterinarians and 100 husbandry and logistics personnel at a time have been needed. Veterinary technicians are essential for the myriad of tasks—from record keeping to pharmacy maintenance to laboratory testing and treatments—that need to be performed. For large confiscations with high numbers of predicted deaths, a pathologist is invaluable.

Triage and Initial Care

When confiscated animals are first encountered by governmental authorities, nongovernmental organizations (NGOs), or individuals, the first tasks are to provide all the animals with at least minimal environmental necessities. The provision of heat or cooling depends, of course, on the surroundings. Often just provision of shade is enough cooling in tropical conditions, whereas provision of heat may involve simply placing animals in partially sunny areas protected from wind. Care must be taken to adjust conditions depending on time of day or changing sunlight or

• BOX 58.1 Supplies and Equipment for Chelonian Confiscations

- Syringes
- Needles
- Exam gloves
- Fluids
  - 5% Dextrose
  - 50% Dextrose
  - Normasol
  - 0.9% NaCl
  - 2.5% Dextrose/0.45% NaCl
- Fluid administration sets
- Sterile water for injection
- Antibiotics
  - Amikacin
  - Ceftiofur
  - Ceftazidime
  - Enrofloxacin
- Parasiticides
  - Metronidazole
  - Paramomycin
  - Fenbendazole
  - Levamisole inj
  - Praziquantel inj
  - Provent-a-mite
- Ophthalmic supplies
  - Sterile lubricant
  - Eye wash
  - Fluoroscein stain
  - Triple antibiotic ointment
  - Gentamicin ointment
  - Terramycin ointment
- Topicals
  - Chlorhexidine ointment
  - Silver sulfadiazine cream
  - Skin glue (cyanacrylate)
- Anesthetics
  - Ketamine
  - Medetomidine
  - Alfaxalone
  - Propofal
  - Lidocaine
- Germicidal agents
  - Alcohol-based hand gel
  - Isopropyl alcohol 70%
  - Chlorhexidine solution
  - Povidone-iodine (Betadine) solution
  - Virkon powder
- Wound supplies
  - Hemostats, thumb forceps, iris scissors, bandage scissors
  - Gauze squares
  - Bulk cotton
  - Fingernail brushes
  - Flushing needles and catheters
  - Bandaging tape
  - Roll gauze
- Surgical supplies
  - Sterile wound pack
  - Sterile general pack
  - Sterile gloves
  - Adhesive drapes
  - Suture
  - Cable ties and fiberglass adhesives
  - Gelfoam
- Laboratory supplies
  - Microscope
  - Slides
  - Coverslips
  - Hematology stains
  - Centrifuge
  - Blood collection vials
  - Heparin
  - Sterile swabs
  - Freezer tubes
  - Lab marking pens
  - Pencils
- Nutrition supplies
  - Gavage tubes
  - Feeding needles
  - Powdered formulas
- Necropsy supplies
  - Dedicated instruments
  - Scalpel blades and handles
  - Bone cutter
  - Cutting board
  - 10% buffered formalin
  - Containers for samples: locking plastic bags, plastic bottles
  - Lab marking pens
- Miscellaneous
  - Clipboards
  - Notebook paper
  - Spiral notebooks
  - Pens, pencils
  - 4″ × 6″ index cards
  - Waterproof color markers for shells
  - Waterproof paper
  - Computer or notebook
  - Small electronic scales
  - Hanging scales
  - Cloth hand towels
  - Garbage bags

shade. As animals are unpacked from primary containers (truck beds, boxes, bags, etc.), a quick assessment of triage category is done and animals are separated by species (Fig. 58.2). Triage categories should begin as large groupings and then over time be refined into more individualized records. One of the challenges is keeping track of which animals are in which group, when follow-up treatments need to be performed, and when animals change triage levels. In the case of large numbers of animals, organization is critical in order to provide the best possible care while minimizing the amount of handling and stress on the animals. At minimum, clipboards with papers divided into columns for identification (ID) number, species, triage level, weight, antibiotic, fluids, and so on should be on hand and data recorded.

In cases where animals are being transported to other facilities for long-term care, communication with receiving facilities regarding treatment is important. Something as simple as index cards with summaries of medical records or as complex as extensive electronic communications can be used. Support personnel should be keeping and transcribing the information, freeing up the veterinary and animal care staff to perform the medical care.

In confiscations of greater than 50 animals, time and resources usually dictate that the initial handling be performed efficiently. This often requires estimation of body weights, the treatment of animals that may not need it, and less than complete emergency care for animals that could benefit from it. Years of dealing with confiscations have revealed that the most important factors affecting immediate survival are how long the animals have been in transport/confinement or without food and water. Initially, if dealing with large groups of animals, individualized medicine is minimized.

Short periods of captivity without extreme deprivation of food or water usually result in animals that need the

TABLE 58.1 Chelonian Drugs and Dosages

Drug, Generic	Concentrations Available	Route(s) of Administration	Dosage	Frequency	Reference
Antibiotics					
Amikacin	50 mg/mL, 250 mg/mL	SQ, IM, ICe	Loading dose 5 mg/kg then 2.5–3 mg/kg	q48–72h	9
Ceftazidime	1 g, 5 g	SQ, IM, ICe	20 mg/kg	q72h	10
Clarithromycin	Various	PO	15 mg/kg	q24–72h	11
Danofloxacin	180 mg/mL	IM, SQ	6 mg/kg	q24–48h	12
Enrofloxacin	5 mg/mL, 100 mg/mL	PO, SQ, IM	10 mg/kg PO, 5–10 mg/kg SQ, IM	q48h PO, q24h inj	13–15
Oxytetracycline	50, 100, 200 mg/mL	SQ, IM	40 mg/kg then 20 mg/kg	q72h	16
Ticarcillin	3 g with 0.1 g clavulanic acid	IM	100 mg/kg	q48h	17
Tulathromycin	100 mg/mL	IM	5 mg/kg	q5–7days	18
Analgesics					
Meloxicam	5 mg/mL	IM	0.2 mg/kg	q24–48h for 4 treatments	19
Tramadol	Various	PO	5–10 mg/kg		19
Fluids					
Reptile ringers	1 part LRS + 2 parts 2.5% Dextrose in 0.45% NaCl	SQ, ICe	5–50 mL/kg	q24–72h prn	19
LRS or other balanced solution		SQ, ICe	5–50 mL/kg	q24–72h prn	19
2.5% Dextrose and 0.45% NaCl		SQ, ICe	5–50 mL/kg	q24–72h prn	19
0.9% NaCl		SQ, ICe	5–50 mL/kg	q24–72h prn	19
Anesthetics					
Ketamine	100 mg/mL	IM	10–60 mg/kg		19
Medetomidine	Various	IM	0.1–0.15 mg/kg		19
Ketamine/medetomidine		IM	5–10 mg/kg 0.1–0.15 mg/kg		19
Atipamizole	5 mg/mL	IM	0.5–0.75 mg/kg		19
Alfaxalone	10 mg/mL	IV, IM, ICe	6–9 mg/kg IV, 9–15 mg/kg IM 24 mg/kg ICe		19
Propofol	10 mg/mL	IV	5–10 mg/kg		19
Dewormers					
Levamasol	Variable	SQ, ICe	5 mg/kg	q10–21d	19
Fenbendazole	100 mg/mL	PO	10–25 mg/kg	For 3 days or every 14 days for 3 treatments	19
Praziquantel	57 mg/mL	PO, SQ, IM	5–8 mg/kg	Repeat in 14 days	19
Metronidazole	5, 50, 100 mg/mL	PO	25 mg/kg q24h for 5 days or 50 mg/kg q14d		19

ICe, Intracoelomic; *IM,* intramuscular; *IV,* intravenous; *PO,* orally; *SQ,* subcutaneous.

TABLE 58.2 Chelonian Triage and Actions

Triage Level	Signs	Immediate Action	Treatment
1	Alert, appropriate struggling, hydrated, eyes shiny, no obvious injuries, appropriate weight	ID number on shell; weigh; weight on shell; place in sheltered area with access to drinking water	Antibiotics, anthelminthics
2	5%–10% Dehydration, eyes sunken, eyes dull or closed, weak, abscesses, wounds, broken shells	ID number on shell; weigh; weight on shell; place in separate sheltered area	Antibiotics, SQ or ICe fluids, wound care
3	10% Dehydration, barely responsive; severe life threatening wounds; exposed bones	ID number on shell; weigh; weight on shell; place in separate sheltered area	Antibiotics, SQ or ICe fluids, steroids; wound care
4	Suspected dead or dead	Confirm death with Doppler; place bodies in bags out of sun; place moribund in sheltered areas or euthanize	Necropsy as time is available; ID numbers on shell

ICe, Intracoelomic; *ID,* identification; *SQ,* subcutaneous.

• **Figure 58.2** Temporary holding conditions of confiscated turtles. (A) Turtles in small holding tubs undergoing treatment. (B) Group holding pen for Philippine forest turtles *(Siebenrockiella leytensis)* ready for release. (C) Individual holding for big-headed turtles *(Platysternon megacephalum)* in a large pool. (Photos A and B courtesy Lisa Eidlin, Photo C courtesy Chris Hagen.)

shortest and most straightforward periods of medical and conservatory care. Those animals are usually placed into the level 1 triage enclosures; they often receive only the first triage treatments and then, after being placed in appropriate housing, begin feeding and drinking and require no further care. If animals have been in the trade routes for longer than a week, they will be starting to become dehydrated and should be provided with drinking or soaking water. Sometimes this will be in the form of shallow pools or low-rimmed pans, in the case of terrestrial turtles; for others it may mean pools or tubs of 2–18 in. deep.

Animals that have been in the trade routes for more than a week will often be in triage level 2 or 3. Treatment during the first few days includes not only antibiotics, but subcutaneous (SQ) or intracoelomic (ICe) fluids and also sometimes steroids and analgesics. Treatment of infected eyes, wounds, and shell damage begins and requires ongoing care for varying amounts of time.

Choice of antibiotics depends not only on pharmacokinetic data but also on the advantage of treating only every 3 days with a cephalosporin versus daily injections of a fluoroquinolone and penicillin. There is a temptation to use drugs known for being long-acting in birds or mammals on the assumption that the properties will be similar in reptiles. It is advisable, if at all possible, to start antibiotic treatment with drugs for which there is some level of chelonian pharmacokinetic information.

Syndromes

The syndromes that are commonly seen in confiscated turtles may involve various body systems. In the immediacy of a confiscation, treatments are based on what can be done efficiently with the highest probability of relieving life-threatening conditions. Conditions that are not urgent should be deferred until all animals have been assessed and undergone basic care.

Dehydration

If "reptile Ringers" (see Table 58.1) is available, it is preferred over other fluids, although standard prepackaged fluids (i.e., 2.5% dextrose in 0.45% NaCl) have been used successfully.[7] Fluids may be administered SQ, although the ICe route will correct dehydration more rapidly. Doses of 10–50 mL/kg are tolerated and can be repeated daily if necessary. All animals should be provided with drinking water in low bowls (to prevent drowning) as soon as is practical.

Septicemia, Bacterial Enteritis

Most animals that have been in the trade or held in substandard conditions are susceptible to bacterial infections. Administration of antibiotics via injection at the same time as fluids are given is recommended if there are signs of stress, dehydration, starvation, or wounds. Most of the antibiotics in Table 58.2 have pharmacokinetic data to inform their use in turtles. Drugs with the longest interinjection intervals include tulathromycin 5 mg/kg every 5–7 days, ceftazidime 20 mg/kg every 3 days, amikacin 5 mg/kg once and then 2.5–5 mg/kg every 3 days, enrofloxacin 5–10 mg/kg every 24–48 hours orally (PO), SQ, or intramuscularly (IM); these are the usual initial treatments. Subsequent treatments are based on individual conditions, but consideration should be given to at least following through a 7- to 10-day course of treatment to minimize the development of bacterial resistance.

Endoparasitism

Normal parasite loads in most turtles will be increased to pathologic levels when stressors are applied. In conditions where species are mixed together, it is also possible for turtles that are naive to some parasites to become infected from other species. Superinfections of enteric parasites have been seen subsequent to both conditions. Treatment with injectable levamisole has been employed successfully in a wide range of turtles. Fenbendazole has also been used frequently, although caution should dictate that low, infrequent doses be used in order to avoid complications such as bone marrow suppression. Under no conditions should ivermectin be used. Fecal flotations should be performed to evaluate what type of parasite is present and so that subsequent dewormings may be adjusted. Praziquantal may be used to treat trematode infections. In the case of amoebae or high numbers of flagellated protozoans detected via microscopic examination of fecal or cloacal swab smears, metronidazole should be given via gavage.

Starvation

Many animals will be extremely thin, their fat stores having been used up, often bordering on terminal starvation. Introduction to calories must be done in a slow, measured manner in order to avoid inducing the complication of refeeding syndrome. Slowly increase the amount being gavage-fed over 1–2 weeks until a quantity of approximately 10 mL/kg body weight can be administered. Fluid support will need to be continued during that time. Although gavage feeding may be safely repeated, sometimes it is less stressful on the animals to place a pharyngostomy tube surgically. These tubes can be left in place until the animals start feeding voluntarily.

Syndromes Affecting Eyes

Blepharitis, conjunctivitis, and corneal ulcerations are all seen (Fig. 58.3A). Stain corneas if possible; otherwise avoid treating with steroids. Consider treatment with topical, systemic, and subconjunctival antibiotics. Parenteral analgesics are often also indicated. In the case of aquatic turtles, blepharitis and conjunctivitis often resolve spontaneously when water quality is improved.

• **Figure 58.3** Ocular and shell lesions in confiscated Chelonians. (A) Corneal plaque and edema in a Philippine forest turtle *(Siebenrockiella leytensis)*. (B) and (C) Necrotic shell lesions in aquatic turtles subjected to hard substrates and transport of turtles in overcrowded conditions in a truck. (Photos courtesy Sheena Koeth.)

Syndromes Affecting Skin or Shell

Ulcerations, abscesses, amputations, and ectoparasites are encountered (see Fig. 58.3B and C). Animals that have been held on hard substrates or piled upon each other often have significant shell lesions consisting of damage to the subkeratinized bone. In extreme conditions there may be full-thickness necrosis through the shells. These lesions must be generously debrided and protected with appropriate ointments and the animals then placed on soft substrates or in water in which they can float (if strong enough not to drown). Collect ectoparasites in alcohol; cleanse the wounds with dilute chlorhexidine, povidone-iodine (Betadine), or just soap and water. Debride or open and remove purulent material; flush copiously with sterile water and dilute povidone-iodine. Apply ointment. Allow to maintain contact for 15 minutes if possible before returning to water. Usually amputations of limbs are for those that have been chronically ill and infected, so they are not candidates for immediate surgical intervention. Shell fractures should be temporarily stabilized after cleaning.

After the initial triage and treatments, the animal's conditions and triage categories may change. All animals should be accounted for daily and assessed for change in condition. As soon as is practical, animals in category 1 should be offered food. Records of interest in food, acceptance of food, force-feeding, and amounts offered should be kept if at all possible. Animals in the other categories may be offered food, although the frequency of treatments may not be conducive to feeding responses.

Ongoing treatments will be dictated by the conditions of the animals, laboratory results and necropsy findings. Fecal examinations (direct and flotations) should be performed on subsets of animals of each species and category. Necropsies should be performed on as many animals of each species as is reasonable.

Laboratory Support

During the first stages of a confiscation, minimal laboratory samples are usually collected. As the situations become less acute, samples can be collected and analyzed. Blood samples for genetics, blood counts, determination of hemoparasites, and plasma biochemistries should be performed for animals that are not immediately rereleased or for those that are going to enter an existing collection of animals.[8] If time and resources allow, choanal and cloacal swabs can be collected for molecular diagnostic testing to help determine suitability of release back to the wild. Gross necropsy findings are valuable in determining subacute treatments and should be recorded and shared with whoever provides ongoing care. Necropsy tissue samples should be saved in formalin, alcohol, and frozen, if possible, for future investigations.

Conclusion

Ongoing nutritional support, appropriate housing, and medical care can last for months for some species. Few confiscated animals should be returned to the wild due to individual problems or to having been exposed to potential pathogens from contact with other animals in the trade. However, because of the massive numbers of animals that have been confiscated, less than perfect solutions have been chosen for many of them. Fortunately, many of the rare species are ultimately set up in assurance colonies for future conservation programs.

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59

Medical Evaluation of Crocodilians

PAOLO R. MARTELLI

Introduction

The order Crocodylia (or Crocodilia) is composed of three families and nine genera. All 23 species of crocodilians are listed under Convention on International Trade in Endangered Species of Wild Fauna and Flora, Appendix I or II. Crocodilians and crocodiles will be used interchangeably hereafter. Fifty years ago crocodilians appeared "slated for rapid extermination at the hands of man" from overhunting and loss of habitat.[1] Remarkably, many species are currently recovering or prospering.[2]

Crocodilians and their caretakers contribute to habitat and species conservation, poverty reduction, and education on a scale unique in the field of wildlife management. Thousands of commercial operations on six continents keep, breed, or raise crocodilians in the millions. An annual average of 1.4 million crocodile skins was traded between 2004 and 2013.[3] Crocodilians are well represented in zoological collections. In April 2017 the Zoological Information Management System (ZIMS) reported 462 institutions holding 6345 individual crocodilians of all species.[4] Conservation organizations maintain and breed crocodilians for restocking programs.

In spite of their charisma and their conservation and economic value, only two veterinary textbooks are devoted to crocodilians, and chapters on crocodilians in wildlife textbooks are brief.[5–11]

This chapter is not a review of the diseases of crocodiles but rather a guide for the clinical examination and health evaluation of crocodilians. Table 59.1 links common clinical findings and differential diagnoses.

Medical evaluation may be required for individuals or for populations, for crocodiles under human care, in the wild, or for animals destined to be released to the wild. Crocodilian patients range in size from an ounce to a ton. Some crocodiles will be examined in a hospital setting, while others will be examined in remote locations. Different approaches to health assessment are required depending on purpose, size, and location.[12]

Medical Evaluation

History

The following questions help in identifying diseases and risk factors.

1. What are the species's normal habitats and behaviors, for a given gender and age?
2. What is the history of previous diseases and what is the current (chief) complaint?
3. What are the admission and quarantine protocols? What standards of hygiene and biosecurity practices are in place?
4. What are the seasonal air and water temperatures and rainfall?
5. What is the physical and chemical water quality and where does water originate from (e.g., city supply, well, river with wild crocodilians, or industrial runoffs, others)?
6. What is the enclosure design, including land and water surfaces and water depth? Are there adequate visual barriers, basking sites, nesting sites, etc.?
7. What is the diet and how is it presented: frequency, locations, time of day?
8. Are the size and gender composition (social structure) and stocking density acceptable? Are there signs of runting, competition, or fighting?

Crocodiles spend much of their lives immobile on land or submerged. Nevertheless, careful observation of the external appearance, alertness, movements, and the interaction between animals and the environment may offer valuable insight. The variation in size within cohorts of juveniles must be kept to a minimum. Animals that manifestly have not been in the water for a long time but display a flattened tail or have tail wounds at various stages of healing suggest bullying and chronic stress. The distribution and use of nesting, basking, or wallowing sites has an impact on social harmony and reproductive success.

TABLE 59.1 Common Clinical Signs and Differential Diagnosis

Clinical Signs	Differential Diagnosis
Distended belly	Omphalophlebitis, yolk sac infection, coccidiosis, intestinal obstruction, gastric impaction, follicular recruitment, tympanism, gestation, coelomitis, metritis, oophoritis, obesity
Abnormal gait and abnormal swimming	Calcium or vitamin E deficiency, penile or cloacal prolapse, injured limbs, gout, spinal deformities, stress behavior, osteoarthritis, pansteatitis, neurologic disease, respiratory disease
Stargazing, circling, ataxia, opisthotonos, central nervous system (CNS) signs	Thiamine deficiency, calcium deficiency, head trauma, meningoencephalitis, heat stroke
Swollen joints	Traumatic, infectious (mycoplasma, other sepsis), metabolic (gout, calcium deficiency)
Red eye, white eye, panophthalmitis	Infectious: high morbidity and mortality bilateral lesions affecting numerous very young animals associated with liver and other organs pathology (chlamydiosis, herpesvirosis, mycoplasma). Trauma: unilateral lesions affecting only few animals
Nasal discharge, wet rales, malodorous breath	Infectious pneumonia, regurgitation after capture while mouth is taped
Dermal and oral lesions	Macular (caiman or crocodile poxvirus), patchy discoloration or ulcers (stress dermatitis, inadequate sanitation, low temperatures, poor water quality, new stock or new enclosure), trauma

Physical Examination of the Patient

General Considerations

Capture and restraint must minimize stress and trauma. Alertness and fitness can be further assessed in the course of capture. Well-restrained crocodiles of all sizes surrender rapidly. Sedatives, anesthetics, nondepolarizing muscle relaxant, and electrical immobilization can be used to assist capture and examination.[13-19] Additional literature and notes on crocodile immobilization can be found on the veterinary webpages of the International Union for Conservation of Nature and Natural Resources Crocodile Specialist Group.[20] The choice of restraint depends on operators' experience and field realities. Following chemical immobilization, it is advisable to confine the crocodile on land until fully awake to prevent drowning or predation.

The skin is inelastic and the vertebral ribs and the abdominal ribs (gastralia), located ventrally between the sternum and the pelvis, create a rigid casing preventing the thorough palpation of internal organs. Hematology and biochemistry do not reliably reveal inflammation or organ damage.[5,21,22] Ultrasonography is used to overcome these limitations inherent to crocodiles and to objectively identify signs of illness or good health. We advocate that ultrasonography is an integral part of the general examination of crocodilians, much in the same manner that radiology is part of the avian general examination. Four windows allow visualization of most internal organs (Fig. 59.1).

Physical examination should be carried out after 3–5 days of fasting because a full stomach interferes with ultrasound examination, and postprandial increases in lipemia, plasma bicarbonates, and uric acid interfere with blood tests and interpretation.[5,21,23]

Sex

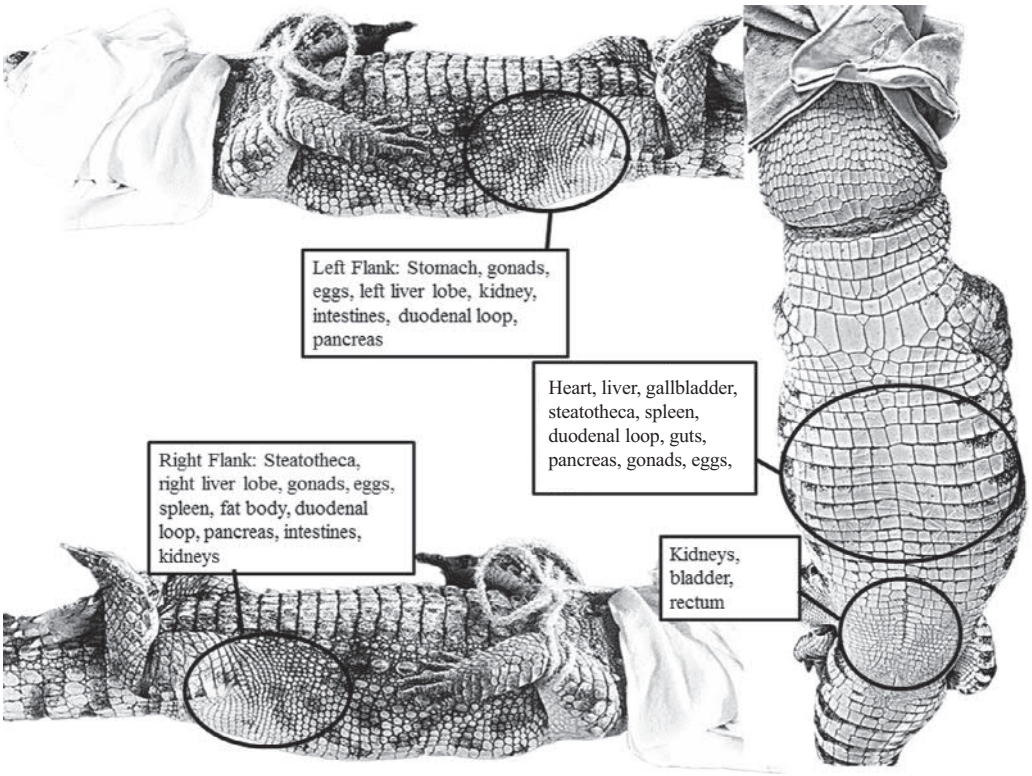
The gender of crocodilians is incubation temperature dependent.[24,25] The cliteropenis is located at the cranial commissure of the slit-like cloaca and may be palpated, exteriorized, or visualized by spreading open the cloaca. Errors in gender determination are not uncommon in immature specimens because cliteropenis dimorphism increases with age. Intersexuality was reported in an African dwarf crocodile *(Osteolaemus tetraspis)*.[26] Environmental pollution may result in hermaphroditic primary sex organs, masculinization of the cliteropenis, and delayed renal development.[27] Therefore medical evaluations of wild crocodiles must document cliteropenis morphology.

Age

Age is difficult to determine in the absence of exact records because growth rates may be very variable. Curves relating age to length exist for a number of species and can be used for reference.[28,29] The author has encountered 4-year-old estuarine crocodiles weighing less than 1 kg, whereas animals of that age in the same facility averaged 30 kg. Abnormally slow growth, referred to as runting, is associated with adrenal and osseous pathologies, as well as immune deficiency (see later). Chronic stress is a significant cause of runting.[22]

Body Score

Crocodiles deposit different types of adipose tissues.[30] The fat that accumulates subcutaneously, between muscles, and intracoelomically gives the crocodile a more or less rounded



• **Figure 59.1** Ultrasound windows in a generic crocodilian.

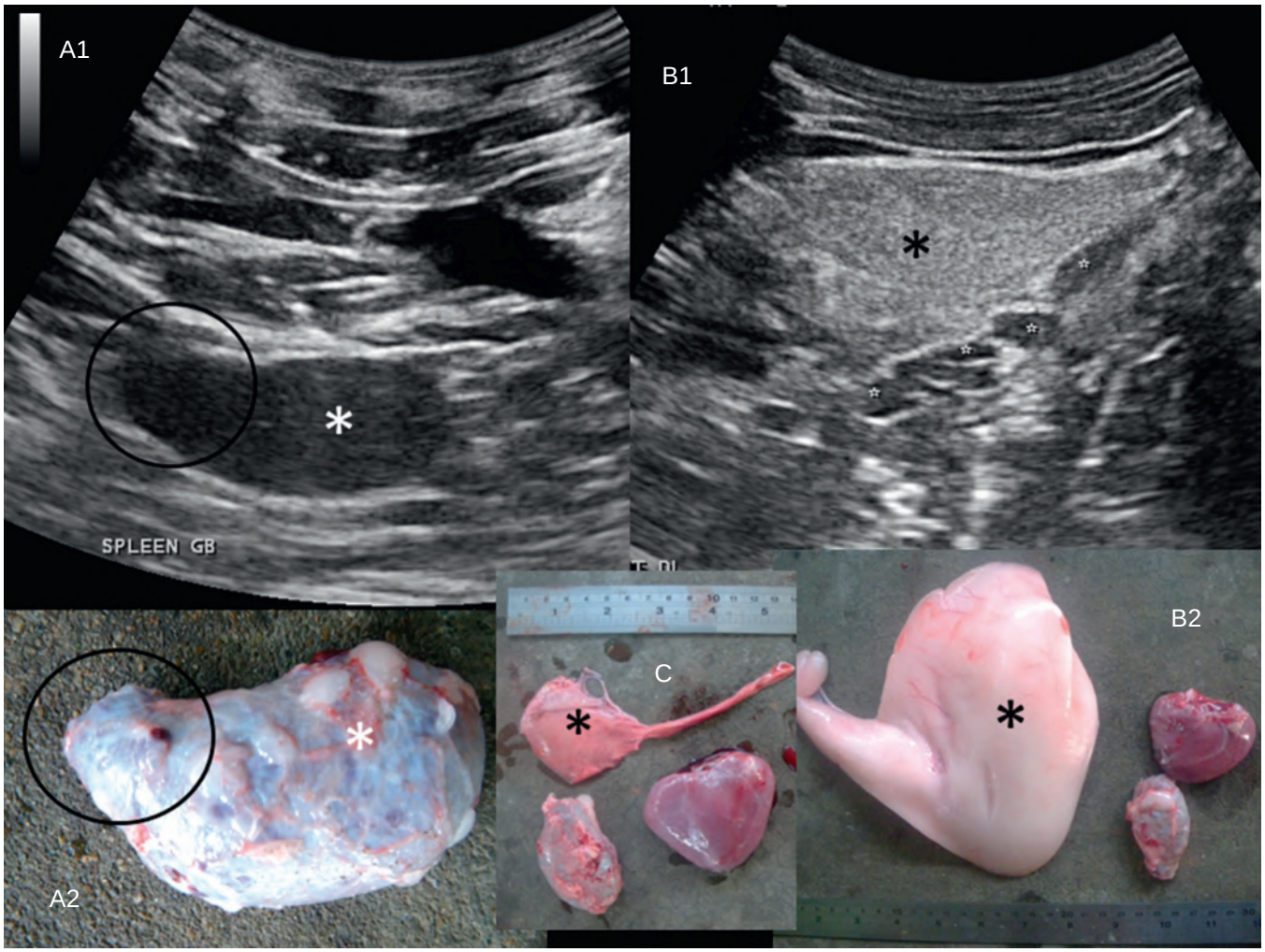
appearance but is not an accurate marker of the body condition. That storage fat is poorly vascularized and does not mobilize rapidly in response to starvation.[5,30] Crocodiles also possess an intracoelomic well-vascularized sack of readily available adipose tissue, known as the steatotheca or fat body.[5,30] The steatotheca mobilizes promptly in response to metabolic requirements; thus measurement of the fat body is a more accurate score of the metabolic status of crocodilians.[5] A steatotheca to heart ventricle (S:V) mass ratio of 5 or greater indicates an excess energy store. An S:V ratio lower than 0.5 indicates very poor body condition.[5] The steatotheca is visible by ultrasound against the abdominal wall of the right flank. The volume or maximum dimensions of the steatotheca are compared with the ventricular measurements to estimate the state of nutrition, using the previous ratio (Fig. 59.2).[5,31]

Integumentary System

Healthy crocodile skin is smooth, shiny, and dry. Scale morphology and the distribution of osteoderms and integumentary sense organs (ISOs) vary between species. Cuvier dwarf caimans *(Paleosuchus palpebrosus)* are very heavily armored with thick osteoderms in all scales, whereas estuarine crocodiles *(Crocodylus porosus)* only have osteoderms in the larger dorsal scales. ISOs in the alligators and caimans (Alligatorinae) are confined to the head. Infectious skin diseases may present as macules, papules, crusts, or ulcers and may be viral, bacterial, or fungal in origin, although multiple pathogens are generally present.[5,32–34] Chronic stress and poor hygiene are determining factors in the occurrence of skin diseases (see Table 59.1).

Immune System

Ascertaining the immune status of the patient is a basic function of the clinical examination. Crocodiles do not possess lymph nodes. Three lymphoid organs are accessible to the clinician—the tonsils, spleen, and thymus. The tonsils consist of lymphoid tissue nested within mucosal folds behind the soft palate.[5,35] The tonsils can be visualized by lifting the soft palate and using an angled mirror or telescope. Tonsils become reactive in a number of infectious diseases, including chlamydiosis and herpesvirosis, two significant epidemic diseases of hatchlings.[5,6,36–38] The spleen may be appraised by ultrasound from the right flank beyond the fat body and below the gallbladder or from the ventral window below and to the right of the gallbladder. It is a well-defined oval organ with a homogeneously granular texture. The spleen reacts to sepsis by enlarging, deforming (budding), and changing texture (see Fig. 59.2). The liver also provides important clues regarding the septic state of the patient (see later). Hematology and serum biochemistry are of minor help, and at times misleading, in assessing the immune state of crocodiles and are secondary to clinical findings including ultrasound. Immunocompromise is the result of chronic stress caused or aggravated by poor management and is associated with moderate anemia, mild



• **Figure 59.2** Ultrasound and gross appearance of the spleen and steatotheca of Siamese crocodile *(Crocodylus siamensis).* (A1 and A2) Ultrasound and gross appearance of the spleen *(white star)* with inflammatory deformity *(circle)*. (B1 and B2) Ultrasound and gross appearance of a well-stocked steatotheca *(black star).* (C) Depleted steatotheca *(black star).* Heart ventricle and spleen are shown for reference.

hypophosphatemia, mild hypoalbuminemia, and increased serum corticosterone.[5,22] Tonsillar, splenic, and thymic lymphoid populations are reduced in immunosuppressed crocodiles.[22]

Circulatory System

The crocodile cardiovascular anatomy and physiology are probably the most sophisticated of all vertebrates.[9,39–41] Crocodilian hearts beat 30–50 beats per minute at rest on land and 5–8 beats per minute when diving.[39] Heart rate decreases under anesthesia.[40] Studies on crocodile clinical cardiology are lacking. Heart rate can be counted by observing or sensing below the costal arch for cardiac movements. Ultrasound allows clear cardiac visualization and measurements. Cardiac contractility of anesthetized or moribund animals may be evaluated by ultrasound. Pericardial effusions occur in chlamydiosis or septicemia. Pericardial and epicardial accretions of uric acid (visceral gout) may be diagnosed and sampled by ultrasound.

Respiratory System

Crocodiles have two symmetrical saccular lungs, no air sacs, and unidirectional air flow.[42,43] Respiratory diseases often become evident only in advanced stages. "Open-mouth behavior" is part of thermoregulation or social behavior and is not a sign of respiratory distress. Respiratory signs include reduced stamina, listing in the water, abnormal swimming, foul smell on exhalation, nasal expulsion of stained exudate, and gurgling rales. Angry animals may emit rumbling or hissing noises. A water-soaked towel placed between the skin and the stethoscope facilitates auscultation. Percussion of the lung field complements auscultation and may suffice to diagnose pulmonary consolidation or collapse.

Latero-lateral and dorso-ventral radiographs allow diagnosis of obvious conditions in all species, but finer interpretation may be hampered by the outline of the osteoderms. Computed tomography is the imaging tool of choice for the evaluation of the crocodile respiratory system.[44]

Bacterial and fungal pneumonia typically present as granulomatous focal or disseminated infections.[5,44,45] Bronchoscopy and bronchoalveolar lavages may be processed as in other species.[46] Parasites are reported from lungs and pulmonary arteries.[47] Pentastomidae infect crocodiles through the consumption of fish and may cause verminous pneumonia.[48–51]

Digestive System

Teeth are replaced continuously but progressively slower with age. Diaphanous teeth may be a sign of stress-induced osteoporosis.[5] Nutritional bone disease is common in young animals and is diagnosed by pressing the two lower jaws together and bending the upper jaw upwards (rubber jaw).[5]

The stomach is located in the left flank and when full may occupy much of the coelomic cavity, interfering with the ultrasound examination. On ultrasound, the stomach wall is thick with identifiable layers. Crocodiles routinely ingest stones and other foreign bodies. Gastric parasites are considered harmless, although studies linked gastric parasites with histopathology findings.[52,53]

The duodenum is arranged in a double loop, and it and the pancreas located between its coils are visible on ultrasound medial to the empty stomach. The intestines are uniformly thick and widen when reaching the rectum. On ultrasound the rectum can be recognized because it abruptly ends where the hypoechoic urodeum (urinary bladder) begins. Coccidiosis in juveniles may manifest as a paradoxical obstipation due to accumulation of fibrin in the lumen of the inflamed intestines.[5]

Normal feces are cylindrical, capped by white urates within a varying degree of liquid urine. Fresh feces may be obtained for parasitology and cytology examination by digital stimulation of the cloaca or by rectal wash. Crocodilian parasites have been catalogued.[47]

The large, symmetrical, bilobed liver envelops the heart laterally and dorsally. Crocodiles have a gallbladder. Herpes virus, West Nile virus, chlamydiosis, and mycoplasmosis cause severe diffuse hepatitis. During sepsis, the liver filters circulating pathogens, causing focal or diffuse hepatitis. Therefore hepatitis may be a sequel and an indicator of sepsis (Fig. 59.3). Hepatitis is best diagnosed by ultrasound. Changes within the hepatic parenchyma seen on ultrasound are verifiable on gross necropsy and histopathology (see Fig. 59.3).[31] Hepatic neoplastic lesions are reported, but neoplasia is rare in crocodilians,[54] and expanding masses are more often exuberant granulation tissue or abscesses.[5,55]

In hatchlings and yearlings, the yolk sac occupies a large part of the coelom. Omphalophlebitis and yolk sac infections are associated with inadequate posthatching hygiene. On ultrasound the healthy yolk sac has a homogeneous appearance.

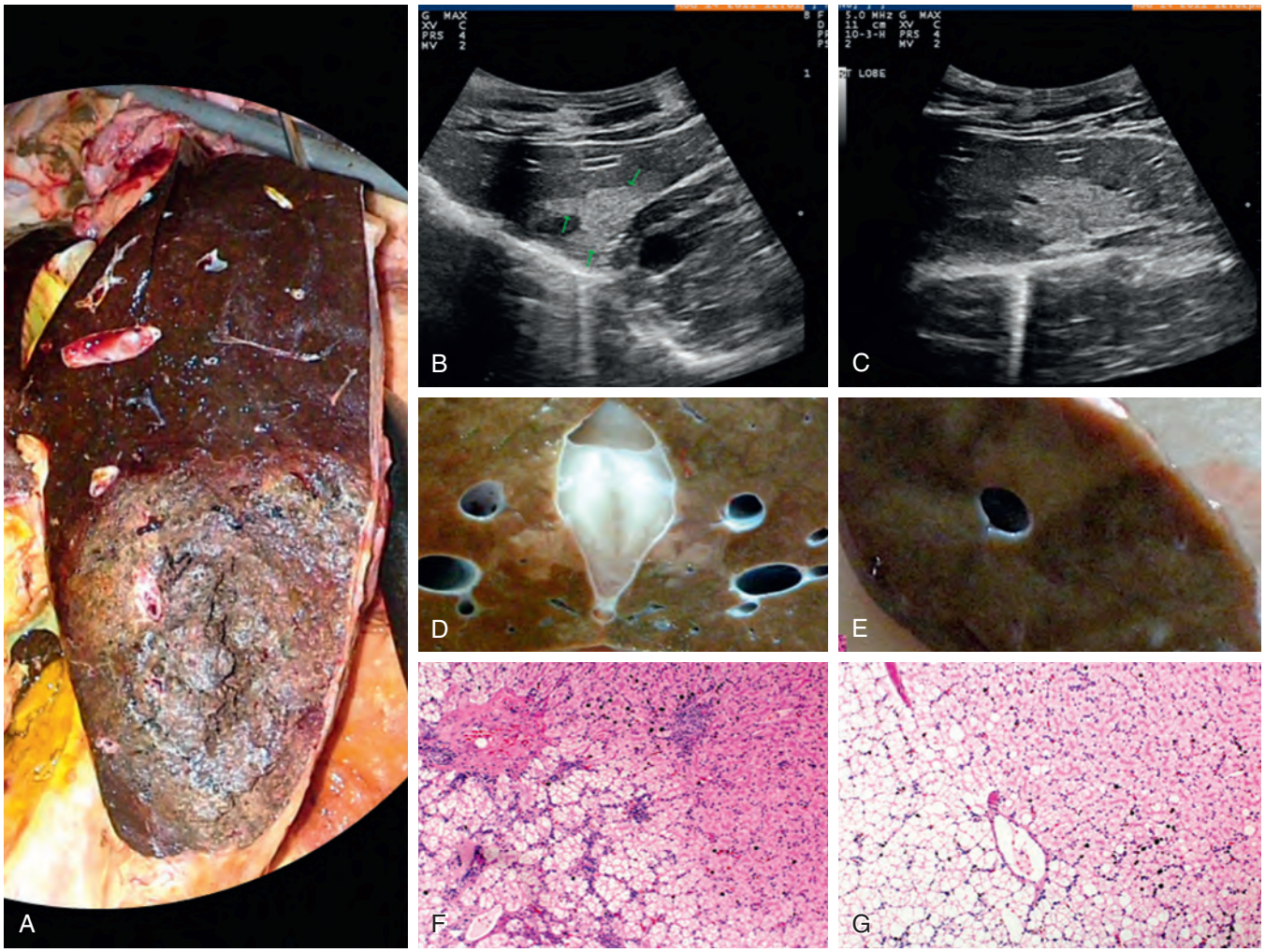
Urinary System

Crocodiles excrete ammonia, uric acids, and small amounts of urea. The crocodile kidney does not concentrate urine, and water conservation takes place in the mucosal lining of the distal intestines or in the urodeum.[56,57] The kidneys are isoechoic with the fat they are encased in and are difficult to identify on ultrasound. They may be visible from the pubic window cranial to the urodeum or from the lateral windows directing the beam ventral and cranial to the sacrum. Bacterial and parasitic nephritis are reported.[5,47] In fasted crocodiles, serum uric acid values higher than 12 mg/dL (750 µmol/L) accompanied by a calcium and phosphorus ratio less than 1 suggest renal disease. Gout is a common manifestation of renal disease.[5] In the winter, crocodiles are predisposed to bouts of gout because urate crystals are less soluble at low temperatures and crocodiles are dehydrated due to reduced feeding.[58] Overfeeding may also cause gout. Urine may be obtained by inserting a semirigid catheter in the cloaca past the proctodeum into the urodeum.[59] Further studies are needed to understand how urine composition may reveal systemic and organ changes. Urinary calcium is elevated in stressed crocodiles.[5,22] Urine samples may also be used to investigate environmental pollutant metabolites and stress levels.[60,61]

Reproductive System

Sexing is described earlier. The reproductive system of the crocodile can be thoroughly assessed by ultrasound.[62–64] In hatchlings the minuscule paired gonads are nearly impossible to locate. As the animals mature, the ovaries present as clusters of anechoic or hypoechoic spheres of variable size depending on reproductive status. Eggs are elongated and calcified in crocodilians and easily distinguished from follicles. Oophoritis presents on ultrasounds as clusters of thick-walled heterogeneous cysts. Gestation or egg retention can be diagnosed by radiographs. Estrogen and calcium serum levels in the Chinese alligator increase 20-fold and 3-fold, respectively, during follicular growth (Martelli, unpublished data). Reproductive success in females requires suitable males, adequate nesting sites and nesting material, tolerable intragender competition, and functional intact ovaries and oviducts.

Testicles are elongated homogeneous paired organs visible on ultrasound from both flanks. Reproductive performance in males requires a peaceful disposition toward females and an intact penis. Semen can be collected by manually milking and stimulating the penis during the breeding season.[65] Serum testosterone levels increase 30- to 380-fold during the mating season in the Chinese alligator (Martelli, unpublished data).



• **Figure 59.3** (A) Postmortem gross appearance of the liver of an adult wild male Nile crocodile *(Crocodylus niloticus)* with extensive necrotizing hepatitis attributable to chronic sepsis of traumatic origin from a tail injury. Ultrasound (B and C), gross (D and E), and histopathology (F and G) appearance of the liver in two farmed Siamese crocodiles *(Crocodylus siamensis).*

Nervous System

See also Table 59.1. Neurologic conditions may manifest as unusual resting positions, listlessness, opisthotonos (star-gazing), excitability, tremors, and convulsions. Thiamine deficiency brought about by consumption of fish rich in thiaminase is a common cause of neuropathy in crocodilians, captive or wild.[5,66,67] Septicemia can result in meningoencephalitis, as can infections by crocodile herpes virus, West Nile virus, and a variety of parasites.[5,6,8,36,37,68]

Clinical presentation of metabolic bone disease, spinal trauma, and steatitis (due to vitamin E deficiency combined with rancid fatty acid in the diet) may be mistaken for diseases of the CNS.[5]

Laboratory Examination

Fecal and urinary sampling is discussed earlier. Excreta analysis is carried out as in other species. Noninvasive fecal glucocorticoid metabolites accurately reflect adrenal activity of crocodilians.[61]

Blood may be collected from the ventral tail vein and from the supravertebral venous sinus in the cervical spine or in the dorsal tail.[5,9,69] The ventral tail vein is accessed as in other reptiles, laterally or ventrally. The author also uses a venous sinus located in the mandibular shelf at the tip of the lower jaw (Fig. 59.4). This site has not been described but has proven advantageous in small patients, heavy animals deep in mud, or whenever the oral cavity presents the simplest access (Martelli, unpublished data).

Current studies do not find significant correlations between hematology, biochemistry, and observed pathologies.[22,70] Changes in leukogram may be presumed to reflect response to treatment.[21] Hematology and biochemistry reference ranges may be found on ZIMS and in the literature.[4,71–74] Medical evaluations of populations should include hematology and biochemistry as reference for longitudinal studies. Hemoparasites, including hemogregarines

• **Figure 59.4** Venipuncture sites in crocodilians. (A) Mandibular shelf in estuarine crocodile *(Crocodylus porosus)*, (B) mandibular shelf in Chinese alligator *(Alligator sinensis)*, (C) dorsal venous sinus in *(Crocodylus porosus)*, and (D) ventral tail vein in gharial *(Gavialis gangeticus)*.

and trypanosomes, are common in crocodiles and do not appear to cause disease.[49,75] An improvement in packed cell volume (PCV), total protein, and albumin, in the absence of leukopenia, is of positive prognostic value in patients recovering from long-term debilitating conditions.

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Reptile and Amphibian Analgesia

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Introduction

Do amphibians and reptiles feel pain? Can we recognize pain in amphibians and reptiles? Is the perception of pain by an amphibian or reptile equivalent to that of a mammal? Does it make anatomic or physiologic sense that the ability to perceive and respond to an aversive stimulus is evolutionarily limited to mammals? We will never be able to answer these questions objectively because amphibians and reptiles simply cannot tell us. However, as in the case of nonverbal human infants or nonhuman mammals, should the inability to communicate dictate whether pain is being perceived or whether an analgesic drug should be administered? By anthropocentric definition, the word "pain" implies higher-level cortical processing of information; therefore the words *nociception* and *antinociception* are used in referring to pain and analgesia in most nonmammalian species. This stems from the controversy concerning whether or not nonmammalian species have the appropriate central and peripheral nervous system structures and pathways, capable of "receiving and processing" noxious stimuli and responding appropriately. In other words, can nonmammals "experience" pain, or are they merely capable of demonstrating a "reflexive" response to a noxious stimulus—that is, nociception?[1] Some would argue that nonmammals cannot experience pain simply because such species lack a neocortex.[2] The thinking is that animal species lacking a neocortex can respond only reflexively to a painful stimulus; they cannot "process" such information in any other part of the forebrain or midbrain, therefore making the "perception" of pain impossible. I would argue that "absence of evidence is not evidence of absence".[3] In other words, merely making a declaration that the experience of pain does not exist in non-mammals simply because it is unknown, unproven, or immeasurable, does not mean that there is indisputable evidence that the experience of pain truly does not exist in a given individual animal or species because it lacks a neocortex. It is my contention that, based on published neuroanatomic, neurophysiologic, and behavioral data, there is considerable and plausible evidence to suggest that, structurally and functionally, amphibians and reptiles have the capacity to experience pain.[1,4] Therefore it is my contention that, because of our limited understanding of pain and analgesia in reptiles and amphibians, we, as clinicians, should err on the side of health and well-being of the herpetile patient by making the assumption that conditions considered painful in humans and other mammals should be assumed to be painful across all other vertebrate species, including amphibians and reptiles.

Amphibian and Reptile Nociception of Pain: Neuroanatomic and Neurophysiologic Evidence

Nociceptive Pathways

It is adaptive for all animals to avoid aversive stimuli in the environment as both proximate and ultimate mechanisms for survival; therefore all vertebrates have specialized sensory receptors, or nociceptors (e.g., thermal, mechanical, and chemical receptors), which are capable of detecting noxious stimuli and afferent pathways relaying this information to the central nervous system. Consequently, for any vertebrate organism to "perceive" a painful stimulus, there must be the following: a peripheral sensory receptor (e.g., nociceptor), a sensory pathway to the spinal cord, initial processing of the painful stimulus within the spinal cord's dorsal horn, ascending pathways to the brain, final processing of the painful stimulus by the brain, and presence of descending pathways, which exert control over the withdrawal, escape, defensive, or immobile responses.

Nociceptors are highly conserved across phyla and have been identified in aquatic and terrestrial invertebrates, teleost fish, amphibians, and birds.[1,5] Functionally, all nociceptors do not respond to the same stimuli. Some may be specifically mechanoreceptors, chemical receptors, or thermoreceptors, and some nociceptors may be multimodal and respond to a variety of different noxious stimuli. In amphibians and reptiles, a variety of nociceptors have been identified, including thermoreceptors and pain receptors (mechanical, chemical, and multimodal).[1,5,6] Efferent pathways exist to initiate a response, typically a movement away from the noxious

stimulus. As with mammals, amphibians and reptiles have myelinated and unmyelinated afferent fibers running together in sensory nerves: large myelinated A fibers (Aβ); small myelinated A fibers (Aδ); and small unmyelinated C fibers (C).[1,7] In amphibians, small, slowly conducting fibers (Aβ and C) transmit the majority of all impulses induced by noxious heat, pinching, pinpricks, and the application of dilute acetic acid to the skin.[7] In addition, a multitude of biochemical mediators (e.g., substance P, catecholamines, cytokines, prostaglandins [PGs], etc.) are involved in the sensation, signal transmission, and perception of pain and or inflammation, which adds to the complexity associated with our understanding of nonmammalian pain.[8,9] Like mammals, amphibians and reptiles have all of the anatomic structures considered critical for the recognition of pain: peripheral nociceptors, appropriate central nervous system structures and pathways, opioid receptors and endogenous opioids, reduction of nociceptive response with analgesics (although data are sparse), pain avoidance learning, and suspension of normal behavior with pain.[10] Recent research in fish, amphibians, reptiles, and birds has demonstrated the transmission of peripheral sensory signals via the spinal cord to midbrain and forebrain regions that are homologous to mammalian cortical and limbic structures (anterior dorsal ventricular ridge and posterior dorsal ventricular ridge of the pallium in amphibians and reptiles).[1,5,11,12] Thus the physiologic and anatomic requirements for pain and analgesia appear to be remarkably similar among all vertebrate species.

Opioid Receptors and Endogenous Opioids

The opioid receptor gene family is highly conserved across multiple vertebrate orders (e.g., bovids, chickens, bullfrogs, and teleost and elasmobranch fishes).[13] An extensive published literature is available with respect to amphibian opioid receptors in the central nervous system. Four opioid receptors have been cloned and sequenced in the northern leopard frog *(Rana pipiens)*[14,15] and three main opioid receptors, μ, δ, and κ, were cloned and sequenced in the rough-skinned newt *(Taricha granulosa)*.[16] In an extension of this work, the same laboratory compared binding of μ-opioid agonists to human brain μ-opioid receptors and frog brain μ-opioid receptors and found that most μ-opioid agonists have higher affinity for binding with the human μ-receptor compared with the frog μ-receptor.[17] In reptiles, there is limited information on opioid receptors. In aquatic turtles, μ- and δ-opioid receptors are located throughout the brain, and δ-opioid receptors are more abundant than μ-opioid receptors.[18] With respect to endogenous opioid-related neurotransmitters, proenkephalin-derived peptides are present in turtles with a distribution similar to that in mammals and birds.[19] In addition, the reptilian brain (aquatic slider turtles, American alligators *[Alligator mississippiensis]*, and anole lizards) was found to contain large quantities of endogenous enkephalins, also known as endorphins.[20] In amphibians, β-endorphin, met-enkephalin, and endomorphin were found in the central nervous system of the African clawed frog *(Xenopus laevis)*.[21]

Measurement and Quantification of Pain and Analgesia in Amphibians and Reptiles

Measuring pain in any species, particularly amphibians and reptiles, is the most difficult hurdle in the study of pain and analgesic efficacy. In mammals, it is well established that perioperative pain management facilitates recovery and healing, reduces morbidity and mortality, and contributes to more rapid return to normal behavior.[4] An objective understanding of normal behavior of a particular species and the ability to differentiate the presence of abnormal behavior indicative of discomfort are critical to the study of pain and analgesia. Methods for assessing and measuring pain in amphibians and reptiles have been described previously.[4,7] Ideally, a combination of appropriate behavioral and physiologic parameters might best be employed to measure pain and analgesia in amphibians and reptiles. Along those same lines, the development of a species- and context-specific ethogram for each species being evaluated would provide the best method for distinguishing normal from abnormal (e.g., painful) behaviors. Most commonly, animal pain or lack thereof is assessed before and after an invasive procedure, such as a surgical procedure. This method requires the development of a behavioral ethogram, which, in turn, requires the observer to become well versed in subtle behavioral differences through many hours of observation and analysis (videotaped or live observation). Behaviors must be operationally defined, which will provide objectivity and reproducibility.[4] For example, in our laboratory we developed a behavioral ethogram to evaluate preoperative and postoperative behavioral responses to food intake, willingness to swim, and breathing in red-eared slider turtles *(Trachemys scripta elegans)* following a unilateral orchidectomy.[22] In a study using ball pythons *(Python regius)*, feeding behavior was used as an indicator of pain associated with the administration of capsaicin.[23] An alternative to studying postsurgical pain is to measure pain under strictly controlled laboratory conditions using established behavioral models, during which noxious stimuli (e.g., mechanical, thermal, and chemical) are applied to an anatomic location on the reptile subject.[24,25]

The application of a noxious thermal stimulus provides a well-established behavioral model for assessing pain and analgesia in rodents.[4] In our own studies, we have successfully adapted this classic thermal nociception model developed for use in rodents and determined that amphibians and reptiles show unambiguous, easily quantifiable withdrawal responses indistinguishable from those of rodents.[24,25] This method has also been applied to amphibians.[26] Although some might argue that a withdrawal response to a noxious stimulus is not equivalent to pain, there is evidence in a variety of species that noxious thermal stimulation caused molecular and cellular changes in the central nervous

system.[27] In amphibians, the majority of nociception and antinociception studies have relied on the use of the acetic acid wipe test, predominantly in northern leopard frog, with some studies using African clawed frog.[7,28] This method has been used to test the efficacy of a variety of opioid, nonopioid, and nonsteroidal anti-inflammatory drugs (NSAIDs).[7] The primary problem associated with the acetic acid wipe test is that it causes inflammation in addition to being a noxious nociceptive chemical, which is a confounding factor in attempts to discriminate only nociception.

Physiologic changes have been used to quantify stress and pain in mammals, and this approach has been adapted for amphibians and reptiles as well.[29,30] For example, heart rate significantly increased in ball pythons after subcutaneous (SC) capsaicin administration.[30] In this study, opioid analgesic drugs did not alter this physiologic response. Recently, in a comparative anesthesia/analgesia study using Oriental fire-bellied toads *(Bombina orientalis)*, heart and respiratory rates were depressed in toads immersed in alfaxalone-butorphanol, but the same toads immersed in alfaxalone-morphine had normal heart rates and depressed respiratory rates.[31] In another amphibian study, the number of eye blinks as well as gular respiration rate were used to measure pain associated with intracoelomic (ICe) administration of concentrated saline in Indian bullfrogs *(Rana tigrina)*.[32] Changes in the resulting behaviors were then quantified following the administration of opioid drugs and NSAIDs in order to determine analgesic efficacy.

Analgesic Drugs

Opioids and Opioid-Like Analgesics

Opioids, the most effective drugs for controlling pain in mammals, are classified according to receptor subtypes—μ (mu), κ (kappa), and δ (delta). μ, δ, and κ opioid receptors—abbreviated MOR, DOR, and KOR—mediate the analgesic effects of opioids, whereas the role of the fourth type of opioid receptor, the nociceptin or orphanin FQ receptor (ORL), is less clear.[7] For pain management in mammals, many clinicians prefer administering either a μ-opioid receptor agonist (e.g., morphine, fentanyl, hydromorphone, etc.), a partial μ-opioid receptor agonist (e.g., buprenorphine), or a mixed-opioid, κ-receptor agonist–μ-receptor antagonist (e.g., butorphanol). In order for exogenous opioids to be effective analgesics in amphibians and reptiles, opioid receptors must be present. The gene family for opioid receptors (μ, κ, and δ) is highly conserved across multiple vertebrate orders.[13] Two snake species have endogenous brain opiates, and red-eared slider turtles have both proencephalin-derived peptides and functional μ- and δ-opioid receptors in the brain.[18,19] In the northern grass frog, μ-, κ-, δ-, and the ORL opioid receptors were cloned.[13] Although opioid receptors are expressed in amphibians and reptiles, we are just beginning to understand the efficacy of commonly used opioid drugs (see also Chapter 26). Tables 60.1 and 60.2 summarize commonly used analgesic protocols in amphibians and reptiles.

Butorphanol tartrate, a κ-opioid agonist/μ-opioid antagonist, was once considered the analgesic of choice in reptiles. However, our laboratory demonstrated that this has no clear analgesic properties in red-eared slider turtles, bearded dragons, corn snakes *(Pantherophis guttatus)*, or ball pythons.[24,25] Consistent with our data, intramuscular butorphanol (1 mg/kg) had no analgesic efficacy as determined by use of a thermal noxious stimulus method (Fleming, 2012)[32a] and no isoflurane-sparing effect in green iguanas *(Iguana iguana)*.[33] In ball pythons, butorphanol administered at 5 mg/kg intramuscularly (IM) had no effect on physiologic parameters compared with saline.[34] Conversely, one study demonstrated that butorphanol (1.5 and 8 mg/kg IM) provided analgesia in green iguanas exposed to a noxious electrical stimulus.[35] In amphibians, eastern red-spotted newts *(Notophthalmus viridescens)* were described as showing more normal postoperative behavior (e.g., feeding, body posture, and response to observer) after limb amputation if they were exposed to butorphanol via immersion bath (0.5 mg/L water) during recovery from tricaine methansulfonate anesthesia.[36] However, when butorphanol was added to alfaxalone for sedating oriental fire-bellied toads in an immersion bath, there was no antinociceptive response to a mechanical noxious stimulus.[31] On the other hand, morphine sulfate, a μ-opioid agonist, was demonstrated to be analgesic in amphibians and reptiles.[4,7] In the previously mentioned oriental fire-bellied toad study, there was a clear antinociceptive response to a noxious mechanical stimulus when frogs were sedated with alfaxalone and morphine in an immersion bath.[31] Using multiple experimental models—including the acetic acid wipe test, the thermal noxious withdrawal stimulus test, and a mechanical noxious stimulus test (von Frey filaments) model of nociception—morphine, at very high doses was antinociceptive in northern leopard frogs across all nociceptive models.[37] Using the thermal noxious stimulus method, morphine was an effective analgesic in bearded dragons (1 and 5 mg/kg) and red-eared slider turtles (1.5 and 6.5 mg/kg), but data were not as clear in corn snakes (even when morphine was administered at an extremely high dose of 40 mg/kg.[25] Similarly, morphine (5, 7.5, 10, and 20 mg/kg) and pethidine (10, 20, and 50 mg/kg), a synthetic short-acting μ-opioid agonist, provided analgesia in Speke's hinged tortoises *(Kinixys spekii)* exposed to formalin administered into a limb, which was reversible after naloxone administration.[38] In further support of μ-opioid agonist analgesic efficacy in reptiles, morphine increased limb withdrawal latencies in Nile crocodiles *(Crocodylus niloticus)*[39] and increased tail flick latencies in anole lizards *(Anolis carolinensis)*.[40] Related to morphine, hydromorphone is a semisynthetic, μ-opioid receptor agonist that was demonstrated to provide analgesia in red-eared sliders using the thermal hind limb withdrawal nociception model at 0.5 mg/kg SC for up to 24 hours.[41]

TABLE 60.1 Analgesic Protocols for Use in Amphibians

	Dosage/ Concentration	Route	Frequency	Comments	References
Opioids					
Buprenorphine	50–75 mg/kg	ICe, SC	NDA	Resumption of normal behavior after limb amputation, eastern red-spotted newt	36
Butorphanol	0.2 mg/kg	IM	NDA	No efficacy data, postoperative in African clawed frog	70
	25–33 mg/kg	SC	q8–12h	Experimentally antinociceptive, northern leopard frogs	7
Butorphanol/alfaxalone	0.5 mg/L	Bath	NDA	Resumption of normal behavior after limb amputation, eastern red-spotted newt	36
	0.5 mg/100 mL	Bath	NDA	No evidence of analgesia in Oriental fire-bellied toads	31
Fentanyl	0.5 mg/kg	SC	NDA	Presurgical analgesia, American bullfrog	71
	0.8 mg/kg	SC	q24h	Experimentally antinociceptive, northern leopard frogs	7, 14
Methadone	10–300 nmol/g	SC	NDA	Experimentally antinociceptive, northern leopard frogs; 1000 nmol/g was fatal	54
Morphine	10–300 nmol/g	SC	q8–16h	Experimentally antinociceptive, northern leopard frogs; 1000 nmol/g was fatal	54
	40.0–100.0 mg/kg	SC	NDA		7
	10.0 mg/kg	ICe	NDA	Antinociceptive in northern leopard frogs; not analgesic in African clawed frogs	28
	10.0 mg/kg	ICe	NDA	Analgesic in northern leopard frogs	72
Naloxone	0.01–10.0 mg/kg	ICe	NDA	Antagonizes μ-opioid analgesia in northern leopard frogs	72
Naltrexone	0.10–10.0 mg/kg	ICe	NDA	Antagonizes μ-opioid analgesia in northern leopard frogs	72
Oxymorphone	200.0 mg/kg	IM	q48h	Analgesic in AAT in edible frogs	42
NSAIDs					
Flunixin meglumine	25.0 mg/kg	SC	NDA	Analgesia in African clawed frogs and northern leopard frogs	28
Local Anesthetics					
Lidocaine	2–4 mg/kg 50 mg/kg	Topical, SC	NDA	No systematic data on local analgesia; systemic effects at high dose	4, 57
Proparacaine Ophthalmic Solution (0.5%)	1–2 drops	Topical Topical	NDA	Effective for intraocular pressure measurement	60
Other Anesthetics					
Dexmedetomidine	0.1–3.0 nmol/g	SC	NDA	Analgesia for at least 8 h even at lower concentrations, northern leopard frogs	64
Dexmedetomidine + alfaxalone	0.3 + 20 mg/100 mL	Bath	NDA	Evidence of analgesic efficacy but minimal sedation	31, 54

AAT, Acetic acid test; *h,* hours; *IM,* intramuscular; *ICe,* intracoelomic; *NDA,* no data available; *NSAIDs,* nonsteroidal anti-inflammatory drugs; *SC,* subcutaneous.

TABLE 60.2 Analgesic Protocols for Use in Reptiles

	Dosage	Route	Frequency	Comments	References
Opioids					
Buprenorphine	0.075–0.1 mg/kg 0.02–0.1 mg/kg 0.1–1 mg/kg	SC, IM	q24h	No evidence of analgesic efficacy; plasma concentrations equivalent to those effective for analgesia in mammals; respiratory depression not studied	35, 41, 45
Butorphanol	1–20 mg/kg 0.4–8 mg/kg	SC, IM	NDA	No evidence of analgesic efficacy; significant respiratory depression in red-eared slider turtles (>10 mg/kg NOT RECOMMENDED)	4
Hydromorphone	0.5 mg/kg	SC, IM	q24h	Good analgesic efficacy in red-eared sliders; respiratory depression not studied, but thought to be significant	45
Fentanyl	12.5 µg/h 2.5 µg/h	TC	One patch for 24–72 h	Transdermal patch; provided antinociception in ball pythons and cornsnakes; plasma concentrations detected in ball pythons and prehensile-tailed skinks	4, 43, 44
Meperidine (pethidine; demerol)	1–5 mg/kg 10–50 mg/kg	SC, IM	q2–4h	Analgesic efficacy short-lived in red-eared slider turtles; respiratory depression not studied	4, 38, 39
Methadone	5 mg/kg	SC, IM	q24h	Good analgesic efficacy in red-eared slider turtles; administered without evidence of efficacy Australian Krefft's river turtle *(Emydura macquarii krefftii)*; anecdotal evidence in lizard species; respiratory depression not studied	4
Morphine	1–40 mg/kg	SC, IM	q24h	Good analgesic efficacy in red-eared slider turtles and bearded dragons *(Pogona vitticeps)*; unknown efficacy in snakes; significant respiratory depression (>5 mg/kg NOT RECOMMENDED)	24, 25, 35, 38, 39, 40
	0.1–0.2 mg/kg	IT		Antinociceptive for >24 h in red-eared slider turtles	58
Naloxone	0.04–2 mg/kg	IM, SC		All species; antagonizes µ-opioid agonists (e.g., morphine)	4, 22, 24, 25
Tapentadol	10 mg/kg	IM	NDA	Analgesic efficacy in yellow-bellied sliders; pharmacokinetics indicated 10-h duration of effect	51, 52
Tramadol	10 mg/kg	PO	q48–72h	Good analgesic efficacy with relatively long duration when administered PO in chelonians; less respiratory depression than other opioids in red-eared slider and yellow-bellied slider turtles. In loggerhead sea turtles, 10 mg/kg PO q48–72h	48, 49, 50
NSAIDs					
Carprofen	0.11–4 mg/kg	SC, IM	NDA	No data regarding analgesic efficacy, safety, or pharmacokinetics/ pharmacodynamics in any reptile species	4
Ketoprofen	2 mg/kg	PO, SC, IM	NDA	Pharmacokinetic data in green iguanas. No evidence of analgesic efficacy in any reptile species, but an NSAID frequently used by sea turtle clinicians as an anti-inflammatory due to apparent safety	4, 70

Continued

TABLE 60.2 Analgesic Protocols for Use in Reptiles—cont'd

	Dosage	Route	Frequency	Comments	References
Meloxicam	0.1–0.5 mg/kg	IV, SC, IM, PO	NDA	Pharmacokinetic data in loggerhead sea turtles at 0.1 mg/kg; did not reach plasma concentrations indicative of analgesia in mammals. Not recommended at these dosages	67
				Pharmacokinetic data in green iguanas at 0.2 mg/kg IV. No evidence of analgesic efficacy in any reptile species; no physiologic changes in ball pythons administered post-operatively	66
				Aquatic turtles (0.2–0.4 mg/kg) IM, IV.	69
				Ball pythons (0.3 mg/kg) IM	34
				Pharmacokinetic data in red-eared slider turtles	68
Local Anesthetics					
Lidocaine (1% or 2%)	1–2 mg/kg (keep <5 mg/kg) 4 mg/kg	SC, IM, IT	NDA	Appears to be a good local nerve block and effective IT in chelonians	58, 59
Bupivicaine (0.5%)	1 mg/kg (keep <2 mg/kg) 1 mg/kg	SC, IM, IT	NDA	Appears to be a good local nerve block and effective IT in chelonians	58
Mepivicaine (2%)	1 mg/kg	SC	NDA	Used as a mandibular nerve block in American alligators	73
Proparacaine ophthalmic solution (0.5%)		Topical corneal	NDA	Blocked corneal sensitivity within 1 min of application with duration of effect at least 45 min in Kemp's ridley turtles *(Lepidochelys kempii)*; Green iguanas; bearded dragons; caiman *(Caiman crocodilus)*	61, 74, 75
Other Anesthetics					
Medetomidine/ dexmedetomidine	0.05–0.3 mg/kg 0.1–0.2 mg/kg	SC, IM	NDA	No evidence of analgesic efficacy; frequently combined with ketamine, midazolam or an opioid in sedation/ anesthesia protocols	4
Ketamine	2 mg/kg	SC, IM, IV	NDA	No evidence of analgesic efficacy; low dose as an analgesic is extrapolated from the mammalian literature; frequently combined with an α-2, benzodiazepine, or an opioid in sedation/anesthesia protocols	4
Midazolam	0.5–3 mg/kg	SC, IM, IV	NDA	No evidence of analgesic efficacy; frequently combined with an α-2, ketamine, or an opioid. Enhances analgesic properties of dexmedetomidine in mammals	4

IM, Intramuscular; *IV,* intravenous; *IT,* intrathecal; *NDA,* no data available; *NSAIDs,* nonsteroidal anti-inflammatory drugs; *PO,* orally; *SC,* subcutaneous; *TC,* transcutaneous.

An analog of oxymorphone, oxymorphazone, a μ-opioid agonist, was demonstrated to be a relatively weak analgesic drug, even at 200 mg/kg, when subcutaneously administered to edible frogs *(Rana esculenta*; now *Pelophylax esculentus)* and subjected to the acetic acid wipe test, but it had an approximately 48-hour duration of effect.[42] There have been no systematic studies of oxymorphone efficacy in any reptile species.

Fentanyl is a synthetic, μ-opioid receptor agonist with 75–100 times the potency of morphine; it can be administered either transcutaneously as an impregnated patch, subcutaneously, intramuscularly, or intravenously.

In amphibians, fentanyl and remifentanil (a similar drug to fentanyl with twice the potency) were determined to be antinociceptive in frogs after either SC or intraspinal administration using the acetic acid wipe test.[14] In fact, of all opioid agonists tested in one study using northern grass frogs exposed to the acetic acid wipe test, fentanyl was the most effective after SC administration.[7] In two reptile species, two separate studies have evaluated the pharmacokinetics of fentanyl. In ball pythons, fentanyl plasma concentrations reached 1 ng/mL within 4 hours of application of a transdermal fentanyl patch (12.5 μg/h)[43] and were detectable by 4–6 hours and for greater than 72 hours in the plasma of prehensile-tailed skinks *(Corucia zebrata)* (the fentanyl dose was applied at 10% exposure of total surface area of a 25-μg/h patch for 72 hours).[44] It remains unclear whether there is any biological significance to these plasma fentanyl concentrations. A recently completed study in our laboratory evaluated the efficacy of transdermal fentanyl patch (12.5 μg/h) administration in ball pythons and found no analgesic efficacy, even after repeating the study.[43] Our laboratory also confirmed that fentanyl was readily absorbed through the skin of the snakes and remained at very high plasma levels during patch application.[43] In the same study, we determined that fentanyl patch application decreased respiration in the same snakes, so we know that fentanyl is biologically active in the snakes even though we cannot definitively determine analgesic efficacy.

Buprenorphine is an effective analgesic in many mammalian species and is used extensively due to its longer duration of action compared with other opioids. Buprenorphine has partial agonist activity at the μ-opioid receptor, partial or full agonist activity at the δ-opioid receptor, and antagonist activity at the κ-opioid receptor. In eastern red-spotted newts subjected to limb amputation surgery, the amphibian subjects were described to show more normal postoperative behavior (e.g., feeding, body posture, and response to observer) after exposure to buprenorphine administered intracoelomically compared with controls.[36] After the acetic acid wipe test was used in northern grass frogs, buprenorphine was weakly analgesic compared with other μ-opioids, such as fentanyl.[7] Buprenorphine pharmacokinetics in reptiles were determined after SC administration in red-eared slider turtles; effective dosages ranged from 0.075 to 0.1 mg/kg, which provided plasma concentrations similar to those associated with analgesic efficacy in humans for approximately 24 hours.[45] Interestingly, plasma concentrations of buprenorphine were reduced by approximately 70% when the drug was administered in the hind limb compared with the forelimb, indicating a significant hepatic first-pass effect. The analgesic efficacy of buprenorphine has not been demonstrated in reptiles. Buprenorphine did not alter responses to an electrical noxious stimulus in green iguanas.[35] Similarly, in our laboratory, buprenorphine (0.1, 0.2, and 1.0 mg/kg SC) provided no analgesic efficacy in red-eared slider turtles exposed to a noxious thermal stimulus.[41]

Tramadol has become a widely used analgesic alternative to other opioids in veterinary medicine because it can be administered orally and due to its relatively long duration of action. Tramadol and its major active metabolite, O-desmethyl-tramadol (M1), produce analgesia in mammals by activating μ-opioid receptors but also by inhibiting central serotonin and norepinephrine reuptake.[46] Respiratory depression, a common side effect associated with administration of other μ-opioid agonists, is significantly diminished with tramadol administration in mammals.[46,47] In mammals, the analgesic effects of tramadol typically begin within 30 minutes after oral administration, and last for 6 hours. In contrast, tramadol (5.0 mg/kg; oral [PO]) administered to turtles significantly increased withdrawal latencies for 12–24 hours post-drug administration, and 6–96 hours after administration of the higher tramadol dosages (10 or 25 mg/kg PO or SC).[48] In loggerhead turtles *(Caretta caretta)*, plasma concentrations of tramadol and M1 remained above the target concentration of ≥100 ng/mL for approximately 48 hours at a dose of 5 mg/kg PO and for 72 hours when tramadol was administered at 10 mg/kg PO.[49] Subjectively, appetite, swimming, and general activity level did not change after drug administration. Most human and nonhuman mammalian studies consider the tramadol target analgesic plasma concentration to be 100 ng/mL. Recently, tramadol pharmacokinetics and efficacy were evaluated in yellow-bellied slider turtles *(Trachemys scripta scripta)* after a single intramuscular dose (10 mg/kg) administered in either the hindlimb or forelimb.[50] Using a thermal hindlimb withdrawal latency test, antinociceptive efficacy appeared to last approximately 48 hours regardless of whether the tramadol was administered in the forelimb or hindlimb, and tramadol and M1 remained above an atypically high target plasma concentration (1 μg/mL or 1000 ng/mL) for approximately 48 hours.[50] Of interest was that the pharmacokinetic trends were similar for tramadol administration in forelimbs and hindlimbs, but the concentrations of M1 was approximately 20% higher in the plasma of the group receiving tramadol in the hindlimbs compared with those receiving tramadol in the forelimbs. The antinociceptive effects of tramadol have not been studied systematically in any amphibian species, although our laboratory is currently evaluating the efficacy of tramadol in tree frogs using a thermal nociceptive stimulus.

With respect to deleterious side effects, respiratory depression associated with tramadol administration in red-eared slider turtles was approximately 50% less than that measured after morphine administration.[48] Therefore tramadol appears to be a promising analgesic alternative to traditional opioids in reptiles. As mentioned, one of the significant deleterious side effects associated with tramadol and all opioid drug administration in mammals is respiratory depression. This problem is paralleled in reptile studies. Our laboratory determined that both butorphanol and morphine caused profound respiratory depression in turtles,[24] whereas respiratory depression was significantly less when turtles were administered tramadol. The bottom line is that it is imperative that clinicians continue to monitor respiration during and after procedures in which any opioid drugs are administered to reptile species.

Tapendatol, similar mechanistically to tramadol, is a human drug that shares μ-opioid receptor activation and norepinephrine reuptake inhibition with tramadol, but tapendatol has only weak serotonergic reuptake and has more potent opioid properties without an active metabolite. Tapendatol was administered to red-eared and yellow-bellied slider turtles in order to determine analgesic efficacy using a thermal noxious stimulus model and pharmacokinetics.[51,52] After intramuscular administration (5 mg/kg), tapendatol plasma concentrations were detectable for approximately 24 hours and the duration of antinociceptive effects was approximately 10 hours in both turtle species.[51,52] The shorter duration of antinociceptive efficacy compared with tramadol may be due to the lack of an active metabolite. Tapendatol has not been studied in amphibians. Although respiratory depression has not been investigated after tapendatol administration in reptiles, in humans it is thought to cause less respiratory depression compared with commonly administered μ-opioid agonist drugs.

Parenteral Anesthetics

There are few published data demonstrating analgesic efficacy associated with administration of anesthetic drugs such as ketamine, dexmedetomidine, medetomidine, midazolam, or propofol in amphibians or reptiles. Whereas α-2-adrenergic drugs are commonly used in combination with ketamine or midazolam for sedation in amphibians and reptiles, few data exist with respect to the analgesic effects of these drugs. Intraspinal dexmedetomidine was administered to northern grass frogs, causing a dose-dependent increase in pain thresholds using the acetic acid wipe test.[53] In a similar study using the acetic acid wipe test in northern grass frogs, systemic administration of dexmedetomidine was analgesic compared with other α-adrenergic agonists.[54] Using the acetic acid wipe test, a thermal noxious withdrawal stimulus test, and a mechanical noxious stimulus test (von Frey filaments) in northern leopard frogs, dexmedetomidine was antinociceptive after exposure to the acetic acid and thermal noxious stimuli but not after exposure to the von Frey filaments.[55] A recently completed study in our laboratory demonstrated that dexmedetomidine (0.1 mg/kg) produced antinociception for up to 24 hours in ball pythons.[56] Although there is interest in the fact that low-dose ketamine provides analgesia in mammals, there are no data in amphibians or reptiles.

Local Anesthetics as Analgesics

Local anesthetics can be used alone or as part of a multimodal anesthetic/analgesic approach. These include lidocaine, bupivacaine, and mepivacaine, which block peripheral nerve transmission to the dorsal horn by inhibiting sodium influx into the neurons and therefore blocking the nociceptive signal from traveling along the nerve fibers. For all local anesthetics, pain transmission is blocked as long as the local anesthetic nerve block lasts, but inflammation and pain will still develop at the site of injury and will be transmitted to the central nervous system after the effect of the block has ceased. Because of its significant analgesic effect, any local block that is correctly executed will significantly decrease the required amount of other anesthetic agents, but additional analgesia is warranted for postoperative pain management in certain cases. The limitations associated with local anesthetic administration include a focal nervous block and short duration of action. However, there is evidence in amphibians that lidocaine will have systemic effects at very high doses.[57] In American bullfrogs *(Lithobates catesbeianus)*, lidocaine (50 mg/kg), administered subcutaneously, caused a significant decrease in respiratory rate, a progressive loss of righting and palpebral reflexes, and contralateral toe pinch withdrawal. However, these systemic effects were not associated with local analgesia; the frogs reacted to a forceps pinch at the site of the lidocaine injection at the time of maximal systemic effects.[57]

With respect to local effects of these anesthetics, there is only one published reptile study in which mepivacaine was used as a mandibular nerve block in an American alligator.[13] In this study, a nerve locator was used to facilitate the procedure. Lidocaine (2%) (up to 5 mg/kg total dose) can be used for local ring blocks or line blocks. Lidocaine may be diluted with bicarbonate or sterile water at a 1:1 ratio or greater to decrease the pain of injection and allow increased volume to be infused without reaching a toxic dose. In addition, topical lidocaine (4%) is commonly administered directly to wounds before and after debridement for local analgesia, particularly in chelonian species. Intrathecal administration of local anesthetics is useful for surgical procedures of the tail, phallus, cloaca, and hind limbs.[58] Lidocaine (4 mg/kg, <1 hour duration), bupivacaine (1 mg/kg, 1–2 hours duration) or preservative-free morphine sulfate (0.1–0.2 mg/kg, duration of up to 48 hours) can be administered intrathecally between the coccygeal vertebrae of turtles. For example, bupivicaine (0.1 mL for each 10 cm of carapace) was administered intrathecally in order to facilitate surgical excision of fibropapillomas from the posterior flippers of a green sea turtle *(Chelonia mydas)*.[4] In a different study, lidocaine (1 mL/20–25 kg) was administered intrathecally in hybrid Galapagos tortoises prior to phallectomy surgery.[59] For topical administration, proparacaine hydrochloride (0.5%) has been used clinically in amphibians and reptiles during eye exams, especially in order to measure intraocular pressure. In American bullfrogs, proparacaine was used to block corneal sensation during a research project in which intraocular pressures were measured for reference ranges.[60] Proparacaine was demonstrated to be effective in blocking corneal sensitivity in Kemp's ridley turtles for up to 45 minutes, with a 1-minute onset to effect.[61] Similarly, intrathecal analgesia was reported in chelonians.[58]

Nonsteroidal Anti-Inflammatory Drugs

Although they are not as potent as the opioids, NSAIDs are used widely in reptile clinical practice as analgesics as well as

for their anti-inflammatory properties but less so in amphibians. NSAIDs provide analgesia in mammals by blocking the binding of arachidonic acid to cyclooxygenase enzyme (COX), preventing the conversion of thromboxane A2 to thromboxane B2 (TBX), thus preventing the production of PG, potent mediators of inflammation.[62] In bullfrogs, administration of meloxicam at a dosage of 0.1 mg/kg once daily suppressed circulating serum PGE2 levels following a controlled injury (i.e., punch biopsy).[63] NSAIDs are often classified based on their relative specificity. There are two COX enzymes, COX-1 and COX-2, which participate in renal and gastric protection and inflammation, respectively.[62] The NSAID ketoprofen is equipotent against both isoenzymes, whereas carprofen is slightly more COX-2–specific and meloxicam is COX-2–specific.[62] Therefore the degree of efficacy and side effects may vary with each of the three NSAIDs.

Although many NSAIDs appear to be relatively safe when used in amphibians and reptiles, there are only a few published studies with respect to analgesic efficacy: one study uses a reptile species and three studies use amphibian species.[7,28,34] Ball pythons administered meloxicam (0.3 mg/kg IM) prior to surgical placement of an arterial catheter showed no physiologic changes (e.g., heart rate, blood pressure, plasma epinephrine, and cortisol) indicative of analgesia. In leopard frogs undergoing the acetic acid wipe test, flunixin meglumine, a nonselective COX inhibitor, provided antinociception after ICe administration, and the results were comparable to morphine administered to the same subjects.[64] In a similar study, two nonselective COX inhibitors, indomethacin and ketorolac, provided weak antinociception in leopard frogs exposed to acetic acid.[65] Although there are no published pharmacokinetic studies of NSAIDs in amphibian species, several reptile studies are in the literature. In a study using the thermal noxious stimulus withdrawal latency model, flunixin meglumine (25 mg/kg), administered in the dorsal lymph sac, provided better analgesia than xylazine hydrochloride or meloxicam.[28] Plasma concentrations of meloxicam (0.2 mg/kg PO) administered as a single dose to green iguanas were at plasma concentrations considered analgesic in mammals, and these concentrations were measurable out to 24 hours postadministration.[66] In loggerhead turtles, meloxicam (0.1 mg/kg) administered both IM and intravenous (IV) did not reach plasma concentrations consistent with analgesia in humans, horses, or dogs, and the half-life was short.[67] In a pharmacokinetic study in which meloxicam (0.2 mg/kg IM and IV) was administered to red-eared slider turtles, the IM dose provided a therapeutic concentration range necessary for meloxicam to provide analgesic and anti-inflammatory effects equivalent in mammals for approximately 48 hours.[68] In a different study evaluating the pharmacokinetics of meloxicam (0.2 mg/kg PO, ICe, and IM) in red-eared slider turtles, only the ICe and IM routes, but not the PO route provided mean blood concentrations of meloxicam that were above those considered effective to induce anti-inflammatory effects in mammals for approximately 8 hours (IM) or 12 hours (ICe).[69] Ketoprofen (2 mg/kg IV), administered to green iguanas, had a long half-life (31 hours) compared with ketoprofen pharmacokinetics in mammals, but the bioavailability after IM administration was 78% with a relatively short half-life (8.3 hours).[70]

Because no efficacy data and few pharmacokinetic data are available with respect to NSAID administration in amphibians and reptiles, appropriate dosages and frequency of administration can only be extrapolated. In addition, clinicians should be aware of the deleterious side effects documented in avian and mammalian species (e.g., renal impairment, gastrointestinal ulceration/inflammation, hematologic abnormalities); assessment of suitability for this class of drugs for the individual patient is prudent.

Multimodal Analgesic Approaches

In amphibians and reptiles, multimodal drug paradigms may be the best approach for managing pain. *Multimodal analgesia* refers to the administration of multiple drugs that have analgesic efficacy at different levels in the central and peripheral nervous system. For example, opioids will have greatest efficacy at opioid receptors in the central and peripheral nervous system, whereas NSAIDs administered at the same time as the opioid will have greatest efficacy as anti-inflammatory agents at the peripheral tissues. Local anesthetics can enhance multimodal analgesic protocols by blocking the initial pain cascade at the peripheral level. In concert, all of these drugs have the potential to minimize the transmission of pain signals to the brain, especially when administered preemptively, before a potentially painful procedure is established (Tables 60.1 and 60.2).

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61

Medical Aspects of Giant Tortoise Relocation in the Galápagos Islands

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The Galápagos Islands are known for their biological uniqueness and as the stimulus for Darwin's *The Origin of Species*. The islands lie 1000 km west of the coast of Ecuador and straddle the equator. Currently, 97% of the land area is National Park and the Galápagos Marine Reserve covers an area of 133,000 square kilometers surrounding the islands.[1] The majority of the native and endemic species of Galápagos are still found there. However, the giant tortoises (*Chelonoidis* spp.) suffered decimation as a result of human influence. Three species of tortoises have gone extinct (Table 61.1) due to humans, and the total numbers of several other species are greatly reduced from historical levels.

Giant tortoises are a keystone species in the Galápagos ecosystem. The Galápagos National Park Directorate (GNPD) oversees the conservation and restoration of the islands. Restoration of tortoises to all the islands where they historically occurred and restoring populations to historic numbers is a current collaborative program of the GNPD, the Galápagos Conservancy, international scientists, and other nongovernmental organizations (NGOs).[2]

Conservation actions for tortoises have evolved from simply releasing juvenile tortoises to habitat restoration through elimination of introduced species and the use of surrogate tortoises to serve as environmental engineers to maintain the delicate balance of this fragile environment.

Rebuilding tortoise populations involves four major program activities: captive breeding and release, head-starting and release of hatchlings from wild nests, release of sterilized hybrid adult animals, and repopulating islands with breeding populations of genetically selected surrogate species. Veterinary involvement in the giant tortoise program is relatively recent. The tortoise program was solidly founded on a basis of good biological science and animal husbandry. As the program matured, additional recognition was given to the potential contributions of nutrition, pathology, health monitoring, disease screening, and surgical and medical care of specific cases.

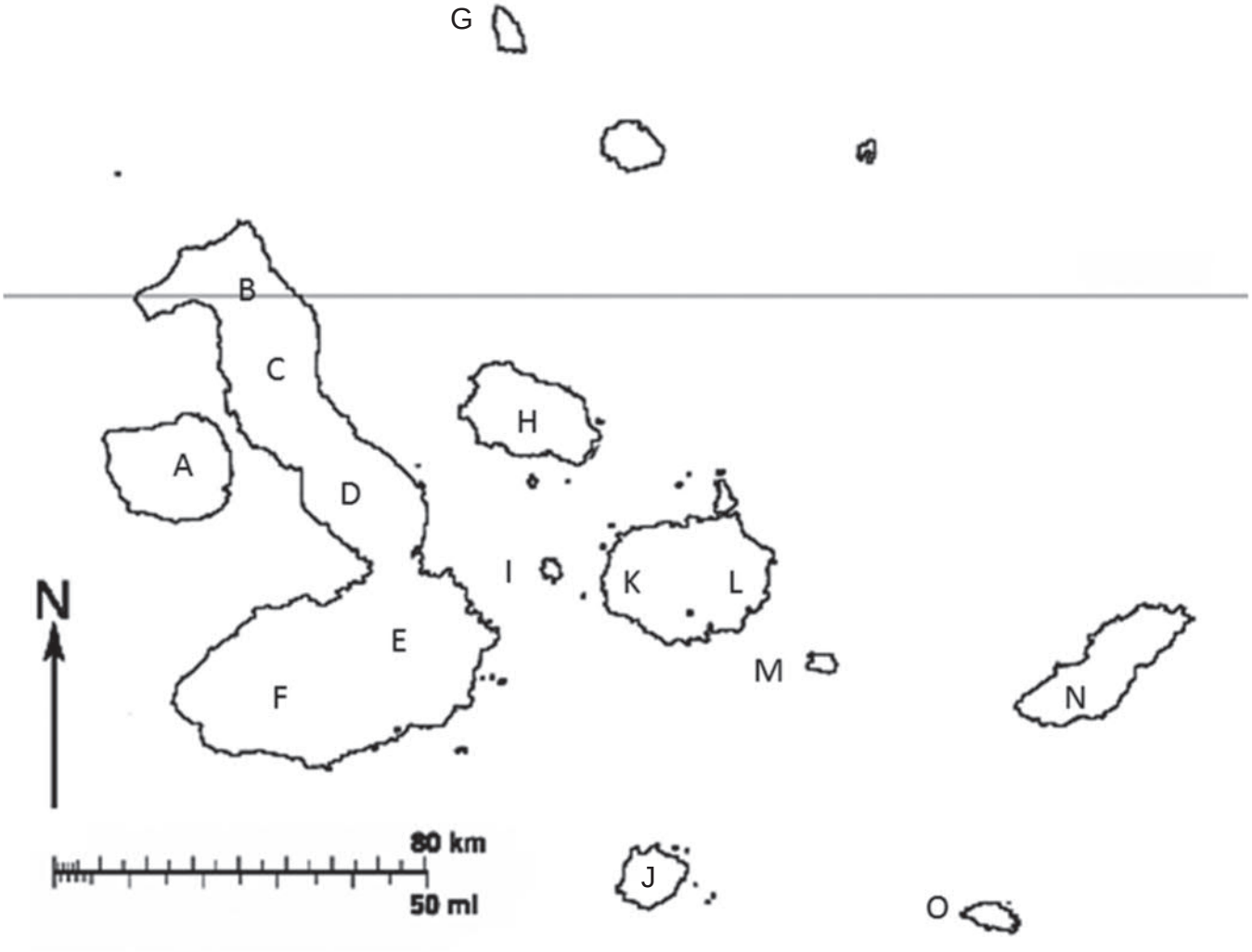
History

The Galápagos Islands were discovered in 1535. Estimates of tortoise populations at the time suggest there may have been as many as 250,000 throughout the islands. There was very little human influence in the first century after their discovery; however, from the 17th through the 19th century, tortoises were harvested by buccaneers, whalers, and fur seal hunters as a source of fresh meat during their long ocean journeys; and in the early 20th century tortoises were harvested by local populations for food and oil to be exported to the South American mainland.[3,4] The tortoise population reached a low point in the 1970s, when populations were estimated to be between 8000 and 14,000 individuals.[5]

Taxonomy of Galápagos giant tortoises has been a topic of debate for almost 200 years. For purposes of conservation management, 15 species are described based on morphologic, geographic, and molecular factors. There are five species from Isabela Island, two from Santa Cruz Island, and one each from the islands of Santiago, Pinzon, Espanola, San Cristobal, Fernandina, Floreana, Santa Fe, and Pinta. Populations on the latter four islands are extinct[6,7] (Fig. 61.1; see Table 61.1). Restoring tortoise populations to historical numbers, including those considered "extinct in the wild," is occurring through a combination of in situ management, breeding and rearing tortoises where appropriate, and, on islands where the endemic tortoise species is extinct, through the use of an analog (closely related) species.[2] Among the 15 species, there are two general morphologies: "dome" (carapace has smooth and rounded contour as seen from the side, typical of most chelonian species) shaped and "saddleback" (front of the carapace is raised and looks like a Spanish saddle in side view). The dome-shaped animals are generally larger (up to 250 kg or more) and are found on islands with higher elevation where the vegetation is denser, the dome shape facilitating movement through thick vegetation, whereas the saddleback tortoises are smaller (males up to 100 kg)

TABLE 61.1 **Galápagos Giant Tortoise (*Chelonoidis* spp.) Population Threats and Conservation Actions**

Map	Species	Morphology	Range	Population	Main Current/ Historical Threats	Conservation Actions	Comment
A	*Chelonoidis phantastica*	Saddleback	Fernandina	Extinct	Volcanic eruption	Potential future surveys	Natural extinction
B	*C. becki*	Mixed	Wolf Volcano	5–10,000	Goats/livestock, genetic contamination	Removal of introduced species	Source of rare/extinct genotypes
C	*C. microphys*	Domed	Darwin Volcano	2000	Goats/livestock	Removal of introduced species	
D	*C. vandenburghi*	Domed	Alcedo Volcano	5–10,000	Goats/livestock	Removal of introduced species	
E	*C. guentheri*	Domed	Sierra negra	300–500	Human predation, introduced species	Education efforts, captive breeding, removal of introduced species	
F	*C. vicini*	Domed	Cerro Azul	400–600	Human predation, introduced species	Education efforts, captive breeding, removal of introduced species	
G	*C. abingdoni*	Saddleback	Pinta Island	Extinct	Human predation, goats	Introduce surrogate species	Sterilized tortoises introduced as ecologic engineers
H	*C. darwini*	Intermediate	Santiago Island	500–700	Goats, pigs, donkeys, black rats	Removal of introduced species, head-starting	Goats, pigs, and donkeys eradicated 2005
I	*C. ephippium*	Saddleback	Pinzon Island	150–200	Black rats	Removal of introduced species, head-starting	Rats eradicated 2012
J	*C. elephantopus*	Saddleback	Floreana Island	Extinct	Human predation, introduced species	Removal of introduced species, surrogate species introduction	
K	*C. nigrita*	Domed	SW Santa Cruz Island	5000	Human predation, introduced species	Education efforts, removal of introduced species, head-starting	
L	*C. donfaustoi*	Domed	Eastern Santa Cruz Island	100	Human predation, introduced species	Education efforts, removal of introduced species, head-starting	
M	*C.* spp.	Saddleback	Santa Fe island	Extinct	Human predation	Analog species introduction	Espanola tortoises introduced as environmental engineers
N	*C. chathamensis*	Saddleback	San Cristobal Island	2000	Human predation	Removal of introduced species	Natural population recruitment
O	*C. hoodensis*	Saddleback	Espanola Island	2000	Human predation	Captive breeding	Founding population 3.12; goats eradicated 1970s



• **Figure 61.1** Map of the Galápagos Islands. Letters indicate the names of the islands or volcanos that are home to the 15 identified species of Galápagos giant tortoises (*Chelonoidis* spp.) and correspond to Table 61.1.

and are found on more arid islands of lower elevation with more open vegetation. The elevation of the front of the carapace allows these animals to reach higher when foraging in dry vegetation. A few island populations are intermediate between dome and saddleback shapes (see Table 61.1; Fig. 61.2A, B).[8]

Human predation had the earliest impact on wild tortoise populations through harvesting of up to 200,000 animals for food and oil in the 17th–20th centuries. Deliberate introductions of livestock (cattle, goats, swine, horses, donkeys) and escape or accidental introduction of dogs, cats, Norway rats, *(Rattus norvegicus)*, black (roof) rats, *(R. rattus)*, and house mice *(Mus musculus)* have continued population pressures on many of the species in their natural habitats.[9] The impacts of introduced vertebrate species on giant tortoises are both direct and indirect. Eggs and hatchlings are predated by rats, dogs, cats, and pigs. Cattle, donkeys, horses, goats, and pigs damage nesting areas, destroy food sources, and destroy or diminish the canopy of vegetation that naturally captures fog and mist precipitation. This restricts tortoise access to freestanding water and affects the microclimate essential to certain life stages. Restoration of giant tortoise populations is fundamentally dependent on the eradication of introduced pest species; and removal of hunting pressure through public education.[10]

Tortoise populations on Southern Isabela *(C. vicina, C. guentheri)*, Pinzon *(C. ephippium)*, Santiago *(C. darwini)*, and San Cristobal *(C. chathamensis)* were severely reduced by human depredation but were also impacted by introduced species. Human exploitation reduced the population on Espanola *(C. hoodensis)* to 2.12 (2 males, 12 females) which were so disperse on the island that breeding had ceased. These animals were gathered and moved into a captive breeding program on Santa Cruz Island in the 1960s. They were joined by a lone male returned from the San Diego Zoo to Galápagos in 1973 to make a founder population of 3.12 animals.[11]

In addition, invasive plants alter the distribution of naturally occurring native food sources and may affect seasonal migration and alter the habitat, making it unsuitable for

• **Figure 61.2** (A) Saddleback morphology of the Pinta Island Galápagos giant tortoise *(Chelonoidis abingdoni)* "Lonesome George" prior to his death and extinction of the species on June 24, 2012. (B) Dome-shape morphology of Santa Cruz Island Galápagos giant tortoise *(Chelonoidis nigrita)*.

use.[12] When essential parts of the home range of individuals are unsuitable, there are frequently no other options available to the individuals dependent on that habitat.

Restoration of Giant Tortoise Populations

Scientists recognized the giant tortoises of Galápagos seemed to be doomed as early as the early 1900s. Tortoise collection expeditions were made from the early 1900s to the late 1950s for ex situ management. Animals from various islands were deposited in zoos throughout the United States, Europe, and Australia, often without identification of the island source or population of the individuals placed into captive collections. Successful breeding outside of Galápagos is relatively rare but occurs in zoos and the private sector.[8]

The Galápagos National Park was established in 1959 to preserve and restore the unique flora and fauna of the archipelago. Early efforts were begun in the 1960s to restore the tortoise population of Pinzon Island, where the total population of adults was estimated to be approximately 200 individuals. No successful recruitment had occurred in nearly 100 years, due to predation of hatchlings by the introduced black rat. Hatching eggs and hatchlings still in the nest were collected and transported to Santa Cruz Island for rearing at the Tortoise Rearing Center until they were large enough to be considered "rat proof" at approximately 5 years of age, when they were returned to suitable habitat on Pinzon.[4]

Other populations were identified for head-starting. These were reduced by human depredation but had significant ongoing population impact from introduced mammals that destroyed nesting areas and predated juvenile tortoises. Eggs and hatchlings from Santiago Island and Santa Cruz were collected and transferred to the rearing center on Santa Cruz for rearing under human care before release when less likely to suffer attacks by introduced predators. In the 1990s the depletion of tortoise populations on the Southern Isabela Island volcanos of Sierra Negra and Cerro Azul was worsened by ongoing human harvest and depredation by introduced species. A new breeding center was built, and a founding population of breeding age animals was brought into captivity, with animals segregated into separate subpopulations from different areas of these two volcano populations.[13]

Head-Starting

Hatchlings and hatching eggs are collected from nesting areas on Pinzon, Santiago, and Santa Cruz islands. Historically, each tortoise population's known nesting sites were visited every year at the time of expected hatching, all observed nests were manually excavated, and all hatchlings and unhatched eggs were removed for transport to Santa Cruz for rearing. Although this practice has been successful in producing high numbers of young tortoises for later repatriation, it may have resulted in overrepresentation of a narrower genetic cross section of the population because the timing and location of collection was consistent from year to year. Recently, the practice changed to visiting one island population per year and attempting to locate a higher number of nests to capture a broader representation of the genetic diversity in each population.

Nests are opened with hand digging and small tools to prevent accidental damage to the nest's contents. Movement of reptile eggs during late incubation is less likely to result in embryonic damage because the membranes are more stable; however, attention must be given to keeping the egg orientation during transport and later artificial incubation the same as it was in the nest.[14] Unhatched eggs and hatchlings from excavated nests are transported in small plastic containers containing lightly moistened vermiculite to maintain humidity and reduce the impact associated with transport. Eggs are carried to a road (Santa Cruz) or to a boat (Santiago, Pinzon) for transport to the rearing center.

Developing eggs are hatched in incubators and hatchlings placed in a "dark box" on moist vermiculite substrate for a month. This mimics the time spent by hatchlings in the nest absorbing their yolk sac before they would emerge when the rainy season begins. Embryonic mortality is encountered when the incubation temperature is too high or too low (<25°C or >33°C). Eggs incubated at lower temperatures have longer incubation times. Causes of embryonic and neonate mortality have not been thoroughly investigated. Observations include unresorbed and inflamed yolk sacs, which are presumed to be infection related, but factors could include incubation problems.[14]

Hatchlings are reared in rodent-proof enclosures, outdoors, on a substrate of crushed lava with approximately 50% shade. Hiding shelters are available with adequate space for all hatchlings in the enclosure. Water is available at all times in a shallow container that has small rocks so hatchlings have good footing to facilitate egress. Lava rocks are placed in the enclosures to provide visual barriers between individuals, and climbing structures that provide exercise. The diet consists almost exclusively of the leaves of two species of locally grown plants: Arrowleaf elephant ear *(Xanthosoma sagittifolium)* and coral bean *(Erythrina smithiana),* which are offered three times per week. At 1½ to 2 years of age they are moved into a larger "preadaptation" enclosure, where they have a substrate of lava rocks and soil with natural vegetation to provide shade. Here they have constant access to water and are able to interact with other juveniles up to 5 years of age.[14]

Poor growth and soft shells are seen during prolonged periods of cool, wet weather; or when structures or vegetative growth results in too much shade, reducing the animal's ability to thermoregulate. Problems are more frequently seen toward the end of the cool season, which occurs July through December. Resolution of these problems occurs with time when shade is reduced, allowing for improved basking and increased ambient temperatures. Bite wounds are seen when hatchlings have restricted access to food or minimal ability to avoid enclosure mates.

Individual hatchlings are identified using painted numbers; the color of paint is chosen to indicate the population of origin, and they are numbered sequentially as they hatch. As they grow, painted numbers are refreshed until juveniles are old enough for placement of a passive induced transponder (PIT) tag in the left hind leg, which will then identify them permanently. Prior to release, each individual is branded using red-hot metal on the carapace. This is a readily visible mark that may be seen in the field, indicating the animal has a PIT tag. In certain field-monitoring situations, animals are branded on the carapace with a number and/or are marked with a numbering system that involves placing V-shaped notches in the marginal scutes to form numbers from 1 to 9999. These procedures are typically performed without anesthesia or analgesia.[14]

Animals are weighed and measured every 3 months in captivity. They are released when they have reached a curved carapace length of 20 cm, at approximately 3–4 years of age. Prior to release, each animal is examined. They should have good body condition and be capable of negotiating rough terrain to find food and shelter and must have reached a size that is likely to survive the risks they will encounter in the wild. Their diet is restricted to only leafy items, lacking seeds for at least 30 days prior to release. This prevents the inadvertent dispersal of seeds of plants from Santa Cruz Island to the site of repatriation.[12]

Release occurs in suitable habitat near the nesting zone from which hatchlings were collected (or in the case of animals bred in captivity, at historical nesting zones for that population) during the wet or rainy season to ensure the availability of food and water for the juveniles as they acclimate to the wild environment.[13,14]

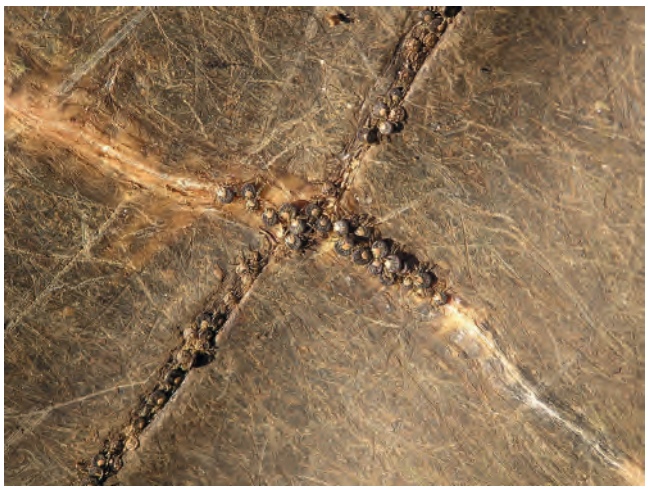
Ecosystem Restoration

Giant tortoises on Wolf Volcano of Isabela Island demonstrate the full spectrum of tortoise shell morphologies found historically in the Galápagos Islands. The larger population, found primarily at higher elevations, is a dome-type tortoise that reaches a size of up to 250 kg. At the lower elevations on the western slope of the volcano, there is a diverse population that includes full saddleback morphologies as well as animals showing intermediate shapes ranging to fully domed. Genetic analysis shows that animals demonstrating the saddleback morphology have genes matching museum specimens originating from Floreana and Pinta Islands.[15] Some of these animals show hybridization with the species native to Wolf, and some show genetic ingression from other island populations. Certain individuals sampled during surveys in 2008 and 2015 have a high degree of purity to those extinct species and have the potential to be used in a breeding program to produce tortoises for reintroduction with the genetic material that evolved on those islands.

The GNPD intends to reintroduce tortoises where they have been driven to extinction, with the goal of restoring ecosystems. Goat eradication programs throughout the islands have removed a major threat to tortoise survival and to ecosystem health but left these habitats without a large herbivore to control woody vegetation. Thick growth of woody vegetation prevents the growth of cactus (*Opuntia* spp.), a species essential to healthy tortoise populations. Woody vegetation obstructs tortoise movement and shades the ground, reducing opportunities for tortoises to feed and thermoregulate. Tortoises consume grasses and forbs, disperse seeds, and disrupt soils and serve to maintain a healthy ecosystem balance.

Without this large herbivore, scrub grasslands change to woody forested habitat. Pinta, Floreana, and Santa Fe Islands have been effectively without tortoises for a century or more.[16]

To some extent, feral goats have controlled woody vegetation but at the same time threatened native and endemic vegetation. Feral goats have recently been removed from the uninhabited islands, and these islands now need tortoises



• **Figure 61.3** Ticks of the genus *Amblyomma* are found attached to the skin and shell of Galápagos giant tortoises (*Chelonoidis* spp.).

to restore the natural balance. The ideal goal is to return genetically pure tortoises to these ecosystems, but pure animals are not known to exist. However, hybrid animals with ancestry to Pinta and Floreana, found on the western slopes of Wolf Volcano, adjacent to Banks Bay, have been collected and will establish the nuclei of captive breeding colonies used to repatriate giant tortoises on these islands.[3]

Field surveys were done in 2008, 2014, and 2015 to collect genetic samples, mark individuals, and photodocument morphologies within the population. Animals identified with high genetic purity were sought during later surveys. These and other unmarked and untested animals were collected in 2015 for the captive breeding effort. Animals selected for captive breeding will be chosen based on having a high degree of genetic purity.

In the collection expedition of 2015, animals were identified for collection by field workers, based on morphology or because of previously identified genetic status. Animals were secured in a cargo net and transferred by helicopter directly to the deck of a ship. On arrival, each animal was examined for physical condition and bled to repeat and/or confirm genetic purity. Ticks of the genus *Amblyomma*,[17] present on the majority of animals, were collected for later identification (Fig. 61.3). Each individual was sprayed with 0.5% permethrin, with care taken to wet all skin and shell surfaces to prevent the translocation of ectoparasites along with the tortoises. Once dry, animals were given fenbendazole solution at 50 mg/kg orally based on estimated body weight. After treatment, animals are placed in a holding space in the hull of the ship. No food was offered during transport, but all feces were collected and incinerated to eliminate parasites and other pathogens and prevent the translocation of seeds from the island of origin to the destination at the rearing center.

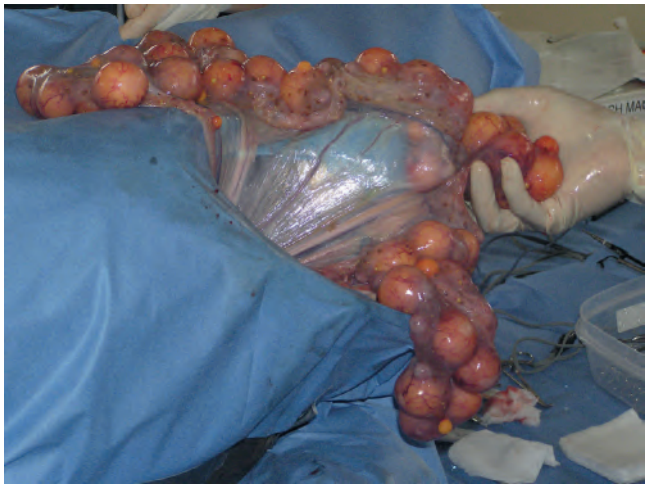
Animals were transported by ship to the breeding center on Santa Cruz Island, where the sexes were segregated until the genetic identity could be confirmed. Treatment with permethrin and fenbendazole was repeated 2 weeks after arrival into captivity. All feces were collected and incinerated for 2 months to ensure that no parasite ova might survive to introduce a novel species of parasite to the local population of tortoises in the rearing center or to the wild native tortoises. No additional screening for infectious diseases was performed. There are no documented detections of chelonian herpesvirus, adenovirus, anellovirus, mycoplasmas, ranavirus, intranuclear coccidiosis, or rickettsia in Galápagos tortoises[18]; and there have been no morbidity or mortality events of tortoises in Galápagos to suggest any of these diseases are present. There are only a handful of tortoises that have ever been returned to the islands, so the risk of introduced disease is very small. However, future management actions may need to incorporate disease screening into translocation protocols.

Surrogate Species Use as Ecologic Engineers

There is hope that tortoises with Pinta Island ancestry will one day be released onto Pinta to reestablish a wild tortoise population in that ecosystem. However, an effective population of Pinta tortoises is not likely to be available any time soon. The National Park chose to use sterilized surrogate mixed heritage tortoises to fulfill the role of ecologic engineers until a breeding population can be introduced.

In the early years of the Captive Rearing Center, giant tortoises of unknown ancestry were surrendered by private owners to the center. These animals were housed together and eventually produced hatchlings. The young produced were raised and held for display for several decades.[14] The program to breed and rear tortoises has expanded, and the housing of these genetically hybrid tortoises occupied valuable space and was a drain on resources in their care and feeding. Recently performed genetic assays showed these animals to be hybrids and were unsuitable for release to bolster native populations on any of the islands. However, if sterilized, they could be released on an island such as Pinta, where there was an immediate need for a large herbivore to consume woody vegetation, disperse seeds, and interact with the remainder of an otherwise intact ecosystem.[19]

Fifteen female and 24 male hybrid tortoises were selected for introduction to Pinta. Female tortoises were anesthetized using ketamine 10 mg/kg and medetomidine 0.1 mg/kg administered intravenously (IV) in either the jugular or brachial vein. Propofol at a dose of 1.7 mg/kg IV was used as a supplement in some individuals to deepen or extend anesthesia time based on tortoise response. A local block using buffered lidocaine (2% lidocaine: 7.5% sodium bicarbonate in a 5:1 ratio) was performed in the prefemoral fossa at the anticipated site of the surgical access. The total volume of lidocaine injected did not exceed 2 mg/kg. Animals were oophorectomied using an endoscope-assisted technique (Fig. 61.4). A bilateral prefemoral fossa approach was used to access and exteriorize ovarian tissues. The mesovarium was transected and blood vessels ligated using



• **Figure 61.4** Endoscope-assisted oophorectomy in a Galápagos giant tortoise (*Chelonoidis* spp.).

• **Figure 61.6** Galápagos giant tortoise (*Chelonoidis* spp.) phallectomy procedure showing placement of clamp and ligatures.

• **Figure 61.5** Needle placement for intrathecal injection in a Galápagos giant tortoise (*Chelonoidis* spp.).

vascular clips or 2-0 polydioxanone suture. The coelomic membrane and muscle wall were closed in a simple continuous pattern using polydioxanone suture impregnated with the antimicrobial triclorsan. The skin was closed with 0 or 2 polydioxanone suture in a horizontal mattress pattern. Anesthesia was reversed with 0.5 mg/kg atipamezole given intramuscularly (IM). Preoperatively, animals were given oxytetracycline at 10 mg/kg IM to reduce the likelihood of bacterial infection, and meloxicam at 0.2 mg/kg IM for analgesia and anti-inflammatory effects.[20]

Male tortoises were phallectomized using epithecal anesthesia. Animals are placed into dorsal recumbency with the head higher than the tail and the plastron angled at an approximately 30-degree angle to the ground. The dorsal tail surface is aseptically prepared with chlorhexidine surgical scrub; 40–80 mg of 2% lidocaine is injected into the epithecal space by placing a 3-cm, 20-G needle between caudal vertebrae at approximately one-fourth to one-third the distance from the end of the carapace to the tip of the tail (Fig. 61.5). The intercoccygeal intervertebral space is located using digital palpation. The needle is advanced until it is possible to aspirate clear fluid with minimal pressure. The full volume of lidocaine is injected at a slow, steady pace. If there is pressure or difficulty in injection, the needle should be repositioned. A properly placed injection will result in a flaccid tail and cloacal sphincter, without any evidence of effect on the pelvic limbs. However, if the pelvic limbs are affected, recovery can be expected in 4–6 hours.

The phallus is exteriorized and a towel clamp or Allis tissue forceps used to retract and extend the organ to its fullest extent. A clamp is placed at the base of the phallus near the attachment to the cloaca. Two transfixational ligatures are placed around the corpora cavernosae, using two polydioxanone sutures placed at the base of the exposed phallus approximately 1.5–2 cm apart (Fig. 61.6). The phallus is transected approximately 1 cm distal to the more distally placed ligature. The mucosa is then closed over the centrally located erectile tissue using 0 polydioxanone suture in a simple continuous pattern. Aerosol aluminum powder protective bandage (Aluspray; Neogen) was placed to inhibit bacterial contamination. Animals were given oxytetracycline at 10 mg/kg IM as a broad-spectrum antibiotic at the time of surgery and a second dose given 3 days later. Meloxicam was given at 0.2 mg/kg IM to reduce inflammation and provide analgesia associated with the surgery.[21] A phallectomy will render the male tortoise incapable of delivering semen into the cloaca of female tortoises. It will not have any effect on hormone or sperm production, so animals can be expected to demonstrate normal mating behavior.

Tortoises were held for several months to allow for healing and while transportation to the release site could be arranged. Five months after surgery, tortoises were released into suitable habitat and began eating and moving through the habitat within minutes of release, despite living their entire lives in captivity. At a future time, if a breeding population of tortoises is introduced to Pinta Island, these

animals will not be able to contribute to the genetics of the introduced population.

Summary

Despite centuries of decimation at the hands of humans and from the impact of introduced animals, the tortoise populations in Galápagos are increasing. It will be decades before numbers will approach aboriginal levels, and that will occur only with active management. Restoration of tortoises is one step in the recovery of Galápagos ecosystems. Additional work must be done in the removal of introduced vertebrates, invertebrates, and plant species that present ongoing threats.

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SECTION 12

Avian

62

Antifungals in Birds

KATHRYN C. GAMBLE

Due to immunosuppression and reduced or lapsed husbandry standards, fungal infections in birds housed in managed care remain a frequent veterinary presentation.[1] Systemic fungal infections with oxyphilic and thermophilic *Aspergillus* predominate in the respiratory tract, due to spore inhalation from environmental fungal overgrowth from damp, protein-rich substrates.[2,3] Vague signs of early infection may go undetected, establishing chronic disease challenges of direct lesion management and requirement for long-term treatment.[2,4] Similarly, the other primary fungal pathogen of concern for avian patients, *Candida*, is associated with poor nutrition, antibiotic treatment, or hand-rearing juveniles.[5] Other fungal pathogens are more limited in scope but warrant pharmacologic treatment. It has been suggested that measurement of antifungal concentration in target tissues may be more useful than plasma concentrations.[6] To achieve optimal treatment success, and especially for dimorphic fungi, antifungal sensitivity or concentration measurements should be considered at available sources (http://strl.uthscsa.edu/fungus/).

Aspergillosis Treatment (Table 62.1)

There are four core classes of antifungal drugs with activity against *Aspergillus*; the polyenes, including amphotericin B (AmpB); triazoles, including itraconazole (ITRA), fluconazole (FLU), and voriconazole (VRC); allylamines, including terbinafine (TERB); and fluocytosine.[7]

Polyenes

Produced by *Streptomyces* spp., these poorly absorbed macrolytic polyketides act through interference with membrane barrier function by sterol binding that results in fatal leakage of fungal cells through creation of transmembrane channels.[1,7,25,26] Due to its lack of specificity, this binding may occur not only in fungal ergosterol but also vertebrate cholesterol, which contributes to patient toxicity.[1]

Amphotericin B

Available as a parenteral, systemic formulation, AmpB has poor gastrointestinal absorption.[1,7,26] Generic deoxycholate AmpB presented an increased risk of patient toxicity, as compared with newer cholesterol and liposomal formulations.[7,25,26] It is highly protein bound, but tissue distribution is good, although limited concentrations occur in cerebrospinal fluid (CSF) and bone.[1,7,26] Direct application of AmpB to the infected area as nebulization (NE) or lavage improves its efficacy and reduces toxicity potential.[7] Metabolism is poorly understood for AmpB, but elimination is biphasic, and nearly 15 days in mammals, although much shorter in birds.[1,26]

Dose-dependent nephrotoxicity, as a result of renal vasoconstriction and direct nephron effect, is lessened in birds due to their faster avian elimination rate.[1,7,26] Slowly administered parenteral AmpB (1–1.5 mg/kg intravenous [IV] twice daily [BID] to three times daily [TID] 3–5 days) can be combined with other antifungals for weeks.[1,2,26] Topical lavage (1–1.5 mg/kg BID) for lesion rinse, or NE (1 mg/mL for 15 minutes BID to four times daily [QID] can be considered for 3–7 days.[1,2] Even though poorly soluble in water, AmpB should be diluted before administration by any route, but balanced electrolytes or saline as the diluent will inactivate the drug.[1,26] Protected from light, sterile water dilutions are stable for 24 hours at room temperature; 1 week under refrigeration; or 30 days at –4°F (–20°C).[26] Some *Aspergillus* spp. have elevated minimum inhibitory concentrations (MIC) beyond typical targets (0.5–2 μg/mL), and increasing resistance is reported.[1,25,26]

Azoles

By interference with sterol-14α-demethylase, this antifungal class interrupts biosynthesis of ergosterol needed for fungal cell membrane function, therefore depleting cell stores while accumulating the unneeded sterol.[7,11,25] More specific for ergosterol, this class has less interference with vertebrate sterols, especially with the newer triazoles.[7,25] These drugs are metabolized by hepatic cytochrome P-450, and concern exists for potential outright hepatotoxicity and hepatic metabolism changes in concurrent polypharmacy.[1]

Itraconazole

This synthetic triazole optimally is absorbed at acidic pH, and variable impact of feeding or fasting has been

TABLE 62.1 Published Avian Aspergillosis Antifungal Regimens Supported by Pharmacokinetics or Tissue Concentrations

	Dose (mg/kg)	Formulation*	Route	Species	Comment
Itraconazole	20 single dose[PK,TC]	CAP-W; CAP-OJ	Gavage	Mallard	[8]
	6 BID[PK]	CAP	PO	Racing pigeon	[9]
	20 SID[PK]	CAP	In food	Humboldt penguin	Compounded product not equivalent to commercial product[10,11]
	8.5 BID[PK]				
	5 SID[PK]	CAP-OJ	Gavage	Blue-fronted Amazon	Same dose potentially fatal to African grey parrot[12]
	10 BID[PK]				
	5 SID[PK]	CAP-OJ	Gavage ± food	Red-tailed hawk	[13]
	10 SID[PK,CD]				
Voriconazole	20 BID-TID[PK]	SUSP	Gavage ± food	Mallard	[14]
	10 BID[PK]	TAB-S	Gavage	Racing pigeon	Hepatotoxicity[15]
	20 SID[PK]				
	5 SID[PK]	TAB-SU	Gavage	African penguin	[16]
	5 SID[PK]	TAB-W	In food	Magellanic penguin	Administered clinically for 5 days with 2 day rest—rest may not be necessary[17]
	12–18 BID[PK]	TAB-W/-SU	Gavage	African grey parrot	Polyuria[18]
	18 TID[PK]	TAB-W/-SU	Gavage	Hispaniolan Amazon	Polyuria[19]
	10 BID[PK]	SUSP	Gavage + food	Red-tailed hawk	[20]
	15 single dose[PK]	POW	In food	Red-tailed hawk	Maximum concentration delayed with food but consistent, regardless of feeding status[21]
	12.5 BID[CD]	TAB-W	In food	*Falco* spp.	Therapeutic concentrations achieved but troughs unmeasurable[22]
Terbinafine	15 SID[PK]	TAB-W	Gavage	African penguin	Absorption not altered by food[3]
	60 single dose[PK]	TAB-W/ TAB-SU	Gavage + food	Hispaniolan Amazon	15 mg/kg dose had no measurable plasma concentrations[23]
	22 SID[PK,TC]	TAB	In food	Red-tailed hawk	Absorption not altered by food; lungs had poor concentration[24]

*-(crushed into); *CAP*, capsule; *OJ*, acidic solution (e.g., orange juice); *PK*, Pharmacokinetics; *POW*, oral powder; *-SAL*, saline; *-SU*, suspending agent; *SUSP*, suspension; *TAB*, tablet; *TC*, tissue concentrations; *-W*, water.

demonstrated in birds.[25,27] In general, the oral suspension produced more rapid absorption, and higher bioavailability when patients were fasted, whereas capsules had improved absorption with food intake.[27] Commercial formulations contain proprietary cyclodextrin, which improves absorption, and has marginalized use of compounded product due to both markedly reduced ITRA concentrations measured in these products and treatment failures.[10,11,25–28] A commercially available subcutaneous controlled release gel was assessed in mallards that produced undetectable plasma concentration of ITRA.[8]

Despite extensive protein binding, and due to its extreme lipophilicity, ITRA is well distributed throughout the body, although brain concentrations were lower than those of plasma.[26,27] Its principal metabolite hydroxy-ITRA is active and contributes markedly in some avian species.[1,11,26–28] Metabolites are excreted through bile and urine, whereas unmetabolized ITRA is excreted via bile.[1,27] Without loading doses, the long elimination half-life may protract achievement of steady state.[1,26,27] With a broad spectrum of efficacy,[26] ITRA remains a primary choice for avian aspergillosis treatment,[3,28] with target plasma concentration of 0.25 µg/mL in humans associated with clinical success,[11,12,28] although some acquired resistance recently has been documented.[1,13,27]

Routinely administered ITRA doses (5–10 mg/kg by mouth [PO] once or twice a day [SID-BID]) may be administered as loading doses of increased frequency or high end of dose range, then maintained for weeks to months until clinical resolution at the lower dose ranges and frequency.[3,26] However, plasma ITRA concentrations demonstrate species variability even at the same doses and consistent feeding schedule. In particular, granivore and carnivore gastric acidity differences may play a role in degree of absorption and maximum concentration achieved.[12,13] In addition, potential ventricular koilin binding has been reported in granivores.[12] Body fat composition was proposed as the explanation for the lower plasma concentrations achieved in anseriformes at even much higher than standard doses (20 mg/kg).[8] In similar dose studies, tissue ITRA concentrations have been measured in several avian species. Especially the lung and brain, where systemic aspergillosis is most prevalent, repeated documentation of their lowest concentrations for all body tissues were noted regardless of the dose, even where other tissues were more reflective of or exceeded plasma concentrations.[1,8,9,13,27]

Of species-specific note, ITRA is not recommended for African grey parrots *(Psittacus erithacus timneh)* due to frequent patient inappetance and depression and sometimes death, although reduced oral dose recommendations (2.5–5 mg/kg PO SID) have been published.[1,6,26] Raptors also may present significant anorexia.[27] However, generally minimal impact to clinical pathology or hepatic function is noted in birds.

Fluconazole

This synthetic triazole specifically inhibits demethylation of lanosterol to ergosterol.[28] Because it is highly water soluble and has low protein binding, FLU is well absorbed orally, unaffected by feeding or gastric pH.[1,7,26] It extensively penetrates to CSF,[25,26] although it has better effects outside the central nervous system.[1,28] In contrast to all other azoles, FLU is metabolized minimally and excreted essentially unchanged by the kidney; therefore caution with renal insufficiency should be exercised.[7,25,26,28] Compounded oral suspension from commercially available tablets crushed with water and commercial suspending agents have been shown stable for 14 days when light protected and stored at 41°F (5°C).[29] Although appropriate treatment for infections with *Candida*, dimorphic fungi, or dermatophytes, it is not effective against *Aspergillus* species.[1,3,25,26,28] In African grey parrots, two doses (10 mg/kg and 20 mg/kg PO) were reported from both single doses and multiple every other day dosing from a variety of commercial and compounded formulation without apparent changes in FLU disposition.[29] It is reportedly toxic to budgerigars *(Melopsittacus undulatus)* at doses administered safely to other psittacines.[26,30]

Voriconazole

A synthetic derivative of FLU, VRC is a second-generation, broad-spectrum antifungal available in both IV and oral formulations.[1,17,21,26,28,31] It has additional demethylation activity that broadens its spectrum of activity.[26] High VRC bioavailability following oral dosing occurs, and it distributes widely, including to the central nervous system.[17,26] Unlike humans, who present a 30%–60% reduction in bioavailability with food intake, plasma concentrations achieved in birds do not seem uniformly affected by feeding or fasting, but effects are observed on time to reach those concentrations and duration that therapeutic concentrations are maintained.[1,6,14,21,22] *Falco* spp. fed at the time of VRC dosing presented 20% reduction in maximum plasma concentrations as compared with fasted individuals.[1,22] African penguins *(Spheniscus demersus)* presented variability with food intake with a graduated elongation of elimination half-life.[16] Chickens had exceptionally poor VRC bioavailability (<20%) and did not achieve clinically relevant plasma concentrations.[1,33] Marked interspecific differences in the maximum plasma concentrations achieved from the same dose administered are noteworthy and unpredictable; for example, following 10 mg/kg PO, African grey parrots achieved 1.6 µg/mL and pigeons 4.4 µg/mL at 90 minutes.[6] Furthermore, despite generally shorter elimination half-lives in avian species, this actual parameter is quite variable between taxa.

Even with standard avian dose recommendation (12.5 mg/kg PO BID × 60–90 days), and as compared with all other azoles, VRC has the most nonlinear pharmacokinetics and metabolic saturation, although essentially no active metabolites are produced.[3,14,21,28,31] This characteristic markedly minimizes interspecies extrapolation of doses, or limits interpretation of extended regimens from single dose or short-term studies.[18,19,31] Autoinduction of its own metabolism is sufficient to require intraindividual adjustments in long-term treatment regimens.[1,17,19–21,26,28,31,34] When compounded from tablet formulation into water or commercial suspending agent, VRC has been confirmed as stable under refrigeration for 14 days.[18,26,35]

Primarily used for *Aspergillus* spp. treatment, VRC targeted to MIC of ≤0.38–≤1 µg/mL is very effective,[18,31] but it is time, rather than concentration, dependent.[21] It has shown little resistance to date and is increasingly used as front line treatment not only for aspergillosis, but also *Candida* sp. and dimorphic fungi, but not for zygomycetes.[3,28,31] Controlled clinical VRC treatment of *Falco* spp. (n = 20) confirmed with aspergillosis was associated with complete clinical resolution in 70% of the birds with limited clinical signs, but confirmed hyphal presence, using VRC orally (12.5 mg/kg BID × 44–100 days) and NE (20 mg/mL of saline SID × 60 minutes), and occasional intraoperative lavage.[4] However, NE VRC (10 mg/mL IV solution × 15 minutes) in pigeons resulted in no measurable plasma concentrations.[37]

Respiratory tissues have been evaluated during nonsurvival VRC treatment studies and often found lacking in the in vivo efficacy or direct measurement within target tissues. In racing pigeons experimentally inoculated with *Aspergillus* and then VRC initiated at the onset of clinical signs (10 mg/kg BID or 20 mg/kg PO SID × 14 days), both doses reduced clinical signs and pathology, although the lower dose eliminated the *Aspergillus* while the higher dose only reduced isolation.[15] Following intratracheal inoculation of *Aspergillus*, domestic quail were treated successfully by two doses (20 or 40 mg/kg PO SID 5–10 days). The higher dose had prolonged survival and fewer colony-forming units (CFUs) than control birds, but it was the lower dose that had significantly fewer pulmonary fungi.[35] However, in treated mallard *(Anas platyrhynchos)* (20 mg/kg PO SID × 21 days), lung, liver, and kidney concentrations were at or less than detection concentration and no increase occurred with treatment progression.[14]

Neurologic dysfunction, including seizures and visual disturbances, and death have been observed in six penguin species for individuals that presented plasma concentrations greater than 30 µg/mL that were achieved with published recommendations for this taxon.[1,31,33] Gross and histopathologic hepatic changes including oval cell proliferation, but not fibrosis, were evident in racing pigeons, including clinical pathologic impact of hepatic function noted at higher doses.[31,34] Longer-term treatment in falcons has

demonstrated food flicking, anorexia, weakness, polyuria, and potential effect on visual acuity sufficient to warrant flight restrictions during treatment.[1,4,31] Polyuria also was observed consistently in African grey[18] and Hispaniolan Amazon[19] *(Amazon ventralis)* parrots.

Allylamines

Terbinafine

Represented by TERB, this class of antifungals inhibits ergosterol synthesis by blocking squalene monooxygenase, causing intracellular accumulation of toxic squalene.[1,3,7,25,26,28,36] It is available as topical cream, liquid spray, or oral formulations.[36] Good oral bioavailability is reported that is unaffected by feeding, although a biphasic absorption has been noted due to particle size during tablet dissolution, and TERB's highly lipophilic and keratinophilic nature.[3,24] Its distribution at higher doses can become limited due to saturation of tissue, although clearance was not affected.[3] Its metabolism is not mediated by hepatic cytochrome P-450 metabolism, but it is metabolized rapidly in the liver followed by predominantly renal excretion, so it is subject to first pass effects.[1,7,25,26,28] In addition, it does present a biphasic elimination half-life in some avian species, where the initial phase is less than a day but terminally extends greater than 5 days.[3,36] Primarily used for dermatophytosis due to rapid accumulation within keratinocytes, other fungal organisms may be susceptible, and resistance is quite rare.[3,25,36] However, African grey parrots receiving oral TERB (15 and 30 mg/kg) did not achieve therapeutic drug concentrations against *Aspergillus* species.[1,36] In addition, documented TERB NE (1 mg/mL solution for 15 minutes) in Hispaniolan Amazon parrots using suspensions made from either crushed tablet or raw drug powder persisted above the *Aspergillus* sp. MIC only for 1 hour or 4 hours, respectively,[37] due to the known poor dissolution of TERB and its settling in suspension.[1,36]

Flucytosine[7,25,26]

In fungi that contain cytosine permease, this pharmaceutic is converted cytoplasmically to 5-fluorouracil and inhibits RNA synthesis. Hepatotoxicity or bone marrow suppression can occur in the patient as a result of this conversion within the gastrointestinal tract. Orally, it is effective against yeast only, although it is well-absorbed and distributed, including extensively to the CSF. However, it rapidly develops resistance and must be used only in combination with other antifungal agents.

Non-*Aspergillus* Fungi Treatment

Mucosal and Dermal Fungal Infections

The most common fungal infections of mucosal surfaces of the cranial gastrointestinal tract and nasal sinus result from *Candida* species. Treatment is most successful when the antifungal agent has direct contact with the fungus.[5] Although quite toxic parenterally, the polyene antifungal nystatin given orally is poorly absorbed, excreted entirely unchanged in feces, and therefore essentially nontoxic at doses of 200,000–300,000 IU/kg BID to TID × 7–10 days with recommendation of fasting before administration.[5,25,26] Azole recommendations include ITRA (5–10 mg/kg PO BID × 7–21 days); 2% miconazole gel applied directly; or considered most effective, FLU at 2–5 mg/kg PO SID × 7 days[5] or 5–10 mg/kg SID × 6 weeks.[26] As a flock treatment, cockatiels *(Nymphicus hollandicus)* were provided FLU as crushed commercial tablets at 100 mg/L of drinking water and shown by measured plasma concentrations to exceed the MIC of 90% of *Candida* species with comparable clinical results with individual oral dosing as 5 mg/kg SID or 10 mg/kg every other day (EOD).[30]

Microsporum spp. and *Trichophyton* spp. infections are seldom reported in birds. Typically, dermatophytosis is managed by removal of infected keratin debris and using standard mammalian topical treatments.[38] The earliest antifungal agent, griseofulvin (GF), acts by microtubule inhibition, thus preventing mitosis, and has action limited solely to dermatophytes. Following oral dosing, which is enhanced by a fatty meal, or reduced pharmaceutical particle size, GF concentrates in the stratum corneum and is effective.[7,25] Published avian antifungal doses for dermatophytosis include systemic azoles (ITRA 10 mg/kg SID × 20 days) or topical polyenes and azoles.[38] Topical azoles, including miconazole, clotrimazole, and eniconazole, are minimally absorbed systemically following topical absorption.[1,7,25] Clotrimazole as lavage or by commercial spray or ointment has been published as effective.[1,26] Eniconazole is produced in a premise treatment formulation that should not be used extralabel as a pharmaceutic, although compounded topical products are available outside the United States.[7,26]

Macrorhabdus Ornithogaster[39]

This anamorphic ascomycetal yeast grows exclusively at the proventricular-ventricular junction and produces maldigestion and weight loss. In reported treatments the measure of success was cessation of fecal shedding of organism. By gavage, AmpB (25 mg/kg PO BID × 14 days) has been used and appears safe and rapidly effective when compounded from commercially available powder, although some failure has been reported at much higher doses (100–150 mg/kg PO BID × 30 days).[26] Nystatin also has been used as a flock treatment at 3,500,000 IU/L of drinking water × 2 days and then for another 28 days at 2,000,000 IU/L. Although FLU at 100 mg/kg PO was successful experimentally in treatment of chickens, this dose was toxic in budgerigars and not effective at lower doses in this species.

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Medical Management of Walk-Through Aviaries

MEREDITH MARTIN CLANCY

Walk-through aviaries are an expanding part of the interactive experiences seen at zoological institutions around the world. Perhaps the first walk-through aviary was built by Smithsonian Institute to house birds at the St. Louis World's Fair in 1904. Thought to be the largest walk-through aviary at the time, it was purchased by the Saint Louis Zoo, where it currently remains.[1] Now, walk-through aviaries are common and represent a shift in focus toward guest-animal interaction where, through an emphasis on education, guest engagement and entertainment may drive conservation missions at the heart of zoos' higher purpose.[2–4] This chapter will cover the medical management of walk-through aviaries, especially addressing the complexities added in those aviaries with an interactive guest component. Walk-through aviary medical management combines preventive care of the flock and interventional medicine of the individual bird with public health best practices. A successful walk-through aviary starts with appropriate exhibit design for the level of interaction desired, safe animal sourcing and quarantine, and an evidence-based flock surveillance protocol with adequate medical intervention capabilities on an individual and population level. It must also include planning with the appropriate public health entities for guest education, zoonotic disease prevention, and disease outbreak management.

Species Choice

Species choice is an important step in designing a successful exhibit. Zoo standards and accreditation guidelines may direct aviary exhibit design and population choices. Although guests must always be supervised in animal contact settings, even with the best observation, certain bird orders, such as Falconiformes, Accipitriformes, and Strigiformes are inappropriate for aviaries where guests share space with the birds.[4–5] Species of birds managed in interactive aviaries are primarily small psittacines, generally of the subfamily Loriinae: budgerigars *(Melopsittacus undulatus)* and lories and lorikeets (tribe Loriini). Exhibits with these brightly colored Australian birds are found throughout the United States and around the world.[6–7] Budgerigars are smaller with a different feeding strategy than lorikeets (*Trichoglossus* spp.). Budgerigars are granivorous,[8] with interactive aviaries typically offering seed sticks that are prepared in-house.[6–7] With a Latin name that describes their brushy tongue specially designed to feed on nectar and pollen, lorikeets are fed cups of commercially prepared nectar by guests at many institutions. Other walk-through aviaries may house other small-to-medium-sized psittacines like cockatiels *(Nymphicus hollandicus)* in guest interactive displays similar to those described for budgies.[6] Wider variety of bird species, such as passerines like Australian finches (Estrildidae) and weavers (Ploceidae), columbiformes, and ground birds like galliformes and anseriformes, are found in walk-through aviaries without structured guest feeding. Although human interaction is possible in these aviaries, the focus is primarily on safe interactions between guests and birds and providing species-appropriate design for the exhibit.[8–11]

Exhibit Design

Veterinary involvement in the design aspects of a walk-through aviary may mitigate future medical and husbandry concerns. Examples include recommendations that reduce pathogen persistence and transmission and the development of husbandry protocols to refine and improve health and nutrition. Adequate provision for space and understanding the species' natural history may reduce competition over nesting sites, feeding stations, protection from inclement weather, and other resources and allow for desired social behavior, including with guests. During aviary operation, Association of Zoos and Aquariums (AZA) and European Association of Zoos and Aquaria standards require zoo staff supervision.[4–5] This helps to safeguard both guests and birds from preventable trauma and inappropriate interaction. Traumatic injuries from conspecific aggression, exhibit structures, or, sadly, inadvertent guests, should be cataloged so that keepers, exhibit operations, and other stakeholders may mitigate problems. Given the predilection for

• BOX 63.1 Sarcocystosis

Etiology: Primarily *Sarcocystis falcatula*, also *S. calchasi*
Susceptible: All, Old World Psittaciformes particularly susceptible
Clinical signs: Three distinct disease manifestations
- Acute/pulmonary: Acute death, upper respiratory disease, pneumonia, emaciation
- Neurologic: Abnormal mentation, lethargy
- Muscular: Weight loss, elevated muscle enzymes, weakness

Pathophysiology: Inflammatory response to protozoa, pneumonia and air sacculitis; meningitis, myositis
Diagnostics: Protein electrophoresis (hyperglobulinemia), serology, muscle biopsy
Treatment: Pyrimethamine (0.5–1.0 mg/kg PO SID)/ponazuril (20 mg/kg PO SID × 30 days), trimethoprim-sulfa (large dose ranges), antiinflammatories, supportive care
Prevention: Pest control to limit vector (*Didelphis* spp.)

• BOX 63.2 Clostridial Disease

Etiology: *Clostridium* spp. toxin, *C. perfringens* most common
Susceptible: Nectivorous birds (e.g., lorikeets)
Clinical Signs: Acute death, weight loss, diarrhea, bloody stool, regurgitation, cloacitis
Pathophysiology: Necrotic enteritis, cholangiohepatitis
Diagnostics: Definitive diagnosis challenging
- Complete blood count/biochemistry panel (CBC/chem): Leukocytosis, elevated liver enzymes
- Fecal Gram stain: Gram-positive sporulated bacteria may be seen
- Culture with toxin assays; polymerase chain reaction (PCR) panels

Treatment: Supportive care, metronidazole, amoxicillin + clavulanate, azithromycin
Prevention: Hygienic food practices

psittacines to explore, caging material must be monitored and cleaned to prevent zinc toxicosis from galvanized wire, and water structures should be emptied of guests' misplaced coin donations.[8,12] Water structures can also serve as a source for mosquitoes that transmit vector-borne diseases like West Nile virus (WNV). Appropriately designed water structures should not allow for mosquito breeding, and standing water should be eliminated as part of a thorough WNV prevention program and to mitigate other water-borne infections.[13–16]

In addition to mosquito control, pest control can reduce exposure to pathogens carried by other animals.[13] Sarcocystis has been reported in walk-through aviaries and in multiple psittacine species.[6,17–21] Sarcocystis can be present in psittacines as both an incidental finding and as a cause of death, but its antemortem diagnosis is a challenge.[22] Prevention rests on pest control to halt transmission.[13,17,21–22] Budgerigars are acutely susceptible to this disease, so antemortem diagnosis or treatment is not often possible. Treatment can be challenging due to severity of disease and variable presentation (Box 63.1).[17–19,21]

Exhibit design may also work to minimize pathogen exposure from the other birds.[15,23–27] Many of the important pathogens in avian species are spread through fecal-oral transmission, making feeding sites an important source of pathogen spread.[11,28–32] Feeder design may help to disrupt the fecal-oral transmission by reducing inadvertent introduction of fecal material from birds walking through or perching above food trays. Feces from nectivorous birds may be sticky with high sugar content, providing the ideal environment for microbial proliferation if not regularly cleaned.[8,33] Clostridial disease in nectivores is most commonly associated with inappropriate food or feeder hygiene (Box 63.2). The high sugar content of the nectar is ideal for bacterial growth, and so it is recommended to replace the nectar every 4 hours in hot weather and use appropriate disinfection of food containers and utensils daily.[8] Specialized feeder design helped to reduce fecal contamination at San Diego Zoo Safari Park (SDZSP) Lorikeet Landing during a salmonellosis outbreak (Fig. 63.1). By designing feed

• Figure 63.1 This nectar feeder design at the San Diego Zoo Safari Park's Lorikeet Landing reduces fecal-oral contamination through reduced ability of the bird to walk through the food or defecate into the nectar and is easily washed and disinfected. (Courtesy Michael Mace, San Diego Zoo Global.)

stations, housing, and other parts of the exhibit that can be easily dissembled, cleaned, and disinfected, the spread of pathogens may be drastically reduced.[8,24] Adequate cleaning to remove organic debris should be followed by disinfection specific to the pathogen of interest, acknowledging that there is not a single perfect disinfectant agent that reaches all avian pathogens (Table 63.1).

The ability to manage the flock for preventive medicine or during a disease outbreak should not be an afterthought in exhibit design but be planned with areas for off-exhibit housing that may also provide protection for breeding pairs and fledging chicks or isolation for medical or behavioral reasons.[6,7] As important as interaction with guests may be from an operational standpoint, animal health and welfare must always be at the forefront of the zoo veterinarian's mind. Those exhibits that provide areas where birds can remove themselves from guest access with or without visual barriers can meet both the operational needs of a guest interactive walk-through aviary while maximizing animal welfare.[4]

TABLE 63.1 Avian Pathogens and Their Appropriate Disinfectants

Disease (Etiologic Agent)	Oxidizing Agents	Quaternary Ammonium Compounds	Aldehydes	Phenols/ Phenolics	Alcohols
Psittacine beak and feather disease (BFDV, Circoviridae)	Chlorine, Sodium hypochlorite,[8] e.g., Virkon S[31]		Glutaraldehyde[8,44]	Effective[77]	
Exotic Newcastle disease (APMV-1, Paramyxoviridae)	Chloramine 1%, Sodium hypochlorite[8]		2% Formalin[8]	Effective,[77] e.g., 1% Lysol[8]	
Avian influenza (AIV, Orthomyxoviridae)	Effective[77]	Effective[8,10]	Effective[77]	Effective[77]	
Avian polyomavirus (APV, Polyomaviridae)	Sodium hypochlorite,[8,44] stabilized chlorine dioxine[44]		Effective[77]	Synthetic phenol,[44] phenolics[8,77]	70% ethanol[44]
Avian mycobacteriosis (*Mycobacterium avium* complex, Firmicutes)			Formaldehyde[50,77]	Effective,[9] e.g., Sodium-o-phenylphenol[50]	
G+ve bacteria (e.g., *Erysipelothrix, Clostridium, Staphylococcus* spp., Firmicutes)	Clorox[9]	Effective,[50] e.g., Roccal[9]	Effective[77]	Effective,[77] e.g., sodium-o-phenylphenol,[50] One-stroke[9]	Effective[77]
Bacterial Spores (e.g., *Clostridium* spp.)	Variable[77]		Effective[77]		Effective[77]
G-ve bacteria (e.g., Enterobacteriaceae, *Pasteurella*, Proteobacteria)	Sodium hypochlorite[8] Clorox[9]	Limited,[75] including Roccal[9]	Effective[77]	Effective,[77] e.g., One-stroke[9]	
Chlamydiosis/Psittacosis (*Chlamydia psittaci,* Chlamydiae)	3% hydrogen peroxide,[8] 1:32 dilution of sodium hypochlorite[49]	Effective,[8] e.g., benzalkonium chloride[28]		1% Lysol[8]	70% ethanol[8]
Cryptosporidiosis (*Cryptosporidium* spp., Apicomplexa)	Oxine*			Chloro-m-cresol (more than bleach)[39]	

*Used at the San Diego Zoo Safari Park.

Flock Nutrition and Husbandry

Feeder design is equally as important as what goes in the feeder because nutrition is an important factor in avian health.[12,34] Food items offered by guests may represent only one aspect of the birds' diet or may be the complete diet. The interactive portion allows for increased variability in amount and, if guest offerings comprise only one aspect of the diet, proportion of the balanced diet.[5] Birds in walk-through aviaries may become obese or develop mineral imbalances through selection of high-energy seeds or through consumption of high-concentration nectar from guests.[6,8,35–36] Provisions for complete diet and a viable means to monitor individual body condition and nutritional status must be made by the veterinarian, in conjunction with the nutritionist and animal care managers.

Another mechanism of maintaining bird health is through maintaining a balance in the normal gastrointestinal flora. Avian probiotics are gaining favor with some practitioners as a means of managing proliferation of a single organism over traditional medical management with broad-spectrum antibiotics.[34,37–39] Although product quality and labeling accuracy have been called into question, probiotic use has been increasing in avian medicine.[40] An outbreak of attaching and effacing *Escherichia coli* in an interactive budgerigar aviary was managed through use of probiotic and exhibit and husbandry modifications. The goal of this management strategy was not to eliminate the pathogen in the aviary, but to reduce shedding and morbidity in the flock (Box 63.3).[15]

Continuous reassessment and refinement of aviary protocols may uncover problems and help to navigate solutions. An increase in morbidity and mortality in an interactive

• BOX 63.3 *Escherichia coli* Sepsis/ Gastrointestinal Disease

Zoonotic

Etiology: *Escherichia coli*
Susceptible: All species
Clinical signs: Acute death, weight loss, diarrhea
Pathophysiology: Enteritis, hepatitis, sepsis
Diagnostics: Enteric culture gold standard
Treatment: Reduce shedding via antibiotics
Prevention: Probiotics, reduce fecal-oral transmission

budgerigar aviary was initially ascribed to trauma. Thorough review of nutrition and husbandry and evaluation of individuals antemortem and postmortem identified the true cause as feed-related hypervitaminosis D. Manufacturer error in vitamin D3 supplementation resulted in soft tissue mineralization causing nonspecific clinical signs. Persistence was required to identify the true cause of mortalities in the flock. This underscores the importance of close scrutiny of exhibits and husbandry practices, with added value to written protocols that may be routinely evaluated.[41]

Walk-through aviaries with guest interaction have a relatively high density of animals in the aviary space, allowing for more reliable and repeatable guest experiences.[6] This flock density increases the ability to transmit pathogens and the rapidity with which pathogens may spread. Understanding the flock's population health and following ideal avicultural principles through appropriate quarantine and animal sourcing are paramount to reducing risk and establishing a successful aviary.[9–10,42]

Animal Sourcing and Quarantine

Zoos often source flocks for walk-through aviaries from private breeders or commercial entities.[7,41,43] If a private or commercial source is to be used to stock a zoo's aviary, one that adheres to the Model Aviary Program (MAP) would be ideal to establish a baseline for medical and husbandry practices.[7,10,42] The MAP was developed to educate aviary owners and veterinarians about reducing risk of chlamydiosis spread, but it may be used to mitigate risks from any infectious disease. It calls for maintenance of accurate records of aviary transactions and avoidance of mixing birds from multiple sources, along with isolation and testing of any bird with abnormal clinical presentation.[42] If medical history of the institution or breeder or preshipment testing is inadequate or unavailable, the quarantine period should be extended beyond the incubation period of avian pathogens of concern or allow for sufficient screening of a representative segment of the flock.[29,42–43] Because some of the species are too small to allow for serology or even routine complete blood count and biochemistry panels, quarantine screening should be risk-based and target diseases specific to the species or institution's collection.[9,43–45] Given the guest interaction component of the aviaries in focus here, zoonotic disease screening should be a priority. AZA accreditation guidelines specifically highlight chlamydiosis in birds of the orders Psittaciformes, Galliformes, and Columbiformes and salmonellosis in all avian species as the predominant concerns for guest contact zoonosis risk.[4]

Screening for Zoonotic Agents

Chlamydiosis shedding via nasal secretions and feces is exacerbated by stress, including shipping and crowding, making quarantine a high-risk period.[46] Although screening tests remain imperfect, the standard serologic test for chlamydial antibodies is reported to be the modified direct complement fixation (CF), with a fourfold increase in paired titer samples considered diagnostic and clinical signs, such as upper respiratory disease, with a single high titer adequate for presumptive diagnosis.[28,46–48] Budgerigars and cockatiels are among the most commonly reported avian species testing positive for chlamydiosis.[28] In lieu of flock screening, some institutions will routinely treat high-risk or public-contact birds.[28,41,47,49–51] Traditionally, treatment has consisted of a tetracycline for 45 days, a duration that exceeds two complete avian macrophage replication cycles, allowing for chlamydial organisms to be released from the macrophage during division where—at adequate plasma concentrations—inhibition of *C. psittaci* occurs.[28,47] Although the risk of zoonotic spread of chlamydiosis from a walk-through aviary is considered low, institutions should invite input from appropriate public health officials prior to instituting a screening or treatment protocol.[47] However, any bird with clinical signs should be isolated from the flock and guests and screened to reduce risks both medical and legal (Box 63.4).

Zoonoses such as salmonellosis are generally screened for via fecal testing.[29–30,52] Given the risk presented to the public and keepers, screening tests with high sensitivity are preferential. Group or pooled samples decrease cost and may increase likelihood of identifying the disease at the population level to counteract the reduced sensitivity introduced by intermittent individual shedding.[9,53] At SDZSP, *Salmonella* fecal PCR is used to screen for salmonellosis in lorikeets both as a routine and medical diagnostic test. Sensitivity is increased by collecting daily samples for 3 days. Although the molecular testing may result in a false positive due to pass-through of a nonviable organism, the benefit of increased sensitivity and quicker turnaround time as compared with fecal culture helps to guide more rapid intervention to reduce risk to other birds and guests (Box 63.5).[54]

Additional Disease Surveillance and Medical Intervention

Surveillance of the flock should be evidence-based, guided by species- and institution-specific health concerns.[9–10] Because whole flock testing may prove logistically challenging, often

• BOX 63.4 Chlamydiosis/Psittacosis

Zoonotic

Etiology: *Chlamydia psittaci*
Susceptible: All, especially Psittaciformes, Columbiformes, Galliformes
Clinical signs: Upper respiratory disease, conjunctivitis, weight loss; signs may be variable
Pathophysiology: Epithelial cell damage affecting respiratory epithelium first, multiple organ systems affected
Diagnostics: Definitive antemortem diagnosis challenging; culture is gold standard
- Confirmed: Bacterial isolation or identification in macrophage, immunofluorescent antibody (IFA) positive tissue biopsy, fourfold titer increase 2 weeks apart
- Probable: Single high titer in bird with clinical signs, antigen identified in feces via PCR
- Suspect: Compatible illness without lab confirmation, single high titer without clinical signs
- Serology: Complement fixation, elementary body agglutination
- CBC/chem: Leukocytosis with heterophilia and monocytosis, increased liver enzymes

Treatment: Tetracycline 45-day treatment (e.g., doxycycline in seed[41,43] or water once daily or parenteral (intramuscularly or subcutaneously) q7d × seven doses)
Prevention: Isolation of suspected cases/adequate quarantine, prophylactic treatment

• BOX 63.5 Salmonellosis

Zoonotic

Etiology: *Salmonella enterica* serovars, *S. enterica* ser. Typhimurium reported commonly in lorikeets
Susceptible: All, high morbidity in Psittaciformes, Passeriformes, and Galliformes
Clinical signs: Acute death, fluffed, lethargic, gastroenteritis, hepatitis, sepsis
Pathophysiology: Enteritis, hepatitis, septicemia
Diagnostics: Culture gold standard
- Confirmed: Positive culture
- Probable: Positive fecal PCR, monocytosis with or without leukocytosis, compatible clinical signs
- Serology: Titers may gauge immune response and exposure

Treatment: Enrofloxacin (20 mg/kg by mouth once daily (PO SID)) 21-day treatment,[52,54] trimethoprim-sulfa also used;[78] fluid support
Prevention: Reduce fecal-oral transmission, vaccination

a subset of birds is screened at regular intervals. At SDZSP, lorikeet routine preventive medicine includes WNV vaccination (West Nile Virus Innovator, Zoetis, Parsippany, New Jersey) annually, and more recently, vaccination with an inactivated *Salmonella* sp. bacterin (Infectious Disease Laboratory, University of Georgia College of Veterinary Medicine, Athens, Georgia). Zoonotic disease surveillance occurs quarterly, including physical exam with body condition scoring, PCR testing in feces, and *Salmonella* serology performed on 25% of the bird population, with individuals

• BOX 63.6 Trichomoniasis

Etiology: *Trichomonas gallinae* most common
Susceptible: All, "frounce" in birds of prey, "canker" in columbiformes
Clinical signs: Anorexia, weight loss, dyspnea, dysphagia
Pathophysiology: Caseous lesions in oropharynx, blunted choanal papillae, ptyalism, and regurgitation in budgies
Diagnostics: Crop wash cytology, PCR
Treatment: Benzimidazoles, e.g., metronidazole (500 mg/L × 21 days[79] in water or oral 15–30 mg/kg PO twice daily × 5–7 days[69,79]), reduce but may not eliminate shedding
Prevention: Difficult to stop bird-to-bird transmission but may reduce food contamination

ineligible to be screened two sequential quarters. Similar to other serology, distinguishing *Salmonella* titers indicative of vaccine-induced protective humoral immunity from response to active infection can be challenging; this shows the importance of continued monitoring and multimodal testing.[54]

For a flock with known disease risks, diagnostic and treatment algorithms may guide intervention. Empirical treatment is that which is based on observation or experience and may be the best available course prior to return of diagnostic test results. Multiple sources detail excellent information on diagnostic testing algorithms for surveillance screening of pathogens not detailed in this chapter.[9–10,14,25,28,31,38,46,48,55]

The ultimate disease surveillance for any flock ends with necropsy. Reports of other pathogens that affect the health of the species found in walk-through aviaries abound, and often the first diagnosis of the disease in the flock comes from necropsy.[17–20,26,41,49,51–52,54–65] Necropsies provide more information than many antemortem tests and may give the veterinarian a significant lead on infectious and noninfectious diseases present in their managed population.[66–67]

The knowledge of common diseases of the species and the individual aviary's history may maximize empirical treatment success. Supportive care and baseline diagnostics are a good place to start, but medical intervention should be tailored to the species and aviary. Shortly after the salmonellosis outbreak in 2014 at SDZSP's Lorikeet Landing, a diagnostic and treatment algorithm was developed to streamline individual medical intervention (Kehoe, unpublished data).

Other medical concerns seen in birds in walk-through aviaries include flagellate-related upper gastrointestinal disease (Box 63.6)[68–69] and reproductive and neonatal issues.[6,9] Flagellate-related upper gastrointestinal disease may be transmitted from bird to bird in an aviary, especially with communal feeding and breeding.[68–69] Budgerigar egg-binding has been commonly reported, often due to hypocalcemia from constant laying.[6] Mites can cause disease in nestlings due to anemia.[29] The tropical fowl mite *(Ornithonyssus bursa)* has been found on lorikeets and other birds at SDZSP associated with morbidity and mortality in chicks (O'Connor, personal communication). Treatment targets

the environment because these are not obligate ectoparasites and can include nonpharmaceutical alternatives, such as diatomaceous earth, predatory mites, and botanical deterrents (Lotz and Zuba, personal communication).

Public Health

This chapter provides insight into some of the pathogens and noninfectious diseases that may affect birds in walk-through aviaries, but the list is not exhaustive. Each aviary will need an individualized approach developed through collaboration between the veterinarian and the husbandry team. The attending veterinarian should also coordinate with state and local public health officials regarding the aviary's preventive medicine practices and flock surveillance.

A systematic process for managing zoonotic disease concerns should be designed and documented for each institution with contact animals.[50] The National Association of State Public Health Veterinarians (NASPHV) has developed signage and education materials that may be used for many animal contact exhibits.[47] Well-established Centers for Disease Control and Prevention (CDC) and NASPHV recommendations are defensible standards of care when dealing with the legal implications of zoonoses in contact animal exhibits.[70] Apart from federally reportable diseases, laws differ from one jurisdiction to the other on what constitutes a reportable disease or animal injury/bite. Most zoos have an onsite human health official or a contracted health service, and coordination with these individuals, as well as local public health officials, may keep guidelines up to date.

Hand-washing is an essential first line of defense for guests in an interactive aviary.[29,47] Even with adequate signage and staff monitoring, adherence to preventive practices by guests may be variable.[71–72] Although from a public health official perspective these guidelines are established to protect the guests from the bird, the attending veterinarian must consider protecting the birds from disease introduction from guests. Avian influenza and exotic Newcastle disease (END) are viral agents that may be introduced to a flock inadvertently (Box 63.7).[73] Significantly heightened biosecurity was implemented at SDZSP during a local END outbreak.[74] Through the Zoo and Aquarium All Hazards Preparedness, Response, and Recovery (ZAHP) Fusion Center, avian influenza zoo preparedness exercises are available, along with contingency planning for more than just disease outbreaks.[75–76]

The operational value to the institution of a walk-through aviary is an important consideration when managing a suspected or confirmed zoonotic disease. Established and documented triggers may make the difficult decision to close the aviary to guests less clouded by financial or other implications. The long-term success of the aviary depends on effective management of the problems as discussed in this chapter through public health vigilance, flock surveillance and medical management, and appropriate aviary husbandry. The veterinarian's role in maintaining a healthy flock and fostering a thriving guest experience cannot be underestimated.

• BOX 63.7 Reportable Avian Viruses

Avian Influenza

Zoonotic

Etiology: Avian influenza virus (AIV), type A influenza virus, Orthomyxoviridae
Susceptible: All, Charadriiformes and Anseriformes considered reservoirs, Galliformes more susceptible
Clinical Signs: Low-path avian influenza (LPAI)—respiratory disease; high-path AI (HPAI)—acute death, systemic illness
Pathophysiology: Respiratory or gastrointestinal disease; HPAI systemic
Diagnostics: Reverse-transcriptase PCR (RT-PCR) matrix (M) protein

Exotic Newcastle Disease

Zoonotic

Etiology: Avian paramyxovirus 1 (APMV-1), virulent Newcastle disease virus, Paramyxoviridae
Susceptible: All, Suliformes, Psittaciformes, Columbiformes, Galliformes most susceptible
Clinical signs: Respiratory disease, nervous signs, diarrhea, depression, high mortality
Pathophysiology: Respiratory, gastrointestinal, neurologic, reproductive disease; lymphoid necrosis
Diagnostics: Viral isolation, hemagglutination inhibition, RT-PCR
Treatment: Supportive care, including antibiotics to treat secondary bacterial pathogens
Prevention: Reduce contact with reservoir species to stop transmission; vaccination may be available to control outbreak

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Systemic Isosporosis in Passerine Birds

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Systemic isosporosis, formerly known as atoxoplasmosis, is a significant disease of captive passerine birds worldwide that can cause mortality in all age classes but is of particular concern in hatchling and fledgling birds. Disease affects a variety of species, and mortality rates may be high.[1–7] Presence of infection and clinical disease have limited the success of captive breeding and reintroduction programs for threatened and endangered passerines, in particular the Bali mynah *(Leucospar rothschildi)*, which appears to be particularly susceptible to disease.[8,9] Systemic isosporosis has been diagnosed in a wide variety of free-ranging passerines worldwide.[5,10–18] With notable exceptions discussed in more detail later, systemic isospora infections in wild birds have been asymptomatic or incidental findings at the time of sampling.

Systemic isosporosis has been known by numerous names, including *Lankesterella*, *Toxoplasma,* and most recently *Atoxoplasma*. Intestinal and extraintestinal forms were historically thought to be separate parasites, which has contributed to confusion and inconsistent diagnoses. Morphologic studies have suspected that fecal oocysts and extraintestinal merozoites within the same host are due to the same parasite.[19–22] Molecular characterization of protozoa from different tissues (e.g., liver, spleen, and intestine) within individual birds has identified identical protozoal gene sequences within visceral organs and the intestines, providing additional evidence that the same parasite may inhabit intestinal and extraintestinal niches.[16,23]

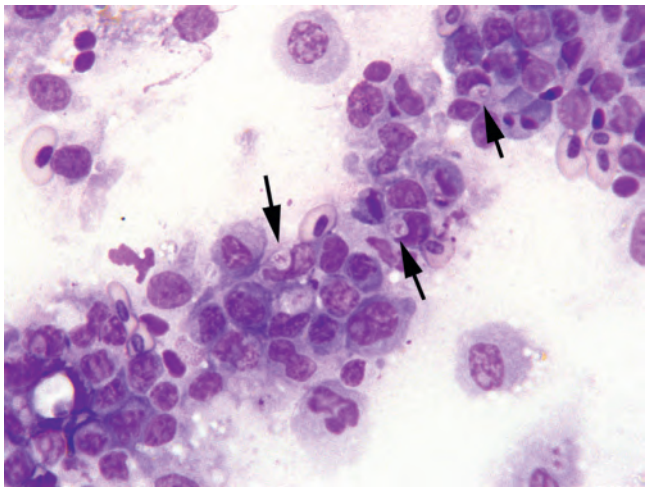
Differences in morphologic and phylogenetic relatedness to other coccidia have further complicated classification. Systemic *Isospora* of passerine birds are genetically similar to *Eimeria* sp. and have a Stieda body similar to other *Eimeria,* unlike mammalian *Isospora* which lack this structure within their sporocysts. However, they have tetrasporozoic diplosporocystic oocysts consistent with *Isospora*. Recent molecular research has confirmed that *Isospora* is a polyphyletic clade with two distinct monophyletic groups, those infecting mammals and those infecting birds.[16,24] Because *Isospora* is the oldest name, *Atoxoplasma* (Garnham 1950) is now considered a junior synonym of the genus *Isospora* (Schneider 1881).[24–26] There are multiple described and named species, plus even more that have not been thoroughly classified beyond *Isospora* sp. It has been hypothesized that individual *Isospora* infect only specific host species or closely related species, and some data suggest that most avian species have one or more unique lineages.[16] However, genetically divergent parasites can cause disease within the same species (mixed infections), and the same parasites can be present in genetically divergent hosts.[16,23,27–29] Additional research into the phylogenetics and host specificity of this group of parasites is needed to provide clarity on the epidemiology of infection among passerines. It is not certain if only some (and which) species of avian *Isospora* produce systemic infections.

Transmission of *Isospora* is via a fecal-oral route. Asexual replication (merogony) occurs within intestinal epithelial cells followed by gametogony, fertilization, and shedding of unsporulated oocysts within feces.[30–31] The oocysts sporulate within the environment and then become infectious. In the species of *Isospora* that cause systemic (visceral) disease, extraintestinal merogony is common within circulating mononuclear cells and gametogeny may occur, albeit rarely.[16,19,29] These circulating cells may serve as a source for reinfection of intestinal epithelial cells and shedding.[19,27] The life cycle is likely direct, because parasites with identical genotypes have been identified in all replicative phases (gametogony, merogony, and sporogony).[16] Vertical transmission has been suggested in blue-crowned laughing thrushes *(Dryonastes courtoisi)* following diagnosis of the parasite in chicks hatched from disinfected eggs that were artificially incubated.[7] Insect vectors may serve as paratenic hosts, although data are not consistent.[10,16,32,33]

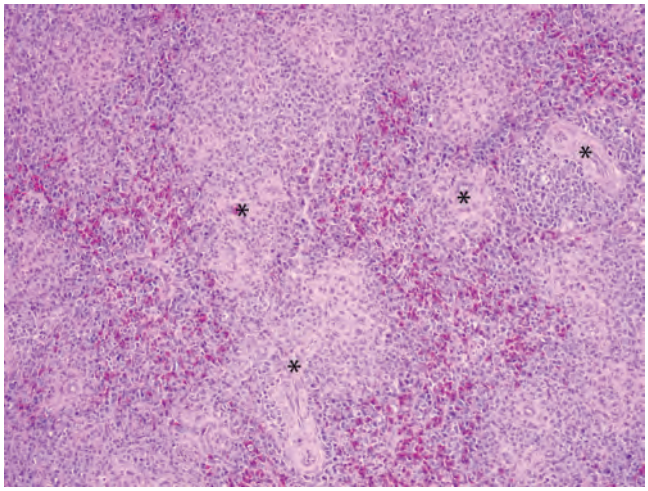
Clinical Signs and Lesions

Many birds are presumed infected with little to no clinical signs or outward evidence of disease.[3] Difficulties in antemortem diagnostics (see later) limit the ability to identify carriers. When present, clinical signs are generally nonspecific and may include ruffled feathers, dyspnea, tachypnea, anorexia, hyporexia, weight loss, diarrhea, dehydration, and death. Juvenile and fledgling birds are most susceptible, but disease may also occur in adults.

The characteristic gross lesion of systemic isosporosis is splenomegaly; however, this finding is variable. In some cases, small tan foci of necrosis and/or inflammation may



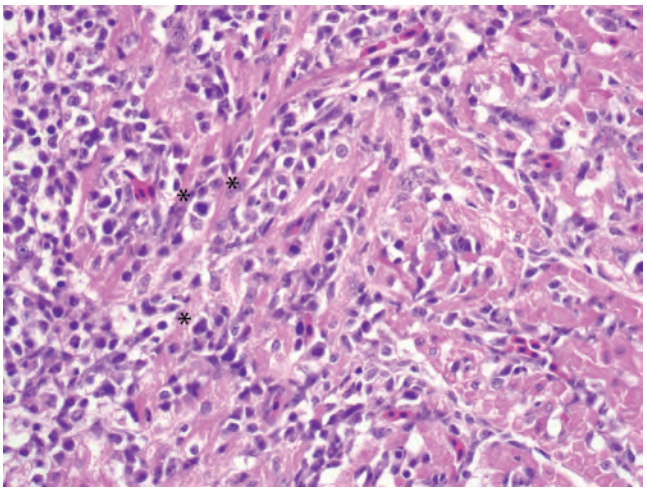
• **Figure 64.1** Diff-Quik–stained impression smear of spleen from an amakihi *(Chlorodrepanis virens)*. Note *Isospora* sp. within the cytoplasm of mononuclear cells indenting the nucleus *(arrows)*.



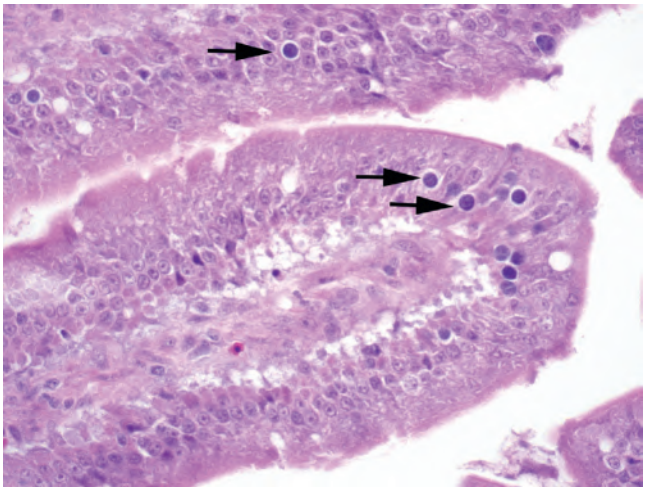
• **Figure 64.2** Hematoxylin-eosin–stained histologic section of spleen from a Taveta golden weaver *(Ploceus castaneiceps)* with lymphohistiocytic splenitis associated with systemic isosporosis. Increased numbers of lymphocytes and histiocytes are present surrounding splenic ellipsoids or Schweigger-Seidel sheaths (examples highlighted with *asterisks*).

be noted in the spleen and liver. Other lesions may include thickened and/or dilated intestines, an enlarged darkened liver ("black spot" because it can be viewed through the body wall), and pale streaks within the heart and skeletal muscle. On splenic, lung, or liver impression smears stained with Wright-Giemsa (or other Romanowsky-type stains such as Diff-Quik), lymphocytes and histiocytes may contain one to multiple 2–3 µm oval-shaped intracytoplasmic pale basophilic to eosinophilic merozoites surrounded by a colorless capsule (parasitophorous vacuole) that indents the nucleus (Fig. 64.1).

Histologically, necrosis and/or lymphoid, histiocytic, or lymphohistiocytic inflammation is common (Figs. 64.2 and 64.3).[1,2,4–6,8,10,29,34–37] Inflammation is often in vascular and/or perivascular spaces. The most commonly affected sites include spleen, liver, heart, lung, and intestine; however, as



• **Figure 64.3** Hematoxylin-eosin–stained histologic section of heart from a red-vented bulbul *(Pycnonotus cafer)* with myositis associated with systemic isosporosis. Myocytes are fragmented (examples highlighted with *asterisks*) and surrounded by inflammatory cells.



• **Figure 64.4** Hematoxylin-eosin–stained histologic section of intestine from a Taveta golden weaver *(Ploceus castaneiceps)*. Multiple *Isospora* sp. microgamonts *(arrows)* are present within the intestinal epithelium.

with other systemic diseases, any organ may be potentially infected. Infected mononuclear cells can be adherent to blood vessel endothelium within the lung.[4] Cytoplasmic merozoites may be difficult to visualize within histologic sections, and inflammation alone is nonspecific; therefore impression smears are critical for diagnosis. In one case series a captive population of red-vented bulbuls *(Pycnonotus cafer)* had significant and fatal skeletal and myocardial lesions (Fig. 64.4) in the absence of characteristic splenic and hepatic lesions.[23] In American goldfinches *(Spinus tristus)* and house sparrows *(Passer domesticus)*, T-cell lymphocytic infiltrates with cytoplasmic protozoal meronts effacing the normal small intestinal mucosa and infiltrating through the intestinal wall into the coelomic cavity suggestive of a T-cell lymphoma have been reported.[6] However, further research is needed to demonstrate the oncogenic potential of the parasite. Other immunohistochemical studies have identified the majority of parasitized cells as B lymphocytes.[35]

TABLE 64.1 Reported Treatment Protocols for Reducing the Shedding of Systemic Isosporosis in Passerines

Drug	Amount	Frequency	Species	Comments	Reference
Sulfadimethoxine	One drop of 100 mg/mL suspension/ 30 mL water*	Once daily	Canary *(Serinum canarius)*		35
Sulfachlorpyrazine (ESB3)	1 g of 30% powder/L of drinking water*	Treat 5 days, 3-day break, repeat 4 times	Canary, Bali mynah *(Leucospar rothschildi)*	Treatment recommended 3 times per year	Norton 2007 (unpublished)
Sulfachlorpyridazine (Vetisulid)	300 mg/L of drinking water*	Treat 5 days, 3-day break, repeat 4 times	Golden-breasted starling *(Cosmopsarus regius)*	Treatment recommended 3 times per year	Norton 2007 (unpublished)
Toltrazuril (Baycox)	12.5 mg/kg PO	Treat 2 days, 5-day break. Treatment cycle was repeated for 3 months.	Blue-crowned laughing thrush *(Dryonastes courtoisi)*	Shedding reduced and clinical signs resolved within 7 days.	40
Toltrazuril (Baycox)	25 mg/kg PO	Treat 2 days, 5-day break. Treatment cycle was repeated for 10 weeks.	Blue-crowned laughing thrush		7
Ponazuril	25 mg/kg PO	Treat 5 days, 9-day break. Treatment cycle repeated 3 times.	Various species of tanagers, starlings, bulbul	Drug top dressed over diet	Adkesson 2017 (unpublished)
Ponazuril	25–50 mg/kg PO; 1–2 mg per chick PO	Daily for 7–14 days	Various species of tanagers, starlings, bulbul	Clinically ill fledgling chicks	Adkesson 2017 (unpublished)

*Offer as sole source of drinking water.

Some infected birds have no associated gross or histologic lesions. In studies of systemic *Isospora* in wild birds, clinical manifestations were lacking in most cases. Unrecognized stressors in artificial environments may precipitate disease in previously infected wild birds. In one study of wild-caught passerines, approximately half of the captured birds died within 15 days of confinement, many of which had systemic isosporosis, although they were also concurrently infected with *Leukocytozoon* (Gill et al., 2008).[5] Concurrent *Leukocytozoon* infection may also have impacted disease development in free-ranging rose-breasted grosbeaks *(Pheucticus ludovicianus)* with systemic visceral infection.[10]

Diagnosis

The "gold standard" for diagnosis remains impression smears of visceral organs, specifically spleen, liver, and lung. Within these impressions, two separate stages may be identified—larger single merozoites within a single parasitophorous vacuole ("waiting" merozoite) and multiple smaller merozoites sharing a common parasitophorous vacuole ("proliferative" merozoites).[5,27,36–38] It is the merozoite within mononuclear cells that causes the characteristic appearance of an "indentation" in the nucleus (see Fig. 64.1).

However, impression smears are not useful as a screening tool in live birds. Cytologic evaluation of whole blood smears and buffy coat smears may identify merozoites within peripheral mononuclear leukocytes; however, organisms can be rare even in fulminant, fatal infections.[5] Consistent complete blood count (CBC) and chemistry findings are lacking, and alterations may represent secondary conditions such as dehydration. Organisms may be identified in feces by routine floatation followed by sporulation, if they are shed within the sample. Because shedding is intermittent, false negatives may occur. Challenges of antemortem diagnosis in juvenile passerines are compounded by limited access to diagnostic samples due to small size of the animal and disruption of the next. Polymerase chain reaction (PCR)-based assays are more likely to be of utility due to increased sensitivity, although, because fecal shedding and systemic phases of disease can be intermittent, studies of shedding patterns are needed to determine optimal sampling intervals.[3,16,39]

Treatment and Management

Antiprotozoal drugs are used for both treatment of clinical disease and suppression of shedding (Table 64.1). Extensive

study on therapeutic dosages and treatment efficacy is lacking. Treatment is generally aimed at reducing shedding during breeding season through chick fledging. Sulfonamide drugs (e.g., sulfachloropyrazine, sulfachlorpyridazine, sulfadimethoxine) in the water are reported to reduce disease by limiting shedding, but controlled studies are lacking and purported efficacy is based primarily on decreases in chick mortality. Toltrazuril has emerged as an effective treatment for reducing shedding of oocysts, although the drug is not commercially available in the United States.[7,40] Ponazuril has been used to reduce shedding and successfully treat clinical cases, although controlled studies of this drug are also lacking. Treatment can effectively eliminate shedding and reduce intestinal and systemic stages, but elimination of infection is unlikely.

Husbandry and sanitation are extremely important components of any plan for controlling systemic isosporosis infections in an aviary setting, particularly with breeding birds. Substrates should be cleaned on a regular basis, and fecal contamination of food and water sources should be minimized through the use of elevated, covered feed stations. Minimizing stress and disturbance of breeding birds may aid in minimizing shedding and infection. Prophylactic treatment with antiprotozoal medications during times of stress has been proposed, but the efficacy of such intervention is not known. Regular diagnostic testing of feces may also aid in identification of individual birds that are chronically shedding at high levels so they can be treated or removed from breeding populations.

If high levels of chick mortality are present from systemic isosporosis, breeding adults should be administered antiprotozoal medications to reduce shedding. Treatment should commence prior to egg laying and continue in pulsed form while the parents continue to feed fledglings. The physiologic stress of fledging likely predisposes chicks to infection, and earlier cessation of treatment may lead to disease because fecal shedding of oocysts has been shown to return to pretreatment levels within as little as 10 days.[40] However, untested negative correlations with clutch size and laying frequency have been reported with toltrazuril at higher dosages.[7] The toxicity, side effects, appropriate dosage, and efficacy of various treatments are areas that warrant significant investigation. Advances in molecular testing are allowing investigation of shedding patterns and offer promise for better monitoring and assessment of treatment efficacy. Disinfection of eggs and artificial incubation may be indicated in certain situations, although it is unknown if a lack of exposure to the organism early in life may actually increase susceptibility to clinical disease later in life. As noted previously, vertical transmission may also be possible.

Conclusion

Conservation and breeding programs for passerines require improved medical management of systemic isosporosis. Despite considerable prior confusion over phylogeny (e.g., *Atoxoplasma, Lankesterella*), these parasites are now classified as *Isospora* sp. and have both intestinal and extraintestinal phases. Lesions vary but classically are associated with lymphohistiocytic inflammation. Differing disease manifestations may be due to differences in host-parasite interactions among species of *Isospora,* as well as species of passerine. Current gold-standard diagnostics (postmortem impression smears) are unrewarding for clinical management, but the advent of molecular tools will allow more in-depth studies into the epidemiology and disease pathogenesis so that management and treatment protocols can be further improved.

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65

Bornaviruses in Birds

DALE SMITH

The discovery of the first avian bornavirus in 2008 provided the key to solving a mystery that had been plaguing avian veterinarians since the late 1970s, when macaw wasting disease, as it was then called, was first identified in macaws imported into the United States from Bolivia.[1–3] This disease, now most commonly known as proventricular dilation (dilatation) disease (PDD), became the most feared and least understood infection of captive psittacine birds. Although there has been debate in the veterinary community regarding the relationship between bornaviral infection and PDD, researchers working with this group of viruses are convinced the relationship is causal, although complex. For clarity, the term PDD is used to describe cases showing the classic disease syndrome, based on clinical and/or pathologic features, and bornaviral infection is confirmed by the presence of viral RNA, viral antigen, or viable virus.

Prior to the discovery of this first avian bornavirus, the only member of the family Bornaviridae was Borna disease virus (BDV), a cause of neurologic disease in mammals in central Europe, particularly horses and sheep.[4] Bornaviruses are members of the order Mononegavirales and are enveloped viruses characterized by the presence of nonsegmented, linear, single-stranded negative-sense RNA genomes. Bornavirus infections are often persistent and are noncytopathic in cell culture; hence the need for molecular diagnostic techniques in viral discovery.

Since 2008 there has been an explosion in the discovery of new members of the Bornaviridae, particularly those affecting avian species (Table 65.1).[5] Additional viral genotypes await formal classification. Several excellent reviews summarize knowledge current to their publication dates.[2,6,7] Active research is ongoing, and thus each year brings new insights into the epidemiology and pathogenesis of avian bornaviral infections.

PDD is recognized worldwide in a broad range of psittacine species; global spread is thought to have occurred through the trade in captive birds. Disease has been most frequently associated with infection by parrot bornavirus 2 (PaBV-2) and 4 (PaBV-4). The natural reservoir (i.e., the source of entry of virus into the captive population) is not known. The only report of bornaviral infection in free-ranging psittacine birds is from Brazil, where seropositivity and shedding of bornaviral RNA were identified in parrots, including nestlings, recently taken into rehabilitation centers.[8] Preliminary research suggests that there are differences in pathogenesis among the different parrot bornaviruses, but the relevance of this to managing clinical situations is not yet clear.[9]

Two species of bornavirus affect passerine birds. Canaries *(Serinus canaria)* infected with canary bornaviruses 1-3 *(Passeriform 1 bornavirus)* can show PDD-like clinical signs and pathologic lesions.[10,11] Experimental infection did not result in clinical disease or significant pathology; however, horizontal transmission to in-contact birds did occur.[10] Twelve of 30 German canary flocks were positive for virus, suggesting a high prevalence of disease in canaries in that country.[10] *Passeriform 2 bornavirus* (estreldid finch bornavirus 1, EsBV-1) was described in 2014 from three estreldid finches in a single flock in Germany as part of an extensive screening program.[12] Because there were a number of disease processes ongoing in the flock, the significance of EsBV-1 could not be determined.

An investigation into the cause of encephalitis of unknown origin in Canada geese *(Branta canadensis)* and trumpeter *(Cygnus buccinator)* and mute swans *(Cygnus olor)* in Canada led to the discovery of *Waterbird 1 bornavirus* (aquatic bird bornavirus 1, ABBV-1).[13] Histopathologic lesions were similar to those of PDD, as were gross necropsy findings and clinical history in the birds for which this information was available.[14] A prospective serologic study in these species by the same authors and extensive surveillance by researchers in the United States showed that antibodies and viral RNA were present at very high prevalence in seemingly unaffected birds, as well as in culled, apparently healthy gulls of several species.[15–17] Infection with ABBV-1 thus appears to be widespread in free-ranging waterfowl and gulls in North America, but associated with disease only in a subset of cases. ABBV-1 viral RNA was also identified in the brains of hunter-killed free-ranging waterfowl in Denmark.[18] ABBV-1 was identified in the embryos of Pekin ducks in eggs purchased commercially in the United States for laboratory use; however, there are no associated surveys of commercial duck flocks.[7]

TABLE 65.1 Members of the Family Bornaviridae, Genus *Bornavirus*, Associated With Disease in Avian Species

Species	Virus	Abbreviation
Passeriform 1 bornavirus	Canary bornavirus 1	CnBV-1
	Canary bornavirus 2	CnBV-2
	Canary bornavirus 3	CnBV-3
Passeriform 2 bornavirus	Estrildid finch bornavirus	EsBV-1
Psittaciform 1 bornavirus	Parrot bornavirus 1	PaBV-1
	Parrot bornavirus 2	PaBV-2
	Parrot bornavirus 3	PaBV-3
	Parrot bornavirus 4	PaBV-4
	Parrot bornavirus 7	PaBV-7
Psittaciform 2 bornavirus	Parrot bornavirus 5	PaBV-5
Waterbird 1 bornavirus	Aquatic bird bornavirus 1	ABBV-1
	Aquatic bird bornavirus 2	ABBV-2

From Amarasinghe GK, Bào Y, Basler CF, et al: Taxonomy of the order Mononegavirales: update 2017. *Arch Virol* 162:1–12, 2017.

A second waterfowl-associated bornavirus, aquatic bird bornavirus 2 (ABBV-2), was identified from the brains of hunter-killed mallards *(Anas platyrhynchos)* and a wood duck *(Aix sponsa)* from Oklahoma, United States.[19] Viral antigen was present in retina and in brain, where it was accompanied by mild perivascular cuffing and gliosis in the few specimens examined histologically. The significance and prevalence of ABBV-2 are unknown.

The bornaviruses affecting birds are quite consistently associated with given taxonomic groups, but there are some notable exceptions. ABBV-1 was identified by reverse transcription polymerase chain reaction (RT-PCR) from the brain of a bald eagle *(Haliaeetus leucocephalus)* with encephalitis, the presumption being that the eagle became infected by eating infected waterfowl.[2] ABBV-1 was similarly identified, using immunohistochemistry (IHC) and RT-PCR, in the brain of a captive emu *(Dromaius novaehollandiae)* with encephalitis and lymphoplasmacytic infiltrates in autonomic ganglia of the gastrointestinal tract.[20] The bird's outdoor enclosure was frequented by Canada geese in a population shown to have a high prevalence of ABBV-1 infection; fecal-oral transmission was proposed. PaBV-4 was identified in a Himalayan monal *(Lophophorus impejanus)* with clinical signs and pathologic lesions consistent with PDD that shared an aviary with parrots.[21] PDD had previously been identified in psittacines in the collection, and PaBV-4 was subsequently identified from a diseased white-bellied caique *(Pionites leucogaster)*. The avian bornaviruses should thus be considered in the differential of any case of nonsuppurative encephalitis in a bird, particularly when appropriate lesions are present in other components of the nervous system.

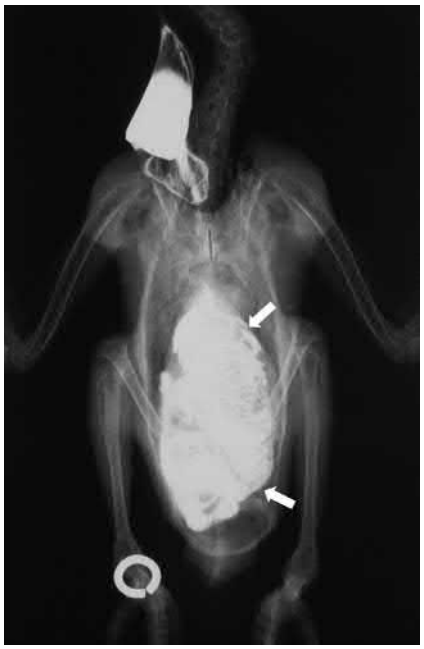
Although BDV is recognized as a pathogen of mammals, BDV RNA was found in the feces of mallards and jackdaws *(Corvus monedula)* in Sweden.[22] Borna disease virus, or a very similar virus, caused a paralytic syndrome with high mortality in young ostriches *(Struthio camelus)* in Israel.[23] Both reports predated the identification of the first avian bornavirus and, in the ostrich outbreak, the use of RT-PCR and sequencing as tools for virus identification.

Antemortem Clinical Diagnosis

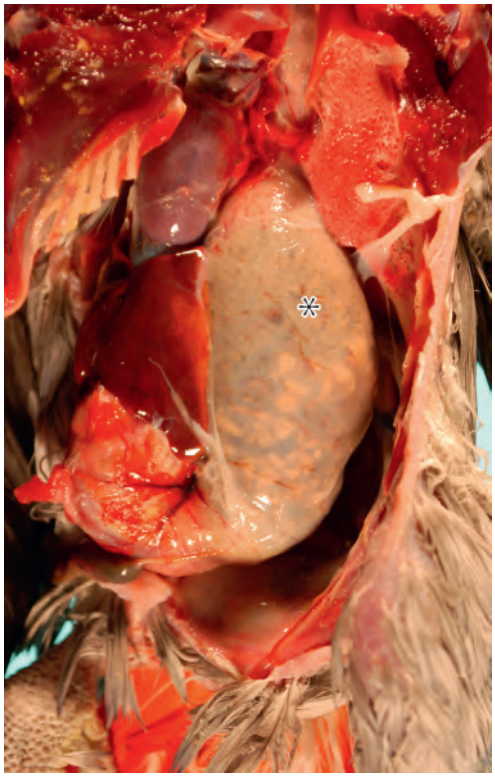
Diagnosing the clinical condition known as PDD and confirming the presence of bornaviral infection in the live bird are not always clear-cut. Infection and disease can occur in birds of all ages. The classic clinical signs in psittacine birds are weight loss, often with continuing appetite; the passage of poorly ground or undigested food items, such as seeds and nuts; and neurologic abnormalities. However, birds may have nonspecific signs of ill health, show some but not all of the previous signs, or be subclinically affected with no overt clinical signs. There are also reports of sudden death as a result of myocardial disease and of blindness. The development and course of clinical disease in an infected parrot is completely unpredictable, in terms of both time to onset and duration of clinical signs. Parrots have developed clinical PDD years after contact with any other bird.[6] There are no consistent patterns in complete blood count or plasma/serum biochemical profiles; a transient increase in gamma globulins was seen in African grey parrots *(Psittacus erithacus)* experimentally infected with PaBV-4.[24] Imaging findings that support a diagnosis of PDD include dilation of the crop, proventriculus, or ventriculus; and reduced or abnormal gastrointestinal motility as viewed on contrast fluoroscopic examination (Fig. 65.1).

The gold standard for diagnosis of PDD in a live bird has historically been the observation of a lymphoplasmacytic ganglioneuritis in a biopsy of the crop wall. This technique has poor sensitivity because biopsy specimens vary considerably in the number of nerves and ganglia present for evaluation, and lesions vary in their distribution.[25,26] The application of IHC and RT-PCR to either fresh, frozen, or formalin-fixed paraffin-embedded (FFPE) tissue to identify the present of bornaviral RNA or antigens increases the sensitivity and specificity of diagnosis based on crop biopsy.[27]

Many veterinary diagnostic laboratories offer either conventional (gel-based) or real-time (quantitative) RT-PCR that targets, most commonly, the gene for the matrix (M) protein or the nucleoprotein (N). Sequencing of products is required to determine the specific virus present. Bornaviral RNA is present in choanal-cloacal, crop, and cloacal swabs; feces; and urine of infected birds.[6,27,28] However, shedding is intermittent, particularly in cloacal swabs and feces, and thus false-negative results may be common.[6,27] It has been suggested that bornaviral RNA is present in the feather calami of infected birds, that plucked, mature contour feathers can be used as diagnostic material, and that this material is stable for at least 4 weeks at room temperature.[29]



• **Figure 65.1** Ventrodorsal barium contrast radiograph of a cockatoo (species unrecorded) with proventricular dilation disease. The massively dilated proventriculus and ventriculus are outlined by luminal barium and indistinguishable from each other. *Arrows* mark the cranial and caudal borders. Barium is also visible in the crop and intestines.



• **Figure 65.2** Dilated, impacted proventriculus *(asterisk)* in a Congo African grey parrot *(Psittacus erithacus)* with proventricular dilation disease. Lymphoplasmacytic infiltrates were present in the ganglia and nerves in multiple tissues. The brain was positive on reverse transcription polymerase chain reaction for parrot bornavirus M protein gene. (Photo credit ML Brash).

Attention must be paid to preventing environmental contamination of the outer surface of the feather, which might reflect the presence of virus in the aviary, but not necessarily in the patient in hand.[30]

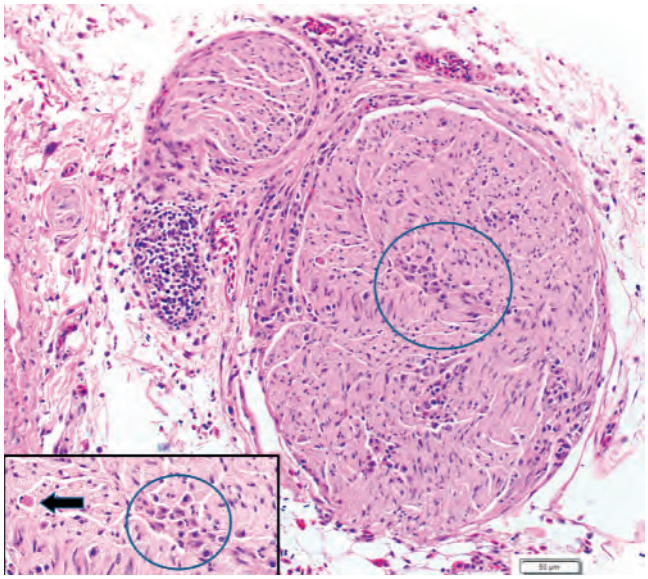
Serology is used in research settings and is available through some diagnostic laboratories.[6,30] Antibodies against various avian bornaviruses have been identified in blood from a variety of psittacine birds, canaries, and several species of wild waterfowl; and in egg yolk from sun conures *(Aratinga solstitialis)*.[6,15,31] Indirect fluorescent antibody testing and Western blot techniques are used primarily in research laboratories, whereas enzyme-linked immunosorbent assays are preferred for practical reasons in diagnostic laboratories.[6] Serologic evaluation has limitations when used in the diagnostic setting; although there is cross reactivity among bornavirus species and genotypes, antigenic diversity does exist and will affect test sensitivity.[32] In addition, commercial secondary antibodies do not uniformly recognize immunoglobulins from different classes and species of birds, making it impossible to determine standardized "cut-off" values that are valid for a range of avian species against a range of bornaviruses.[6,15] Further investigation is necessary to develop reliable and commercially viable serologic tests with wide applicability; in the meantime, it is critical to interpret serologic results in conjunction with the laboratory that performs the testing.[6,30]

Necropsy Diagnosis

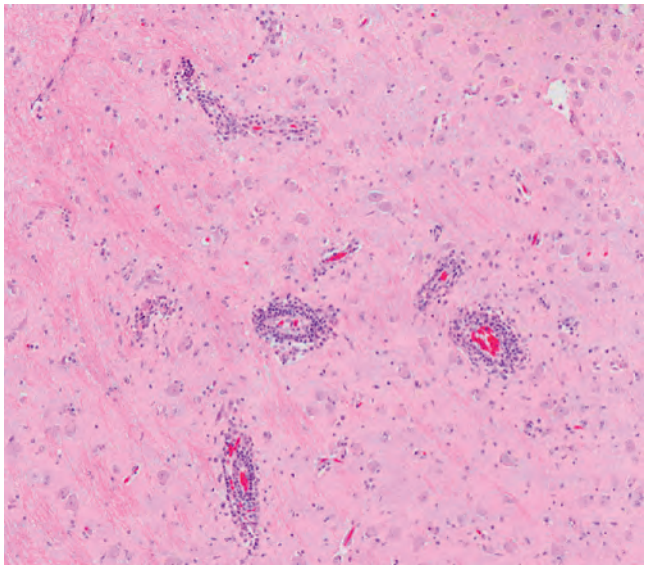
The classic gross necropsy lesions of PDD are enlargement, distension, and impaction of the proventriculus and to a lesser extent the ventriculus, with thinning of their walls (Fig. 65.2). In birds fed seeds or nuts, poorly ground ingesta will be present. Dilation of the crop and of segments of the intestines may also be seen. Birds are frequently in poor condition with marked muscle wasting and minimal to no fat stores.

The characteristic histologic lesion upon which the diagnosis of PDD rests is a lymphoplasmacytic infiltrate in the nerves and ganglia of the peripheral, central, and autonomic nervous systems.[25,27,33] Necropsy should include collection of a complete range of tissues, including brain, eye, and multiple peripheral nerves (i.e., vagus, brachial, sciatic). Infiltrates are often most easily identified in brain; nerves and ganglia in crop, proventriculus, and ventriculus; and adrenal gland and periadrenal ganglia (Fig. 65.3). Lesions may be extremely subtle or can be overwhelmingly blatant, for example with heavy infiltrates of inflammatory cells coursing through the muscularis of the ventriculus or forming large perivascular cuffs in the brain (Fig. 65.4). There can also be considerable variation in the presence and intensity of lesions among nerves and ganglia within a given tissue, as well as among tissues.

Confirmation of infection with an avian bornavirus can be achieved by RT-PCR on fresh or FFPE tissues, or by IHC.[6,14,30] Viral RNA and or/viral antigen are widespread in tissues, with the highest amounts found in



• **Figure 65.3** Microscopic appearance of the autonomic nerves supplying the ventriculus of a Derbyan parakeet *(Psittacula derbiana)* with proventricular dilation disease. The bird was positive on reverse transcription polymerase chain reaction for parrot bornavirus M protein gene. A lymphoplasmacytic infiltrate is present within and surrounding the nerve (main image and inset, *circles*), as well as a single Mott cell (inset, *arrow*).



• **Figure 65.4** Microscopic appearance of the brain from a Canada goose *(Branta canadensis)* that was positive on reverse transcription polymerase chain reaction for aquatic bird bornavirus 1. Note the prominent lymphoplasmacytic perivascular cuffing.

brain, proventriculus, kidney, and colon in one study on psittacine birds.[34] Bornaviruses can be cultivated in cell culture; however, because the Bornaviridae are noncytolytic, molecular or immunologic techniques are required to identify infection.[30,35] Viral culture thus tends to be used in research rather than in diagnostic settings.

Epidemiology and Pathogenesis

The epidemiology and pathogenesis of bornaviral-related infection and disease in birds are incompletely understood.[36] PDD has long been recognized as transmissible, with an assumption of horizontal transmission through direct contact or contamination of the environment. Outbreaks can occur in association with the presence of an infected bird.[37,38] However, naturally and experimentally infected psittacine birds and canaries shedding viral RNA can live with uninfected birds for long periods of time without spread of infection.[10,27] The factors that affect the infectivity of shed virus and the susceptibility of a new host are unknown.

Experimental infections of birds with various avian-origin bornaviruses were initially undertaken to fulfil Koch's postulates and prove a causal association between viral infection and PDD. More recent work has investigated the relative pathogenesis of various parrot and canary bornaviruses to better understand the epidemiology of infection. Infection and clinical disease can be consistently induced using various invasive routes of experimental inoculation (e.g., intramuscular, intravenous, subcutaneous, intracerebral) alone or in combination with a more natural route (e.g., oral, intranasal).[6] However, experimental infection of cockatiels with known virulent PaBV-4 via the oral and the intranasal routes only (i.e., not in combination with an invasive route) did not result in infection.[39] There is a continued need for studies that will explain the variations seen among different experimental investigations.

There is a potential for vertical transmission of avian bornaviral (ABV) infection. Bornaviral antigen or RNA has been identified in the gonads of adult psittacine birds and in eggs and/or embryos from various psittacine species, canaries, a Canada goose, and Pekin ducks.[6,7,27] Cockatiel *(Nymphicus hollandicus)* embryos have been experimentally infected, but none were taken through to hatching.[40] One 5-week-old, artificially incubated, and hand-fed Indian ring-necked parakeet *(Psittacula krameri)* from an infected aviary was positive for ABV genotype 4 (PaBV-4) on cloacal swab, but infection post hatching could not be discounted.[41] The hatching of viable, vertically infected chicks remains undocumented.

The pathogenesis of bornavirus infection is unknown but may mirror that of Borna disease virus in mammals, where experimental work suggests that lesions are a result of T cell–mediated damage to infected neurons, increased levels of glutamate as a result of astrocyte dysfunction, and release of proinflammatory cytokines by activated microglia.[4] Loss of nitrergic neurons in the esophagus and isthmus has been postulated as the proximate cause of proventricular dilation in birds.[36] The relationships between viral infection and viral localization, distribution of pathologic lesions, clinical disease, and seroconversion remain to be clarified and may in fact vary among species of birds and of viruses.

Treatment

There are two aspects of treatment of birds with bornavirus infection: reducing or clearing viral load and ameliorating clinical signs. Ribavirin inhibits replication of PaBV-4 in cell culture, but preliminary pharmacokinetics indicated it would be difficult to obtain and maintain therapeutic levels.[42,43] Favipirivir (T-705) strongly suppressed the growth of PaBV-4 in cell culture; this drug is licensed for oral use in humans, but there are no reports of its in vivo use in birds.[44]

Drugs targeting inflammation appear to be the most logical treatment to reduce clinical signs, which are assumed to result at least in part from an immune reaction to the presence of the virus. Treatment with celecoxib (10 mg/kg by mouth, once daily) has been reported to dramatically reduce clinical signs in some birds with PDD.[45] Unexpectedly, more severe disease was noted in experimentally infected cockatiels treated with meloxicam, and there is little evidence of improvement using cyclosporine.[2,46] General supportive care, including the provision of an easily digested diet, can often improve the general well-being and body condition of birds showing clinical signs of PDD and extend their life span; however, the potential for transmission of virus to uninfected birds must always be considered.

Control

Effective quarantine protocols and separation of infected and uninfected birds remain the sole methods of preventing the horizontal spread of bornavirus infection. No specific information is available on the environmental survival or sensitivity to disinfectants for the avian bornaviruses; they are assumed to have the same stability as other similar enveloped RNA viruses. Disinfection protocols using hypochlorites (bleach), phenols, and formaldehyde are suggested.

Given the difficulties in identifying infected birds, particularly in the early stages of infection or when there is a chronic carrier state, prevention of new infection and elimination of disease within a flock remain problematic. Combined use of RT-PCR evaluation of cloacal swabs and serology is recommended, with repeated cycles of testing and separation of infected, possibly infected, and apparently uninfected birds until at least two sequential negative results are obtained for the presumed negative birds.[6] Collection of eggs for artificial incubation and hand-raising chicks in a clean facility have been proposed as components of conservation programs dealing with endangered species, with awareness of the possibility of vertical transmission.[47]

Research into the development of vaccines is ongoing. Recently, vectored virus vaccines expressing N protein and phosphoprotein genes from PaBV-4 provided partial protection against experimental inoculation with PaBV-2 in cockatiels.[48] An effective, widely available vaccine is still a long way from reality.

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The Use of Prosthetic and Orthotic Limbs in Avian Medicine

LAURA M. KLEINSCHMIDT

Introduction to Prosthetics and Orthotics

Prosthetics and orthotics are a continually evolving treatment modality in veterinary practice, and have been developing in human medical practice for centuries.[1] Prosthetics replace a missing limb or body part while orthotics support or protect an injured limb. Two major categorizations of prosthetic devices include internal coaptation (direct skeletal attachment, surgical implants) and external coaptation devices. Orthotic devices used in veterinary medicine consist of external coaptation devices. The devices themselves are called a "prosthesis" or "prosthetic device" (prosthetics) or an "orthosis" or "orthotic device" (orthotics). Use of prosthetics and orthotics in veterinary medicine has been reported in the literature for dogs, cattle, goats, horses, tortoises, birds, and micropigs.[2–21] Many different types of devices have been used including titanium bone implants, joint replacement implants, braces, boots, slings, carts, and even devices made with three-dimensional printers. With improvements in the quality of care provided for veterinary patients, prosthetics and orthotics are becoming a viable treatment option for these animals.

The use of prosthetics in avian medicine reported in the literature is more limited than in mammalian counterparts. Several case reports describe beak prostheses.[4,9,10,15] These cases primarily involved a single psittacine (Moluccan cockatoo *[Cacatua moluccensis]*) describing maxillary (rhamphotheca) fracture and prosthetic placement, and long-beaked birds including a black-neck aracari *(Pteroglossus aracaris)*, a channel-billed toucan *(Ramphastos vitellinus)*, an African ground hornbill *(Bucorvus abyssinicus)*, and a Marabou stork *(Leptoptilos crumeniferus)*, with both mandibular and maxillary devices described.[4,9,10,15] Overall, beak prostheses were successful in addressing the injuries, even if some were removed later in life. Sporadic reports of limb prostheses also exist in the literature and conference proceedings, including several crane species (a white-naped crane *[Grus vipio]*, a Siberian crane *[Grus leucogeranus]* and a whooping crane *[Grus Americana]*), a buzzard *(Buteo buteo)*, a Secretary bird *(Sagittarius serpentarius)*, a bald eagle *(Haliaeëtus leucocephalus)*, a mallard duck *(Anas platyrhynchos)*, and a domestic goose *(Anser anser)* with both implantation devices and external prostheses described.[5,12,13,18–21] The long-term success rate was variable in these cases, though most of these patients showed significant improvement immediately following treatment even if long-term prognosis was poor.

In clinical practice, limb prostheses are not routinely considered a good option for treatment of avian species due to a higher likelihood of patient refusal and guarded long-term prognosis. In waterfowl, raptors, long-legged, and other large-bodied birds, solely the need for surgical amputation of the legs results in euthanasia in most cases. However, limb prostheses provide an alternative to euthanasia that would improve animal welfare for select individuals under human care. This also has implications for the conservation of highly endangered species that are genetically valuable and may otherwise be removed from the breeding population without alternative options for limb replacement.

Choosing the Ideal Avian Candidate and Device

In considering the use of these devices, clinicians must weigh the pros and cons in each individual patient. Candidates for a prosthetic or orthotic device include birds with an injury to the legs resulting in neurologic damage, fracture malunion, loss of appendages, or surgical amputation with resultant inability to ambulate normally. The best candidates for orthotic or prosthetic devices are large-bodied or long-legged birds that require two functional limbs for effective mobility and normal day-to-day function. These birds include birds of prey, waterfowl, galliformes, storks, ibises, herons, cranes, flamingoes, and penguins. Most smaller birds (<200 g), including most passerine species, are not good candidates for prosthetic or orthotic devices due to their low body weights and very small limbs. Although prostheses/orthoses may be made with lightweight materials, construction of a

prosthetic/orthotic device to accommodate for such a low body weight and still allow a small bird to move freely presents significant challenges. These challenges may be overcome as new technologies become available. Furthermore, small bird species including passerines and psittacines may often compensate with only one functional leg and may learn to utilize their remaining limb and their beak to move around their enclosures effectively, making limb amputation without prosthetic device placement a more viable treatment option in these species. Birds may also survive adequately without a supportive device if they rely more heavily on flight as a means of locomotion, although they may experience some difficulty during take-off or landing. Birds housed outdoors will be at a higher risk for predation.

An animal with inherent genetic value, such as endangered species, may be a more desirable candidate in the captive breeding setting when considering the time and resources needed for success. Companion birds carry substantial emotional value with their bonded owners and may be good candidates as long as the owner is well informed upon deciding to pursue this treatment option. When choosing a candidate, the bird's temperament must come into consideration. Most prosthetic or orthotic devices will not be accepted by an individual or species that is prone to stressful behavioral responses, and complications may include myopathy, immunocompromise secondary to stress, or death. An animal accustomed to human contact (educational ambassador, hand-reared, well trained, companion animal, etc.) would make an ideal candidate, as frequent patient handling may be required. Some bird species (i.e., psittacines) are more apt to pick at external coaptation devices. Psittacines may also be less likely to tolerate anything attached to their body externally and may refuse to move until the offending item is removed, making these animals less ideal candidates. Though any bird may react negatively to a novel external device, many birds will adapt over time with the appropriate training and physical therapy. As with any medical treatment, the clinician must decide if this treatment would be appropriate for each individual patient.

Maintaining prosthetic and orthotic devices in animal patients does require significant dedication and attention to detail from staff or owners wishing to utilize these devices. The immediate post-placement period requires intensive physical therapy in most cases (e.g., physical therapy protocol provided in Box 66.1), with gradual increases in time spent with the patient wearing the device until the patient will tolerate the device for the desired length of time. Some birds may not tolerate being handled multiple times a day, and thus sessions in the device may occur less frequently but still follow a protocol of gradually increasing durations wearing the device. Each case is different and physical therapy must be tailored to each individual to accommodate progress made and the resources/staff available. Additional physical therapy modalities like those used in companion mammal medicine may have applications in avian cases (Box 66.2).[22] Caretakers must monitor patients closely for subtle signs of discomfort or gross lesions indicative of skin inflammation, infection, ulceration, or pressure sores under the device due to excessive use, inappropriate fit, or poor hygiene. The contralateral limb must be routinely monitored for muscle damage/breakdown or pododermatitis. The device must be cleaned regularly to avoid dermatitis secondary to poor hygiene. The device must be serviced regularly to avoid device failure due to poor maintenance or lack of replacement after excessive wear. Devices may also be modified over time if the patient's needs change or be replaced if newer, more innovative devices become available. With a dedicated and attentive caretaker, birds

• BOX 66.1 Example of Intensive Physical Therapy Schedule

- Weeks 1–2: Allow the patient to wear the prosthetic device for 30 minutes up to four sessions per day, with at least a 30-minute break between sessions. Every other day, if the bird is tolerating four sessions, add one additional session. Continue increasing the number of sessions every other day up to eight sessions a day.
- Weeks 3–4: If the patient has well-tolerated wearing of the prosthetic device for 30 minutes for eight sessions per day, move on to this step. Allow the patient to wear the prosthetic device for 1-hour sessions, with at least a 30-minute break following, starting with up to four sessions per day. If the bird tolerates the four sessions of 1 hour each, every other day add one additional session. Work up to the patient wearing the prosthetic for eight sessions of 1 hour each with at least 30 minutes between sessions per day.
- Weeks 5–6: If the patient has accepted the device for 1-hour sessions, increase to 2-hour sessions with at least a 30-minute break in between. Start with two sessions per day and gradually increase every other day to up to four sessions per day.
- Weeks 7–8: If the patient accepted the device for 2-hour sessions, progress to 4-hour sessions with at least a 1-hour break in between sessions. Start with one session per day. If the bird has tolerated the 4-hour session once per day for 1 week, increase to two sessions per day for week 8 with at least a 1-hour break between.
- Progressing from Week 8: Once the animal has accepted the device for longer periods without secondary complications, consider increasing the time to 8-hour sessions per day (or more per patient needs).

• BOX 66.2 Examples of Supplemental Physical Therapy Modalities

- Hydrotherapy (swimming)
- Range-of-motion exercises
- Massage
- Acupuncture/electrotherapy
- Laser treatment (i.e., affected joints)
- Thermotherapy (heat/cold packing)
- Balance, coordination, or strengthening exercises

It is recommended to consult with a certified veterinary physical therapist for further information on these modalities.

with prosthetic/orthotic devices may do very well and have an excellent quality of life.

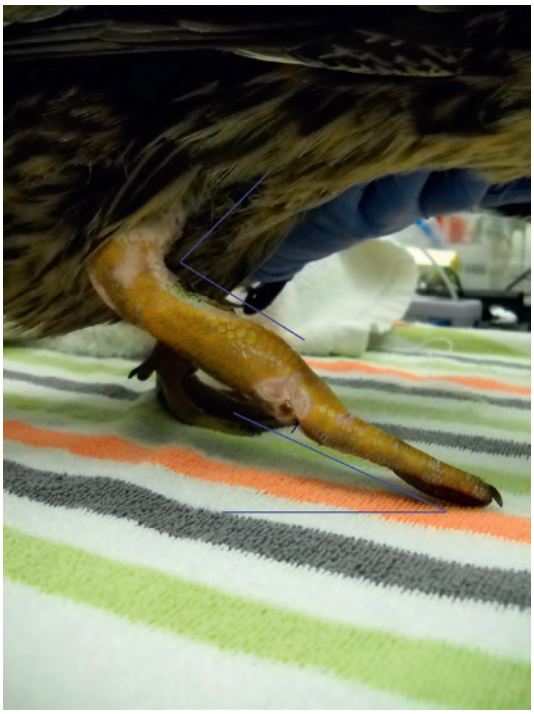
Lastly, when choosing a prosthetic/orthotic device for a patient, a clinician must determine the ultimate goals and realistic expectations for any device or individual. By combining the bird's clinical condition and determination of what ideal daily use entails, the clinician may better decide which type of device is appropriate for any given patient. For example, a bird that is routinely handled may do well with an external coaptation device that is easily taken on and off. However, a different patient that requires less frequent handling, such as an exhibit or breeding animal, may do better with a permanent internal coaptation device or more robust external coaptation device. The decision to try a prosthetic device over humanely euthanizing an injured bird may depend on the long-term plans for that individual. Is the animal a crucial contributor to the population? Will the animal be able to successfully breed with the use of the supportive device if that is the goal for the individual? How will the device affect this individual's quality of life? How much additional longevity after device placement is a reasonable expectation of success in exchange for the time and resources devoted to this individual? Unfortunately, the data available thus far on long-term prognosis with prosthetic devices in birds are variable by individual and species; there are not enough data with which to make definitive conclusions. It is only with further collaboration and discussion between colleagues regarding case successes and failures that veterinarians will be able to make definitive conclusions about long-term prognosis for any given type of patient or device.

Fabrication and Care of a Prosthetic/Orthotic Device

Once a clinician has made the decision to try a prosthetic/orthotic device, the animal must be prepared accordingly prior to fabrication and placement of the device. Any external wounds or surgical incisions should be treated in accordance with standard wound care principles and should have healed completely prior to device placement to avoid irritation, delayed wound healing, or secondary infection due to mechanical trauma from the device. The placement of a supportive device prior to the resolution of external wounds could also result in increased pain or discomfort during placement or while wearing the device, and therefore could contribute to patient aversion to the device. Bone fractures should also be treated and allowed to heal prior to measuring for the device, as the mechanics of the limb may change during the orthopedic healing process. In addition, an external device could alter bone healing and result in angular limb deformities or other complications if utilized prior to complete fracture calcification. Any other concurrent medical issues should also be addressed and either resolved or stabilized prior to moving forward with the fabrication of a prosthetic device. Animals with injuries that necessitate prosthetic devices often have additional injuries or ailments, such as myopathy or shock, which require treatment prior to proceeding. Anesthesia, sedation, or prolonged manual restraint may be required during the fabrication process; thus, only a stable patient should undergo these procedures. Animals with unrelated advanced disease processes are likely not ideal candidates for use of prosthetic devices in general due to their potential co-morbidities or imminent mortality.

Once a patient is stabilized, clinicians are encouraged to collaborate with veterinary orthopedic surgeons to plan and place internal coaptation prosthetic devices, such as titanium bone implants or joint replacement devices. Licensed prosthetists and orthotists (veterinary or human medical professionals) are an invaluable resource to developing an innovative solution for each individual patient and should be consulted from inception to implementation of an external coaptation device. The easiest way to find a prosthetist/orthotist nearby is to consult with a veterinary specialty referral or university-based orthopedic surgery practice who are most likely to have collaborated with these consultants on companion mammal cases; these consultants would also likely be more willing to help with an avian case than a prosthetist/orthotist who has never before worked with veterinary patients. However, local human hospitals, orthopedic surgery, or physical therapy practices may also be able to direct veterinary clinicians to an available licensed prosthetist/orthotist in the surrounding area. Consultants will likely want to examine the patient and identify the specific needs of each individual in order to personalize the device accordingly; they also have the infrastructure to create professional-grade devices cost effectively. During the developmental stage, species-specific and individual needs also should be discussed in accordance with previously set expectations for the patient. For example, a waterfowl species that swims regularly will need a device made of waterproof materials that allows drainage and dries quickly to prevent moist dermatitis under the device. Devices should be made with a similar color pattern to the natural markings of the species involved. The weight-bearing surface of the device should have adequate traction to prevent patient slippage on wet or smooth surfaces. A professional with experience and knowledge of material options and device configurations is crucial to developing a device with the highest likelihood of success.

The first step in fabrication of a device is to determine the ideal "normal" for the individual. Preferably with the animal awake and standing in normal anatomical position, accurate measurements of the angles of the joints of the limbs should be taken of both the normal limb and the affected limb (Fig. 66.1). Clinicians should utilize the normal contralateral limb as a frame of reference for ideal/normal limb mechanics for the individual. Alternatively, similar-sized conspecifics also may be used as a reference of normal anatomy at this stage. Once angle measurements are obtained, the affected limb must be cast using malleable thermoplastic casting material (Fig. 66.2). A prosthetist/orthotist will then make



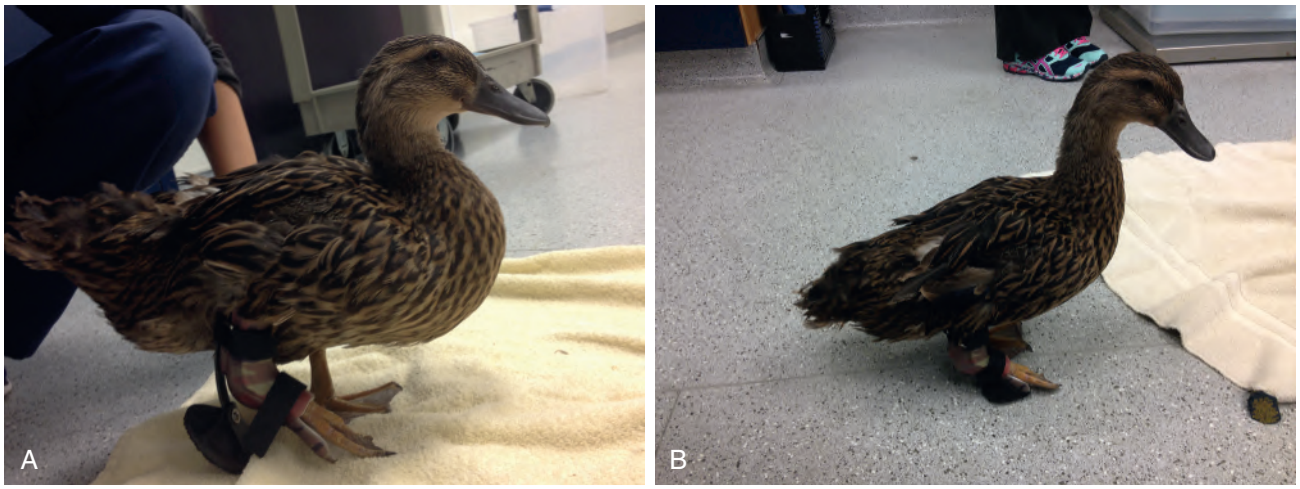
• **Figure 66.1** Prior to fabrication of a supportive device, accurate measurements of the angles of the joints of the limbs should be taken of both the normal limb and the affected limb. These angles should include the angle at which the limb makes contact with the ground and all other acute angles of the joints that will be involved in the prosthesis/orthosis.

• **Figure 66.2** The affected limb must be cast using malleable thermoplastic casting material so that the prosthetist/orthotist may make a mold of the leg to which the device will be fitted. Depending on the patient, anesthesia or sedation may be necessary to create a cast of the leg.

• **Figure 66.3** A 1-month-old male domestic goose *(Anser anser)* presented to a rescue agency missing its right foot. The affected limb was cast under manual restraint (Fig. 66.2), and a prosthetic device was created using polypropylene plastic that was heated and vacuum molded to a solid plaster mold made from the hollow original cast. Thermofoam padding and diabetic-grade silicone were used for internal cushioning, Velcro strapping was used to attach the device to the patient's limb, and a nonskid rubber sole was affixed to the distal end of the device for traction.

a mold of the leg to which the device will be fitted during the building process. Depending on the patient, anesthesia or sedation may be necessary to take accurate limb angle measurements and/or to create a cast of the leg. Examples of anesthesia or sedation protocols in birds have been discussed elsewhere.[23–26] The measurement and casting process should not be painful, and thus using more than minimal analgesia is unnecessary in most cases.

Once a cast mold of the affected leg has been made, the orthotist/prosthetist will create a device tailored to the unique case and individual using the previously discussed parameters. Materials such as thermofoam padding, medical-grade silicone (as used for human diabetic patients), and lightweight polypropylene plastics may be used to increase patient comfort and mobility with a lighter weight device (e.g., device pictured Fig. 66.3). Orthotists/prosthetists heat and vacuum-mold these materials to a solid plaster mold made from the hollow original cast. Materials used to affix the supportive device to the affected leg may vary depending on durability but may be as simple as Velcro strapping. The bottom of the device should be made with materials resulting in a nonskid sole. Clinicians should not be surprised if additional modifications are necessary after fabrication of the original device. Some trial and error should be expected until the ideal version of the device has been achieved (Fig.



• **Figure 66.4** (A) Trial and error should be expected to attain the ideal version of a device for each individual patient. This example shows the first orthotic prototype for a mallard duck *(Anas platyrhynchos)*. The height of the device was too tall, resulting in the animal leaning heavily toward the unaffected limb. The angle of the weight-bearing surface was inaccurate, resulting in only the cranial edge of the weight-bearing surface touching the ground. Finally, the proximal portion of the brace was too long, resulting in difficult and uncomfortable attachment to the patient due to apposition with the thigh musculature. Modification of the device was necessary to create a functional orthosis. (B) The final version of the orthotic device used in this patient following modification for appropriate fit. Note the more normal body posture and accurate angulation of the weight-bearing surface, resulting in full traction with the ground. The device was also shortened proximally to prevent apposition with the thigh musculature to prevent patient discomfort during use.

66.4A, B). Depending on the extent of modifications necessary and the patient's stress level under manual restraint, additional anesthetic or sedation episodes may be needed during the fitting process. The device should fit snugly to prevent contact sores or skin ulcerations but should not be too tight as to cut off circulation to the distal portions of the leg. The device may need to be modified depending on the surrounding soft tissue structures or directionality of the feathers to avoid patient discomfort. Additionally, the animal should be observed standing/walking in the device to ensure the correct angulation and resultant weight-bearing surface are present to allow the patient to ambulate with a near-normal gait. The closer to a normal gait, the better, as this will increase normal weight-bearing on the affected limb and prevent or delay contralateral limb breakdown or pododermatitis. Once an ideal device has been created, the animal should start a physical therapy regime as previously described. Also, the animal should be gradually introduced to a variety of substrates, most importantly those present in its natural enclosure. For example, a waterfowl species may have hydrotherapy performed with and without the device during physical therapy; sessions with the animal wearing the device should be used to test its compatibility with submersion and swimming in a controlled setting (i.e., a large trough filled with water) prior to being introduced to a natural-type pond in an exhibit or other outdoor setting. Social species should also experience a gradual return to the flock to avoid the other animals ostracizing the patient or picking at the device. Depending on the species involved, attention to detail during the device design process may help to prevent these negative social interactions by avoiding certain colors or hardware on the device. These modifications may also be made in the trial and error process to improve overall patient success.

Prosthetic and orthotic devices should be maintained with proper hygiene and repaired or replaced as needed. External coaptation devices (see Figs. 66.3 and 66.4) should be cleaned regularly with a damp cloth and mild antibacterial soap, and then dried with a towel before use. A less frequent deep cleaning should also be done using a 50/50 mix of water and rubbing alcohol before allowing the device to air-dry prior to use. Extreme temperatures could damage polypropylene plastic prosthetic/orthotic devices and thus should be avoided. Heating elements or devices, such as hair dryers, should not be used to dry these devices to prevent thermal damage. Devices should not be left in direct sunlight (e.g., in a hot car) to prevent their disfiguration. Care instructions will vary depending on the building materials used to create the device, and thus clinicians should consult with their prosthetist/orthotist on specific care instructions for each unique device prior to use. The need to repair or replace a device over time will vary depending on the degree of use (amount of daily wear and tear) and the environment in which the device is used, and thus will be patient dependent. Clinicians may also choose to replace devices with innovative models as newer technologies become available.

Conclusion

Prosthetics and orthotics are a viable treatment option for exotic animal practitioners to use to improve quality

of life and longevity for their animal patients, including avian patients. As with any treatment option, the presiding clinician must decide if this treatment would be appropriate for each individual patient by scrutinizing the species-specific requirements, individual temperament, population sustainability, and support staff/caretakers and other resources available in each case. The obvious benefits of using prosthetics and orthotics include that they are an alternative to euthanasia and will allow more normal weight-bearing and enhanced mobility and thus improve quality of life, especially in patients who cannot ambulate without them (bilateral amputations in quadrupeds, large mammals that require distribution of their weight on four limbs to ambulate, or bipedal animals such as birds). Possible risks include patient refusal to use the device despite physical therapy—the physical therapy itself being time and labor-intensive—underlying skin inflammation, infection, or ulceration due to poor hygiene or inappropriate fit, contralateral limb muscle damage/breakdown or pododermatitis, and device upkeep, including regular cleaning, maintenance, and replacement of devices over time. Clinicians interested in using these devices are encouraged to collaborate with other veterinary (or human) medical professionals, especially licensed prosthetists/orthotists. Any prosthetic/orthotic device will require a level of innovation and creativity because each device must be individualized for each patient and a unique situation. Collaboration and discussion between veterinary professionals of both case successes and failures are crucial to the further development of prosthetic and orthotic device use in avian patients.

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67

Avian Spirurids

CARLES JUAN-SALLÉS AND MICHAEL M. GARNER

Introduction

Spirurids (order Spirurida) are a diverse group of nematodes that includes an extensive list of genera and species, a number of which infect birds. Spirurids are characterized morphologically by the production of small, thick-shelled, oval embryonated eggs in most genera, a variety of cuticular ornamentations in the cephalic area, lateral chords that can be large, and often an eosinophilic fluid in the pseudocoelom.[21] Known life cycles usually involve an ingested arthropod or crustacean intermediate host.

For a comprehensive review of all avian spirurids, readers are referred to recent reviews and a vast bibliography for this group of nematodes. This chapter will focus on the most frequently encountered spirurid-induced diseases of free-ranging and captive birds. Tables 67.1 and 67.2 list the main spirurids reported to cause disease, with their definitive and intermediate hosts, tissue targets, clinical findings, lesions, and treatment if available. Captive birds appear to be overrepresented in the literature of spirurid-associated morbidity and mortality. However, disease can occur also in wild birds and in some cases has been associated with the decline or mortality events of some populations, including endangered avian species.[11,12,52]

Main Diseases Caused by Spirurids in Free-Ranging and Captive Birds

Acuariids, characterized by cuticular cordons located in the cephalic/anterior portion (Figs. 67.1 and 67.2) (only absent in the genus *Paracuaria*) or other cephalic ornamentations (in subfamily Schistorophinae),[2] include several of the most pathogenic spirurid genera in birds, and usually infect the proventriculus, ventriculus, or upper digestive tract. *Dispharynx, Echinuria, Acuaria,* and *Streptocara* appear to be the most relevant genera in birds. Dispharynxosis is an important cause of disease in zoo and aviary birds. Affected animals have diffuse proliferative or polypoid, adenomatous proventriculitis (see Fig. 67.2) that can be complicated by exacerbated catabolism, aspiration pneumonia, and gastric bleeding,[7,22,26,39,43] and rarely can be associated with gastric neoplasia.[26] Psittacine birds, especially Oceanian species, are commonly affected[7,22,26] and may present similarly to proventricular dilatation disease (PDD).[26] The association of proventricular spiruridiasis with neoplasia is not surprising as other spirurids, particularly *Spirocerca lupi* in carnivores, have been shown to cause or be associated with sarcomas and other neoplasms.[4,8,13]

Streptocara incognita is mostly a mucosal pathogen of ducks, swans, and flamingos.[1] The ventriculus is the primary target, although any segment of the upper alimentary tract can be infected. Swans have been reported to develop severe necrotizing pharyngitis and esophagitis with *S. incognita* nematodes embedded in the affected mucosae (Fig. 67.3).[1] *Gongylonema* (superfamily Spiruroidea) has also been reported to cause upper digestive tract disease, including necrotizing stomatitis in scops owl *(Otus scops)* fledglings,[18] and esophageal obstruction in horned owls *(Asio otus)*.[30] *Echinuria uncinata* also causes proventricular disease in young ducks and swans. Lesions consist of proventricular thickening due to glandular hypertrophy and dilatation as well as parasitic granulomas that can become large enough to cause obstruction.[52] Crowding and drought may exacerbate parasitism.[52]

The acuariid *Acuaria skrjabini*, which targets the ventriculus in finches, and *Procyrnea* spp. (Habronematidae) are pathogenic in passerine birds by burrowing between the koilin and ventricular glands[25,34,38]; nonpasserine species such as woodpeckers and toucans can also be affected with *Procyrnea*.[45] These nematodes, along with other habronematids with identical organ tropism including *Sicarius uncinipenis* in greater rheas *(Rhea americana)*[9,53] and *Hadjelia truncata* in pigeons, Coraciiformes, and helmeted guinea fowl *(Numida meleagris)*,[36,44] cause weight loss, anorexia, diarrhea, and ventriculitis, with koilin degeneration and thickening, ulceration, hemorrhage, secondary bacterial and fungal infections, or combinations thereof.[9,25,34,38,45,53]

Tetramerids are characterized by a marked sexual dimorphism.[27] Tetrameridosis can be caused by numerous species in the genera *Tetrameres* and *Microtetrameres*, as well as a few species of *Geopetitia* (in particular, *G. aspiculata*), which are cosmopolitan and infect the proventriculus of a wide range of avian hosts; *Geopetitia* also targets the esophagus and ventriculus.[27] *Tetrameres* and *Microtetrameres* burrow within

Text continued on page 476

TABLE 67.1 Main Pathogenic Spirurids Described in Wild and Captive Birds, With Avian and Intermediate Hosts, and Organ Targets

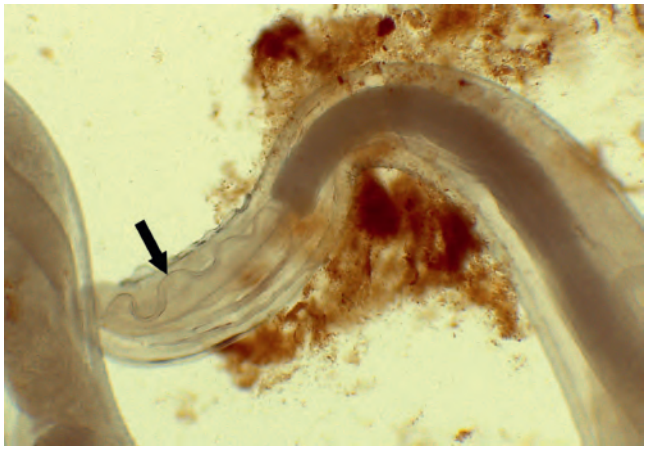
Family (Superfamily)	Species	Avian Hosts	Intermediate Hosts	Organ Targets	References
Acuariidae (Acuaroidea)	*Dispharynx* spp. (particularly *D. nasuta*)	Wide range of orders	Pillbug, sowbug	Proventriculus, esophagus	7, 22, 26, 39, 42, 43
	Acuaria skrjabini	Finches	Unknown	Ventriculus	25, 34
	Cheilospirura hamulosa	Game gallinaceous birds, pheasants	Grasshopper, beetle, weevil, sand hopper	Ventriculus	14
	Echinuria uncinata	Ducks, geese, swans	Water fleas	Proventriculus, intestine, body walls	52
	Streptocara crassicauda, *S. incognita*	Wild ducks; geese	Amphipod crustaceans (*Gammarus* spp., *Hyalella azteca*)	Ventriculus, esophagus, pharynx	Reviewed in 1
	S. incognita	Chilean flamingos *(Phoenicopterus chilensis)*		Proventriculus, ventriculus, esophagus	Reviewed in 1
	S. incognita	Mute swans *(Cygnus olor)*		Oropharynx, esophagus	1
Tetrameridae (Habronematoidea)	*Tetrameres* spp. (wide range of species, disputed)	Wide range of species, usually aquatic	Mostly coleopteran and orthopteran insects, also crustaceans	Proventriculus	27, 35, 46
	Microtetrameres spp. (wide range of species)	Wide range of species, usually terrestrial	At least orthopteran insects	Proventriculus	19, 27
	Geopetitia aspiculata	Passerine birds, Charadriiformes, Coraciiformes	Crickets and cockroaches	Distal serosal surface of esophagus, proventriculus and ventriculus	27
Habronematidae (Habronematoidea)	*Procyrnea* sp.	Passerine birds; black-backed woodpecker *(Picoides arcticus)*	Insect suspected but not identified	Ventriculus; also proventricular and intestinal lumen	38, 45
	Hadjelia truncata	Domestic pigeons; also Coraciiformes, helmeted guineafowl *(Numida meleagris)*	Beetles	Ventriculus	36, 44
	Sicarius uncinipenis	Greater rhea *(Rhea americana)*	Unidentified arthropods	Ventriculus	9, 53
Onchocercidae (Filaroidea)	*Sarconema eurycerca*	Swans and geese *(Branta*, *Anser)*	Biting louse	Heart	33, 51
	Pelecitus sp., *P. tercostatus*, *P. calamiformis*	Psittacine birds, channel-billed toucan *(Ramphastos vitellinus)*	Lice, mosquitoes, other arthropods	Subcutaneous tissue around joints (especially hock)	2, 10, 32
	Chandlerella quiscali	Emus *(Dromaius novaehollandiae)*, northern crested caracara *(Caracara cheriway)*	Midges (*Culicoides* spp.)	Brain, spinal cord	16, 31
	Paronchocerca ciconarum	Storks	Unknown	Heart, pulmonary vessels	15
	P. tonkinensis	Rufus-crested bustards *(Eupoditis ruficrista)*	Unknown	Pulmonary arteries (adults); microfilariae in tissues	37

Diplotriaenidae (Diplotriaenoidea)	*Serratospiculum* spp.	Falcons; also other raptors and passerine birds	Beetles, grasshoppers, locusts, wood lice, crickets	Air sacs	23, 40, 41, 49
	Serratospiculoides amaculata	*Falco* spp., Cooper's hawk *(Accipiter cooperii)*, great tit *(Parus major)*	Grasshoppers, crickets	Air sacs	24, 28, 47
	Monopetalonema alcedinis	Belted kingfisher *(Megaceryle alcyon)* and other kingfishers	Unidentified insects	Air sacs	6
Thelaziidae (Thelazioidea)	*Oxyspirura mansoni*	Wide range of orders	Surinam cockroach	Conjunctival sac	50
	O. petrowi	Over 80 species worldwide	Unidentified arthropods	Eyelids, nictitating membrane, nasolacrimal duct, Harderian gland, intraorbital tissues	11, 12
	Thelazia anolabiata, *Thelazia* spp.	Numerous species; disease in Andean Cock of the Rock *(Rupicola peruviana)* and Senegal's parrot *(Poicephalus senegalus)*	Dipteran flies	Periocular tissues	17
	Ceratospira inglisi	Pigeons, doves, cockatoos	Presumably flies	Periocular tissues	48
Gongylonematidae (Spirudoidea)	*Gongylonema* sp.	Scops owl *(Otus scops)*, presumed accidental host	Around 50 species of arthropods (mostly coprophagus)	Oral cavity	18
	Gongylonema sp.	Horned owls *(Asio otus)*		Esophagus	30
Gnathostomatidae (Gnathostomatoidea)	*Gnathostoma* spp.	Fish-eating birds	Copepods first, then fish and amphibians; birds are parathenic hosts	Skeletal muscles	20
Physalopteridae (Physalopteroidea)	*Physaloptera* sp.	Bobwhite quail *(Colinus virginianus)*	Orthopterans, beetles	Skeletal muscles	5
	P. alata	Eurasian bittern *(Botaurus stellaris)*		Stomach	29

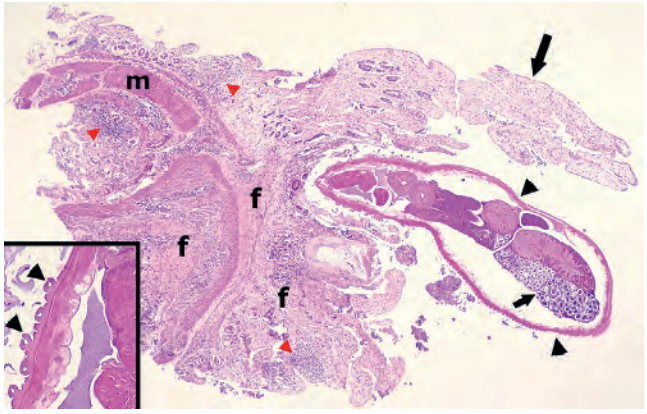
TABLE 67.2 Clinical Findings and Lesions Commonly Reported With the Main Pathogenic Spirurids of Wild and Captive Birds. Treatment Protocols, When Proved to Be Effective, Are Also Included

Species	Clinical Presentation	Lesions	Treatment	References
Dispharynx spp. (particularly *D. nasuta*)	Weight loss, debilitation, proventricular dilatation	Diffuse proliferative proventriculitis, proventricular adenomatous polyps, proventricular dilatation, proventricular mucosa covered by abundant mucus or mucohemorrhagic material; also nematodiasis without lesions	Ivermectin 0.4 mg/kg SC once a month resolved egg shedding and proventricular filling defect in jacanas[43]	7, 22, 26, 39, 43
Acuaria skrjabini	Lethargy, inappetence, diarrhea, weight loss	Necrotizing ventriculitis, koilin degeneration and thickening, secondary bacterial infection; nematodes burrow in the interface between koilin and mucosal glands		25, 34
Cheilospirura hamulosa	Anemia, emaciation	Ventriculitis with leiomyositis		14
Echinuria uncinata	Emaciation, anemia, eosinophilia, heterophilia	Proventricular thickening with excessive mucus; granulomas with intralesional nematodes		52
Streptocara crassicauda, *S. incognita*	Emaciation, debilitation	Ulcerative or necrotizing ventriculitis, esophagitis or pharyngitis		Reviewed in 1
S. incognita	Found dead (debilitating condition, cachexia)	Ulcerative ventriculitis and proventriculitis with intralesional nematodes and blood-filled cystic lesions in ventricular muscular layers		Reviewed in 1
S. incognita	Anorexia, lethargy, reluctance to move	Fibrinonecrotizing pharyngitis and esophagitis with intralesional nematodes		1
Tetrameres spp. (wide range of species, disputed)	Weakness, loss of appetite, diarrhea, eosinophilia, anemia; associated with mortality in wild cranes	Proventricular thickening, compression atrophy of glands by female nematodes, necrosis; fistulated proventricular nodules		27, 35, 46
Microtetrameres spp. (wide range of species)	Generally not described; anorexia, weight loss, lethargy, and leukocytosis in hornbills	Glandular compression atrophy, ductal hyperplasia/goblet cell metaplasia of proventricular glands, mild glandular necrosis, proventriculitis and hemorrhage; female nematodes hematophagous		19, 27
Geopetitia aspiculata	Weight loss, distension of coelomic cavity	Granulomatous proventriculitis and visceral coelomitis around encysted nematodes; posterior end of some nematodes in the lumen of proventriculus	Ivermectin, fenbendazol	27
Procyrnea sp.	Lethargy, perching low or on ground, emaciation	Koilin fragmentation and thickening with intralesional nematodes and secondary bacterial and *Candida* infections, ventriculitis, ulceration, hemorrhage; associated with amyloidosis in passerine birds		38, 45
Hadjelia truncata	Weight loss, poor food consumption, diarrhea, weakness, reduced packed cell volume (PCV) and total proteins, mortality (pigeons)	Ventricular enlargement, koilin disruption/degeneration with clear spaces, abundant nematodes between koilin and mucosa, secondary bacterial colonization (pigeons)	15 mL of levamisole solution (37.5 mg levamisole hydrochloride/mL) in 1 L of drinking water[36]	36, 44
Sicarius uncinipenis	Weight loss, hyporexia/anorexia	Koilin ulceration/degeneration, ventriculitis, red nematodes embedded in the mucosa		9, 53

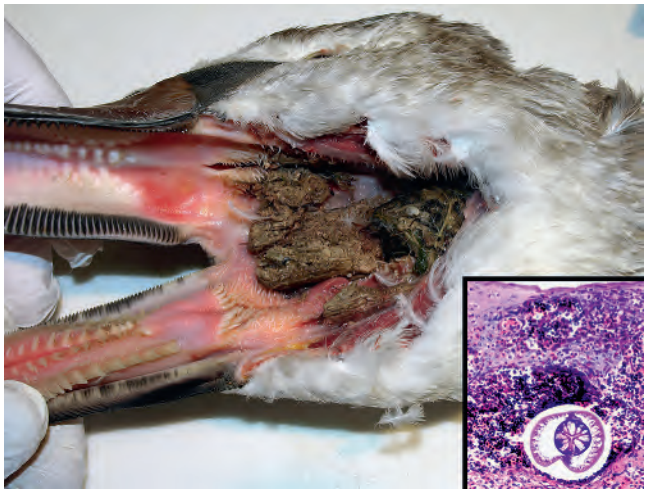
Sarconema eurycerca	Emaciation, cardiac failure	Myocarditis with hemorrhagic tracts, vasculitis/fibrinoid necrosis, mineralization, adult nematodes and microfilariae		33, 51
Pelecitus sp., *P. tercostatus*, *P. calamiformis*	Lameness, nodular thickening of soft tissues around joint	Nodular thickening of soft tissues around joint, fibrinous tenosynovitis		2, 10, 32
Chandlerella quiscali	Torticollis, progressive ataxia	Encephalitis		31
Paronchocerca ciconarum		Myocardial degeneration, thrombosis and thickening of pulmonary arteries		15
P. tonkinensis		Pneumonia; association with cardiac rupture, myocardial degeneration and fibrosis		37
Serratospiculum spp.	Dyspnea, reduced fitness in flight, weight loss, lethargy; also vomiting, bone fractures	Air sacculitis, pulmonary necrosis, bronchopneumonia, emaciation; bone fractures noted in all infected wild raptors in one retrospective study in Italy	Melarsomine (0.25 mg/kg IM for 2 days) followed 10 days later by a single dose of ivermectin (1 mg/kg IM) for *S. seurati*[49]	23, 40, 41, 49
Serratospiculoides amaculata	Subclinical if parasites not abundant, but can cause signs similar to *Serratospiculum*; anorexia, recumbent and hindlimb deficits (falcon with atypical parasite migration into vertebral canal)	Air sacculitis with fibrosis, pneumonia; meningomyelitis with spongiosis and axonal degeneration in a falcon with atypical parasite migration into vertebral canal		24, 28, 47
Monopetalonema alcedinis	Not available	Air sacculitis, pneumonia		6
Oxyspirura mansoni	Conjunctivitis, association with blindness and cataracts	Conjunctivitis, bilateral cataracts (histopathological findings not provided) in zoo pheasants	Removal of nematodes, topical 0.5% iodine or 0.5% Lysol, 0.5% diethylcarbamazine in drinking water	50
O. petrowi	Ocular inflammation, corneal opacity	Harderian adenitis with ductal dilatation, fibrosis and gland atrophy; corneal ulceration and opacity		11, 12
Thelazia anolabiata, *Thelazia* spp.	Keratoconjunctivitis	Keratoconjunctivitis, hyphema, blepharospasm	Removal of nematodes, ivermectin 0.2 mg/kg IM single dose, topical ciprofloxacin and epithelial regenerator	17
Ceratospira inglisi	Chemosis	Chemosis, subclinical	Removal of nematodes, topical Ivomec 1% (1 drop), triple antibiotic ophthalmic ointment	48
Gongylonema sp.	Starvation, emaciation, plaques in the oral mucosae	Necrotic stomatitis	Fenbendazol 50 mg/kg PO, daily debridement, and supportive therapy	18
Gongylonema sp.	Weakness	Obstruction of esophagus with masses of nematodes attached to the mucosa	Fenbendazole 33 mg/kg PO for 3 consecutive days	30
Gnathostoma spp.	Not reported	Rhabdomyositis with fibrosis		20
Physaloptera sp.	Not reported	Necrotizing and granulomatous rhabomyositis with intralesional physalopteroid larvae		5
P. alata	Not available	Erosion of gastric mucosa, covered with mucus		29



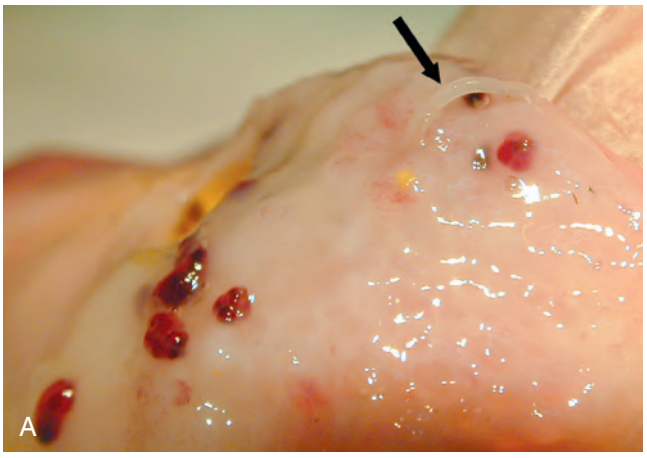
• **Figure 67.1** Note cuticular cordons *(arrow)* in the anterior end of a specimen of *Dispharynx* obtained from a parrot *(Barnardius zonarius semitorquatus)* with adenomatous proliferative proventriculitis. (Photo courtesy Dr. Nuhacet Fernández [Loro Parque, Spain].)

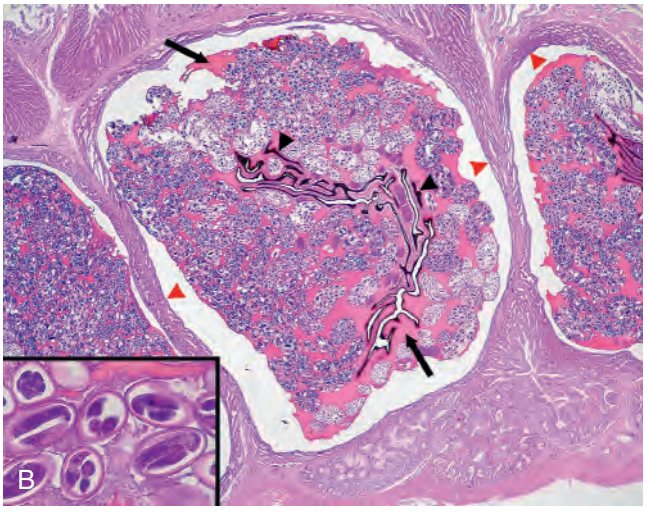


• **Figure 67.2** Photomicrograph of a polypoid proventricular lesion *(long arrow)* around a female acuariid spirurid *(black arrowheads)* attached to the mucosa in a musk lorikeet *(Glossopsitta concinna)*. Note abundant eggs in the uterus *(short arrow)*. Fibrosis *(f)* and inflammation *(red arrowheads)* are prominent. *Inset:* detail of cuticular cordons *(arrowheads)*. *m*, Muscular tunics. Hematoxylin and eosin.



• **Figure 67.3** Note severe necrotizing pharyngitis in a mute swan *(Cygnus olor)* with streptocariasis. *Inset:* a cross section of *Streptocara incognita* nematode is embedded in the mucosa and associated with hemorrhage. Hematoxylin and eosin. (Photo courtesy Dr. Amer Alić [University of Sarajevo, Bosnia and Herzegovina].)

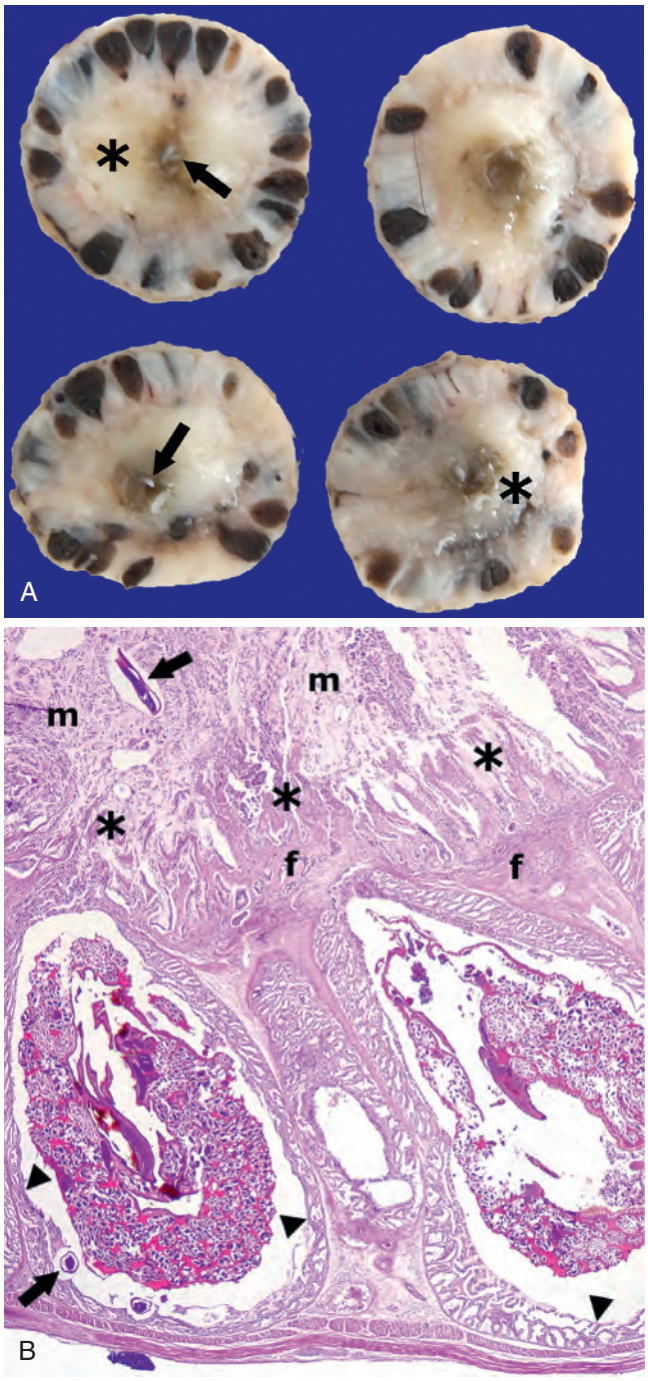




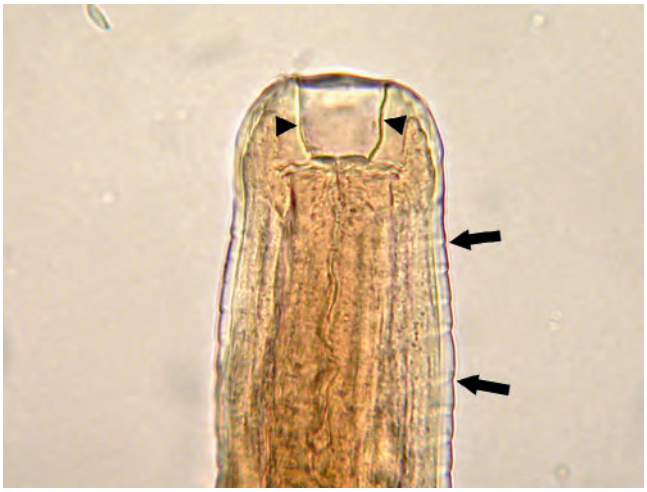
• **Figure 67.4** (A) Gross appearance of low numbers of female *Tetrameres* as raised reddish foci scattered in the proventricular mucosa of a rock pigeon. Note also an unidentified nematode attached to the mucosa *(arrow)*. (Photo Courtesy Dr. Andrés Montesinos Barceló [Centro Veterinario Los Sauces, Spain].) (B) Photomicrograph of the proventriculus depicted in Fig. 67.4A, in which proventricular glands are dilated due to the presence of tetramerid, globoid nematodes with abundant eggs, eosinophilic fluid *(arrows)* in the pseudocoelom, and heavily pigmented intestinal brush border *(black arrowheads)*. Glands are severely atrophied *(red arrowheads)* due to compression by the large, gravid *Tetrameres* females. *Inset:* higher magnification of characteristic oval, thick-shelled embryonated spirurid eggs. Hematoxylin and eosin.

the lumen of proventricular glands, while *Geopetitia* encysts on the serosal surfaces. While infection can be subclinical (Fig. 67.4), severely parasitized birds may develop anorexia, weight loss, diarrhea anemia, and proventriculitis (Fig. 67.5).[27] Infection with *Tetrameres* can be suspected grossly because of the presence of slightly raised, red foci in the proventricular mucosa that may resemble hemorrhage (see Figs. 67.4A and 67.5A) but actually consist of the globular gravid females.[27]

The spirurids *Oxyspirura*, *Thelazia* (Fig. 67.6), and *Ceratospirura* (family Thelaziidae) uniquely target periocular tissues (conjunctival sac, eyelid, Harderian gland, nasolacrimal duct) and may cause mainly conjunctivitis, corneal opacity or ulceration, and Harderian adenitis.[11,12,17,48,50]



• **Figure 67.5** (A) Cross sections of formalin-fixed proventriculus from a rock pigeon with fatal *Tetrameres* parasitism showing abundant females (dark foci) and marked thickening of the mucosa *(asterisks)* with luminal narrowing. Visible in the lumen are the smaller males *(arrows)*. (B) Photomicrograph of the proventriculus depicted in Fig. 67.5A, in which prominent hyperplasia *(asterisks)* and fibrosis *(f)* of the mucosa with accumulation of abundant mucus *(m)* and debris in the lumen are observed. Two glands are dilated and atrophied *(arrowheads)* with females. The small males *(arrows)* are also present. Hematoxylin and eosin.

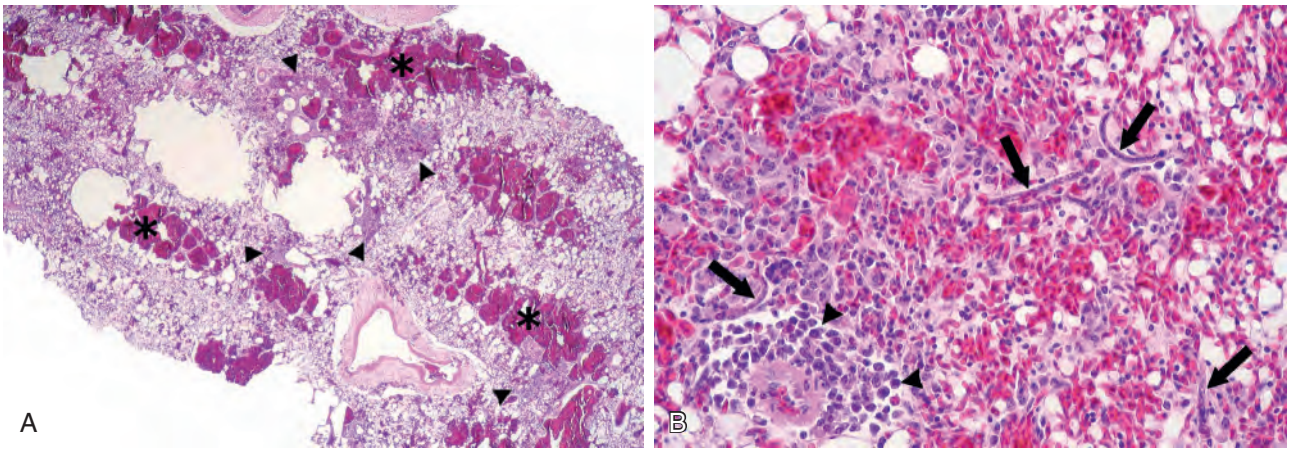


• **Figure 67.6** Anterior end of *Thelazia anolabiata* obtained from an Andean cock the rock *(Rupicola peruviana)*. Note the cylindrical buccal apparatus without labia *(arrowheads)* and the cuticular annulations *(arrows)*. (Photo courtesy Dr. Víctor Javier Mamani Palomino [Universidad Peruana Cayetano Heredia, Perú].)

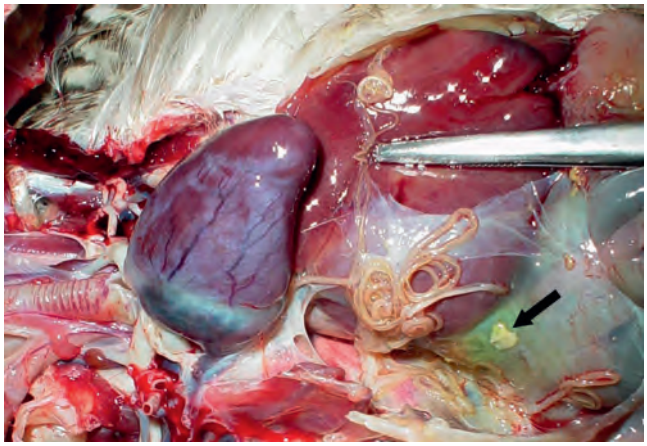
Oxyspirura may contribute to the decline of Northern bobwhites *(Colinus virginianus)* and lesser prairie-chicken *(Tympanuchus pallidicinctus)*.[11,12]

Filarids of birds include numerous genera and about 160 species that are transmitted by hematophagous arthropods, although with few exceptions are considered nonpathogenic.[2,3] Filarids in the family Onchocercidae produce microfilariae (Fig. 67.7), which can be seen in the bloodstream and skin, while adults may be difficult to find if present at all and this often precludes parasite identification.[2,3] The onchocercids *Sarconema*, *Pelecitus*, *Chandlerella*, and *Paronchocerca* have been associated with disease that may become apparent clinically. *Sarconema eurycerca* has been reported to cause myocarditis, vasculitis, fibrinoid necrosis, and hemorrhagic tracts in the heart with intralesional adult filarids and microfilariae.[33,51] *Pelecitus* causes inflammation of the periarticular soft tissues in psittacine birds and toucans.[10,32] In the United States, *Chandlerella quiscali* is widely distributed in common grackles *(Quiscalus quiscula versicolor)* and other passerine birds, in which adults live in the cerebral pachymeninges.[2,3] In emus *(Dromaius novaehollandiae)*, however, this filarid can cause torticollis and ataxia due to encephalitis.[31] Granulomatous encephalomyelitis due to this parasite has been recently reported in a wild northern crested caracara *(Caracara cheriway)*.[16]

Diplotriaenoid spirurids are cosmopolitan air sac parasites (Fig. 67.8); unlike filarids, adults produce ova with fully differentiated first-stage larvae in the air sacs, which are coughed up and swallowed. Intermediate hosts include a variety of arthropods that may be ingested by the definitive avian host, but the life cycle and intermediate hosts in the wild are not fully understood.[41,47] *Serratospiculum* is the main diplotriaenoid of birds, mostly falcons, but also other raptors and crows.[23,40,41,49] Some geographic variation exists for the predominant species involved: for example, *S. tendo* is the usual species in Europe, and *S. seurati* in the



• **Figure 67.7** (A) Photomicrograph of the lung of a bearded barbet *(Lybius dubius)* with severe parabronchial hemorrhage *(asterisks)* associated with microfilariasis. Note foci of pneumonia and atelectasis *(arrowheads)*. Hematoxylin and eosin. (B) Higher magnification of a pneumonic focus in Fig. 67.6A. Areas of perivascular *(arrowheads)* and interstitial inflammation with collapse of air capillaries (atelectasis) and hemorrhage contain intralesional microfilariae *(arrows)*. Hematoxylin and eosin.



• **Figure 67.8** Gross image of serratospiculosis in a falcon from Colombia. Several adult nematodes are present in the lumen of the right caudal pulmonary air sac; parasitism is associated with moderate air sac opacity and fibrin deposit *(arrow)*. (Photo courtesy Dr. Delio Orjuela [Fundación Paz Animal, Colombia].)

Middle East.[40,41,49] In North America, serratospiculosis has been reported to occur with *Serratospiculoides amaculata*.[24,47] While some studies question the clinical impact of serratospiculosis, others point to relevant disease or lesions.[41,49] A recent study of serratospiculosis caused by *S. seurati* revealed an incidence close to 9% in over 1700 falcons, with over 70% presenting primarily with dyspnea and reduced fitness on flight; anorexia, lethargy, and vomiting can also occur.[49] Affected wild raptors frequently are found with fractures, and it has been suggested that serratospiculosis may predispose to trauma and decreased predatory capacity.[41,47] Lesions of serratospiculosis consist of air sacculitis (see Fig. 67.8) with fibrosis as well as air sac muscular, epithelial, and mesothelial hyperplasia associated with nematodes and their eggs, which may also be present in the lungs and cause pneumonia with necrotic foci.[41,47] A combination of melarsomine and ivermectin has proved effective in resolving symptoms with elimination of dead adult nematodes in some falcons, and discontinuing egg shedding.[49] *Serratospiculoides amaculata* can cause similar lesions in falcons and other raptors[24,47] and has recently been reported in a great tit *(Parus major)* with similar lesions.[28] Aberrant parasite migration into the vertebral canal of a falcon caused meningomyelitis with hind limb neurologic deficits and recumbency.[24] Prevention and control of serratospiculosis in captivity should include limiting of access to intermediate hosts, as well as fecal and clinical examination of falcons to detect affected specimens.

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68

Selected Medical Aspects of Bird Reproduction in Ex Situ Conservation

DANTE LUIS DI NUCCI

The knowledge of aviculture techniques has had and continues to have a significant role in the pet bird industry, commercial poultry farming, zoos, and ex situ bird conservation programs. Captive breeding is already underway or recommended for consideration as a conservation action for a total of 257 avian species (2.6% of extant species).[1]

Establishing a successful program of captive breeding and incubation begins with familiarization with the natural history and biology of the species, selection of individuals and compatible breeding pairs, knowledge of minimum needs for captive management (enclosures, enrichment, nutrition, health programs), artificial incubation techniques (equipment, understanding of avian egg and embryo development, protocols for sanitation, incubation, egg management, record keeping, and egg necropsy), and knowledge of pathologies of reproductive organs, which can cause infertility, affect the normal development of the embryo, or lead to neonatal diseases.

This chapter summarizes information on medical aspects to aid in the diagnosis and understands possible causes of infertility, embryonic deaths, and consequent neonatal complications of captive breeding.

Reproduction

Infertility can be defined as a lack of egg production or increased frequency of infertile eggs. Although the majority of infertility problems are due to poor management, diseases must be ruled out as well.[2] Common causes of infertility include incompatibility of breeding pairs, same-sex pairings (mostly occurring in birds without sexual dimorphism), lack of or difficulty copulating (e.g., underlying disease or physical disorders), breeding immature birds, poor nutritional status, nutritional deficiencies, obesity, poor body score, lack of environmental stimuli (e.g., artificial lighting), stress, inadequate genetic management of the population, cloacal abnormalities, and other reproductive disorders.[2–4] Diagnosing infertility problems requires an in-depth review of management and breeding records, as well as performing physical examinations, hematology, biochemistry, and other necessary diagnostic testing such as imaging techniques (radiography, laparoscopy, or ultrasonography).[3,5–8]

Avian reproductive disorders are a result of a complex combination of hormonal, physiologic, and behavioral actions related to photoperiod, food availability, and suitability of nest sites, among other factors.[9] There are several comprehensive works on the anatomy of the reproductive tract and reproductive disorders, and their diagnosis, treatment, and prevention observed in Psitaciformes, Galliniformes, and Anseriformes.[3,5,6,9–12] Box 68.1 describes the most common female and male reproductive disorders.

Artificial Incubation

Artificial incubation should be considered an essential tool for the conservation of rare and endangered species. Many bird species will lay replacement eggs if the first clutch of eggs is removed or lost, creating an opportunity for increasing reproductive productivity. In some cases a combination of natural and artificial incubation may yield the best results. In this case, protocols for removal of eggs for artificial incubation should be carefully planned so as not to negatively interfere in embryonic development and hatching.[13]

Those contemplating the implementation of an artificial incubation and hand-rearing program should plan on an investment in time and economic costs because there are demanding tasks in effort, equipment, and building infrastructure requirements that must be considered to maximize the success of the program. Separate rooms or facilities are recommended for storage of eggs, incubation, nursery, and grow out rooms. Areas of egg storage should have humidity and temperature controlled. Incubation rooms must be equipped with incubators, hatchers, and egg candlers. The nursery must be equipped to handle altricial and precocial species. Animal intensive care units are essential for successful rearing of altricial species. Sufficient space and setting according to type of bird to be raised are needed for precocial species. A unidirectional work flow throughout the facility

• BOX 68.1 Common Avian Reproductive Disorders

Reproductive Disorders in Females

Dystocia, egg binding, and egg bound
Chronic egg laying
Egg-related coelomitis and coelomitis of reproductive origin
Ectopic ovulation
Oophoritis
Cystic hyperplasia of the oviduct and ovary
Retained cystic right oviduct
Ovarian torsion
Ovarian and oviductal neoplasia (adenocarcinoma, granulosa-theca cell tumor, arrhenoma and arrhenoblastoma, ovarian sertoli cell tumors, dysgerminomas, leiomyoma)
Oviductal impaction
Oviductal prolapse
Oviductal rupture
Oviductal volvulus
Salpingitis, metritis, and oophoritis
Prolapse and miscellaneous disease of the cloaca

Reproductive Disorders in Males

Orchitis, or epididymitis
Cystic dilation of the seminiferous tubules and testes
Testicular neoplasia (sertoli cell tumor, seminomas, mixed germ cell–sex cord–stromal tumor, arrhenoblastomas, testicular teratoma)
Phallus inflammation and prolapsed

Data from references 3, 5, 6, 9–12.

from incubation and hatching to hand-rearing is essential as part of biosecurity protocols. At all stages of development, it is critical to have the ability to control temperature, humidity, and airflow. Strict sanitation protocols must be used to minimize the movement of infectious agents that may lead to mortality of eggs and chicks. Biosecurity protocols should include restriction of personnel, foot baths, clean clothing, gloves, and a rigorous hygiene plan of the rooms and equipment (chemical compounds, ultraviolet radiation, ozonization). The facility will be fully operational after all the necessary equipment, standard operating procedures, and personnel training have been established.[13–15]

Two key factors in the success of artificial incubation are fertility and hatchability. Fertility is defined as the ratio of fertile eggs to total eggs laid, and hatchability is the ratio of eggs that hatch to the total number of fertile eggs. Factors affecting both should be considered when evaluating eggs that do not hatch and may be divided into three periods: prior to oviposition, preincubation, and incubation.[15] In the first period, causes of infertility include: behavioral (e.g., immaturity, pair incompatibility, sexual inexperience, improper imprinting, infrequent mating); environmental (e.g., incorrect photoperiod, incorrect design of the nest box or nesting materials, improper design of the enclosure, lack of visual barriers); and medical (e.g., obesity, endogamy, musculoskeletal, neuromuscular or reproductive disease, nutritional deficiencies or excesses, parasitic disease).[3]

There are three critical points during the preincubation period: collection and handling, storage, and disinfection of eggs. Careful handling helps prevent injury (shell breakage) and microbial infection of eggs. Proper collection and handling techniques include protection from physical damage (cracked shells, freezing, or overheating), preventing damage from sudden or rough movement, and speed of transfer. For incubated eggs a rapid transfer (less than 5 minutes) between nests and/or incubators is recommended.

The most frequent pathogens in poultry hatcheries are *Pseudomonas* sp., *Escherichia coli, Salmonella* sp., *Mycoplasma* sp., and *Aspergillus fumigatus.*[16] In a study in rheas *(Rhea americana)*, 13 bacterial agents (*Acinetobacter* sp., *Aeromonas* sp., *Alcaligenes* sp., *Bacillus* sp., *Cedecea* sp., *Citrobacter freundii, E. coli, Proteus* sp., *Pseudomonas* sp., *Salmonella gallinarum, Serratia ficaria, Serratia marcescens, Staphylococcus aureus*) and four fungal agents (*Alternaria* sp., *Aspergillus* sp., *Fusarium* sp., *Mucor* sp.) were isolated from eggs with microbial contamination during the incubation process.[17]

Options for egg disinfection include dry cleaning, spraying, and dipping. The dry cleaning method is the recommended form of disinfection and is preferred because it is safer for the embryos. Dry cleaning can be performed by gently, with a dry cloth, swabbing the surface of the egg. Disinfection by egg dipping or spraying can be performed using various commercial disinfectants (e.g., dilute chlorine, iodine, or quaternary ammonium compound solutions). Possible detrimental effects of egg dipping include damage or removal of the cuticle layer. The cuticle somewhat seals the pores and is useful in reducing moisture losses and in preventing bacterial penetration of the egg shell. The use of formaldehyde for egg dipping is not recommended because of the potential for toxicity to the developing embryo.[18] After the eggs have been collected and disinfected, they can be stored until ready for incubation. Incorrect storage of eggs can have significant impact on hatching. The storage room should have a temperature range of 12.8°C–15.6°C to prevent the onset of embryonic development (which does not begin until the egg temperature is greater than 21°C). The relative humidity should be maintained at 70%–80%. The eggs should not be stored more than 7 days, and it should be done in such a way that the air chamber is facing upward (there is no need to rotate the eggs in storage).[13]

Environmental factors are critical to the normal development of the embryo during the incubation and hatching processes. These factors include incubation temperature and humidity, egg orientation, egg turning, and ventilation.

The appropriate incubation temperature for different avian species is reported and outlined in the literature.[15,19] During incubation, a continuous variation of 0.5°C in the dry bulb temperature will result in a significant increase in congenital malformation and embryo mortality.[20] Prolonged temperature extremes beyond that recommended for the species can have significant effects on embryo development. At higher temperatures, embryonic development will be accelerated at different rates and in different tissues. If chicks hatch, they are likely to be reduced in size. At low

temperatures, embryonic development will be retarded, also at different rates in different tissues. Slight temperature deviations are not usually lethal, unless it is due to an excessive degree or duration. The chicks often have incomplete yolk sac retraction or partially open umbilical seals. In these cases, although embryos may survive the early stages, some late mortality is likely to occur.[13]

Avian eggs lose 15% ± 3% of their weight during incubation due to water evaporation through the pores of the eggshell. Because this is a physical process (not a metabolic process), it is not affected by the stage of embryonic development and follows a linear pattern throughout incubation. Under artificial incubation conditions, this weight loss is managed by controlling the humidity in the incubator.[13] Domestic poultry are normally incubated at 55%–60% relative humidity, and this is a good starting point for most nondomestic species. However, there are species with more specific needs for humidity requirements; for example, very low values (25%–28%) are necessary for ostriches *(Struthio camelus)*[21] and higher (84%–86%) values are needed for roseate spoonbills *(Platalea ajaja)*.[22] To evaluate weight loss, the egg is weighed at the beginning and then at least once or twice a week during the incubation process. If the humidity is too high, weight loss will be insufficient and embryos are likely to become edematous, malpositioned, and/or have residual albumen or fluids that could result in drowning. In addition, some may present with unretracted yolk sacs, and/or open umbilical seals are also possible. When humidity is too low, eggs will undergo excessive weight loss that could cause poor bone mineralization, through alteration of calcium transport, and weak, dehydrated chicks.[13]

The absence of egg turning has been shown to result in the adhesion of the embryo to the inner shell membrane. Premature or abnormal adhesion of the embryonic membranes to the inner shell membrane or other structures can increase the incidence of malpositioning, decrease albumen use, cause abnormal fluid distribution, decrease oxygen exchange at the surface of the chorioallantois, or lead to a poorly developed yolk sac.[23] The eggs positioned with the air cell up hatch best when they are turned to 90 degrees, resting at a 45-degree angle, whereas those set horizontally hatch best when turned approximately to 180 degrees, alternating sides. Horizontally set eggs turned in only one direction will cause rupture of the membranes and blood vessels, resulting in embryo mortality. Setting eggs with the air cell down causes embryonic malposition.[23]

Proper ventilation and gas exchange is another key component of artificial incubation. Maintaining an adequate egg concentration inside the incubator will help to maintain normal levels of oxygen and carbon dioxide inside the incubator as the embryos breathe throughout incubation period.[13]

An understanding of embryo development is important for assessing its health. Fifty-five stages of the chicken embryo development have been classified and described in detail (Hamburger and Hamilton Stage—H&H stage).[24] It is possible to extrapolate these stages of development of chicken embryos, and use this information as a basis to understand the development in other bird species (Table 68.1).[24–26] A number of techniques may be used for assessing embryonic health via external egg appearance. These techniques include candling, egg monitoring, egg weight loss management, and floating the egg.

Candling is the primary method for monitoring embryonic development. This method is based on the visualization of the internal structures of the egg when illuminated with a powerful light (preferably cool) inside a dark room. In general, to assess fertility of the egg, candling may be performed at approximately 7–10 days post incubation. Before this stage, the movement and temperature fluctuations associated with candling may cause embryonic mortality.[27] After this stage, candling can be done every 48 hours to assess continued embryo growth. In a healthy embryo, candling is usually sufficient once a week.[27] This technique is useful for detecting very early blood vessels, small cracks and other defects in the shell that should be recorded and, eventually, repaired. In addition, it is important to detect early embryo death as evidenced by the presence of a blood ring. Any eggs with a dead embryo must be removed from the incubator so as not to be a continuous culture medium for bacteria or fungi. Other uses for this technique are to define and mark the air cell to assess drawdown and to define the approach to enter the egg in an assisted hatch or for its necropsy.[27] This technique is not appropriate in thick-shelled or pigmented eggs (e.g., cranes). In these species, floating the egg is an acceptable alternative. This method can be used to determine fertility and viability of eggs after approximately 21 days of incubation. At this stage, eggs float nearly vertically, with the large end up. From 21 to 23 days, only a slight rotational movement of the egg is noticeable. Close to hatching, twitching and stronger movements are apparent. To pursue this technique, it is necessary to float eggs in a mild disinfectant solution (10% povidone-iodine) at 43°C and observe the egg for movement for 1 minute or less. It is not recommended to float the egg for long periods of time because this increases the risk of asphyxiation and overheating of the embryo. In addition, it is not recommended to float the eggs in cool water because the egg contents will contract and may draw bacteria through the shell.[28]

Other monitoring techniques include a digital egg monitor called "Buddy" (Avian Biotech International, Tallahassee, Florida). This unit uses infrared beams to detect blood flow within the developing egg (indicates the detection of heart beat and pulse rate of the chick). The monitor appears to be excellent for assessing viability of the near-hatch chick but is not functional for all avian eggs. Xeroradiography of ostrich eggs is another method that has been reported.[29]

The chick embryo assumes the proper position to pip (penetrate) the air cell 2–3 days before hatch.[30] Recognizing the normal positioning of the embryo before hatching will aid in the decision making for the need for hatching assistance. Seven malpositions with different degrees of embryo lethality have been described (Table 68.2).

Each unhatched egg must be examined postmortem to document potential causes for lack of hatching. This information can then be used in the decision-making process of evaluating the artificial incubation protocols. An egg necropsy technique has been described.[31] The first step is the examination of membranes in situ, sampling for bacterial and fungal culture, followed by exposure of the internal egg contents (Fig. 68.1). Embryonic death is classified into three stages: early dead embryo (EDE—H&H stage 1–19), mid-dead embryo (MDE—H&H stage 20–39),

TABLE 68.1 Avian Embryonic Development and Possible Causes of Embryo Death

H & H Stages*	Hr/day (16 DI**)	Hr/day (21 DI)	Hr/day (35 DI)	Hr/day (57 DI)	Development Landmarks	Causes of Death or Abnormalities in Embryos
1	0	0	0	0	Embryonic shield	**Egg handling**
2	4–5	6–7	10–12	16–19	Initial primitive streak	Eggs stored too long
3	9–10	12–13	20–22	33–35	Intermediate primitive streak	Eggs stored under incorrect conditions
4	14–15	18–19	30–32	49–52	Definitive primitive streak	
5	14–17	19–22	32–37	52–60	Head process, notochord	Incorrect egg fumigation or sanitation (dirty hands)
6	18–19	23–25	38–42	62–68	Head fold	
7	18–20	23–26	38–43	62–71	1 somite + neural folds	Excessive vibrations (jarring)
8	20–22	26–29	43–48	3–3.5 days	4 somites + blood islands	Rapid temperature change
9	22–25	29–33	48–55	3.5–4	7 somites + optic vesicles	High temperature in early incubation
10	25–29	33–38	55–63	4–4.5	10 somites + cranial flexure	**Incubation faults**
11	30–34	40–45	67–75	4.5–5	13 somites + distinct neuromenes	Temperature, humidity, turning Cooling after development has begun
12	34–37	45–46	3–3.5 days	5–5.5	16 somites + head rotated onto left side	Suffocation due to incorrect ventilation
13	37–40	48–52	3.5	5.5–6	19 somites + head fold of amnion over brain	**Inbreeding** Chromosome abnormalities
14	38–40	50–53	3.5	5.5–6	22 somites + visceral arches	**Egg-transmitted infectious diseases**
15	38–42	50–53	3.5–4	5.5–6	24–27 somites but becoming hidden	**Parenteral nutritional deficiencies** **Abnormal or aged sperm**
16	39–43	51–56	3.4–4	6	Tail and wing buds forming	**Idiopathic developmental abnormalities**
17	40–49	52–64	4	6–7	Leg buds forming, amnion closing	**Drugs, toxins, pesticides**
18	55	3 days	5	8	Allantois forming	**Cracked eggs or small holes in eggs**
19	55–64	3–3.5	5–6	8–9.5		
20	55–64	3–3.5	5–6	8–9.5	Leg buds larger than wing buds	**Parenteral nutritional deficiencies**
21	64	3.5	6	9.5	Faint eye pigment	Riboflavin, vitamin B12, folic acid, biotin, manganese, pyridoxine, pantothenic acid, phosphorous, boron, linoleic acid, vitamin K, vitamin D
22	64–73	3.5–4	5–7	9.5–11	Distinct eye pigment, limbs elongating	
23	3 days	4	7	11		
24	3.5	4.5	7.5	12		**Secondary vitamin deficiencies**
25	3.5–4	4.5–6	7.5–8	12–14	Elbow and knee joints distinct	Antibiotic therapy destroying vitamin-producing flora
26	4	5	8	14		Diet imbalances, inadequate food intake
27	4	5–5.5	8–9	14	Digital grooves distinct	
28	4–4.5	5.5–6	9–10	14–16		**Viral diseases**
29	4.5–5	6–6.5	10–11	16–18		Newcastle diseases, infectious bronchitis
30	5–5.5	6.5–7	11–12	18–19	Egg tooth, 2 scleral papillae, limbs bent	**Bacterial infections**
31	5.5–6	7–7.5	12	19–20	Dorsal feather germs	*Salmonella, staphylococcus, streptococcus,* and *E. coli*
32	6	7.5	12.5	20	6 scleral papillae	
33	6	7.5–8	12.5–13	20–22	13 scleral papillae	**Fungal infections**
34	6	8	13	22	Nictitating membrane visible	**Poor handling of egg before or during first days of incubation**
35	6.5–7	9	14–15	23–24	Ventral feather germs, eyelids begin closing	Egg jarring or shaking in the first trimester
36	7.5	10	17	27	Flight feather germs	
37	8	11	18	30	Scale primordia visible	**Incubator faults**
38	9	12	20	33		Incorrect turning, temperature, humidity, and ventilation
39	10	13	22	35	Eyelids nearly closed	
						Inbreeding resulting in lethal genes

TABLE 68.1 Avian Embryonic Development and Possible Causes of Embryo Death—cont'd

H & H Stages*	Hr/day (16 DI**)	Hr/day (21 DI)	Hr/day (35 DI)	Hr/day (57 DI)	Development Landmarks	Causes of Death or Abnormalities in Embryos
40	11	14	23	38	Nails cornified, plantar papillae	**Lethal malpositions** Inadequate or incorrect turning—Abnormal egg size or shape—Incorrect incubator temperature
41	11.5	15	25	41		**Incubator faults** (Poor incubator ventilation—Egg cooling early in incubation—Inadequate/incorrect turning, temperature, or humidity)
42	12	16	27	43		**Incorrect hatcher temperature or humidity**
43	13	17	28	46		**Long storage time preincubation**
44	14	18	30	49	Yolk sac external	**Infectious disease**
45	14–15	19–20	32–33	52–54	Yolk sac halfway in	**Nutritional deficiencies** (Vitamin A, D, E, K, pantothenic acid, folic acid)
46	16	21	35	57	Hatched	**Lethal genes**
						Chromosomal abnormalities
						Idiopathic developmental abnormalities

*H&H stages (Hamburger and Hamilton Stages).[24]
**DI (Day of Incubation).
Modified from references 24 and 25.

TABLE 68.2 Types of Late Stage Embryo Malpositions

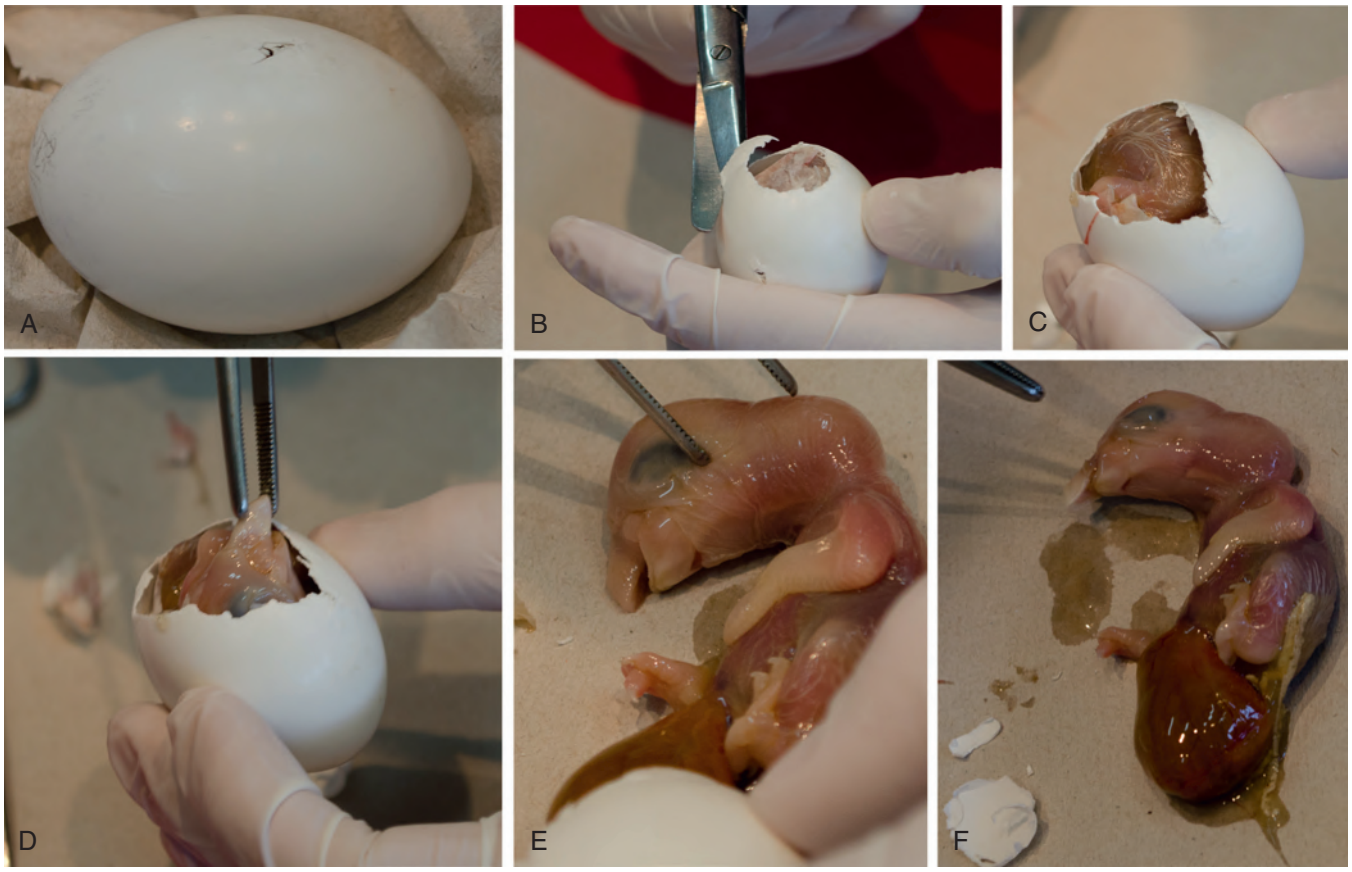
Description of correct positions: Head at large end near air cell, head tucked under right wing upside down

Avian embryo malpositions.

Malposition	Description	Lethality	Cause
1	Head between thighs. Failure to lift, turn head right in last trimester	Lethal	High incubation temperature
2	Head, small end of egg, chick upside down	50% lethal, assist hatch	Incubator egg position, low temperature
3	Head under left wing, rotates head left (not right). Body rotated on egg long axis	Usually lethal	Incubator egg position, temperature, parent malnutrition
4	Beak (maxilla egg tooth) away from air cell	Slightly low hatchability	Incubator position
5	Feet over head	Usually lethal	Embryo cannot kick/rotate for hatching
6	Head over right wing. Head in same plane as wing psittacines	Slightly low hatchability	Parent malnutrition
7	Embryo crossways in egg, often have other defects	Fatal	Small embryos, spherical eggs

Modified from reference 27.

and late dead embryo (LDE—H&H stage 40–45). Different types of causes that are related to possible death are associated with the different development stages (see Table 68.1). Bacteria associated with embryonic death include species of *Salmonella, Mycoplasma, Staphylococcus, Chlamydophila,* and *E. coli.* Of the fungal causes, *Aspergillus* is the most common cause of egg death. Of the viral causes, herpes viruses and avian paramyxovirus are the most commonly reported. Toxic causes of egg death include oil, insecticides, herbicides, nicotine, and selenium.[27] Antibiotic use, including chloramphenicol, penicillin, tetracycline, oxytetracycline, aminoglycosides, and sulfas, has also been implicated in embryonic death.[27]



• **Figure 68.1** Hyacinth macaw *(Anodorhynchus hyacinthinus)* egg necropsy with malposition 5. (A) Pip-to-hatch from the middle of the egg and not the air cell. (B) Creating an aperture with scissors for the air cell. (C) Malposition 5 (upside down). (D) Extraction of the dead embryo. (E) Prominent head and neck edema. (F) Failure to absorb the yolk sac. (Photography courtesy Temaiken Foundation.)

Neonatology

Birds may be classified according to their state of maturity at hatching as either precocial or altricial. Precocial (nidifogous) birds, such as Rheiformes, Galliformes, Gruiformes, Tinamiformes, and Anseriformes, are covered entirely by feathers, have eyes and ear canals open, are capable of moving with great ease and independence, and feed themselves at hatching. Altricial (nidicolous) species, such as Psitaciformes, Passeriformes, Columbiformes, Piciformes, Falconiformes, Strigiformes, and Ciconiiformes, are helpless at hatch. Most altricial birds are born without feathers, with their eyes closed, and depend totally on their parents for food and warmth.[32]

Hand-raising birds is a technique that has several applications. The most common are (1) breeding for pets (individuals tend to be tame and sociable with humans), (2) increasing egg production by encouraging birds to lay additional clutches, (3) raising offspring hatched from artificially incubated eggs, (4) saving sick or abandoned offspring, and/or (5) reducing the burden of parental care on a compromised parent and prevent or reduce the transmission of diseases from the parents to the neonate.[32] Strategies for carrying out the hand-rearing of a bird depend largely on which of the two groups it belongs to. When establishing a hand-rearing protocol, specific species needs should be considered based on previous knowledge and experience. Recognizing if a bird is precocial or altricial will determine basic management guidelines for this type of bird, such as the selection of heat source during the first weeks of life (incubator or infrared lamp), choice of housing environment (intensive care units or different sizes of plastic boxes), substrate (tissue paper, wood shaving, nonslip mats), space for walking to exercise, provision of water, and feeding tools (syringe, spoon, feeding tubes, or clamp).

The feeding protocol must be detailed in terms of composition, temperature, quantity, and frequency. The infant should be monitored and weighed daily (always at the same time), keeping a daily record and developing a growth curve. Subnormal weight gain is the first nonspecific sign of growth problems. Sick neonates are often presented in critical condition, regardless of the underlying cause. They are frequently hypothermic, dehydrated, or hypoglycemic and may be septicemic.[32] The approach and treatment are

• BOX 68.2 Common Problems of Neonates

Failure to absorb the yolk sac
Stunted growth
Leg and toe deformities
Constricted toes
Stifle subluxation
Beak malformations (lateral deviation or mandibular prognathism)
Break trauma
Regurgitation
Esophageal or pharyngeal trauma
Crops stasis
Crop burns and fistulas
Air in the crop
Foreign body ingestion or impaction
Aspiration/brooder pneumonia
Eyelid malformation
Occluded ear opening
Malformed feather or feather stress bars
Intestinal intussusceptions
Hepatic hepatomas
Gout
Wine-colored urine
Hepatic lipidosis
Neck deformities
Congenital abnormalities
Viral diseases (polyoma; psittacine beak and feather; proventricular dilatation disease; Pacheco disease [herpes]; pox virus)
Microbial diseases (gram-negative infections with *E. coli, Klebsiella* sp., *Enterobacter* sp., *Salmonella* sp. and *Pseudomonas* sp.). *Chlamydophila* sp.
Parasitic diseases *(Trichomona*, *Giardia*, *Atoxoplasma)*
Fungal diseases (*Candida* sp.)

Data from references 2, 32, 33.

to address the clinical signs following the basic principles of avian emergency medicine and ensuring that the underlying cause is identified and corrected.[34,35] Box 68.2 details the pathologies most frequently observed in neonates. If the purpose of the hand-rearing is to reintroduce the bird to its natural habitat, it is recommended to limit as much human contact as possible during the different rearing steps, especially during feeding, because this will decrease the chances of imprinting. The use of special puppets or clothing for this purpose has been successful.

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SECTION 13

Marsupials

69

Tasmanian Devil Facial Tumor Disease

CAROLYN J. HOGG AND KATHERINE BELOV

The Tasmanian devil *(Sarcophilius harrisii)* is the world's largest marsupial carnivore. Devil populations have declined by up to 95% in some areas of the Australian island state of Tasmania since the emergence of the infectious cancer, devil facial tumor disease (DFTD) in 1996.[1] Since that time, a second clonal, infectious cancer in Tasmanian devils has been described, known as DFT2,[2] with the original form known as DFT1.

Signs and Symptoms

DFT1 tumors present as large, solid, soft tissue masses that ulcerate, first appearing on the head and/or neck regions (Fig. 69.1). Histologically, they form subepithelial expansile masses of round spindloid cells with abundant eosinophilic cytoplasm encased within a pseudocapsule. The tumor is locally aggressive and metastasizes in 65% of cases, 57% of these to the local lymph nodes. Tumors similar to those on the face may occur later in other parts of the body.[1] In contrast, the tumors from DFT2 are characterized by sheets of pleomorphic (amorphic to stellate and fusiform) cells arranged in a solid pattern. Periaxin is a known histochemical marker that is diagnostic for DFT1[3]; DFT2 tumors appear to be negative for periaxin, an immunohistochemical marker that is diagnostic for DFT1.[2]

Male and female devils are equally affected by DFTD, with animals younger than 2 years rarely affected. It is possible young animals are bitten less often or do not develop tumors due to the length of the incubation period of the disease. However, it has also been proposed that young devils are protected from DFTD by the presence of antimicrobial peptides in mother's milk and pouch,[4] as well as the interplay between puberty and the devil's immune system.[5] In areas where the disease has been present for a long period of time, populations of Tasmanian devils are still persisting (Save the Tasmanian Devil Program [STDP], personal communication).

Once clinical, the course of DFTD is rapid, with tumors enlarging from small nodules to large friable masses over the course of 2–3 months, and death usually occurs within 6 months.[6] Mortality is almost always 100%, mostly because of starvation as the tumor destroys facial bones and dental arcades. An immune response to DFTD has been noted in six wild devils at West Pencil Pine (northern Tasmania), with four of these devils showing signs of tumor regression, although these incidences are extremely rare.[7] At this time there is no treatment for DFTD, although new immunotherapy trials are giving promising results.[8]

Cause

DFT1 is a peripheral nerve sheath tumor that arose from a Schwann cell or Schwann cell precursor, because tumors produce the Schwann cell–specific myelio protein, periaxin.[9] Due to its recent discovery, the epidemiology of DFT2 is currently unknown. Tasmanian devils have 14 chromosomes. The cytogenetic profile of DFT1 differs markedly from the normal devil karyotype and is characterized by the absence of identifiable chromosome 2, missing X and Y sex chromosomes, and the presence of four marker chromosomes. The DFT1 tumor is evolving over time, with a number of different strains now present.[10] DFT1 tumor studies have the same chromosomal rearrangements, indicating their origin as clones from a rogue cell line and transferred between individuals as allografts.[11] The DFT2 cytogenetic profile is distinct from DFT1, in which all tumors described showed identical structural abnormalities, including the presence of additional material on chromosomes 1, 2, and 4, a deletion involving chromosome 5, and monosomy for chromosome 6. Both X and Y sex chromosomes are present.[2]

In theory, because the tumor cells are different from the host cells, they should be recognized as foreign and rejected. However, this is not the case, with lymphocyte infiltration into DFTD tumors rarely observed. Research has shown that devils do have a competent immune system, similar to that of other mammals.[12] Initially, it was thought that, due to low genetic diversity at the major histocompatibility complex (MHC) locus, the devil's immune system would not recognize the tumor as being foreign.[13] However, recent research has shown that the tumor is able to downregulate its MHC so it is undetectable by the devil's immune system.[14]

• **Figure 69.1** Tasmanian devil (*Sarcophilius harrisii*) devil facial tumor disease.

In the cases of DFT1, direct exposure to tumor cells is necessary for development, but that alone is not sufficient for the disease to occur. Damage to the skin or mucous membranes around the head and neck, as a result of fighting, scratching, and biting, is also required before DFTD may develop.[15] Most biting injuries occur between adult males and females during the mating season (February to March[16]). Aerosol and vertical transmission do not appear to occur.[17] The incubation period of DFT1 and DFT2 is unknown, but one animal developed DFT1 after 15 months in captivity without apparent exposure to a diseased devil (STDP, personal communication). It is for these reasons that there is a stringent biosecurity categorization protocol in place that requires devils to be at each biosecurity category for a period of 15 months.[18]

Devil Populations

Before the arrival of DFTD, Tasmanian devils did not usually breed before their second year,[16] with the majority of males and females breeding between 2 and 5 years of age. As devils age, their immune function decreases and their capacity to mount an immune response to DFTD declines[19]; as a result, mature devils are the first devils to be lost from a population with the arrival of the disease.[20] In areas where disease is present, there has been a rise in precocial (1 year old) females breeding.[20] It is unknown if this is driven by a reduction in devil density or an increase in resource availability, or a combination of factors.

DFTD was first observed in the northeast of the state and has progressively spread south and west, with affected devils now being found over much of Tasmania. The northwest of Tasmania still remains disease free as of 2017, and due to its remote location, the occurrence of the disease in southwest Tasmania is currently unknown. Initial modeling indicated that DFTD would cause the extinction of the Tasmanian devils within 25–30 years.[21] Although DFTD has been present in the Tasmanian ecosystem for more than 20 years, it does not disappear at low population densities. Populations in the northeast of the state where the disease was first documented are still persisting.

In response to the rise of DFTD, the STDP was established in 2003 and is a joint initiative between the Tasmanian and Australian federal governments. The STDP is currently undertaking an intensive annual monitoring program (2015–2020) across 10 locations to determine if devil populations are persisting, recovering, or going extinct. Concerns now exist over the devil's ability to recover in light of small population sizes, low species-wide diversity, and fragmentation across the habitat. The loss of the devil to the Tasmanian ecosystem would be catastrophic because it would allow for increases in feral cat numbers. Recent work has shown that the presence of Tasmanian devils does not change feral cat abundance but rather feral cats have more nocturnal foraging behavior in areas where devils have declined.[22] The impact of feral cats to Australian native fauna is well documented on the Australian mainland, with 22 mammal extinctions attributed to cats and an additional 75 mammal species threatened.[23] The overarching goal of the STDP is to ensure that devils remain an ecologically functional part of the Tasmanian ecosystem.

Initially, the progression of DFTD from east to west led researchers to assess the differences in genetic diversity across the state.[24] Despite the devil's low genetic diversity, some of the animals from the northwest area are genetically distinct from eastern animals, and it was thought that suitable habitat connecting the two areas had resulted in physical and genetic barriers.[25] However, because the latest research into landscape genetics of Tasmanian devils has shown that their dispersal and gene flow are not influenced by landscape features, the disease will keep moving across all areas of their range.[26]

Work into the development of a vaccine has been ongoing since the commencement of the STDP. Initial work into the study of two western devils that were injected with dead tumor cells showed that one devil mounted an immune response but the other did not. The devil that responded had MHC genes that were different than those of the tumor, thus recognizing it as foreign. Because the MHC genes of the other devil were similar to the tumor, it was believed that this was the reason for the lack of response. When challenged with live tumor cells, the devil that responded remained clinically unaffected, whereas the other devil developed signs of DFTD 12 weeks later. Since this work, there have been significant advances in our understanding of the disease and its interplay with the devil immune system.[27–33] Recent trials of an immunotherapy, consisting of deactivated DFTD cells, with captive devils has shown promising results,[8] and these trials are now being conducted with devils that are being released to mainland Tasmania. This may open the way for potential development of a vaccine; however, delivering that vaccine to the wild population will be challenging.

Studies to control the disease through selective culling began in 2004, when infected devils on the Forestier Peninsula were trapped and euthanized. After 12 months, fewer

• **Figure 69.2** Tasmanian devil *(Sarcophilius harrisii)* released onto Maria Island as part of the metapopulation in 2012.

animals with large tumors were being found and population density remained at 1.6 devils/km^2 compared with a similar area with no culling where devil density decreased from 0.9 to 0.6 devil/km^2 over this time period.[34] Analysis of the tumors from the commencement of the culling program and the end of the program showed that culling enhanced the evolution of the disease, making it more aggressive and difficult to identify.[31] Culling as a management tool for DFTD has now been abandoned.

Moving Forward

An insurance population of Tasmanian devils was commenced in 2006, with a goal of maintaining 95% wild-sourced genetic diversity for 50 years to enable reintroduction once wild populations had gone extinct.[35] Since the first devils were moved to the Australian mainland in 2005, the insurance metapopulation has grown to more than 700 devils (as of 2017) housed in zoos, group-house enclosures (0.5–3 ha), free-range enclosures (22 ha), Maria Island (115 km^2) (Fig. 69.2), and a fenced peninsula, Forestier Peninsula (300 km^2).[18] Recent studies into the management of the captive population have indicated that there is a reduction in productivity in individuals held in captivity for multiple generations,[36,37] that inbreeding is increasing,[38] and that generations in captivity impact a devil's ability to avoid vehicle strikes when released to the wild.[39]

If the Tasmanian devil does go extinct, there will be significant consequences for the Tasmanian ecosystem. Preventing the loss of this iconic marsupial carnivore requires careful planning and management and will need to use a variety of available options. A program in which conservation managers and research scientists have been working together to provide real-time data for management practice has been in effect since 2013.[40] This has allowed conservation management teams responsible for the persistence of the Tasmanian devil in the landscape to make adaptive management decisions based on empirical evidence. The development of a new adaptive metapopulation strategy will assist the STDP to achieve its goal of an ecologic, functional Tasmanian devil population persisting in the landscape. Research into both strains of DFTD is ongoing, with new information appearing frequently, and management practice adapting as new information comes to light. To keep abreast of the latest developments, please consult the STDP website www.tassiedevils.com.au.

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70

Medical Aspects of Potoroid Marsupial Conservation Translocations

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Introduction

Potoroids (bettongs and potoroos) are small (weight range, 875–3250 g), largely nocturnal, and principally mycophagous marsupials within the Family Potoroidae (Superfamily: Macropodoidea). There are eight extant species in Australia (Table 70.1), with a further three species that have become extinct since European settlement of Australia.[1] All extant potoroid species have undergone significant population declines and range contractions as a result of habitat degradation, fragmentation, and loss; altered fire management regimes; predation by introduced carnivores; and historical persecution as agricultural pests.[1]

Conservation Management

Gilbert's potoroo *(Potorous gilbertii)* and the brush-tailed bettong *(Bettongia penicillata)* have extremely restricted distributions in southwestern Australia. Gilbert's potoroo, Australia's most endangered mammal, was thought extinct until 1994, when a single population was discovered at Two Peoples Bay Nature Reserve in Western Australia. Two additional populations have been established through conservation translocations, and there were approximately 100 individuals in 2012. However, recent wildfires have been responsible for reducing the population to approximately 50 individuals.[2] The brush-tailed bettong, formerly one of Australia's most widespread marsupials, was reduced to four small remnant populations in Western Australia by 1970. Subsequent intensive conservation efforts saw significant increases in its population and distribution. However, since 1999 the population has declined by more than 90%. The leading hypothesis for the decline is increased vulnerability to predation by feral cats *(Felis catus)* as a result of disease.[3,4] The burrowing bettong *(Bettongia lesueur)* was extirpated from mainland Australia and found only on offshore islands prior to recent reintroductions to fenced reserves on the mainland.[2] The Eastern bettong *(Bettongia gaimardi)* was also extirpated from mainland Australia by the early 20th century and was found only on the island of Tasmania prior to recent mainland reintroductions. Conservation translocations have emerged as a key component of conservation management strategies for these species, and potoroids are among the marsupial species for which conservation translocations are most frequently undertaken.

The International Union for Conservation of Nature (IUCN) defines conservation translocations as "the intentional movement and release of a living organism where the primary objective is a conservation benefit"; conservation translocation is an overarching term that encompasses a range of conservation-related animal movements.[5] The most important conservation translocations in potoroid conservation management strategies are reintroductions, most frequently to predator-free fenced reserves, and assisted colonizations, most frequently to predator-free offshore islands. Reintroduction is defined as "the intentional movement and release of an organism inside its indigenous range from which it has disappeared" and assisted colonization as "the intentional movement and release of an organism outside its indigenous range to avoid extinction of populations of the focal species."[5]

Welfare Considerations

Conservation translocations are inherently stressful for the animals involved, and animal welfare should be accorded high priority and be an overarching theme during planning and implementation. Overall animal welfare outcomes may be improved by monitoring parameters that may be indicative of welfare such as morbidity and mortality, dispersal, and fecundity—parameters that are also indicative of overall conservation translocation success. Additional parameters that have been specifically monitored in potoroids include body weight; body condition index; physiologic variables such as hematology and biochemistry; and fecal glucocorticoid metabolite concentrations as a measure of stress physiology.[6–10] Monitoring physiologic variables provides baseline data that may be used for selection of suitable candidates for translocation and to adapt and refine conservation translocation methodologies—both of

TABLE 70.1 Extant Potoroid Species, Their Conservation Status, and Distribution

Common Name	Scientific Name	IUCN Red List Classification[5]	Current Distribution
Rufous bettong	*Aepyprymnus rufescens*	Least concern	Widespread in New South Wales and Queensland
Eastern bettong	*Bettongia gaimardi*	Near threatened	Tasmania, reintroduced to mainland Australia
Burrowing bettong	*Bettongia lesueur*	Near threatened	Islands off Western Australia, reintroduced to mainland Australia
Brush-tailed bettong	*Bettongia penicillata*	Critically endangered	Southwestern Western Australia
Northern bettong	*Bettongia tropica*	Endangered	Limited distribution in northeastern Queensland
Gilbert's potoroo	*Potorous gilbertii*	Critically endangered	Limited distribution in Western Australia
Long-footed potoroo	*Potorous longipes*	Vulnerable	Limited distribution in New South Wales and Victoria
Long-nosed potoroo	*Potorous tridactylus*	Near threatened	Fragmented distribution in southeastern Australia

IUCN, International Union for Conservation of Nature.

which enhance animal welfare outcomes through reduction and refinement. Factors influencing fecal glucocorticoid metabolite concentrations have been evaluated in captive brush-tailed bettongs and subsequently applied to a reintroduction program to evaluate stress physiology and host–parasite interactions.[6,7] Elevated fecal glucocorticoid metabolite concentrations have been demonstrated in eastern bettongs subjected to a period of captivity as part of a delayed release tactic during reintroduction (W Batson, personal communication, February 23, 2017). Evaluation of stress physiology is important in identifying key stressors in the conservation translocation pathway, and results may be used to inform adaptive management strategies and improve translocation outcomes.

Restraint, Anesthesia, and Analgesia

Free-ranging potoroids are typically caught in small, wire, cage traps baited with a mixture of rolled oats and peanut butter. Internal ceiling padding and external visual barriers are recommended on cage traps to reduce trap-associated trauma and minimize stress. On removal from traps, potoroids should be placed into soft cloth bags for subsequent handling. Although potoroids will tolerate short periods of manual restraint (up to 10 minutes) for examination and minimally invasive sampling (Fig. 70.1) without apparent adverse effects, anesthesia is recommended to minimize stress and allow for complete physical examination and sample collection. Inhalation anesthesia using isoflurane or sevoflurane in oxygen administered by mask is the preferred method of anesthetic induction; premedication is generally not required, but intramuscular (IM) administration of diazepam (1 mg/kg) or midazolam (0.3 mg/kg) may be considered in fractious or unduly stressed individuals. Potoroids are recovered from anesthesia in soft cloth bags but must be monitored and managed to prevent airway occlusion until sufficiently recovered.

• **Figure 70.1** Short periods of restraint for minimally invasive sampling such as blood collection from the lateral coccygeal vein are possible with potoroids restrained in soft cloth bags.

Ejection of pouch young is a risk when trapping, handling, and transporting female potoroids. Females with large pouch young should be excluded from conservation translocations, and the pouch of females with furless pouch young should be secured with adhesive tape for handling and transportation. The tape is left in situ at release with females invariably removing the tape after they have settled. In addition, females with two active mammary glands should be precluded due to the likelihood of an untrapped emergent or young at-foot joey that remains dependent on milk.

Administration of analgesic drugs is indicated where potoroids have sustained trapping-related injuries or been subjected to invasive sampling procedures during the conservation translocation process. Meloxicam (0.2 mg/kg subcutaneously (SC) q 24 hours), buprenorphine (0.01 mg/kg IM q 12 hours), and tramadol (5 mg/kg IM q 12 hours),

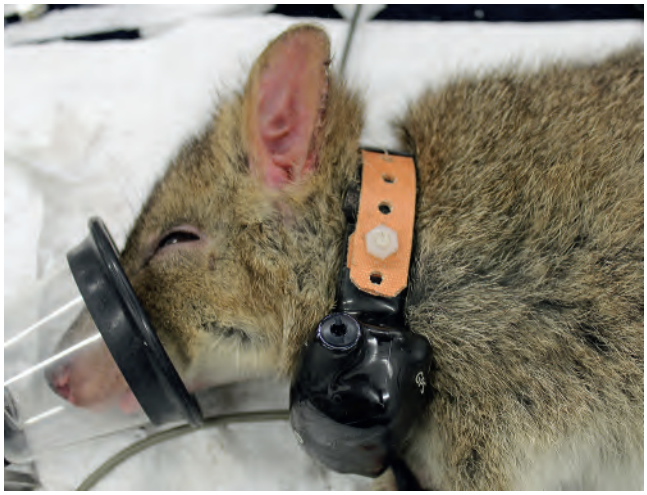
although unsupported by pharmacokinetic data, appear to be safe and efficacious choices for providing analgesia in potoroids. A topical proprietary preparation containing lignocaine, bupivacaine, epinephrine, and cetrimide has recently been used to provide hemostasis, analgesia, and antisepsis following ear-tagging and collection of ear punch biopsy samples for genetic analysis in reintroduced eastern bettongs.

Transportation

Potoroids are best transported in soft cloth bags suspended within adequately ventilated transport containers. Transportation should be timed to avoid high environmental temperatures. Although potoroids have been transported without the use of sedative or neuroleptic drugs, a study demonstrated marked elevations in creatine kinase in eastern bettongs transported by road and air for reintroduction.[9] The use of diazepam (1 mg/kg IM) in these animals appears to have ameliorated the effects of exertional myopathy.

Telemetry

Telemetry (very high frequency [VHF] and/or global positioning system [GPS]) is frequently used for postrelease monitoring, and veterinarians should have input into the design and be involved in the application of telemetry devices. Neck-fitted telemetry collars are most commonly used in potoroids (Fig. 70.2). The relatively short neck and prominent shoulders of potoroids predisposes them to collar-associated injuries, including alopecia, dermatitis, and ulceration associated with tight-fitting or excessively wide collars. Other potential injuries include forelimb entrapment in loose collars and entanglement of poorly designed antennas in vegetation. Careful attention should be paid to collar fit during application, with follow-up examination to assess for collar-associated injuries. Where possible, collars should be applied and assessed in captivity, where close monitoring can occur prior to field application.



• **Figure 70.2** GPS telemetry collar fitted to an anesthetized eastern bettong *(Bettongia gaimardi)*. (Courtesy Adrian Manning.)

Disease Risk Assessment

Although the principal reason most potoroid conservation translocations fail is predation by introduced predators, disease has the potential to significantly impact the success of these programs.[1] A comprehensive disease risk assessment is therefore recommended but beyond the scope of this chapter. Readers are referred to Chapter 2: Risk Analysis Framework Guidance for Wildlife Health Professionals by Travis and Smith in this volume and several recent reviews for detailed approaches to conducting disease risk assessments in conservation translocations.[11–13] Table 70.2 outlines some known and potential pathogens and parasites of potoroids that may warrant consideration during disease risk assessments for conservation translocations.

Health Evaluation and Disease Surveillance

Baseline health and disease data have been established for Gilbert's potoroo, brush-tailed bettongs, and eastern bettongs, but such data are limited for other potoroid species.[9,10,14–16] Comprehensive health evaluations serve several functions and are recommended for all potoroid conservation translocations. Baseline health and disease parameters may be applied more broadly to the conservation management of the potoroid species in question. These data can also be used to inform disease risk assessments and mitigation strategies and form the basis for longitudinal monitoring of translocated populations. Finally, health evaluations can be used as a basis for selecting candidates of an appropriate health status, maximizing both conservation and animal welfare outcomes.

Standardized assessment protocols and accurate record keeping are vital to ensure consistency throughout the process and for accurate comparisons during experimental evaluation of conservation translocation techniques. Physical examinations should include collection of morphometric data such as body weight, pes, and head length; collection of blood for hematologic and biochemical analyses; collection and identification of ectoparasites; collection of hair or feces for stress physiology studies; collection of feces for evaluating endoparasite ova/oocysts; and collection of tissue samples for genetic analysis.

Disease screening in potoroid conservation translocations should be based on the outcome of a project-specific disease risk assessment. However, the availability of validated testing methodologies for a given pathogen and logistical and resource limitations will also influence the choice of diseases screened for. The small size of potoroids limits the volume of blood that may be safely collected, further necessitating prioritization of diagnostic testing.

TABLE 70.2 Known and Potential Pathogens and Parasites of Potoroids That May Warrant Consideration in Disease Risk Assessment During Conservation Translocations

Pathogen or Parasite	Diagnostic Testing Options
Viruses	
Potoroid herpesvirus-1 (eastern bettong) Novel gammaherpesvirus (brush-tailed bettong)	Conjunctival, nasal, oropharyngeal, and urogenital swabs—pan herpesvirus PCR
Orbiviruses (Wallal, Warrego, Eubenangee serogroups)	Serum—serum-virus neutralization assay (Wallal and Warrego serogroups) Fresh tissue samples—PCR, virus isolation Formalin fixed tissue samples—PCR, immunohistochemistry
Bettongia penicillata papillomavirus type 1	Fresh or frozen skin samples—PCR
Encephalomyocarditis virus	Serum—serum neutralization test Tissue samples—virus isolation
Bacteria	
Treponema species (Gilbert's potoroo)	Urogenital swabs—bacterial culture, darkfield microscopy
Bordetella bronchiseptica	Nasal swabs, tracheal wash, bronchoalveolar lavage—bacterial culture
Salmonella species, *Campylobacter* species	Tissue samples, feces—bacterial culture
Mycobacterium species (typically captive animals)	Tissue aspirates, bronchoalveolar lavage samples—acid fast stains, culture, and PCR
Mycobacterium ulcerans (long-footed potoroo)	Tissue samples—histopathology, culture, and PCR
Leptospira species	Serum—microscopic agglutination test, indirect hemagglutination assay, ELISA Urine—darkfield microscopy, culture, PCR
Fungi	
Cryptococcus neoformans, *C. gattii*	Serum, cerebrospinal fluid—latex cryptococcal antigen agglutination test Tissue samples—culture, PCR, immunohistochemistry
Protozoa	
Trypanosoma copemani, T. vegrandis, T. noyesi	Blood smears—light microscopy (low sensitivity) Whole blood—PCR combined with Sanger sequencing
Theileria gilberti, *T. penicillata* Uncharacterized *Theileria species*	Blood smears—light microscopy (low sensitivity) Whole blood—PCR targeting the 18S rRNA gene
Toxoplasma gondii	Serum—modified agglutination test, indirect fluorescent antibody test, ELISA Formalin fixed tissue—histopathology, immunohistochemistry, PCR
Eimeria potoroi, E. mundayi, E. aepyprymni, E. gaimardi, Eimeria burdi	Feces—fecal floatation
Helminths	
Eucoleus potoroi	Feces—fecal floatation to identify capillarid eggs Bronchoalveolar lavage—microscopy
Angiostrongylus cantonensis	Fresh brain or meninges—microscopy Fixed brain—histopathology
Arthropods	
Ixodes species, *Haemaphysalis* species, *Amblyomma* species	Physical examination—microscopy
Thadeua greeni	Skin scraping—microscopy
Heterodoxus species, *Paraheterodoxus* species	Physical examination—microscopy
Echidnophaga species, *Pygiopsylla* species	Physical examination—microscopy

Diseases of Captive and Free-Ranging Potoroids

Management of pathogens and parasites in potoroid conservation translocations is important to reduce morbidity and mortality in individuals subjected to the multiple stressors of conservation translocation and to protect populations of conspecifics (if present) and sympatric species in the recipient ecosystems. Potential pathogens and parasites of concern will vary depending upon the origin of the source population (e.g., captive bred, free-ranging, or a mix of both). Decision making around management of pathogens and parasites must be made in light of the fact that many have coevolved with their hosts and the growing recognition of the important ecologic role played by pathogens and parasites. The decision to exclude an animal from a conservation translocation or initiate treatment due to evidence of exposure or the presence of a particular pathogen or parasite should be determined based on the results of a project-specific disease risk assessment.

Diseases of Captive Potoroids

Diseases that have been associated with significant morbidity and mortality in captive potoroids include herpesviral hepatitis characterized by a peracute course and high mortality in brush-tailed bettongs and rufous bettongs *(Aepyprymnus rufescens)*[17]; pulmonary and systemic cryptococcosis in Gilbert's potoroos, long-nosed potoroos *(Potorous tridactylus),* and brush-tailed bettongs[18]; mycobacteriosis associated with various nontuberculous *Mycobacterium* species, including *M. avium* and *M. intracellulare,* in brush-tailed bettongs, long-nosed potoroos, and long-footed potoroos *(Potorous longipes)*[19]; and verminous bronchitis, bronchiolitis, and pneumonia associated with the capillarid nematode *Eucoleus potoroi* in long-nosed potoroos, rufous bettongs, and brush-tailed bettongs.[19] These diseases are most likely the result of factors associated with captivity that alter the host–pathogen and host–parasite relationship, such as higher stocking densities, suboptimal nutrition, exposure to novel pathogens, anthropogenic stressors, and other husbandry-related factors. Where captive-bred individuals constitute the source population for a conservation translocation, screening for these diseases should be considered.

Diseases of Free-Ranging Potoroids

A high prevalence of *Trypanosoma* species has been observed in brush-tailed bettongs, and trypanosomiasis has been postulated as a potential contributing factor to the recent decline of this species, although causality has not been proven. *Trypanosoma copemani* (clade A) and mixed infections with multiple *Trypanosoma* genotypes have been associated with pathologic changes, including degeneration and necrosis of skeletal and cardiac muscle.[3] Variable spatial prevalence of *Trypanosoma* species across geographically isolated populations of brush-tailed bettongs and demonstrated pathology in infected bettongs suggest these parasites should be carefully considered in brush-tailed bettong conservation translocations.

Other pathogens that have been associated with disease in free-ranging potoroids include balanoposthitis and dyspareunia associated with an uncharacterized *Treponema* species in Gilbert's potoroo[14]; *Mycobacterium ulcerans* associated with ulcerative skin lesions in long-footed potoroos[19]; papillomatous skin proliferations associated with *Bettongia penicillata* papillomavirus type 1 in brush-tailed bettongs[20]; and pulmonary cryptococcosis in eastern bettongs. In some instances these pathogens have not been well characterized and their prevalence may not be established across populations, warranting their consideration in conservation translocations.

Postrelease Monitoring

Longitudinal monitoring of health and disease has been infrequently conducted in potoroid conservation translocations. Investigation of postrelease morbidity and mortality is important because it informs decision making within an adaptive management framework in subsequent phases of the project. Monitoring of physiologic variables may also provide information on the response of individuals and the population to conservation translocation beyond the traditional metrics of survival, dispersal, and fecundity. Longitudinal health monitoring in a population of reintroduced eastern bettongs demonstrated changing health status of bettongs over time, including changing ectoparasite assemblages, changes to body weight, and changes to various hematologic and biochemical parameters indicative of changing nutritional status.[10] Such findings highlight the value of health monitoring beyond the immediate postrelease and establishment phases of potoroid conservation translocations.

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Macropod Pediatric Medicine

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Introduction

Macropod pediatric medicine is concerned with the health, growth, and development of neonatal, juvenile, and subadult members of the marsupial Family Macropodidae. This Family includes kangaroos, wallabies, wallaroos, pademelons, and the quokka *(Setonix brachyurus)*.[1] The spectrum of health problems encountered in pediatric patients differs from that of adults and the animal's response to illness and stress varies with age, stage of development, and method of rearing.

Given the highly altricial nature of macropod young at birth and their specialized environmental requirements, factors such as small size, unique physiology, and developing immunocompetence are important considerations in the assessment of health and the diagnosis and treatment of illness.

Normal Postnatal Development

Neonatal macropods lack a functioning adaptive immune system, weigh less than 1 g, and resemble eutherian embryos.[2] After birth they crawl from the cloaca to the pouch where they attach to a teat to continue development within a nonsterile environment. Early pouch young are ectothermic with a relatively low metabolic rate.[3] Suckling is continuous initially, but becomes progressively intermittent after approximately the first third of pouch life. Ambient conditions (e.g., temperature, high humidity) within the pouch are stable. Milk composition changes dramatically over the course of lactation with lipids, protein, and energy content increasing over time and carbohydrates decreasing at the point of pouch emergence.[2,4]

Although cutaneous respiration has been documented in newborn macropods, their simple lungs are capable of gas exchange.[5] The lungs are composed of short branching airways that terminate in large saccules. Lung development is generally slow; the saccules initially become subdivided by septal crests and decrease in size. In the tammar wallaby *(Notamacropus eugenii)*, the first true alveoli are present at 65 days of age and a typical alveolized lung structure, characterized by the presence of respiratory bronchioles, alveolar ducts, and alveolar sacs, can be seen at 142 days of age.[5] The attainment of alveolized lung corresponds with a dramatic increase in lung surface area and an increase in metabolic rate.[6]

At birth, the marsupial liver is actively hematopoietic and a variety of cell types are present at various stages of development including those of the erythrocytic, granulocytic, and leukocytic lineages. Throughout pouch life hematopoiesis declines in the liver and the bone marrow takes over the role while the liver structure matures and becomes largely restricted to gastrointestinal-related functions.[7] Pouch young have a form of hemoglobin adapted to lower oxygen and higher carbon dioxide concentrations than ambient levels. Renal function is limited in the early pediatric phase and is compensated for by continuous oral intake of milk.

Gastric ghrelin secretion, important for regulating appetite, begins shortly after birth in the developing wallaby during a period of rapid hypothalamic growth.[8] The establishment of a population of commensal gastrointestinal microorganisms is expected to protect the host from infection and colonization by pathogenic organisms and is important for normal gastrointestinal development.[9] The microbiome of highly immature pouch young still permanently attached to the teat has been shown to be surprisingly diverse.[10] Milk from later stages of lactation and herbage consumed by the emerging pouch young may play independent roles in the various stages of forestomach maturation, resulting in an effective forestomach fermentation system capable of digesting vegetation.[11]

Recent work has demonstrated that the marsupial immune system is complex and on par with that of eutherian mammals.[12] The pouch contains a broad range of gram-positive and gram-negative bacteria. Complementary protective mechanisms are provided by the innate immune system and an assortment of maternal protection strategies, such as immune compounds in milk, prenatal transfer of immunoglobulins, antimicrobial compounds secreted in the pouch, and chemical or mechanical cleaning of the pouch and pouch young. The paired cervical thymi, which are readily visible on examination of hairless pouch young, and the smaller thoracic thymus play an important role in the maturation of T-cells and in cell-mediated immunity.[9,13] Innate immunity involves the production of antimicrobial peptides such as cathelicidins, which kill a broad range of

bacteria.[14] Pouch young are able to synthesize these soon after birth to complement those present in milk.[15,16]

The development of endothermy in marsupial young begins at least halfway through pouch life concurrent with the initiation of thyroid function. In the tammar wallaby this transition occurs between 55 and 200 days of age at a mass of 70–300 g.[3] A myriad of morphologic, physiologic, and behavioral changes that allow for endothermy and the maintenance of body temperature occurs across this time period.[17]

Approach to the Pediatric Macropod Consultation

History

The clinical evaluation relies heavily on thorough history-taking. Box 71.1 provides a checklist of important factors that should be discussed during initial patient assessment. Mimicking the environmental factors necessary for normal development may be challenging, especially if the owner or carer lacks experience. Identifying husbandry deficiencies can be instrumental in achieving a diagnosis and developing a therapeutic plan.

BOX 71.1 History-Taking Checklist for the Macropod Pediatric Consultation

- Signalment
  - Species
  - Sex
  - Stage of development, for example, pouch-dependent age factor <0.4, 0.4–0.6, 0.7–1.0 (see text); emerged (i.e., at-foot); weaned
- Weigh and take body measurements
- Presenting complaint
  - Duration and clinical course (improving, deteriorating, stable)
- Previous medical problems and/or treatments (drugs, dose and duration)
- If orphaned, reason for orphaning (e.g., maternal death or rejection, illness or injury, unknown, intentional) and age/age factor/weight at time of separation from the mother
- If wild, time since rescue and rescue location
- Housing (e.g., artificial pouch, indoor room, grassed yard)—obtain detailed description
- Temperature (of artificial pouch or ambient if emerged)
- Diet
  - Formula type, dilution rate, volume per feed, frequency of feeds
  - Method of feeding (bottle-fed, lapping from bowl)
  - Types and quantities/proportions of solids offered
  - Supplements
  - Hygiene routine (e.g., frequency of bottle cleaning, pouch changes, disinfectants used)
  - Skin care (if hairless)
  - Toileting routine
  - Contact with other animals (conspecifics; wild and domestic animals of other species)
  - Proposed fate of the animal (zoo collection, return to wild, pet)

For animals undergoing hand-rearing, carer or owner notes on growth, behavior, feeding, and toileting should be inspected. If such notes are not routinely kept, the carer/owner should be encouraged to do so.[4] In circumstances where aspiration of formula is suspected or feeding has been problematic, inspection of the feeding equipment including teat and hole size and observing feeding technique can be informative.[18] Skin moisturization protocols and artificial pouch conditions should be assessed for furless joeys. The length of time in care may be relevant as orphans lose most maternal immunoglobulins by 4–6 weeks after separation from the mother and are left protected only by an underdeveloped active immune system.[19]

Physical Examination and Assessment

The examination should take place in a warm room with pre-warmed hands in the majority of circumstances. For orphaned pouch-dependent young, the bulk of the examination is best conducted while they are held within an artificial pouch. If the joey is still with the mother, maternal sedation may be required.

Estimating age and determining whether the animal's behavior is appropriate for the stage of development are vital steps in the assessment process.[20] Knowledge of expected growth trajectories and developmental milestones of a given species is necessary for interpretation of findings. Species-specific growth charts are available online.[21]

The age factor is defined as the age as a proportion of total expected pouch life, for example, an age factor 0.7 pouch young has completed 70% of expected pouch life. As a general rule, the intensity of care necessary for marsupial pouch young at an age factor of less than 0.4 or while still in the fixed lactation phase is substantial and the chance of successfully hand-rearing a pouch young from this stage to adulthood is extremely low. If an animal of this size cannot be returned to the mother's pouch or cross-fostered (see later), euthanasia should be considered.

A thorough physical examination includes measurement of vital parameters and assessment of skin health (especially if furless), hydration status, and gait (if emerging or emerged). Critical issues that require immediate stabilization are summarized in Table 71.1.

For critically ill animals, obtaining a blood sample for glucose and electrolyte assessment is valuable. Generally additional diagnostic tests such as radiography or other imaging, complete blood count, serum biochemistry panel, and sampling for cytology and/or culture may be postponed until the stabilization process has commenced.

In situations where a pediatric patient can be assessed, treated, and quickly returned to a healthy mother capable of providing adequate care, that is usually preferable to hand-rearing. Partial temporary closure of the pouch orifice with tape may reduce the risk of the pouch young being evicted in the immediate recovery phase. This approach secures the joey while allowing the dam to be able to access the pouch for cleaning.

TABLE 71.1 Critical Issues in Macropod Pediatrics That Require Immediate Stabilization

Problem	Action Required
Hypothermia (cloacal temperature <35°C)	Slowly correct over a 2–3 h period Do not feed as digestion will be impaired Warming devices, for example, heating pads, forced heat blankets, hot water bottles, humidicribs, brooders, and heat lamps may be used to restore normothermia; aim for an ambient temperature of 32°C–34°C; care must be taken to avoid overheating via such methods
Hypoglycemia (blood glucose <3.2 mmol/L or 60 mg/dL)	If alert and normothermic, offer formula If unable to feed, give 1 mL/kg of 12.5% dextrose IV (i.e., dilute 50% dextrose 1:4) followed by a CRI of isotonic fluids supplemented with 1.25%–5.0% dextrose Resume feeds when stable
Dehydration/hypovolemia	If alert and normothermic, offer oral rehydration solution and formula If unable to replace losses through adequate oral intake, SC and/or IV fluids should be given and consider placement of NG tube Note: fluid pumps, Buretrol sets or syringe drivers are recommended as rapid IV fluid administration is not well tolerated
Sepsis	Supportive measures such as warmth and nutrition Parenteral antibiotic cover ideally on the basis of culture and sensitivity (broad spectrum if empirical)
Pain	Provision of adequate analgesia, for example, opioids for moderate to severe pain Avoid nonsteroidal antiinflammatories if dehydrated

CRI, Continuous rate infusion; *IV,* intravenous; *NG,* nasogastric; *SC,* subcutaneous.

Clinical Pathology

Organ function increases during postnatal development, and there are corresponding variations in enzyme levels and products related to normal metabolism and filtration over time. Care should be taken when utilizing adult reference ranges to evaluate clinical pathology parameters. Lower red cell and globulin levels and elevated phosphorus and alkaline phosphatase are common in healthy juveniles compared to adults.[22] Early pouch young have fetal hematologic characteristics with up to 100% nucleated erythrocytes at 1 day of age in some species.[23] Macropod young with a history of dehydration should have their renal function evaluated via serum biochemistry and urinalysis where practical.

Pharmacology/Toxicology

In the absence of specific studies, it is difficult to predict the effect of age on pharmacokinetics and toxicology.[24] Care is advised, however, when prescribing drugs that undergo extensive hepatic metabolism or renal elimination in pediatric patients due to immaturity of organ systems. Differences in albumin levels in juveniles compared to adults may influence the bioavailability and the half-life of chemicals that are highly protein bound. Young brush-tailed rock-wallabies *(Petrogale penicillata)*, for example, have been documented to have higher albumin levels than adults.[25] Additionally, dietary composition (milk/formula and/or different types of solids) may interact with orally ingested toxins and medications and influence bioavailability in unpredictable ways. For this reason the author prefers injectable formulations in most instances.

Anesthesia

Pediatric patients have a tissue oxygen demand that is greater than that of adults, excluding early marsupial pouch young that have yet to achieve endothermy. As such there is a higher risk of hypoxemia and rebreathing of carbon dioxide. To help combat this, higher fresh gas flow rates and non-rebreathing systems should be used. The higher minute volume of young animals can influence volatile anesthetic agent absorption, and thermal extremes need to be avoided as these further increase minute volume.[26]

Inhalant anesthetic agents are most commonly used via mask induction. Pediatric patients are predisposed to hypoventilation and airway collapse in the presence of respiratory depressant drugs such as opioids and inhalational agents, so particular caution needs to be exercised in monitoring and supporting respiration. Smaller airways are more prone to obstruction; intubation is possible but in small macropods may be challenging.

Prolonged fasting prior to anesthesia should be avoided. Similarly a feed should be given as soon as it is safe to do so after recovery. Dextrose supplementation in intravenous crystalloid fluids may be needed to overcome the risk of hypoglycemia during prolonged anesthetic events.

Common Presentations

Common conditions encountered in macropod pediatric practice are summarized in Table 71.2.

TABLE 71.2 Clinical Signs, Diagnosis, and Treatment of Common Macropod Pediatric Conditions

Condition	Clinical Signs	Causes	Diagnostic Tools	Treatment
Failure to thrive	Poor weight gain and/or skeletal growth	Malnutrition • Inappropriate diet offered • Insufficient feedings • Incorrect temperature of feed • Poor suck reflex • Inappropriate thermal environment • Psychological stress* • Inappropriate equipment • Disease, for example, GI disease causing malabsorption	Detailed husbandry and diet review Physical examination Fecal examination CBC, biochemistry Work-up for systemic disease based on other clinical signs, for example, imaging	Modification of diet Nutritional support (may include NG tube feeding) Supplement with Impact (bovine colostrum supplement, Wombaroo, Glen Osmond, South Australia) Modification of environment, for example, additional thermal support Treat underlying disease
Diarrhea (noninfectious)	Soft, loose, or watery fecal matter incompatible with stage of development Increased frequency of bowel motions Cloacal/rectal prolapse	Inappropriate diet, for example, feed containing lactose Poor feeding management (sudden changes in diet, e.g., after orphaning, transitioning between formulae or at weaning; incorrect feed temperature, excessive feed volumes, irregular routine) Poor hygiene Environmental stress* Foreign body ingestion	Obtain detailed description of duration, type, and nature of diarrhea Consider stage of development Investigate possible husbandry issues Abdominal imaging if indicated	Correct hydration deficits Consider electrolyte supplementation (e.g., between formula feeds) Good nursing/hygiene Barrier creams to cloaca to prevent skin maceration Surgery (if obstructive GI disease or prolapse diagnosed)
Diarrhea (infectious)	Soft, loose, or watery fecal matter incompatible with stage of development Increased frequency of bowel motions Cloacal/rectal prolapse	Bacteria • *E. coli* • *Klebsiella* • *Salmonella* • *Clostridium* • *Yersinia* Yeast • *Candida* • *Torulopsis* Protozoa • *Eimeria* • *Cryptosporidium*	Rule out noninfectious causes (see above) Fecal direct exam, Gram (± other) stain, and fecal flotation Fecal culture if not responsive to husbandry modification and supportive care	As above + Treat specific pathogens if identified If antibiotics are prescribed, consider concurrent prophylactic anti-yeast therapy Plasma transfusion from healthy adult of same species
Acute abdomen	Abdominal distension Marked abdominal pain	Bloat Gastrointestinal obstruction Severe ileus	Review husbandry and nutrition Physical examination Plain ± contrast abdominal radiography/ sonography	Stabilize If fails to respond to medical therapy (fluids, analgesia, gastroprotectants, motility agents), consider surgical exploration Dietary correction
Skin disease	Dry, rough cracking scaly skin Localized inflammation Alopecia Poor hair coat Cutaneous swellings	Inadequate moisturization Self-sucking of a body part Environmental stress* Inappropriate pouch environment Malnutrition Ectoparasites Bacterial, fungal, or viral lesions	Signalment History Skin scrapes Biopsy for histopathology and/or culture	Correct husbandry Routine moisturizer application Omega 3 supplementation Provide a dummy (e.g., teat) Bandaging Antimicrobials if appropriate

Continued

TABLE 71.2 Clinical Signs, Diagnosis, and Treatment of Common Macropod Pediatric Conditions—cont'd

Condition	Clinical Signs	Causes	Diagnostic Tools	Treatment
Traumatic fractures	Lameness, swelling Inability to stand	Trauma	Assess environment for risks (substrate, enclosure size) Radiography	Reduce fractures according to small animal orthopedic principles Euthanize if fracture type/location will result in significant dysfunction in a wild animal or long-term welfare concerns for a captive animal
Pathologic fractures	Lameness, swelling in absence of known trauma Inability to stand	Metabolic bone disease • Inadequate calcium intake (inappropriate formula) • GI disease resulting in malabsorption • Vitamin D deficiency • Inadequate or improper exercise for stage of development	History and husbandry/dietary review Review medical history Nutritional analysis of feed	Fracture reduction Pouch rest Calcium/Vit D supplementation and dietary correction Euthanasia may need to be considered for severe cases
Respiratory disease	May be subtle (e.g., lethargy, inappetence) Dyspnea Nasal discharge Respiratory stertor	Rhinitis Aspiration pneumonia Infectious pneumonia	History and husbandry review Physical examination Thoracic radiography Culture and sensitivity Endoscopy	Oxygen therapy Antimicrobials ideally on the basis of culture and sensitivity (oral, nebulized, systemic)
Cataracts	Clouding of the lens of one or both eyes	Trauma Nutritional issues, for example, galactosemia Metabolic disease Infection Congenital	History and husbandry review (especially nutritional) Thorough ophthalmic exam Serum biochemistry Urinalysis	Rarely may resolve spontaneously Surgery but prognosis is guarded due to high risk of post-operative complications in some cases depending on degree of pathology and surgical technique
Oral disease	Inappetence Facial swellings Oral plaques Loose or fractured teeth	Candidiasis Malocclusion Dental or soft tissue bacterial infections	History and husbandry review Thorough oral examination (may require anesthesia)	Fluid and nutritional support if unable or unwilling to feed Treat underlying problem
Sudden death	Death without premonitory clinical signs	Various, for example, tetanus, toxoplasmosis, GI accident, trauma, aspiration	Careful evaluation of husbandry and history Necropsy examination	Correct any predisposing factors for other animals in group on basis of necropsy exam results

*Psychological and environmental stressors include an insecure pouch environment, excessive handling, petting and playing with children, contact with unfamiliar environments and noises, sudden changes in routine (e.g., prolonged pouch deprivation during the process of pouch weaning), sudden changes in feeding schedule, sudden withdrawal of contact with carer or buddy. *CBC,* Complete blood count; *GI,* gastrointestinal; *NG,* nasogastric tube.

Infectious Diseases

The developing immune system has a reduced capacity to respond to infectious insults compared to that of adults. Infectious diseases may be acquired from the environment, from in-contact animals, or from human carers. Vertical transmission of some diseases is also possible (e.g., toxoplasmosis).[27] Hand-reared macropods are considered at increased risk of infectious disease because they do not receive the protective factors present in maternal milk, and the natural pouch environment is impossible to replicate perfectly in a care situation. If an infectious disease is suspected, careful evaluation and elimination of predisposing factors is important.

Preventative Medicine

The focus of a preventative medicine program is to ensure the mother's health is optimized, or in the case of orphans, to encourage meticulous husbandry conditions. Quality

nutrition for both pouch-dependent and emerged young is vital. Macropods have been successfully hand-raised on various formulas.[4,18] Appropriate feed volumes and frequency, utensil hygiene, and maintenance of a quiet, clean, secure, thermally suitable environment are as important as the formula type used. Offering a formula that closely mimics the changes in natural milk composition is considered most appropriate. At the point of emergence, macropods need access to vegetation or forage that nutritionally matches their natural feeding ecology (grazing and/or browsing). Many species are susceptible to serious gastrointestinal disorders, including dysbiosis, at the time of weaning, especially if inappropriate foodstuffs are offered. Orphaned macropod young may benefit from exposure to fresh feces from healthy adult conspecifics. Alternatively such feces may be mixed with water or formula and fed as a slurry.[23]

Vaccination against clostridial organisms with a multivalent preparation marketed for livestock can be given at pouch emergence, with a second dose generally given between 4 and 14 weeks later.

Routine endoparasitic treatment or prophylaxis is not necessary for most species. Juvenile kangaroos often bear reasonable gastrointestinal helminth burdens, but the clinical significance of these appears to be minimal.[28]

Mimicking the gradual natural emergence from the pouch onto natural substrates is considered important for developing bone health, and yards must be kept free of cat feces to reduce the risk of toxoplasmosis.

Cross-Fostering

Cross-fostering is the rearing of the young by a surrogate mother of a different taxon and is a technique that provides an alternative for euthanasia for very small unfurred orphans of threatened species.[29] A coordinated program is required to manage a pool of surrogate animals, and the technique necessitates euthanasia of the surrogate's pouch young.

Factors implicated in successful cross-fostering attempts include relative size of donor and surrogate females, size of pouch young at weaning, differences in length of pouch life between species, and size differences between the donor young and those of the surrogate species at transfer.

Females regulate milk composition and production irrespective of pouch young age. As such, transfer of donor young to species with more immature or advanced mammary glands will result in a slowing or acceleration of pouch young growth and development that can influence the duration of pouch life.[30]

While it is ideal for a surrogate mother to raise a transferred pouch young through to weaning, if the joey can at least be raised to an age at which hand-rearing can be confidently undertaken, then the cross-foster can be considered a success. This has been demonstrated by the transfer of a 47-day-old Goodfellow's tree kangaroo *(Dendrolagus goodfellowi)* orphan to a yellow-footed rock-wallaby *(P. xanthopus)* surrogate.[31]

Euthanasia

For most individuals barbiturate overdose via intravenous injection is the preferred method of euthanasia.[32] Sedation or anesthesia may be necessary to reduce the stress of physical restraint. For very small patients, intraperitoneal or intrahepatic injection of barbiturate is an acceptable alternative. Intraosseous injection may also be used if there is a preexisting catheter or if the animal is anesthetized prior to injection.

In situations where barbiturate is not available, manually applied blunt force trauma techniques for pouch young and lethal shot for at-foot young have been described, but these should be carried out only by adequately trained personnel.[33]

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SECTION 14

Small Mammals

72

White-Nose Syndrome: Cutaneous Invasive Ascomycosis in Hibernating Bats

CAROL U. METEYER AND MICHELLE L. VERANT

Background

North American Perspective

Mortality events in hibernating bats were uncommon until early 2007 when a biologist reported dead little brown bats *(Myotis lucifugus)* in a cave near Albany, New York. The disease was termed "white-nose syndrome" (WNS) due to white fungal hyphae visible on the muzzle.[1] The causal fungus was identified as *Pseudogymnoascus* (formerly *Geomyces*) *destructans* (*Pd*),[2–4] and has since spread from the eastern into central United States and southeast Canada, with an unexpected detection in Washington State in 2016 (Fig. 72.1).

Over the past decade, declines up to 98% have been reported for some hibernating populations of bats affected by WNS in the northeastern United States.[5,6] The northern long-eared bat *(Myotis septentrionalis—Threatened)* has been extirpated from 69% of former known hibernacula and is considered to be at risk of extinction.[7] Little brown bat populations have stabilized at up to 30% of colony sizes.[7] However, little brown bats and other species most affected by WNS (Indiana bat *[Myotis sodalist—Endangered]*, tri-colored bat *[Perimyotis subflavus]*) are not expected to recover to population sizes present prior to WNS.[8] While it is difficult to predict the outcome of WNS in some species and at geographic margins, it is clear WNS has drastically changed the composition of bat communities in the northeastern and midwestern United States. Continued monitoring of bat populations will be important for understanding the long-term impacts of WNS on North American bats (https://www.fort.usgs.gov/science-tasks/2457).

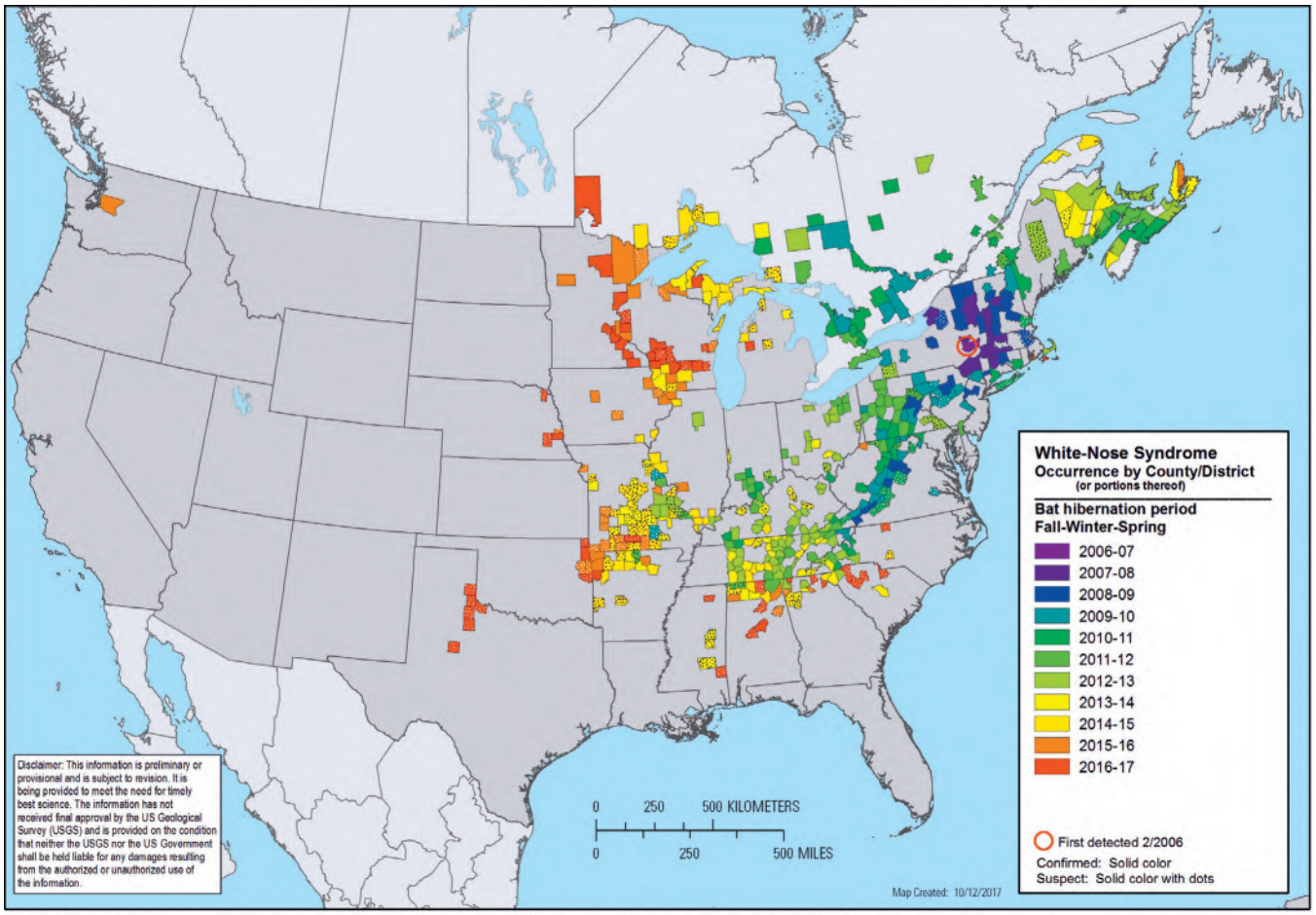
Global Perspective

Following discovery of WNS and *Pd* in the United States, white fungal growth was reported on bats in Europe; the white material was confirmed to be *Pd*, and histologic lesions consistent with WNS were identified as well.[8a] However, WNS in Europe has not been associated with mass mortality.[9] Genome sequencing of *Pd* isolates from Europe has demonstrated the presence of at least eight haplotypes. In contrast, all isolates sequenced to date from North America are of the same haplotype and identical to the most common haplotype found in Western Europe, indicating that *Pd* was likely introduced to North America from Europe.[10] A recent investigation in northeastern China identified *Pd* on hibernating bats and hibernaculum surfaces,[11] and two hibernating eastern water bats *(Myotis petax)* with white fungus *(Pd)* observed on their muzzles were confirmed to have WNS by histopathology. Similar to bats in Europe, there was no associated mortality of bats in the caves in China where *Pd* was detected.

Interdependence Among Pathogen, Host, and Environment

Pseudogymnoascus destructans is a psychrophilic fungus that is closely related to other *Pseudogymnoascus* spp., *Geomyces* spp., and allies commonly found in soil and decaying matter in cool environments, including caves and mines used by bats for hibernation.[4,12,13] Growth of *Pd* is restricted to cold temperatures (0°C–19°C), with maximal growth rates at 13°C–15°C,[14] which is within the range of temperatures selected by bats for hibernation (3°C–15°C).[15]

During hibernation bats downregulate physiologic functions to conserve energy.[16] During these prolonged states of torpor, *Pd* is able to proliferate and invade bat skin without evidence of a cellular inflammatory response.[17] Alterations of the innate immune system and production of antibodies against *Pd* have been demonstrated in infected bats, but these immune responses do not provide protection from



• **Figure 72.1** Detection of white-nose syndrome and the causative agent, *Pseudogymnoascus destructans*, as of October 12, 2017. The black dot surrounded by a red circle is the site of initial mortality. (Updates at https://www.whitenosesyndrome.org/resources/map.)

WNS.[18–20] Once bats become euthermic following spring emergence from hibernation, inflammation may be severe, and result in further damage to the wing membrane.[21]

Differences in susceptibility to WNS infection and disease severity have been observed across species of cave-hibernating bats. Some data suggest that big brown bats are resistant to WNS, and population increases for this species have been reported within regions affected by WNS.[22,23] Differences in species susceptibility to WNS may be correlated with temperature of selected hibernation sites, colony size and tendency to cluster during hibernation, body size and hibernation physiology, native microbial skin communities, and the composition of sebaceous lipids on the skin surface of the bat. It is likely that a combination of factors is responsible for this variable sensitivity to infection with *Pd* and expression of disease in species and populations surviving in areas with WNS.

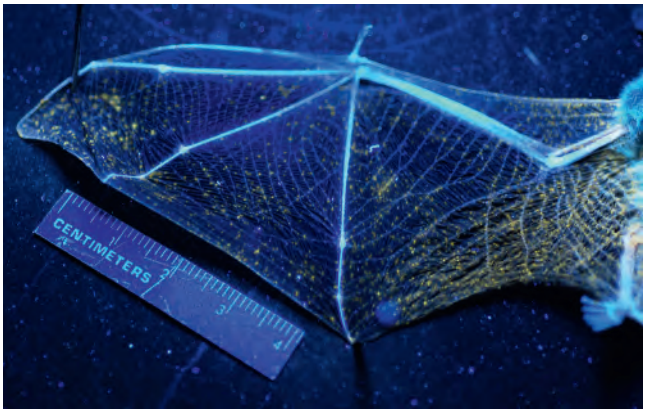
As WNS spreads, mortality of infected bats at lower latitudes and warmer climates may be moderated due to shorter hibernation seasons and more frequent foraging during winter. However, recent studies in Tennessee have documented population declines of up to 98% in hibernating colonies of northern long-eared bats *(Myotis sodalis)*,[24] indicating that this may not be the case for all species or locations.

The invasion of wing membrane and replacement of tissue by *Pd* leads to a complex suite of behavioral and physiologic disturbances that may ultimately lead to death.[25] Increased frequency of arousal from torpor has been demonstrated in hibernating bats affected by WNS.[26,27] These arousals are thought to contribute to higher energy demands, depletion of energy reserves prior to spring emergence, and emaciation often seen in bats with WNS. Other life-threatening physiologic disruptions reported in bats with WNS include electrolyte imbalances, dehydration, and acid-base disturbances, which are also thought to contribute to mortality.[28–30]

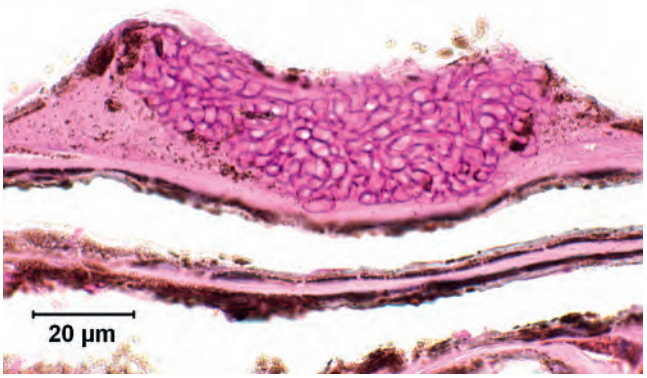
Clinical Signs

Observation of bats flying during the day or on the ground outside of hibernacula in colder winter climates is preliminary evidence that WNS is present in the population. Bats may also be seen moving closer to the entrance of hibernacula, flying into buildings, trembling on the ground, or struggling to fly.[6,25] Healthy hibernating bats in southern

• **Figure 72.2** Muzzle of a little brown bat *(Myotis lucifugus)* with white fungal growth confirmed to be white-nose syndrome by histopathology. Presence of *Pseudogymnoascus destructans* was confirmed by culture and molecular methods. (Photograph by Carol Meteyer, US Geological Survey.)



• **Figure 72.3** Wing of a dead tricolored bat *(Perimyotis subflavus)* lit from above with hand-held 51 LED 385-nm ultraviolet flashlight shows points of orange–yellow fluorescence consistent with sites of white-nose syndrome lesions. (Modified from Figure 1E; Turner GG, Meteyer CU, Barton H, et al: Nonlethal screening of bat-wing skin with the use of ultraviolet fluorescence to detect lesions indicative of white-nose syndrome. *J Wildl Dis* 50:566–573, 2014.)



• **Figure 72.4** Histologic section of wing membrane from the little brown bat *(Myotis lucifugus)* in Fig. 72.2. Periodic acid-Schiff stains hyphae of *Pseudogymnoascus destructans* magenta. Branching, septate hyphae with irregularly parallel walls fill cup-shaped epidermal erosions that have a discreet interface with host tissue. Bar = 20 µm. (Photograph by Carol Meteyer, US Geological Survey.)

latitudes normally forage outside of hibernacula during winter, but colonies of bats with WNS are more active, depart hibernacula in higher numbers earlier in the day, and can demonstrate other behaviors similar to bats affected with WNS in northern locations.[31]

Delicate white hyphae are often seen on exposed skin surfaces of hibernating bats with WNS in hibernacula (Fig. 72.2). However, other noninvasive dermatophytes on bats can produce similar white hyphae,[32] so further study is warranted to confirm WNS. In addition, bats with WNS may not have visible fungus,[33] and handling or removing an infected bat from a hibernaculum generally results in loss of visible hyphae on the skin surface.[17]

Wing membranes are the most common site of *Pd* infection, but with the exception of readily observable visible hyphae that may or may not be present, other gross signs of WNS are often subtle. For example, extended wing membranes may be stiff or sticky with loss of elasticity and areas of tissue contraction. Examining the wing surface under a long-wave ultraviolet (UVA) light (366–385 nm) can highlight pinpoint or coalescing areas of orange-yellow fluorescence (Fig. 72.3), indicating potential presence of epidermal lesions caused by *Pd*.[34] However, UVA light should be used only as a screening tool to identify individual bats for further diagnostic testing to confirm WNS. In the weeks following spring emergence, wing damage such as depigmentation, holes, and tears can be observed in bats infected with *Pd* during hibernation. However, healing of wing tissue occurs within weeks postemergence.[35,36]

Diagnosis

Confirmation of WNS in a bat requires identification of characteristic histologic changes[17] and confirmation of presence of *Pd* by real-time polymerase chain reaction (PCR) or fungal culture analysis. *Pseudogymnoascus destructans* may be cultured from samples using suitable culture medium (e.g., Sabouraud dextrose agar or dextrose-peptone-yeast extract agar) incubated at a cold temperature (approximately 5°C–10°C) for a minimum of 2 weeks.[2,37] Curved conidia produced by *Pd* are morphologically distinct from other fungi generally found on bats,[38] but identification of the isolate by *Pd*-specific PCR or PCR amplification of the rRNA gene region and sequence analysis is necessary for definitive confirmation.

Skin lesions associated with WNS can be unevenly distributed on the wing, and without targeted biopsy sampling (described in "Surveillance"), a whole carcass should be submitted for extensive evaluation of ear, muzzle, and wing membrane.[26] The histologic characteristics of dermal invasion by *Pd* vary as the disease progresses, but the unique pattern that defines WNS consists of dense aggregates of large irregular branching and septate hyphae forming cupping erosions that have a defined interface at the margin with host tissue (Fig. 72.4).[17] Evidence of a cellular inflammatory response is also generally absent in hibernating

bats, but euthermic bats collected outside hibernacula in spring can have abundant inflammation, which completely resolves without fibrosis or scarring.[35]

Surveillance

Conducting surveillance for WNS in hibernating bats is recommended in late winter to decrease disruption of bats during hibernation, and because clinical signs, prevalence and abundance of *Pd*, and skin lesions have been shown to increase in bats over time during hibernation.[33,39] Selecting bats with visible signs of infection can increase the probability of detecting *Pd* in a population.[33] Noninvasive sampling techniques for *Pd* include epidermal swabs from wings and muzzle, and less invasive sampling for histopathology can be done using wing punch biopsies taken from areas of visible white fungus or by targeting areas of orange-yellow fluorescence under UVA light.[40] However, these techniques have reduced sensitivity for detecting *Pd* or WNS, and whole carcasses of fresh dead bats should be submitted to an experienced diagnostic laboratory for testing if clinical signs are seen in new geographic areas or in species for which WNS has not be previously diagnosed.

Sampling bats as they emerge from hibernation in spring is also possible, but the probability of detecting *Pd* or WNS declines over the first few weeks posthibernation as wings heal and the amount of *Pd* on bats declines. Observation of wing damage after spring emergence may reflect presence of WNS but is not specific for the disease.[41,42] Although probability of detecting *Pd* on bats during summer months is low, *Pd* has been detected on bats that use caves and mines for day roosts in summer,[11,42a] and *Pd* is known to persist in the physical environment of hibernacula year-round with no seasonal differences in detection probability.[43] Detection of *Pd* in the environment of a hibernaculum is correlated with presence of WNS in bats at those sites, but sensitivity of environmental sampling for detecting *Pd* is dependent on the design of the sampling scheme and can lag in time behind first detection of *Pd* in bats at a site.[42b,43] For updated information on *Pd*/WNS surveillance, see the US Geological Survey National Wildlife Health Center White-Nose Syndrome web page (https://www.nwhc.usgs.gov/disease_information/white-nose_syndrome/index.jsp).

Disease Mitigation

Management of WNS in bats is a complex challenge similar to other diseases in free-ranging wildlife populations. Current efforts are focused on integrated approaches that reduce infection and mortality in bats, as well as promoting overall health of bat populations to support resistance to and recovery from WNS.[44] Because WNS is not the only cause of bat mortality and population decline,[45,46] recovery and conservation of bat populations will require a holistic management approach.[47]

Supportive care for bats with WNS consists of providing warmth, food, and water, which leads to full recovery within several weeks.[35] Bats in the wild also recover if they survive the potentially fatal wing damage that may occur postemergence.[36] Although bats are generally re-infected with *Pd* each fall as they enter hibernation,[39] band recoveries have shown survival of bats for up to 6 years in spite of WNS.[48]

Other potential disease management options that are being tested include vaccination, ultraviolet (UVA) light, antifungal compounds, and biological agents. Some of these have demonstrated effectiveness against *Pd* in the laboratory[49,50,50a]; however, field trials to assess applicability, safety, and efficacy in wild bats, as well as potential ecologic side effects, are ongoing.

To date, management actions for reducing impacts of WNS on bat populations have primarily focused on reducing disturbance of bats through protection of hibernacula, and minimizing risks of human-assisted spread of *Pd* through education and development of decontamination protocols. Additional information and guidance for implementing these management actions may be found in *Recommendations for Managing Access to Subterranean Bat Roosts to Reduce the Impacts of White-Nose Syndrome in Bats*, and the *National White-Nose Syndrome Decontamination Protocol*. These guidance documents and additional acceptable management practices for bats are available on the White-Nose Syndrome Response Plans web site (https://www.whitenosesyndrome.org/white-nose-syndrome-response-plans).

Alterations of habitats used by bats for foraging or roosting have also been proposed as management actions to increase survival and to support recovery of bat populations susceptible to WNS.[51] Given the long life spans and low reproductive rates of bats, adult and juvenile survival are critically important for long-term population recovery.[47] However, further research is needed on the implementation and effectiveness of these approaches for reversing population declines in bat species affected by WNS.

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A full WNS bibliography can be found at, https://www.whitenose-syndrome.org/wns-bibliography

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73

Naked Mole Rat Management and Medicine

JAN RAINES

Biology

Naked mole rats (NMRs; *Heterocephalus glaber*) are a small (average weight of 35 g) eusocial species with a unique reproductive strategy whereby only the dominant queen reproduces, while other females in the colony assist with pup rearing, nest defense, and colony maintenance. There are only two eusocial mammal species: the NMR and the Damaraland mole rat *(Cryptomys damarensis),* both members of the family Bathyergidae.[1] The eusocietal organization functions much like that of social insects such as bees living in a hive ruled by a single queen who is the sole reproducing female.[2] NMRs live exclusively underground; consequently, they have developed multiple physiologic adaptations for this subterranean lifestyle: a stout, cylindrical body with a robust skull, reduced or absent eyes (considered functionally blind), reduced external ears, scant pelage, and powerful incisors and forelimbs for digging. Other adaptations to living underground are their lack of thermoregulation and high circulating hemoglobin and myoglobin concentrations allowing them to thrive in a carbon dioxide–rich atmosphere.[3,4] Native to Ethiopia, Somalia, and Kenya, NMRs build elaborate tunnel systems (up to 2–3 km in length) for colonies that contain up to 300 individuals. Even in the wild, these animals are long-lived for their size, with an average life span of more than 16 years.[5–7]

Husbandry

Because they are a subterranean species, NMRs may present many husbandry challenges. In the wild, colony members congregate in nesting chambers (Fig. 73.1) to conserve heat, and they use other chambers for urinating and defecating. To replicate their native environment, captive colonies are housed either in naturalistic tanks connected with PVC pipes (Figs. 73.2–73.4) or in more basic facilities devised from acrylic chambers connected with a similar pipe system (Fig. 73.5). Because the animals are poikilothermic, supplemental heat must be provided within the range of 84°F–95°F (29°C–35°C), and humidity levels within the colony should be maintained at 80%–85%.[8,9] NMRs are very sensitive to vibrations, and every effort must be made to reduce loud and extraneous noise near their enclosure. Some institutions minimize disruptions from the visiting public by placing double-paned glass along the enclosure facing or by operating white noise machines. To avoid disturbing the animals, cleaning activities should be kept to a minimum level; however, the modular nature of the enclosures does allow for easy removal of the toilet ("potty") chambers.

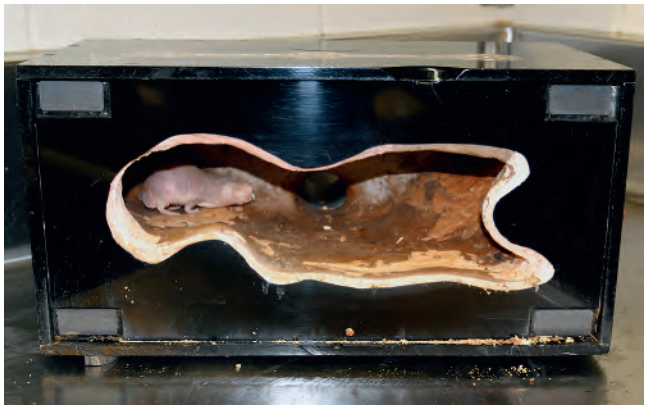
Nutrition

NMRs are herbivorous, hindgut fermenters that also practice coprophagy to maintain gastrointestinal health. The cecotrophs are ingested by adult and juvenile NMRs to support the cecal microbial population.[10] As another sequela of their subterranean existence, NMRs receive all their daily water requirements from their diet; therefore they must receive tuberous vegetables (sweet potatoes, turnips, carrots) daily to meet these needs. This basic diet can be supplemented with corn and other fruits and vegetables. Commercially available pelleted diets may be offered, but the calcium levels within the diet must be evaluated carefully. NMRs are able to absorb calcium passively from their gastrointestinal tract, and they can reach excessive calcium levels rapidly.[11] Without access to sunlight exposure, NMRs have evolved an ability to survive in a functional vitamin D3 deficiency without the typical associated pathologies. Like calcium supplementation, oral vitamin D supplementation should be avoided because it can lead to rapid intoxication and soft tissue mineralization.[8,9,12]

Reproduction

Male and female workers exhibit minimal sexual dimorphism because they are reproductively suppressed; therefore sex determination in NMRs is challenging. Males and females differ by anal to urogenital distance, but the difference can be extremely subtle. The nonbreeding male typically has a

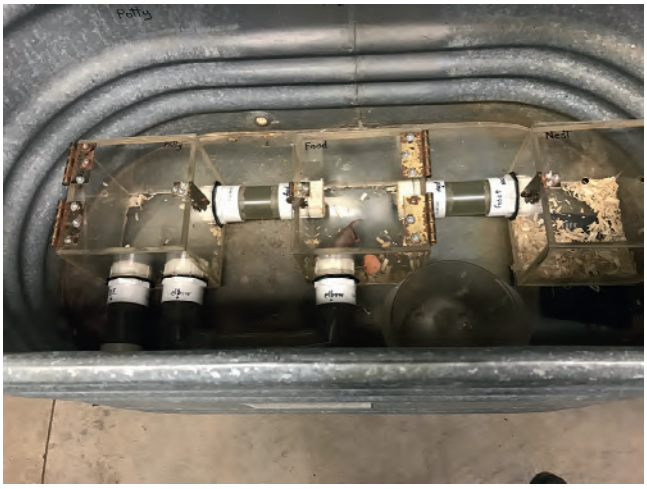
• **Figure 73.1** Naked mole rat *Heterocephalus glaber* colony.



• **Figure 73.2** Naked mole rat *Heterocephalus glaber* exhibit containment box with naturalistic interior.

• **Figure 73.3** Posterior of exhibit demonstrating connecting tubes.

• **Figure 73.4** Chamber, again demonstrating the naturalistic interior; this liner material is designed to allow naked mole rats *Heterocephalus glaber* to chew freely to encourage naturalistic behaviors. As the walls wear away, they may be easily repaired with more material, e.g., USG Hydrostone Gypsum Cement.



• **Figure 73.5** Demonstrating a more basic naked mole rat *Heterocephalus glaber* setup. These may be quite elaborate as well.

more circular anal and urogenital sphincter, whereas a nonbreeding female has a broader and more flattened urogenital area.[9] These differences become much more obvious once the individuals enter the breeding population.

In this eusocial structure, the queen as the single breeding female can have 5–7 L per year with 1–27 pups in each litter. The pups are nursed exclusively by the queen for approximately 4 weeks. Interestingly, NMRs may give birth to and successfully nurse almost twice as many offspring as there are mammary glands on the queen.[13] Other colony members are involved in the care of the offspring and the queen, in addition to the maintenance of the colony. The queen is the largest female, with her body lengthening and growing larger as she becomes queen; this extension of the body is the result of vertebral lengthening.[14] NMRs are spontaneous ovulators maintaining accessory corpora lutea to enhance progesterone secretion, but the physiologic significance of these structures is unknown.[14] Queens are long lived, and some colonies have maintained the same queen for up to 15 years. Only one to three adult males have the ability to mate with the queen, who courts only the largest males in the colony.[15] The other males in the colony have low levels of luteinizing hormone (LH) and testosterone, resulting in small, intraabdominal testes with abnormal sperm production.[16–18] The queen and dominant males suppress other members in the colony through behavioral contact ("shoving") and, to a lesser degree, by urinary pheromones. Nonbreeding females have prepubescent ovaries due to inhibition of gonadotropin-releasing hormone (GnRH)

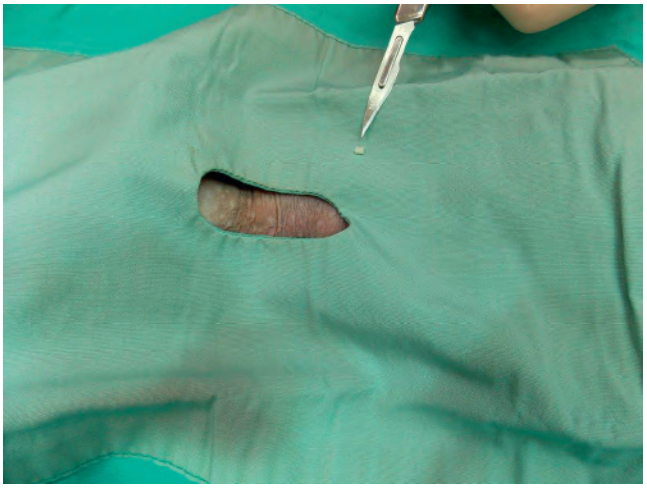
that can become active within 24 hours of the loss of the queen. The reproductive activation of several females and increases in intracolony aggression, social instability, and body weight often follow a queen's death. Females older than 6 months are capable of becoming reproductively active and fight to establish dominance if a queen dies. The death of colony members following queen removal may be an extreme attempt by new queens to establish their dominance and reimpose reproductive suppression on subordinates. During these upheavals, both sexes sustain serious injuries as they battle to establish dominance and become breeding animals.[6,19–22]

In captive colonies, females have produced litters as large as 27, whereas it is reported that litter size is closer to 12 pups in wild populations. Because NMRs are year-round breeders, captive populations may reach carrying capacity very rapidly. In wild colonies, dispersal is a valid option to reduce population size or reduce incidence of inbreeding, with the animals merely sealing off a new colony in a group of tunnels to divide them from the originating group. When a colony splits into two groups, one of the non-breeding females in the new colony will ascend to become queen.[14,23,24] There is a strong indication that rapid colony growth in limited space contributes to high pup mortality and is correlated to the size of the breeding colony.[13] Colony collapse occurs when the colony has exceeded carrying capacity, and pup mortality is high (95%–100%).

In captivity, where there is limited space for the colony to expand, finding a successful method of contraception without causing queen succession has been challenging. Three successful methods have been used to date: surgical sterilization, hormonal manipulation, and vaccination (see also Chapter 22). A tubal ligation and a complete hysterectomy are successful methods of surgical sterilization, but an ovariohysterectomy is not an option because removal of ovarian tissue would cause the queen to lose her status and instigate a succession coup. Not knowing how well an individual could be reintroduced after an invasive surgical procedure, test procedures were performed on other females in the colony. NMRs are highly xenophobic, and they will kill individuals that they do not recognize, as well as animals from their own colony that have been removed for an excessive length of time.[25] Aggression post removal can occur in as brief a timeframe as 30 minutes or not until almost 2 hours; some of this range is dependent on the individual's social standing when removed. Preventative measures may be used to reduce the risk of injury whenever an animal is removed from a colony even for a short period of time (Box 73.1). A flank hysterectomy where the uterus was removed from a single incision sparing the ovaries was accomplished in a subordinate female who was introduced to the colony without incident following the procedure. The tubal ligation surgery has also been performed via both a flank approach and ventral midline, again with successful return to the colony. Flank approaches are recommended to reduce incidence of visceral herniation if colony members chew at surgical sites. As an alternative sterilization method, a second queen was implanted with melengestrol acetate (MGA) (Fig. 73.6). A concern with the use of MGA was the suppression of the pheromones maintaining sterility because its use could stimulate a fight for dominance. MGA is a synthetic progestin affecting contraception by blocking ovulation and thickening cervical mucus; however, follicle growth and estrous behavior continue. An MGA implant (0.15 mg/kg, ZooPharm, Windsor, Colorado 80550) was placed in a routine fashion, and reproduction was curtailed for more than 2 years, but implant failure occurred at 26 months with confirmed pregnancy. A queen in another colony received a porcine zona pellucida (PZP) vaccine in an attempt to curtail reproduction. The PZP vaccine causes antibodies that prevent attachment of sperm to the ova. It does not appear to suppress estrous cycle, and it is easy to administer. A dosing regimen of 0.2 mL PZP emulsion (50 µg) intraperitoneally was administered. The queen received one injection followed by a booster injection 2 weeks later. With the initial series and an annual booster schedule, there have been no pups to date (at time of publication, more than 3 years).

• BOX 73.1 Techniques for Reintroducing Naked Mole Rats *(Heterocephalus glaber)* into Colony Post Procedure

- Attempt to wear exam gloves at all times while handling animals to reduce scent transmission.
- Do not perform surgical prep with fragrance-containing items.
- If an animal does not have a transponder, temporarily identify with a marker for any follow-up monitoring.
- Remove multiple animals from the colony, and reintroduce the lone animal to that group before reintroducing to the entire group.
- The maximum time out of colony should be no more than 2 hours.
- Have soiled shavings available so that the animal may be scented with them prior to returning to the colony.



• Figure 73.6 Naked mole rat *(Heterocephalus glaber)* under fenestrated drape with melengestrol acetate implant indicated at point of scalpel blade.

Restraint and Anesthesia

Manual restraint is adequate for most exams and medication administration in NMRs. Examination gloves must be worn at all times when handling NMRs because any foreign odors may cause the other members of the colony to attack the individual when it is reintroduced. When anesthesia is necessary, NMRs do well with inhalant gases such as isoflurane or sevoflurane. Their oral cavity is too small to allow for intubation, but they can be maintained safely on a facemask. Supplemental heat must be provided.

Therapeutics

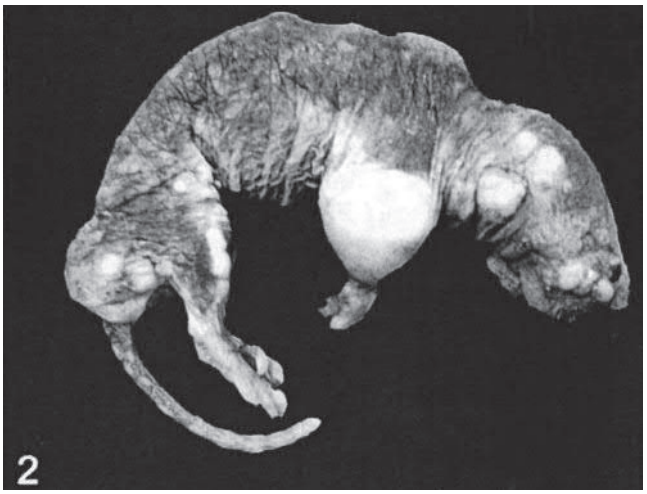
Many oral antibiotics (TMPS at 15–30 mg/kg orally [PO] once or twice a day [SID or BID] and enrofloxacin at 5 mg/kg PO or intramuscularly [IM] SID) and nonsteroidal antiinflammatory drugs (meloxicam at 0.1–0.2 mg/kg PO SID) at standard rodent dosing regimens have been used successfully in this species, with administration volume being the only limiting factor. Response to analgesics may be minimal in this species because they lack C-fibers in addition to substance P and nerve growth factor, both neurotransmitters in pain cascades, which decrease or eliminate any cutaneous or thermal pain sensation. This adaptation is thought to be part of a protective mechanism to reduce acid accumulation in tissues in their hypoxic environment.[8,26,27]

Diseases (Infectious and Noninfectious)

The most commonly reported disease conditions in NMRs are related to trauma secondary to intraspecific aggression. The most common bacteria isolated from bite wound abscesses have been *Pasturella* sp. Noninfectious disease issues are most often directly related to husbandry. Neonatal death from maternal neglect is most often seen with impending colony collapse. Neglected animals are dead within 12 hours of birth, and they are often moved by the worker animals into the "potty" chamber shortly after, and sometimes before, death. Calcinosis circumscripta and cutis has been reported in multiple colonies. The animals present with subcutaneous swellings that range in size from 2 mm to 2 cm and contain a white, opaque, pasty material, and these lesions can be of such size that the animal is incapable of movement (Fig. 73.7). This condition is attributed to inappropriate vitamin D supplementation within the diet; however, even after vitamin D supplementation was discontinued, it was reported that the pups from the subsequent litters born immediately around the time of discontinuation matured to develop these lesions.

Use in Research

Because of their unique biology and physiology, NMRs are valuable models for aging research. NMRs age at a slower rate; they have increased resilience to oxidative stress and mitochondrial injury; they have minimal cellular senescence; and they maintain fertility until death.[28–31] NMRs also have a higher expression of cytoprotective genes allowing them to resist overt disease.[8,26]



• **Figure 73.7** Naked mole rat *(Heterocephalus glaber)* with calcinosis cutis/circuscripta. (From Delaney MA, Nagy L, Kinsel MJ, Treating PM: Spontaneous histologic lesions of the adult naked mole rat *[Heterocephalus glaber]* a retrospective survey of lesions in a zoo population, *Vet Path* 50[4]:607–621, 2013.)

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Immobilization, Health, and Current Status of Knowledge of Free-Living Capybaras

SANTIAGO MONSALVE

Introduction

This chapter is based on work done in the Colombian Caribbean and Orinoquía region in which investigative processes have been undertaken in the area of conservation medicine with capybaras (*Hydrochoerus* spp.) in *in situ* conditions.

The Capybara

The capybara is the largest living rodent species in the world and the only surviving member of the family Hydrochoeridae. This species is found in a geographic distribution area that includes Panamá, Colombia, Venezuela, Ecuador, Perú, Paraguay, Uruguay, Brazil, Bolivia, and northern Argentina.[1] Its semiaquatic behavior is an advantage due to the fact that it can procreate in areas that are subject to marginal hydrologic fluctuations, cattle ranching, and agriculture. Its condition as a native species, adapted to swampy areas and areas subject to flooding, in contrast to bovine and equine domesticated species, provides it with greater efficiency in the utilization of available forage in these environments.[2] The capybara is a species of great economic interest, due to its high fecundity and the excellent quality of its meat and skin, characterized by the fact that countries such as Brazil, Venezuela, and Argentina have established successful programs for management of the species.[3–5]

Geographic Distribution

The capybara, also known as the chigüiro, ponche, capyibara, cacó, and carpincho, belongs to the order Rodentia, genus *Hydrochoerus*. The capybara is autochthonous to the American continent, where there are two species: *Hydrochoerus hydrochaeris* and *Hydrochoerus isthmius*, the first with a distribution in the Llanos Orientales and Amazonian region of Colombia, northern Argentina, Paraguay, Ecuador, Venezuela, Brasil, Perú, Bolivia, and the Guyanas, while the second inhabits northern Colombia, Panama, and Venezuela.[6] The rapid decrease of wild populations in Colombia for decades has led to the taking of diverse measures to ensure their conservation, while at the same time satisfying the interests of the population for exploiting this resource in the different countries in which they are naturally found in situ.

Habitat

The transformation of natural savannahs since the time of colonization and the expansion of cattle ranching have been threats for its habitat. Petroleum exploration and exploitation, the incursions of roads, and the presence of groups on the margins of the law have also generated significant processes of transformation of the ecosystem due to environmental impacts.[7] Likewise, the expansion of monocultures has been another factor that has affected the habitat of capybaras and constitutes a threat to their populations, as this activity implies the use of agrochemicals that deteriorate the quality of water, which is an important resource for this species. In Colombian Orinoquía and Venezuela, great swaths of savannah flood during the rainy season, increasing available habitat for animals; nevertheless, in some areas, flooding may be extreme and these floodwaters may be considered to be a limiting factor, because these animals also require dry zones to rest. During summer, these bodies of water shrink to the point that they completely disappear; at this moment, the available and optimal habitat for these animals is reduced to small strips around remaining water sources (Fig. 74.1).[8] In actuality, thousands of capybaras die during the dry season without scientists having clarity about the population equilibrium that this anthropic condition may be generating or the epidemiologic implications associated with this annual mortality.

Biology and State of Conservation

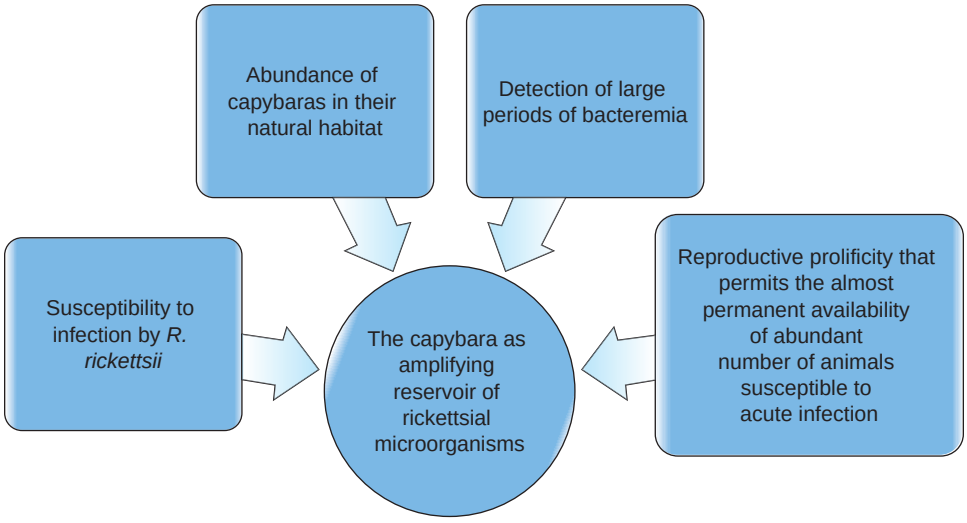
Despite capybaras being rodents and having high reproductive rates, the fear exists that legal and illegal extraction may prevent this species from maintaining sustainable populations over time.[9,10] Capybaras are highly social and their population dynamics are intensely related to reproductive competition in males and females.[11] Demographic changes in capybara populations can be modified due to different social mechanisms and habitual infanticide in which groups of male individuals may kill juvenile competitors in order to assume population control.[12,13] This behavior has been considered to be an ancestral trait in rodents[13,14] and an important estrual inductor in females, reducing the period of time between litters.[12,13] This species counts on reproductive suppression on the part of females,[15] which may have several young in the group; however, not necessarily all the females of the group are reproductively active during certain periods of time, which suggests mechanisms of estrual suppression.[13,15] According to the International Union for Conservation of Nature (IUCN), the species *H. hydrochaeris* is catalogued as a Least Concern species due to its wide geographic distribution, high and prolific population, and permanence in protected areas.[6] Capybaras may be found in habitats close to bodies of water, rivers, and streams,[3] and can be considered to be the most efficient nitrogen recyclers of all animals, as, in a matter of hours, their urine makes important quantities of nitrogen soluble, which are re-integrated into pastures.[16] Sufficient data for the cataloguing of the species *H. isthmius* do not exist, according to the IUCN.[6]

• **Figure 74.1** Wild capybara *(Hydrochoerus hydrochaeris)* habitat. Casanare, Colombia.

Capybara as Microorganism Amplifier

Diseases transmitted by vectors are not circumscribed to definite regions and have been recognized in practically any place they have been investigated.[17] The risks of the majority of diseases contracted from wild animal populations are unknown, as is their impact. Many of these diseases may eventually affect man,[21] increasing epidemiologic alterations, especially when a species such as the capybara is utilized for the consumption of its meat, because it is a species that cohabitates with domestic animals and humans in large areas immersed in extensive agricultural production. Capybaras have been reported to be transmitters of diseases, and it has been determined that populations of this species in the Casanare Department (Colombia) have or have had contact with *Brucella abortus* and *Leptospira interrogans*.[22] Populations of capybaras have been diminished during dry seasons, and combined with greater human intervention in the environment, as is presented in this department, may represent an indicator of increase in density of disease-causing organisms.[23,24]

It has been determined that horses, cattle, tapirs *(Tapirus terrestris)*, and capybaras *(Hydrochoerus* spp.*)* are infected by ticks with the *Amblyomma cajennense* complex, as well as domestic animals and humans.[25] This genus of ticks has been considered to be a vector of diverse microorganisms; thus the capybara has been catalogued as an animal amplifier of rickettsial diseases, as it fits certain requirements that permit disease cycles within its habitat (Fig. 74.2).[26,27]



• **Figure 74.2** Characteristics necessary for an organism to be considered to be a reservoir for the amplification of Rickettsias.

• **Figure 74.3** (A) *Amblyomma* spp. and (B) *Dermacentor* spp. obtained from a capybara *(Hydrochoerus hydrochaeris)*.

Different studies have demonstrated that capybaras are amplifying hosts of *Rickettsia rickettsii* (the microorganism that causes Rickettsiosis, a zoonotic disease with a high percentage of lethality) and are commonly parasitized by ticks of the *A. cajennense* complex, a principal vector of rickettsiosis in South America (Fig. 74.3A)[27]; however, they have also been shown to be infested by other species of ectoparasites such as ticks of the genus *Dermacentor* spp. (see Fig. 74.3B). The increase in cases of maculous fever in Brazil is related to the presence of capybaras in the area due to their condition as primary host amplifiers of these microorganisms.[28]

As they are considered to be susceptible to *R. rickettsii* and by being primary hosts of *A. cajennense*, capybaras may be epidemiologic bioindicators of Rickettsia infections in specific geographic areas.

The ticks that infest wild capybaras simultaneously transmit different microorganisms to individuals of the species, to other wild and domestic animals, and to humans. In studies undertaken in two states of Brazil (Rondonia and Sao Paulo), the DNA of *Ehrlichia* spp. has not been detected by molecular techniques in the amplification of fragments of gen dsb[29]; in Colombia (Casanare Department) the circulation of microorganisms of the family Anaplasmataceae have been found using polymerase chain reaction (PCR) for the amplification of a segment of gen 16S rRNA por tqPCR.[30]

Preliminary studies on ticks of the *A. cajennense* complex have shown identities similar to *Ehrlichia* spp. and *Anaplasma* spp. The results of these studies have permitted the gleaning of preliminary evidence of the circulation of pathogens to the family Anaplasmataceae of capybaras in in situ conditions.[31]

Although in the last century there was important progress made in the control of infectious diseases, these still represent an important problem for public health, principally in terms of zoonotic microorganisms that continue to urgently require a constant epidemiologic vigilance; thus due to the fact that capybaras are considered to be amplifying hosts of diverse microorganisms,[27,28] it is important to take into consideration the diagnosis of zoonotic diseases of rickettsial origin, including the microorganisms *Rickettsia* spp., *Ehrlichia* spp., and *Anaplasma* spp., as pathogens that represent a risk in the transmission of human and animal diseases.

Immobilization

Physical Immobilization

Two methods may be used to physically capture wild capybaras.

Capture Corrals

The purpose of this strategy is to condition capybaras during the space of 1–3 months, generally during the dry season, to approach a corral by baiting it every day with food. These corrals have a guillotine style door that is supported at a distance by means of a string that acts as a latch and remains open, permitting access to the interior of the corral so that they can be captured for investigative means. In studies done in the Colombian Caribbean region for the process of conditioning, rations of sugarcane, carrots, ears of corn, and cut grass have been used, which were moved incrementally each day from the wetlands that the capybaras inhabit toward the interior of the corral. The capture corrals are constructed piece by piece so that the animals gradually become accustomed to the structure. Although it is a relatively costly and time-intensive methodology, it represents a secure methodology for the animals and operators that want to utilize corral capture.[2] This technique is very useful next to lakes and rivers and may yield the capture of between 10 and 20 capybaras per month.

• **Figure 74.4** Physical restriction of free-living capybara *(Hydrochoerus hydrochaeris)*.

• **Figure 74.5** Chemical immobilization of a free-living capybara *(Hydrochoerus hydrochaeris)*.

Roping

This methodology requires the assistance of cowboys in the geographic zones in which physical restraint is to proceed. Immobilization is done by chasing the animals over the savannah on horseback, during which, generally, the cowboys use lariats for restraint (Fig. 74.4). This technique is very useful in large, flat zones and may yield the capture of between 5 and 10 capybaras per day. The cost of this methodology may be less than physical capture using corrals and may yield proportionally a larger number of animals per day.

Chemical Immobilization

Chemical immobilization of wild fauna is indispensable in investigative processes on wild fauna (Fig. 74.5); with capybaras, it has been utilized with the intent of hematologically characterizing species or with the goal of estimating the prevalence of Rickettsia antibodies in order to understand the epidemiologic role of these rodents in Brazilian maculous fever.[28] Some rodents show limbic movements when high doses of ketamine or tiletamine/zolacepam have been used for surgical procedures.[32] Xylazine and other α2 adrenergic agonists are frequently combined with ketamine for short-term immobilization and surgical anesthesia.[33] These combinations, however, produce hypertension, bradycardia, arrhythmias, and respiratory depression[32,33]; the use of ketamine and benzodiazepines together produces less cardiopulmonary depression, analgesia, and good muscular relaxation.[34] The tiletamine-zolacepam combination has been widely reported as an anesthetic combination for the chemical restriction of capybaras; however, long-duration inductions may cause problems in the recuperation of capybaras and the possible risk of postanesthetic suffocation (Table 74.1).

Diseases Reported in Wild Capybaras

In the majority of wild capybara populations, malnutrition (in the dry season) and predation are the principal causes of mortality; however, disease may play a secondary role in the decline of wild populations.[37] Capybaras are known in diverse reports to be animals that play an epidemiologic role in the transmission of diverse pathogens such as *R. rickettsii* through infestation by ticks of the genus *Amblyomma* spp. *Hydrochoerus* spp. has also been considered to be a transmitter of zoonotic diseases such as the diverse ectoparasites *Leishmania* spp., *Leptospira* spp., *Tripanosoma* spp., and multiple enterobacteria (Table 74.2).[38]

Conclusions

The capybara is a wild animal that is resistant to disease and adapts well to rural environments, making it an excellent agricultural alternative (subsistence consumption); however, the use of the species for this purpose presents various challenges, as its habitat changes seasonally. The high variability in the behavior of this forager allows the species to adapt to changes in feeding, which explains the high plasticity of capybaras in dealing with environmental changes and permits them to colonize different types of habitats. Due to its proximity to human populations and consumption of wildlife, the capybara is a species that could be of great importance to the monitoring of emerging infectious diseases and the potential reemergence of zoonotics as ecosystemic bioindicators. Deeper investigations into the distribution and ecology of wild reservoirs of these pathogens and their vectors are required.

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TABLE 74.1 **Immobilization Combinations in the Capybara (*Hydrochoerus* spp.)**

No.	Geographic Region	Samples	Ketamine Dose	Andrenergic Agonists (α2) Dose	Tiletamine Zolacepam Dose	Others	Antagonists	Reference	Comments
1	Colombian Caribbean region	8	—	—	5 mg/kg	—	Not reported	2	Safe anesthetic combination. In higher doses, may have been used to induce anesthesia for a larger period of time.
2	Colombian Caribbean region	8	10 mg/kg	Xylacine 0.5 mg/kg	—	—	Not reported	2	Safe anesthetic combination. May produce some brachycardia and inspiratory dyspnea.
3	State of Minas Gerais, Brazil	10	10 mg/kg	Xylacine 0.5 mg/kg	—	—	Not reported	33	Best analgesic in integument.
4	State of Minas Gerais, Brazil	10	—	—	5 mg/kg	—	Not reported	33	Long-duration anesthesia with low analgesia.
5	State of Minas Gerais, Brazil	10	—	—	5 mg/kg	Levomepromazine 0.5 mg/kg	Not reported	33	Safe anesthetic combination, good analgesic
6	State of Apure, Venezuela	16	4 mg/kg	—	—	Fenotiazine 0.4 mg/kg	Not reported	35	Good immobilization without inducing total anesthesia
7	State of Apure, Venezuela	12	4 mg/kg	Medetomidine 0.04 mg/kg	—	—	Atipamezole 0.1 mg/kg	35	Good immobilization without inducing total anesthesia, risk of postanesthesia suffocation
8	Curitiba, Brazil	22	—	—	3 mg/kg	Morphine 0.3 mg/kg + Azaperone 1.2 mg/kg	Not reported	35	Sedation for opthamalic tests
9	State of Apure, Venezuela	8	—	—	5.1 mg/kg	—	Not reported	36	Safe anesthetic combination, long duration
10	State of Apure, Venezuela	6	—	Medetomidine 0.008 mg/kg	1.6 mg/kg		Not reported	36	Safe anesthetic combination
11	State of Apure, Venezuela	8	—	Medetomidine 0.0075 mg/kg	1.5 mg/kg	Butorfanol 0.075	Not reported	36	Safe anesthetic combination, good analgesic

TABLE 74.2 Infectious Diseases Reported in the Capybara (*Hydrochoerus* spp.)

Classification	Agent	Disease/Reservoir/ Zoonosis	Signs/Symptoms in Capybaras	Reference
Virals	Picornaviridae	Foot-and-mouth disease	Vesicular interdigital lesions	39
	Picornaviridae	Viral encephalomyocarditis	Miocarditis necrotizante	40
	Poxviridae Orthopoxvirus	Zoonosis: fever, lymphadenopathy, headache and malaise	Asymptomatic	41
	Retroviridae	BLV	Not reported	42
Bacterial	*Rickettsia bellii, Rickettsia parkeri*	Spotted fever rickettsiosis	Asymptomatic	43
	Escherichia coli O157	Zoonosis: hemorrhagic enterocolitis. Disease not reported in capybaras	Not reported	44
	Rickettsia rickettsi	Amplifier: Rocky Mountain spotted fever	Asymptomatic	27, 45
	Leptospira Icterohaemorrhagiae, L. grippotyphosa and L. shermani	Reservoir of leptospirosis	Not reported	46
	Brucella spp.	Zoonosis: Brucelosis. Reservoir of *Brucella abortus*	Not reported	47, 48
	Ehrlichia spp. and *Anaplasma* spp.	Zoonosis: Human granulocytic anaplasmosis. In canines: Anaplasmosis and ehrlichiosis	Not reported	31
	Mycobacterium bovis, Mycobacterium intracellulare	Tuberculosis	Tubercular Granulomas, glomerulonephritis, pancreatic fibrosis. Mesenteric granulomatous nodules	49, 50
Parasitic	*Fasciola hepatica*	Fasciolosis	Abortions, infertility, hepatic lesions	51–53
	Protohallidae, pycnotrichidae, cycloposthiidae families	Unreported disease, endosymbiont	Asymptomatic	54
	Tripanosoma evansi	Reservoir	Asymptomatic	55, 56
	Género *Amblyomma* spp.	Amplifier of Rickettsial microorganisms	Asymptomatic	43, 57
	Cryptosporidium parvum	Zoonosis: Nonsanguinary watery diarrhea, anorexia, vomiting	May present acute sanguinary diarrhea	48, 58
	Monoecocestus hagmani	Intestinal parasitism	Weight loss, anemia, abdominal distention	48

BLV, Bovine leukemia virus.

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75

Xenarthra Immobilization and Restraint

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Introduction

The superorder Xenarthra is composed of a unique group of mammals. The different anatomic and physiologic peculiarities that characterize the group present difficulties and complications for veterinary care and management. Considering this great species diversity, it is essential that veterinarians have knowledge of the individual requirements of each group to obtain a diagnosis and administer the most appropriate therapy.

Physical Restraint

Anteaters

Adequate physical restraint techniques for anteaters depend on the species. In general, great care must be taken to avoid injuring the animal and to avoid injuries to staff by the animal while it is trying to defend itself.[1] Physical restraint of giant anteaters *(Myrmecophaga tridactyla)* may be carried out with the aid of leather ties mounted on a long pole or by using nets; in these cases, it is very important to avoid injuries to the animal's lips or mouth during capture.[2] In captivity the lesser anteaters (*Tamandua* spp.) may be more tractable and can be held by the tail for the administrations of injections in the hind limbs.[1] When unaccustomed to contact, the limbs and head should be held using leather gloves or straps, or simply place the animal in a cloth bag.[2] In the case of pygmy anteaters *(Cyclopes didactylus)*, handling in captivity becomes relatively easy and less stressful when performed regularly. First, allow the animal to climb on your hand on a surface of cloth, trying not to catch the animal by the tail and being careful not to pull it abruptly. This way, individuals may be weighed, measured, or transferred.[3] Physical restraint of the pygmy anteater is usually done with the aid of a cloth or towel wrapped around them to prevent them from using their front claws. Once immobilized, they may be held with both hands, securing their claws with the thumb and forefinger and encircling the rest of their body with the other fingers. This procedure may be performed without the use of protective gloves, but it is preferable to use leather gloves to avoid getting injured by the defensive response of the animal.[1]

Armadillos

These animals normally do not try to bite; nevertheless, they possess strong claws on their forelimbs and feet.[4] Most armadillos may be manually restrained by holding the animal by the sides. The use of gloves may facilitate the restraint, in addition to avoiding possible scratches and bites.[2] In cases in which this technique is impossible, the animal may be held by the tail.[4] However, depending on the weight and size of the animal, this method of restraint may be very traumatic and may even break the segment of the tail being held. This type of restraint should be avoided as much as possible. There are reports of six-banded armadillos *(Euphractus sexcintus)* biting handlers. Giant armadillos *(Priodontes maximus)* are usually captured by the use of traps. Handling them must be done with extreme caution because they may inflict strong bites on the handlers if they are not properly restrained.[2]

More stressful species, such as Andean hairy armadillos *(Chaetophractus nationi),* tend to defecate and scream when they are manually restrained, in an attempt to be quickly released.

Sloths

Sloths may be manually restrained with gloves or nets. These animals may move quickly when they feel threatened. They use their powerful arms and legs to approach the hand of their aggressor and are capable of inflicting strong bites with their large, sharp, and coniform incisor teeth.[2] It is not uncommon for a wild sloth to be able to defend itself when it is being restrained by up to two persons. In spite of their gentle appearance, sloths possess great arm and leg strength that complicates their handling. In general, sloths are held for clinical examinations, for blood collection, or to induce anesthesia. They are held in a dorsal decubitus position with

the pelvic limbs extended to the sides on a flat surface; then a second handler may help by restricting the movements of the thoracic limbs by keeping them at a distance where the animal may neither grab nor bite the person performing the clinical examination or sampling.[2]

Chemical Immobilization and Anesthesia

The chemical immobilization of xenarthrans is undoubtedly one of the most complicated areas of wild animal anesthesia and, for various reasons, the least known. The complexity of working with these species is due, among other things, to the lack of knowledge about the anatomy, physiology, and ethology of the different species of xenarthrans and the lack of knowledge of their pharmacologic response to different anesthetics. For an adequate chemical immobilization of xenarthrans, we must know the anatomic and physiologic details of each of the species to be anesthetized, such as heart rate (HR), respiratory rate (RR), basal body temperature, venipuncture sites for support in emergency situations, and how these animals naturally respond to stressful situations.

Chemical Immobilization and Anesthesia in Anteaters

The first thing to consider is the remarkable difference in sizes and weights that exist between the three genera of anteaters, ranging from the giant anteater that may weigh up to 55 kg in captivity, passing through the lesser anteaters that vary from 6 to 10 kg, to small pygmy anteaters that weigh 60 g when born and may reach a maximum of 400 g as adults.[1,3] This helps to estimate the amount of anesthetic to be used, in addition to determining the method of drug administration that is safest for both animals and work team members. The choice of technique or method of anesthetics administration for anteaters depends on various conditions, such as the species, age, behavior of the animal, and the situation of the animal at the time of capture (captive or free living). For giant and lesser anteaters kept in captivity, the use of distance injection techniques using darts propelled by blowpipes or dart guns reduces the stress produced by physically restraining the animal and reduces the risk of the handlers' contact with the animal (Fig. 75.1). In the case of docile or juvenile specimens of the giant and lesser anteaters *(Myrmecophaga* and *Tamandua)*, and in all specimens of the pygmy anteaters *(Cyclopes)*, physical restraint and manual application of the drugs using simple syringes are recommended (Fig. 75.2).[1] In the anteaters, various injectable combinations have been reported for the immobilization of both wild and captive animals (Table 75.1).[1,5–7]

Anesthetic Monitoring in Anteaters

The monitoring of physiologic parameters during the anesthetic period is of the utmost importance and may be performed in a manner similar to that of other mammalian species. It is recommended to record the data every 5–10 minutes, while continuously monitoring the animal during the entire anesthetic process.[1] The cardiac function (HR and cardiac rhythm) may be monitored by use of a standard stethoscope for adults in the case of giant and lesser anteaters and with a pediatric stethoscope in the case of pygmy anteaters.[8] If available, it is recommended to use electrocardiography to better monitor the patient and diagnose alterations that cannot be detected by simple cardiac auscultation.[1] Respiratory function should be monitored continuously; RR may be recorded by thoracic auscultation and visualization of respiratory movements.[8] Oxygen saturation (SO_2) may be monitored by using pulse oximetry. Place the clamp sensor in one of the fingers in lesser and pygmy anteaters, in the plantar portion of the hind, or in the tail.[1] In giant anteaters the pulse oximeter sensor may be placed on the tongue (Fig. 75.3) or an esophageal sensor placed inside the mouth in direct contact with the oral mucosa.[1] Particular consideration should be given, especially when anesthetizing pigmy anteaters, to the importance of recording body temperature and associating it with the environmental temperature because these animals have a partial dependence of their internal temperature in relation to the environmental one.[9]

• **Figure 75.1** Use of blowpipe in free-living giant anteater *(Myrmecophaga tridactyla)*. (Photo by Gianmarco Rojas at Tamandua Project, Brazil.)

• **Figure 75.2** Application of anesthetic drugs in pygmy anteater *(Cyclopes didactylus)*. (Photo by Gianmarco Rojas at Huachipa Zoological Park, Peru.)

TABLE 75.1 Anesthetic Protocols Used in Xenarthrans

Protocol	Species	Common Name	Dose (mg/Kg)	Antagonist/Dose (mg/Kg)	Comments
Ketamine	*Myrmecophaga tridactyla*	Giant anteater	11	None	Low anesthetic quality immobilization[20]
	Dasypus novemcinctus	Nine-banded armadillo	65–90	None	Low anesthetic quality immobilization[20]
	Choloepus hoffmanni	Hoffmann's two-toed sloth	6	None	Low anesthetic quality immobilization[20]
Ketamine Midazolam	*Cyclopes didactylus*	Pygmy anteater	8–12 0.4	None	Short noninvasive procedures[1]
	M. tridactyla	Giant anteater	5–10 0.2	Flumazenil/0.01–0.02	Short nonbloody procedures[2]
	D. novemcinctus	Nine-banded armadillo	5–10 0.2	Flumazenil/0.01–0.02	Short procedures[2]
	C. hoffmanni	Hoffmann's two-toed sloth	5–10 0.2	Flumazenil/0.01–0.02	Short procedures[2]
Tiletamine/ zolazepam	*Tamandua tetradactyla*	Lesser anteater	15	None	Rapid induction but very prolonged recovery[20]
	Dasypus spp.	Nine-banded and long-nosed armadillos	8.5	None	Rapid induction but very prolonged recovery[10]
	Tolipeutes matacus	Three-banded armadillo	4–12 ♂ 4–9 ♀	None	Rapid induction and anesthesia of medium duration (approximately 40 min) Regular muscle relaxation. Tachypnea and apnea[12]
	Zaedyus pichiy	Pichi armadillo	15		Prolonged recovery[31]
	C. didactylus	Linné's two-toed sloth	1.9–6–10	None	Too prolonged and irregular recovery[16]
Tiletamine/ Zolazepam Medetomidine	*T. matacus*	Three-banded armadillo	3 0.06	Atipamezole/0.3	Used with isoflurane. Rapid induction but very prolonged recovery[12]
Ketamine Xylazine	*M. tridactyla*	Giant anteater	5–10 0.5–1.5	Yohimbine/0.12–0.2	Without regurgitation
	T. tetradactyla	Lesser anteater	20 1	Yohimbine/0.12–0.2	Good muscle relaxation, few considerable alterations[5]
	Dasypus spp.	Nine-banded and long-nosed armadillos	40 1	Yohimbine/0.12–0.2	Without regurgitation, frequent salivation[10,11]
	Priodontes maximus	Giant armadillo	5 1	Atipamezole/0.1	Rapid recovery after the antagonist[18]
	T. matacus	Three-banded armadillo	30–32 1	Yohimbine/0.12–0.14	Without regurgitation, frequent salivation. Good muscle relaxation. Tachypnea and apnea[12]
	C. didactylus	Linné's two-toed sloth	10 1	Yohimbine/0.12–0.2	Without regurgitation[16]
Ketamine Medetomidine	*M. tridactyla*	Giant anteater	2–4 0.02–0.04	Atipamezole/ 5× the dose of Medetomidine dose	Good muscle relaxation[31]
	Dasypus spp.	Nine-banded and long-nosed armadillos	7.5 0.075	Atipamezole/ 5× dose of Medetomidine	Good muscle relaxation and rapid recovery after the antagonist[10]
	C. didactylus	Linné's two-toed sloth	3 ± 0.3 0.04 ± 0.005	Atipamezole/0.1	Used in free-ranging sloths[16]
	C. hoffmanni	Hoffmann's two-toed sloth	3 0.04	Atipamezole/ 5× Medetomidine dose	Good muscle relaxation[17]
	Bradypus variegatus	Brown-throated three-toed sloth	5 0.02	Atipamezole/0.1	Good muscle relaxation and rapid recovery after the antagonist[17]

Continued

TABLE 75.1 Anesthetic Protocols Used in Xenarthrans—cont'd

Protocol	Species	Common Name	Dose (mg/Kg)	Antagonist/Dose (mg/Kg)	Comments
Ketamine Dexmedetomidine	*C. hoffmanni*	Hoffmann's two-toed sloth	2 ± 1 0.012 ± 0.006 (for *C. hoffmanni*)	Atipamezole/0.145	Short procedures in free-ranging animals. Requires supplementation[26]
	B. variegatus	Brown-throated three-toed sloth	2 ± 1 0.011 ± 0.005 (for *B. variegatus*)	Atipamezole/0.122	Short procedures in free life. Requires supplementation[26]
Ketamine Acepromazine	*C. didactylusi*	Linné's two-toed sloth	10 0.1	None	Rapid induction but poor sedation and muscle relaxation[16]
	B. torquatus	Maned three-toed sloth	1.3 0.1	None	Mild sedation. For clinic examinations and blood samples[23]
Ketamine Xylazine Midazolam	*Chaetophractus nationi*	Andean hairy armadillo	15 1 0.4	Yohimbine/0.2	Without regurgitation, mild salivation. Rapid recovery after the antagonist[13]
	C. didactylus	Linné's two-toed sloth	3 1 0.2	Yohimbine/0.125 Flumazenil/0.005	Rapid induction and recovery. Mild hypertension[25]
Ketamine Medetomidine Midazolam	*C. nationi*	Andean hairy armadillo	7 0.08 0.1	Atipamezole/0.4	Rapid induction and recovery after the antagonist*
Ketamine Dexmedetomidine Midazolam	*C. didactylus*	Pygmy anteater	4 0.015–0.03 0.1	Atipamezole/0.15–0.30	Rapid induction and recovery after the antagonist[7]
	M. tridactyla	Giant anteater	4 0.015 0.1	Atipamezole/0.15	Rapid induction and recovery after the antagonist[7]
	T. tetradactyla	Lesser anteater	4–5 0.02 0.1	Atipamezole/0.15	Rapid induction and recovery after the antagonist[7]
	Cabassous unicinctus	Southern naked-tailed armadillo	5 0.015 0.1	Atipamezole/0.15	Rapid induction and recovery after the antagonist*
	C. nationi	Andean hairy armadillo	7 0.04 0.1	Atipamezole/0.4	Rapid induction, good quality of anesthesia and recovery after the antagonist*
	D. novemcinctus	Nine-banded armadillo	5 0.015 0.1	Atipamezole/0.4	Rapid induction and recovery*
	Z. pichiy	Pichi armadillo	7 0.05 0.05	Atipamezole/0.4	Rapid induction and recovery after the antagonist[31]
	C. hoffmanni	Hoffmann's two-toed sloth	2.6 0.012 0.1	Atipamezole/0.22	Rapid induction and recovery after the antagonist[24]
	B. variegatus	Brown-throated three-toed sloth	2 0.012 0.1	Atipamezole/0.12	Rapid induction and recovery after the antagonist*
Ketamine Acepromazine Diazepam Buprenorphine	*M. tridactyla*	Giant anteater	8.8 0.06 0.3 0.006		Maintenance with isoflurane[31]
Ketamine Butorphanol Medetomidine	*D. novemcinctus*	Nine-banded armadillo	15 0.1 0.07	Atipamezole/ 5× dose of Medetomidine	Good analgesia and muscle relaxation[11]

TABLE 75.1 Anesthetic Protocols Used in Xenarthrans—cont'd

Protocol	Species	Common Name	Dose (mg/Kg)	Antagonist/Dose (mg/Kg)	Comments
Xylazine Butorphanol Midazolam	*P. maximus*	Giant armadillo	1.2 0.4 0.2	Yohimbine/0.125 Naltrexone/0.25	Maintenance with Isoflurane Rapid induction and recovery after the antagonist[14]
Isofluorane	*C. unicinctus*	Southern naked-tailed armadillo	4%–5% induction 2%–3% maintenance	None	Rapid induction and recovery. Does not generate analgesia*
Ketamine Xylazine Propofol	*Euphractus sexcinctus*	Six-banded armadillo	7 1 5 (IV)	None	For semen collection by electroejaculation[30]
Ketamine Butorphanol Propofol	*E. sexcinctus*	Six-banded armadillo	7 0.4 5 (IV)	None	For semen collection by electroejaculation[30]

*Unpublished personal information.

• **Figure 75.3** Positioning of pulse oximetry sensor on tongue of giant anteater *(Myrmecophaga tridactyla)*. (Photo by Gianmarco Rojas at Huachipa Zoological Park, Peru.)

Anesthetic Recovery in Anteaters

The use of antagonists is recommended to reduce recovery time. In addition, the animal should be monitored until showing signs of adequate recovery. In giant anteaters the animal is monitored directly until it initiates head and thoracic and pelvic limbs movements. The animal is then observed from a safe distance in the case of free-ranging animals or, for captive animals, kept within a suitable transport box until it completely recovers. The recovery box should allow for adequate extension of the animal's neck to avoid problems with airway obstruction or by the presence of secretions. In lesser and pygmy anteaters, animals may be monitored until almost totally recovered because they are easily physically restrained.

Chemical Immobilization and Anesthesia in Armadillos

In general, a variety of anesthetic agents have been used in different species of armadillos (see Table 75.1), and many of these can be used for animals in free-living conditions.[2,10–13] The thigh musculature is the best place for intramuscular injections of anesthetic drugs in armadillos.[10,12,13] For those species that are completely inside the carapace, such as *Tolipeutes,* the musculature of the base of the tail may be used.[12] Cyclohexamines, as in other wild species, is the pharmacologic group most frequently used in armadillos. The addition of alpha-2 agonists or/and benzodiazepines are the most frequently reported combinations in the different armadillo species.[2,10–13] Free-ranging giant armadillos is commonly caught using traps. These individuals are then immobilized with ketamine in combination with xylazine administered intramuscularly.[2] Other reports describe the use of combinations of xylazine and midazolam for sedation followed by isoflurane for anesthesia in captive giant armadillos.[14] Ketamine and xylazine have also been described for anesthesia in the nine-banded armadillo *(Dasypus novemcinctus)*[10,11,15] and greater long-nosed armadillo *(D. Kappleri)*.[10] Another combination that offers a good option for free-living animals is the combination of ketamine with medetomidine because the effects of medetomidine are easily reversed.[10,16,17] There is a report of the use of ketamine, medetomidine, and butorphanol for intraabdominal surgeries of *D. novemcinctus.*[11] Although these animals metabolize the combination of ketamine with alpha-2 agonists very well,

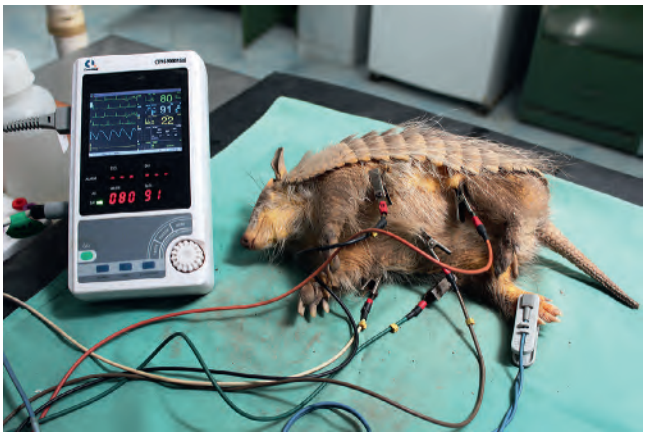
the use of antagonists such as yohimbine or atipamezole can accelerate recovery after the handling.[2,12,13]

The combination of cyclohexamines with benzodiazepines offers safe sedation with variable duration. Combinations of ketamine with diazepam or with midazolam have been described as options for armadillos.[2,4] These combinations have a short induction period, moderate muscle relaxation, and a relatively short period of sedation. The use of tiletamine-zolazepam has also been described for the anesthesia of armadillos of the *Dasypus*[10,15] and for *Tolypeutes*[12] genuses, and the combination provides good muscular relaxation. However, because of its prolonged recovery, it is of limited use for anesthesia of free-living specimens.[2,12] Other injectable combinations that were also relatively successful for armadillo chemical restraint were the combinations of ketamine and acepromazine,[18] and droperidol and fentanyl.[19] Unfortunately, the former has a long recovery time, and the latter is no longer commercially available.

Most armadillo species may be attached and connected to a volatile anesthetic delivery system or placed in an induction chamber.[2] Many volatile anesthetics, such as halothane,[4] isoflurane, and sevoflurane, have been successfully used in different species of armadillos.[2,15,20] However, it is important to note that armadillos have the capacity to hold their breath voluntarily for prolonged periods, thus hindering the use of this anesthetic method.[2,15] The use of injectable premedication followed by immediate sedation of armadillos minimizes the occurrence of respiratory arrest.[18] Another technique that may reduce this problem is the administration of pure oxygen during the first minutes and gradual increase of the concentrations of the anesthetic, verifying that the animal continues breathing spontaneously and avoiding hyperventilation or apnea. Sedation and short-term effective anesthesia have been achieved with isoflurane alone in southern naked-tailed armadillo *(Cabassous unicinctus)* for blood sampling and claw-cutting procedures.

Anesthetic Monitoring in Armadillos

In armadillos the induction process is the time when most anesthetic emergencies occur.[13,20] Attention should be paid to significant changes in body temperature. In these species, it is usually low, varying from 30°C to 35°C.[2,15] In addition, they are strongly influenced by the environmental temperature. Therefore it is recommended to monitor both environmental and body temperatures simultaneously. HR may be monitored by cardiac auscultation using a pediatric stethoscope, by electrocardiogram, or indirectly by pulse oximetry. RR may be assessed by visual counting of respiratory movements (Fig. 75.4).[10,13] HR and RR in anesthetized armadillos may vary by the species and drugs used. There are reports of HR (bpm) and RR (rpm) values, respectively, in southern naked-tailed armadillo of 79–98 and 38–43, Andean hairy armadillo *(C. nationi)* of 81–115 and 28–47, nine-banded armadillo *Dasypus* sp. of 52–168 and 14–70, giant armadillo *(P. maximus)* of 48–60 and 4–20, and southern three-banded armadillo *(T. matacus)* of 90–140 and 60, respectively.



• **Figure 75.4** Anesthetic monitoring in Andean hairy armadillo *(Chaetophractus nationi)*. (Photo by Gianmarco Rojas at Huachipa Zoological Park, Peru.)

Measurement of blood gases in armadillos is complicated by the difficulty in locating and sampling the arteries in these animals; however, blood gases should be monitored when possible.[2] With great care, mixed arterial and venous blood may be collected from the heart. Pulse oximetry is used in armadillos by placing the sensor on the tongue or penis.[10] There are also reports of sensor placement on the thigh of animals.[20] Other locations for placing the oximeter sensor are the jaw, the fingers, or even the palmar and plantar surface in small species.[13] Some technical problems, mainly related to the sensitivity of the sensor, may make it difficult to assess this parameter.

Anesthetic Recovery in Armadillos

Excessive salivation caused by various drugs may be reduced by the use of atropine.[4,18] Armadillos have been reported to recover spontaneously from ventricular fibrillation without treatment; likewise, armadillos may tolerate long periods of apnea and hypoxia, as compared with other mammals.[19] Many cardiovascular abnormalities may occur when using alpha-2 agonists for immobilization procedures.[21] Alpha-2 agonists may be antagonized with atipamezole or yohimbine, both to reverse cardiovascular adverse effects, as well as to initiate anesthetic recovery of the animal.[2] Reversing the effect of benzodiazepines using flumazenil may reduce recovery time. However, the short duration of action of the antagonism of flumazenil (60 minutes) makes it impractical to use in free-living conditions because the duration of benzodiazepine effects may last for more than 4 hours.[2] In general, it is important to keep the armadillos in confinement or under observation until their full recovery before being released.

Chemical Immobilization and Anesthesia in Sloths

Sloths may be manually restrained and injected intramuscularly while they are resting. After the anesthetics are

administered, the animal should be handled as little as possible and preferably left inside a containment box or transport box until the maximum induction effect of the anesthetics has been achieved. When the anesthetics take effect, the sloth may be held with gloves to avoid bites. Although a sedated sloth is unable to grasp or hold on to a branch, it is very possible that it may still bite (although with less force). This is especially true when manipulating sloths of the genus *Choloepus.* Sloths may also be held and sedated by using masks and an inhalation anesthetic; however, sloths may hold their breath for prolonged periods of time, making this a very difficult option.[2,22] A variety of injectable anesthetic combinations have been used in sloths (see Table 75.1).[16,17,23–26] The reports of the use of anesthetic combinations include sodium pentobarbital used alone or in association with acepromazine.[27,28] Currently this combination is not used due to its notable depressant cardiorespiratory effects, prolonged recovery time, and lack of antagonists. Ketamine has also been used alone or in combination with other anesthetics such as acepromazine, benzodiazepines, and alpha-2 agonists.[16–18,24–26,29]

More recently the successful use of alpha-2 antagonists such as yohimbine and atipamezole has been reported[16,24,25] and benzodiazepine antagonists such as flumazenil.[25] These antagonists have greatly reduced anesthetic recovery times in sloths. Combinations of ketamine and diazepam or midazolam are a good choice for short-term immobilizations if the animal is not exposed to painful procedures. The combination of ketamine with alpha-2 agonists may be the best option for immobilization of sloths because of the adequate muscle relaxation, degree of analgesia, and the possibility of reversing their effects. It has been shown that protocols using medetomidine and dexmedetomidine have less deleterious effects compared with combinations that include xylazine.[16,17,24,25] In addition, other combinations such as tiletamine-zolazepam have been used with variable results.[16] Tiletamine-zolazepam is considered safe and effective for captive conditions; however, it has a prolonged recovery.[20,24] This combination promotes deep anesthesia with good muscle relaxation, but in some cases it may lead to respiratory depression. Combination of ketamine and alpha-2 agonists promotes dissociative anesthesia of approximately 40-minute duration.[6,17] Combinations of ketamine, alpha-2 agonists, and benzodiazepines provide more durable anesthesia times reaching greater than 60 minutes and very short recovery times, offering an anesthetic quality superior to those of other pharmacologic combinations.[24,25]

Anesthetic Monitoring in Sloths

The HR may be monitored by auscultation with the help of a pediatric stethoscope or with the support of an electrocardiograph. RR is verified by counting respiratory movements.[17] Blood pressure may be monitored in xenarthrans with indirect methods because the location of intravenous and intraarterial accesses is a challenge in these animals.[2] In sloths, blood pressure may be measured by the placement of a Doppler vascular flow sensor over the radial artery, with a 5-cm pediatric blood pressure cuff placed on the arm of the animal and connected to a sphygmomanometer.[17] Sloths present extreme variation in blood pressure, and stress may have great influence on these values.[23] Pulse oximetry is used in sloths for monitoring cardiorespiratory function; however, their values may be influenced by hypothermia, hypotension, and tissue pigmentation.[2] The positions of the pulse oximeter sensor in sloths include the tongue, jaw,[24] preputial area, and rectal mucosa.[17]

Anesthetic Recovery in Sloth

The use of alpha-2 antagonists and flumazenil is recommended in sloths, both to minimize the negative effects on the cardiovascular system and to initiate the anesthetic recovery of the animal.[2,24] Sloths may require confinement during recovery until they may grab the branches on their own. Good confinement prevents the occurrence of self-inflicted injuries associated with excitement during recovery.

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SECTION 15

Carnivores

76

Update on Field Anesthesia Protocols for Free-Ranging African Lions

PETER BUSS AND MICHELE MILLER

Introduction

Chemical immobilization of free-ranging African lions *(Panthera leo)* is a fundamental procedure used in the conservation of this vulnerable carnivore and allows for the capture, translocation, and treatment of individuals. Cyclohexylamines are the most widely used group of agents in the immobilization of lions and, historically, include phencyclidine and ketamine. Tiletamine-zolazepam (TZ) is commonly used as it results in faster induction, better muscle relaxation, and shorter recovery than with phencyclidine, and it is more potent and requires less dart-volume than ketamine.[1] To reduce recovery times and improve patient safety, partially or fully reversible immobilizing-drug combinations including tiletamine-zolazepam-medetomidine (TZM), ketamine-medetomidine or detomidine, and butorphanol-medetomidine-midazolam (BMM) have been developed.[2,3]

Capture Technique

Lions are preferentially captured at night as they are more active, less wary of people, and more inclined to approach bait. For safety reasons, all capture preparations should take place before nightfall. Two sites are established: a capture site to which lions will be attracted and darted, and a processing site to which the immobilized lions will be transported for veterinary procedures. The distance between these two areas (25–200 m) will depend on how tolerant the lions are to human presence. These sites reduce the potential contact between lions approaching the bait and personnel working at the processing site. The capture site should be open and cleared of all vegetation that may obstruct the flight of a dart or conceal a lion from view. The site should also be large enough to allow easy vehicle access. The processing site should be large enough to accommodate all personnel and equipment, as well as the immobilized lions. The area must be well lit and partially or completely surrounded by vehicles to provide a barrier against intrusion of an awake lion.

Lions are attracted to the capture site using a carcass that is partially eviscerated, dragged behind a vehicle for up to 2000 m, creating a scent trail, and securely fastened to the base of a tree or steel stake driven into the ground within the capture site. Recorded sounds of lions and/or hyenas feeding on a carcass or an animal in distress are played through loudspeakers to attract lions to the capture site. Lions are opportunists. Anticipating a "free meal," they will walk toward the sound. When they encounter the carcass scent trail, they will follow it to the capture site. Once feeding on the carcass, the lion can be darted from the safety of a vehicle that is positioned to dart specific individuals. Immobilized lions are blindfolded and front limbs hobbled as a safety precaution to protect personnel, placed onto a vehicle, and transported to the processing site. Anesthetic depth is determined by administering a painful stimulus from the safety of the vehicle before the lions are approached on foot. Other lions that will not be immobilized are allowed to continue feeding on the bait to keep them occupied and prevent them from moving toward the processing site.

Cardiovascular and respiratory functions, body temperature, and anesthetic depth are monitored at frequent intervals. At the end of a procedure, individuals are observed until fully recovered and protected from potential attack by other lions.[2]

Immobilizing Agents and Antagonists

Tiletamine Plus Zolazepam (Zoletil, Telazol)

TZ has been used for the past two decades as the drug of choice for immobilizing free-ranging lions (Table 76.1).[4] This drug combination has a wide safety margin (2–10 mg/kg) and results in rapid induction with limited adverse respiratory or cardiovascular effects.[5] Tonic convulsions may result and hyperthermia is a potential risk. Recovery is gradual and predictable. Depending on the total dose administered, it may take up to 4 hours before an animal is able to stand and walk. During recovery, an individual

TABLE 76.1 Immobilizing Drugs, Drug Combinations, and Antagonists Commonly Used in Free-Ranging African Lions (Doses Are Given in mg/kg or as a Ratio [Antagonist:Agonist] in mg)

Immobilizing Drugs and Combinations						Immobilizing Drug Antagonists		
Ketamine (IM)	Tiletamine & Zolazepam (IM)	Butorphanol (IM)	Medetomidine (IM)	Detomidine (IM)	Midazolam (IM)	Naltrexone (IM, IV)	Atipamezole (IM, IV)*	Flumazenil (IM, IV)
	3–5[2]							
	4–5 (≤10)[4]							
	0.38–1.32[7]		0.027–0.055				2.5–5× medetomidine	
	1.3–2.3[6]		0.06–0.08				0.2–0.4	
2.5[3]			0.07					
5–7[2]			0.03–0.05				5× medetomidine	
4–5[3]				0.05				
		0.3[8]	0.05		0.2	0.7	0.3	0.0032
		0.2–0.3[2]	0.05		0.15	2× butorphanol	5× medetomidine	

*Atipamezole may be administered IV slowly in emergencies.

is vulnerable to attack by other lions and hyenas, and may suffer injuries as it persistently tries to stand before it is ready.

Cyclohexylamine Plus Alpha$_2$-Agonist

The dose of TZ required to immobilize lions can be reduced by as much as 75% if combined with medetomidine. Recovery times are significantly reduced by antagonizing the medetomidine effects with atipamezole (see Table 76.1).[6] TZM provides a smooth anesthetic induction within 3–10 minutes with good muscle relaxation and sufficient analgesia for minor surgical procedures.[6,7] Decreases in respiration and heart rate are limited and remain stable throughout the anesthesia, and hemoglobin oxygen saturation values seldom decrease below 90%. Rectal temperature may exceed 40°C and should be closely monitored. The palpebral reflex may be present and becomes more prominent as the duration of anesthesia increases.[7] Spontaneous recoveries may occur after approximately 1 hour following induction, and repeat intramuscular (IM) doses of both TZ and medetomidine (one-third of induction dose) are recommended. In the authors' experience, multiple additional TZM doses may result in prolonged recovery times as a consequence of accumulative tiletamine effect and judicious administration is advised.

Recovery to standing and walking is variable depending on initial TZM dose, time between darting and atipamezole administration, and whether additional doses were given. Recovery times are reduced if the antidote is given later in the immobilization period as a result of tiletamine metabolism. Lions immobilized with lower TZM doses recover to walking within 8–30 minutes following atipamezole administration at approximately 45 minutes after induction.[7]

The use of ketamine plus medetomidine or detomidine in free-ranging lions has been reported.[3] These drug combinations resulted in induction times of 6–10 minutes with limited respiratory and cardiovascular adverse effects. Hemoglobin oxygen saturation varied from 85% to 90%. Atipamezole was administered approximately 1 hour after immobilization and recovery took 25–32 minutes. These combinations are recommended for short-duration procedures such as radio-collaring, disease surveillance, or snare removal.[3]

Butorphanol, Medetomidine, and Midazolam

BMM combination (see Table 76.1) provides an effective immobilizing drug combination for lions with a rapid induction (5–10 minutes) and 45 minutes of stable anesthesia.[8] Immobilized lions do not react to minor painful procedures but more invasive surgeries require additional analgesia. Mild bradycardia (<50 beats/min), mild metabolic acidosis, normocapnia, and mean PaO_2 of 87 mm Hg have been reported in BMM-immobilized lions.[8] Complete reversal

is achieved with naltrexone-atipamezole-flumazenil administration, with recovery to standing within 4–8 minutes. An advantage of this combination is that reversal agents can be administered at any time point following induction.[8]

Field experience has shown that BMM-immobilized lions may spontaneously stand up at ≥45 minutes after induction, especially following a loud noise or painful manipulation. Immobilized lions should be administered one-third of the initial BMM dose IM at 45 minutes after induction and every subsequent 30 minutes. The use of blindfolds and hobbles is advocated to limit the possibility of injury to people working with the lions. Antidote dose rates are calculated on the total immobilizing drug doses administered. The authors have not observed adverse central nervous system or cardiorespiratory effects following intravenous atipamezole administration in cases of anesthetic emergencies. Additional atipamezole doses (10× medetomidine dose, mg) may also be given following prolonged procedures to ensure animals do not become resedated. At the suggested midazolam dose rates, the omission of flumazenil does not significantly change the quality or time to recovery.

Darting From a Helicopter

Helicopter-based darting of lions should only be attempted by a capable pilot and experienced veterinarian. This capture method results in a marked sympathetic response in the lion, and the authors have found TZM and BMM to be ineffective unless administered at more than twice the recommended dose. TZ is the suggested drug of choice; however, hyperthermia in the immobilized lion is a significant risk as a result of increased exertion caused by the approach of the helicopter during darting, increased daytime ambient temperatures, and drug-induced muscle tonicity. Alighting from a helicopter and approaching an apparently immobilized lion should be done with extreme caution until the anesthetic depth may be confirmed.

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77

Overview of African Wild Dog Medicine

JENNIFER N. LANGAN AND GWEN JANKOWSKI

Introduction

The African wild dog *(Lycaon pictus)*—also referred to as the African hunting dog, painted dog, and Cape hunting dog—is one of Africa's most endangered carnivores.[1] Owing to its decreasing numbers, it holds an International Union for Conservation of Nature (IUCN) red list priority status for the conservation of canid species in Africa.[2] African wild dogs were formerly distributed throughout sub-Saharan Africa but are now mostly confined to southern Africa and the southern portion of East Africa (Fig. 77.1).[3,4] They require large ranges and live at low population densities. The population is currently estimated at 6600 animals and continues to decline as a result of ongoing habitat fragmentation, conflict with humans, and infectious disease.[1,5] Predation by lions and competition with spotted hyenas also contribute to population suppression.[6] There are approximately 600 African wild dogs in zoos, which serve to educate the public and fulfill an important role as ambassadors aiding the recovery effort for this species.

Biology and Anatomy

The African wild dog is a member of the family Canidae in the order Carnivora; it likely diverged from wolves in the Pleistocene period.[1] Genetic studies demonstrate that it is sufficiently different from other canid species to warrant being classified into a separate genus. Adults weigh 18–35 kg, with males slightly larger than females.[2,3,7] The average age of survival in zoos is 10.3 years[8]; a few animals live 12–16 years.[2,7,9] The African wild dog's most striking characteristic is the tricolored spotted coat, for which it received its Latin name, *Lycaon pictus,* meaning "painted wolf." It has large round ears, lacks a supracaudal (tail) gland, has four digits on each limb (lacks dew claws), and the pads of the middle digits are connected by dermal webbing. Reproductive anatomy is similar to that of domestic dogs in both males and females, but females have 12–14 mammae. They have very sharp, large premolars relative to their body mass, which allow them to consume sizable quantities of meat and bone with impressive speed. The dental formula is I3/3, C1/1, PM3/4, M3/3 = 21, of which the last mandibular molar is vestigial and generally not visualized.[1]

Management, Husbandry, and Behavior

The typical wild pack is composed of an older dominant female paired with a young dominant male and subordinates of both sexes. Dominant males may be displaced as they age or lose strength. Juvenile males are most likely to stay with the pack, whereas females often emigrate. Following the death of the dominant female, significant social changes occur within the group, which can result in pack dispersal in the wild.[1,7]

Social management of African wild dogs under human care is challenging and can have significant health impacts. It involves working across institutions to create and maintain packs that thrive socially, support a healthy population, and sustain genetic diversity. The most stable social groups include a well-established dominant pair with male offspring of any age and young female offspring. The inability to disperse may result in conflict between female offspring above 18 months of age and the dominant female. The decreasing frequency of "hoo-calls" (long-distance communication calls) and distance between resting sites of same-sex groups suggest that unrelated individuals under human care are more likely to integrate into a pack successfully.[10] If animals need to be separated due to social incompatibility, it is recommended that individuals be split up as same-sex packs or with littermates less than 18 months of age.[7] Contraception for reproductively mature individuals intended to reduce aggression has not been successful.[7] Measurement of fecal corticosteroids may be a useful management tool and has demonstrated that dominant animals generally have the highest stress levels.[11–13]

Behavior is a key indicator of social and physical well-being in African wild dogs. "Normal" behavior varies by an individual's status within the pack, and establishing pack hierarchy is essential for avoiding excessive aggression.

• **Figure 77.1** Distribution of the remaining African wild dog *(Lycaon pictus)* populations.

Permanent or even brief temporary removal of an established pack member may have profound social impacts, including changes in social hierarchy with aggression so substantial that reintroduction may not be possible.[7,15] Detailed plans to reduce stress and promote normal behaviors should be implemented if a dog must be isolated, including maintaining olfactory and visual contact. If separation is required, it may be helpful to subdivide the pack and then reintroduce them all simultaneously. Alternatively, introductions of subordinate dogs first, then dominant pairs, may be effective.

Successful implementation of enrichment has included environmental devices, sensory stimulants, and food, behavioral, and habitat variance.[16] Piles of leaves, dirt, and mulch allow natural digging and rolling behaviors. Rotating exhibits with other predators provides habitat diversity and promotes scent-marking behavior. Offering carcass feeds hung from trees or on zip lines, feeding bones, providing several types of enrichment to the pack simultaneously, and permitting breeding when possible are recommended for this species.[15,16]

Enclosures should be large and contain ample space for exercise to meet the animals' physical, social, behavioral, and psychological needs.[7] Specific size and perimeter recommendations may be found in the Association of Zoos & Aquariums (AZA) *Large Canid Care Manual*.[7] Facilities that allow the public to observe the animals should prevent close contact or inadvertent access to the enclosure. Dogs should have access to multiple heated areas if the temperature regularly drops below 4.4°C–7.2°C (40°F–45°F) and should have shelter from the elements.[7] Additionally, a heated den should be provided if breeding is planned. Facilities should have sufficient holding space to accommodate separating animals for long periods. With the exception of fish, housing African wild dogs with other species is not recommended due to their high predatory drive.

African wild dogs should be moved only in sturdy metal or wood crates with good ventilation that meet US Department of Agriculture (USDA) and International Air Transport Association (IATA) requirements for live animal transport. Completing a written transport plan, health evaluation, and crate training facilitates transitions when animals are relocated.

Nutrition

African wild dogs are generalist predators, occupying a range of habitats where they hunt medium-sized antelope.[6] In natural settings, reduced prey populations and competition from other predators inhibit population growth.[3,17] African wild dogs in zoos are fed a nutritionally complete raw meat–based diet (1–1.36 kg/adult/day) and are supplemented with small whole prey, knuckle/rib/shank bones (one to two times week), and carcasses (pig, deer, calf, horse). Lactating bitches require up to three times

maintenance caloric intake. Specific recommendations for kilocalorie requirements may be found in the AZA *Large Canid Care Manual*.[7] Feeding African wild dogs a portion of their diet while separated from the pack aids in monitoring individual animals' food consumption. Packs are generally fasted from their normal meat diet 1 day per week and may be provided with bones.

Reproduction and Contraception

African wild dogs are seasonally monoestrous obligate cooperative breeders with a brief copulatory tie.[18,19] Within a pack the alpha male and female produce the majority of surviving pups annually.[7,20,21] Most successful reproduction occurs after 2 years of age, with senescence around 8–9 years.[8,21] Subordinate females may reproduce, but offspring typically do not survive. More often, subordinate females develop pseudopregnancy and may lactate in order to help care for the pups of the dominant pair.[1] Females produce an average of six to eight pups[22,23] and up to 21 pups[8] in a den after a gestation of 69–71 days.[24–26] Primiparous females have higher estrogen, which is reported to result in more male offspring.[24] Hand-rearing is not recommended due to the extremely aggressive and social nature of these animals.[8] In zoos, the breeding pair is separated from the pack to prevent trauma to the pups. The group is gradually introduced when the pups begin to emerge from the nest box. A birth plan detailing responses to aggression toward the pups, large litter size, and other contingencies is recommended.

Newborn pups weigh about 300 g, open their eyes around 2 weeks of age, and emerge from the den to start taking solid food at 3 weeks. Sex determination is similar to that for other canids. Pups are weaned and start to follow the pack at 11–12 weeks of age. In free-range settings, all members of the pack raise the pups; they regurgitate food while the young are in the den and relinquish kills to the pups and yearlings.[1,3]

Reproductive anatomy is similar to that of other canid species. Owing to social dynamics, most reproduction has been natural; however, semen has been preserved and used for artificial inseminations.[15] Captive *Lycaon pictus* generally reproduce in the fall in the northern hemisphere.[8] Estrus lasts 6–9 days and includes vulvar swelling and sanguineous discharge with interest from the male. Attraction from the male may be observed for 1–2 weeks prior to tying.[14,25] Males show increased testicular development, spermatorrhea, and semen production; therefore the corresponding seasonal ability to collect sperm via electroejaculation is improved.[15]

The progestin-based melengestrol acetate (MGA) implant, previously used in canids, has been associated with uterine pathology.[27] The AZA Reproduction Management Center (formerly the Wildlife Contraception Center) (www.stlzoo.org/animals/scienceresearch/reproductivemanagementcenter) recommends gonadotropin releasing hormone (GnRH) agonists such as Suprelorin (deslorelin acetate) implants or Lupron Depot (leuprolide acetate 4.7 mg implant, Virbac AH, Inc., Fort Worth, Texas). Current estimates show 23% of females have some degree of reproductive pathology,[28] the most frequently reported of which is cystic endometrial hyperplasia (CEH) with or without pyometra and adenomyosis (Fig. 77.2).[27–29] Adenocarcinoma, uterine rupture, and pyometra without other pathology have also been reported (Kinsel, personal communication, April 10, 2017).[23,27,28] The Species Survival Plan Program (SSP) currently recommends that all postreproductive females (>10 years) be spayed.[8] Deslorelin implants have been used for contraception and behavioral alteration in males with variable results (see Table 77.1 for a summary of reproductive information) (see also Chapter 22).[26,30,31]

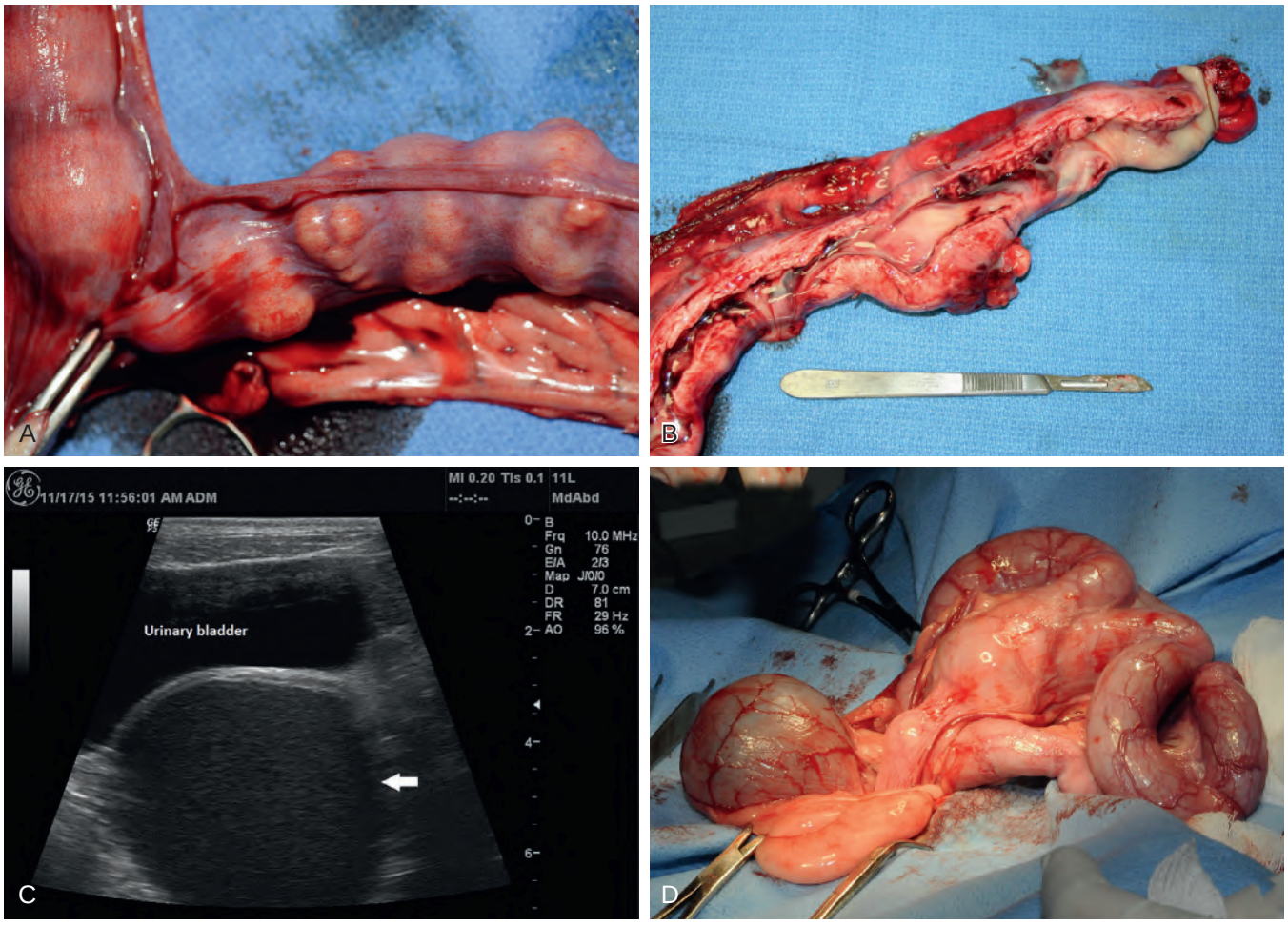
Handling, Restraint, and Anesthesia

Healthy adult African wild dogs are not physically restrained due to safety concerns. Noninvasive procedures including visual examination, hand injections, wound treatment, venipuncture, and crate training may be accomplished with operant conditioning.

Restraint cages are useful and provide a safe, controlled environment to facilitate intramuscular injections of anesthetics. A quiet area away from the pack promotes quick inductions and smooth recoveries. Remote injection systems are recommended for immobilizing free-ranging African wild dogs or in situations when a chute is not available. Anesthetic regimens selected should take into account health status, age, and environmental conditions. In cases where cardiovascular disease has been confirmed or cardiac status is unknown, alpha-2 agonists should be avoided and alternatives such as ketamine-midazolam-butorphanol with propofol or gas anesthesia (isoflurane, sevoflurane) should be considered. Chemical restraint protocols used in African wild dogs may be found in Table 77.2 and have been previously published.[32–34] Drug combinations at higher dosages for free-ranging African wild dogs are available in the literature.[35,36] Reversal of alpha-2 agonists and opioids with atipamezole and naloxone decreases recovery time. To avoid dysphoria, it is advised to wait at least 60 minutes postinduction before administering reversals. Recovery in a crate or nest box may reduce struggles to stand during recovery. Telazol (tiletamine HCL and zolazepam HCL, Zoetis, Parsippany, New Jersey) as a sole agent or used in combination with medetomidine is a reliable option during an emergency response but often results in a prolonged recovery time.[34] Vascular access, intubation, and monitoring equipment are applied as in other canids. Every wild dog anesthesia should include oxygen supplementation, electrocardiography (ECG), and monitoring of pulse oximetry, heart and respiratory rate, blood pressure, and temperature.

Clinical Pathology

As in other canids, the jugular, cephalic, and saphenous veins are commonly used sites for venipuncture. Tables 77.3



• **Figure 77.2** Common uterine pathology in African wild dogs *(Lycaon pictus)*: (A) uterine hyperplasia and pyometra, (B) uterine adenocarcinoma with associated pyometra, (C) ultrasound image of a dilated uterine horn filled with hyperechoic material *(arrow)*, and (D) and corresponding intraoperative image in the same animal with pyometra.

TABLE 77.1 Reproductive Information for African Wild Dog *(Lycaon pictus)*

Reproductive Cycle	Monestrous
Usual age of first reproduction	21–22 months
Copulation (North America)	August–October
Length of estrus	6–9 days
Gestation (from first day of copulation)	69–71 days
Parturition (North America)	October–January
Mean/maximum litter size	6–8/21

and 77.4 show normal hematologic and serum chemistry reference ranges for captive African wild dogs.[9,37]

Diseases

Rabies and distemper have contributed to mortality in African dog populations, occasionally resulting in local extinction events.[38–41] There is serologic evidence of exposure to canine parvovirus, canine distemper virus, adenovirus, rabies virus, coronavirus, rotavirus, and *Ehrlichia canis*.[21,42,43] Outbreaks of anthrax in Kruger National Park occur, but infected animals commonly survive.[43] Contact with domestic dogs has been reported to increase exposure to some canid pathogens, but sylvatic viral strains also pose a significant threat.[5,44]

Parasitic disease and infection has rarely been described.[44–46] *Toxocara canis, Dipylidium caninum, Spirometra* sp., Taeniidae, and *Ancylostoma* spp., as well as two genera of canid protozoan gastrointestinal parasites, *Sarcocystis* and *Isopora,* were identified in fecal samples from free-ranging animals but were not associated with clinical disease.[44,47] Standard anthelmintics at canine dosages have been successfully used to treat internal parasites. It is recommended that African wild dogs be routinely tested and maintained on prophylactic heartworm preventative in endemic areas.

Over the last decade, valvular dysplasia of varying severity has been increasingly recognized as a significant concern in African wild dogs in North America. Sibling groups and offspring have been affected over multiple generations,

TABLE 77.2 Select Chemical Restraint Agents Used in African Wild Dogs *(Lycaon pictus)*

Drug Combination (mg/kg) IM	Reversal Agent (mg/kg) IM	Comments
Medetomidine (0.025–0.04) Ketamine (2.5–3) ± Midazolam (0.1–0.15) ± Butorphanol (0.1–0.2)	Atipamezole (0.14–0.24) ± Flumazenil (0.01–0.05) ± Naloxone (0.02)	Excellent muscle relaxation, quick recovery
Medetomidine (0.03) Midazolam (0.3), Butorphanol (0.3)		Completely reversible
Dexmedetomidine (0.025), Ketamine (3), Midazolam (0.15)	Atipamezole, flumazenil	Generally larger dart volume
Ketamine (2–4), Midazolam (0.15–0.3), Butorphanol (0.3–0.4), ± Supplemental Propofol (0.4–0.5 IV) or Gas anesthesia	Flumazenil, naloxone	Suggested alternative for cardiac cases
Tiletamine-Zolazepam (Telazol) (2–5)		Reliable plane of anesthesia, prolonged recoveries

IM, Intramuscular; *IV,* intravenous.

TABLE 77.3 Hematologic Values for African Wild Dogs *(Lycaon pictus)*

Parameter	Mean	SD
White blood cell count ($\times 10^3/\mu L$)	10.7	3.53
Red blood cell count ($\times 10^6/\mu L$)	7.98	1.61
Hemoglobin (g/dL)	15.1	2.43
Hematocrit (%)	43.7	7.33
MCV (fL)	55.6	4.53
MCH (pg/cell)	19	1.75
MCHC (g/dL)	34.1	2.08
Platelet count ($\times 10^3/\mu L$)	451	183.8
Segmented neutrophils ($\times 10^3/\mu L$)	7.44	3.34
Neutrophilic bands ($\times 10^3/\mu L$)	0.508	0.025
Lymphocytes ($\times 10^3/\mu L$)	1.9	5.37
Eosinophils ($\times 10^3/\mu L$)	0.458	0.396
Basophils ($\times 10^3/\mu L$)	0.014	0.039

General ZIMS database reference: Species360 (2017), ZIMS.Species360.org.

suggesting that the condition has a genetic, inheritable basis, as is well documented in domestic dogs.[8] Cases range from minor to severe valvular insufficiency with congestive heart failure (Fig. 77.3). Mildly affected animals exhibit no clinical signs, making the condition difficult to detect. The extent of this disease is uncertain, but it is highly probable that cardiac disease is underdiagnosed. Incorporating echocardiographic examination with preventative health evaluations for captive wild dogs is recommended by the SSP in order to identify and medically manage affected individuals.[8] Treatment recommendations are based on those for domestic dogs and have included angiotensin-converting enzyme (ACE) inhibitors, diuretics, and inodilators (pimobendan). Despite the challenges associated with a progressive genetic bottleneck, it is strongly recommended that individuals with cardiac disease not be selected for breeding to prevent further incorporation of genetic defects into breeding lines (Briggs, personal communication, April 22, 2017).

Neoplasia has not been extensively reported in the literature but appears to play an important role in the health of captive populations.[48] Apocrine gland tumors have been documented in clinical settings, presenting as single or multiple dorsal cutaneous masses that can progress to large regionally invasive tumors (Fig. 77.4) (Agnew, personal communication, April 25, 2017). Cases from females are overrepresented in pathology reports submitted to the African wild dog SSP (Kinsel, personal communication, April 10, 2017). Surgical excision is recommended, although large tumors may require other forms of treatment. Tumor growth results in ulceration and necrosis and negatively affects an animal's quality of life. Other common neoplasias include hemangiosarcoma, peripheral odontogenic fibroma (fibromatous epulis), adrenocortical adenoma/carcinoma, and mammary and uterine neoplasia.

Other notable conditions diagnosed in African wild dogs include dental disease—particularly fractured teeth—pancreatitis, diabetes, spina bifida, syringomyelia, keratitis, snake bites,[49] and trauma from conspecifics (Kinsel, personal communication, April 10, 2017). African wild dogs mask signs of illness and may have advanced disease by the time a change in behavior or appetite is observed.

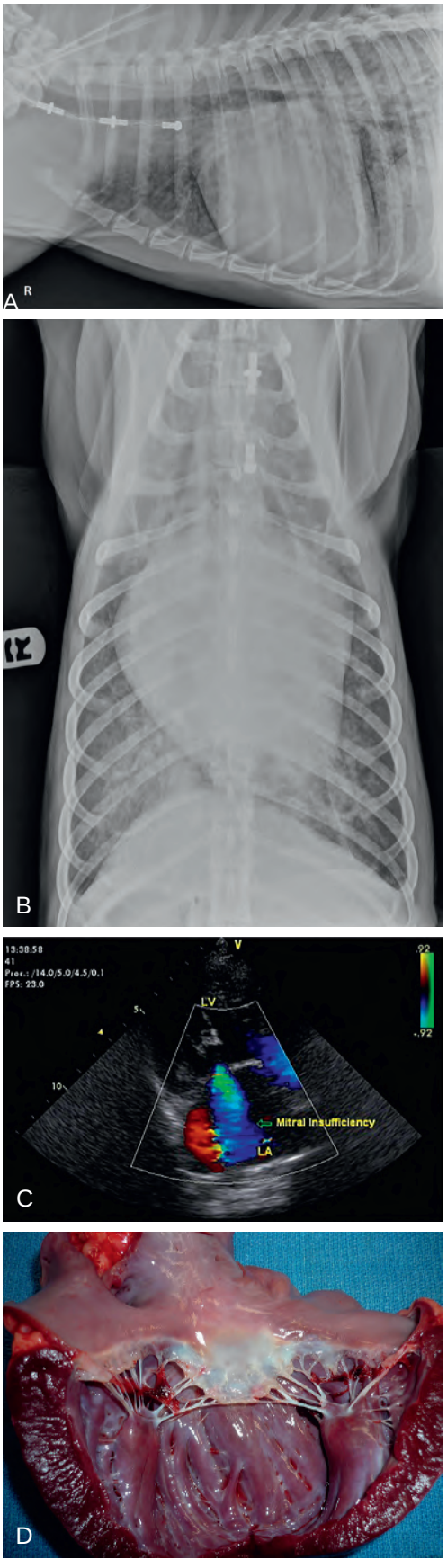
TABLE 77.4 Serum Chemistry Values for African Wild Dogs *(Lycaon pictus)*

Parameter	Mean	SD
Calcium (mg/dL)	10.2	0.78
Phosphorus (mg/dL)	5.5	1.88
Sodium (mEq/L)	148	4.75
Potassium (mEq/L)	4.6	0.75
Chloride (mEq/L)	116	5.25
Bicarbonate (mEq/L)	20	5.25
Carbon dioxide (mEq/L)	19.6	4.25
Blood urea nitrogen (mg/dL)	25	8.75
Creatinine (mg/dL)	1.1	0.35
Total bilirubin (mg/dL)	0.2	0.1
Glucose (mg/dL)	148	39.3
Cholesterol (mg/dL)	260	74
Triglyceride (mg/dL)	69	43.25
Creatine phosphokinase (IU/L)	229	141.75
Alkaline phosphatase (IU/L)	55	45.25
Alanine aminotransferase (IU/L)	50	21
Aspartate aminotransferase (IU/L)	36	15.75
Gamma glutamyltransferase (IU/L)	6	3
Lactate dehydrogenase (IU/L)	181	172
Uric acid (mg/dL)	0.4	0.35
Amylase (IU/L)	375	199.5
Lipase (IU/L)	123	70.5
Total protein (colorimetry) (g/dL)	5.9	0.63
Globulin (colorimetry) (g/dL)	2.8	0.6
Albumin (colorimetry) (g/dL)	3.2	0.4

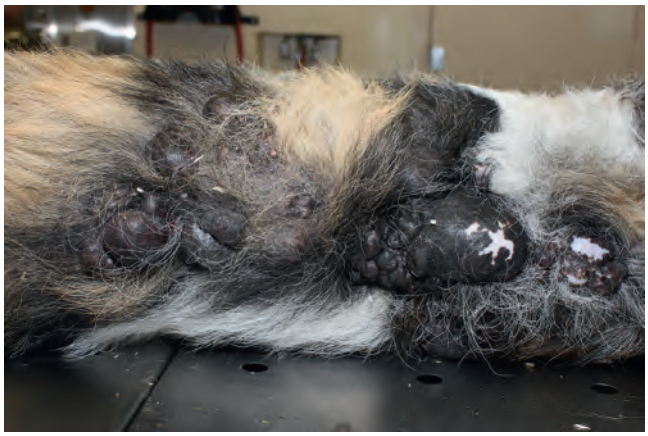
General ZIMS database reference: Species360 (2017), ZIMS.Species360.org.

Preventative Medicine

Preventative health, preshipment, and quarantine examinations should be conducted to determine an animal's health. A complete physical exam—including a dental examination, complete blood count, chemistry panel, heartworm testing, urinalysis, radiographs of the thorax and abdomen, and evaluation for endo- and ectoparasites—should be completed on a routine schedule as part of a preventive medicine plan. Additionally, abdominal ultrasound examination or computed tomography in females and echocardiograms for both sexes are recommended owing to disease predilection in this species.



• **Figure 77.3** Images from African wild dogs *(Lycaon pictus)* with heart disease: (A) Lateral and (B) ventrodorsal radiographs of the thorax consistent with congestive heart failure. (C) Echocardiography image showing mitral insufficiency. (D) Necropsy image of mitral valve dysplasia.



• **Figure 77.4** Advanced apocrine gland neoplasia along the dorsum in an African wild dog *(Lycaon pictus)*. (Courtesy A. Moresco, Denver Zoo.)

TABLE 77.5 Selected Vaccination Schedules Used for African Wild Dogs *(Lycaon pictus)*

Disease	Vaccine	Schedule (Weeks)
Canine distemper	PUREVAX Ferret Distemper Live Canarypox vector vaccine (Merial)	Begin at 6–9 weeks, booster subcutaneously every 2–3 weeks through 16–20 weeks and repeat annually
Rabies	Imrab 3 (Merial)	Initially at 14–16 weeks, booster at 1 year and then every 1–3 years thereafter

External and internal parasites should be treated according to domestic dog guidelines. Fleas, ticks, and ear tip trauma from biting flies have responded well to products containing carbaryl or pyrethrins. Good hygiene, removing standing water, fly traps, and premise sprays help control fly and mosquito populations.

Newly acquired animals should be quarantined away from the collection for a predetermined period based on a thorough risk assessment by the supervising veterinarian and have at least two negative fecal examinations. Animals should be individually identified with microchip transponders placed subcutaneously between the shoulder blades or to the left of midline over the shoulder.

Currently there are no universal recommendations for vaccination protocols. The safety and efficacy of vaccines in African wild dogs have historically been unsatisfactory. Vaccination strategies to conserve free-ranging populations have been reported and continue to be investigated.[50] Modified live canine distemper vaccinations have failed to produce protective antibody levels in some cases[51] and have induced distemper resulting in mortality in other cases.[7,45,52,53] Vaccine-induced distemper can be avoided by using killed vaccines, and at least one vaccine (Purevax ferret Distemper, Merial Inc., Athens, Georgia) has been shown to produce measurable titers after a series of three injections at 2- to 3-week intervals.[54] Subunit canine distemper vaccines (CDV-ISCOM, Erasmus MC, Rotterdam, The Netherlands) stimulated appropriate titer formation, but titers did not endure relative to the Purevax Ferret Distemper vaccine. Vaccine recommendations for domestic dogs have been reduced from yearly to triennially; however, it is unknown whether nondomestic canids maintain titers in a similar manner. In one study, protective titers from the Purevax vaccine persisted in 39%–85% of African wild dogs for a minimum of 1 year.[55] Early studies have indicated that vaccination with killed rabies vaccines may not be sufficient for protection.[5,50,56] However, a recent study showed that a single intramuscular vaccination of dogs older than 14 weeks with Imrab 3 (Merial Inc., Athens, Georgia) provided persistent protective titers.[57] A survey in 2006 showed that most African wild dogs maintained presumably protective titers after vaccination for canine distemper and rabies for 1 year; however, few dogs maintained titers for 2–3 years.[58] There is a paucity of scientific information regarding vaccination against canine parvovirus and leptospiral infection. Protocols should be developed that take into consideration local environmental disease prevalence, animal health, and risk factors. Select vaccination protocols for captive African wild dogs are listed in Table 77.5 (see also Chapter 79).

Acknowledgments

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Medicine of Captive Andean Bears

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Biology

The Andean bear *(Tremarctos ornatus)*, also known as the spectacled bear, is the only living species of the subfamily Tremarctinae within the family Ursidae. This species is the only ursid native to northern and western South America, ranging from the Andes Mountains in Colombia, Venezuela, Ecuador, Peru, Bolivia, Province of Darien in Panama, to the north of Argentina.[1,2] The bears inhabit diverse environments that encompass both tropical and dry areas, as well as humid cloud forests and high elevation grassland and shrubland ecosystems known as "Páramo." The species is listed as Appendix I endangered by the Convention on International Trade in Endangered Species of Wild Fauna and Flora and as vulnerable by the International Union for Conservation of Nature (IUCN).[3]

A diploid number of 52 chromosomes makes them genetically different from other Ursidae, which have 74 chromosomes.[4] Historically, Andean bears were presumed to be nocturnal, although reports from Bolivia and other countries in the last two decades have documented diurnal activity thought to be related to low numbers of bears in the geographic area. Field researchers also have suggested that this species has both daytime and nighttime activity periods, possibly related to food (bromeliads) availability.[5]

Special Anatomic Features

Andean bear body weight ranges from 60–175 kg, with heights of 1.5–2.1 m. Females are two-third the size of males (60 kg). Fur color may be black, dark red-brown, or brown-blackish, and is dense and coarse with long hairs of 55–120 mm. Most individuals have light-brown or ginger-colored markings across their face ("spectacled markings"), as well as on their neck and chest, with reduced hair on the muzzle. They have a short 7-cm-long tail. The dental formula is I 3/3, C 1/1, P 4/4, M 2/3, for a total of 42 teeth.[5,6] Andean bears also have several anatomic features that distinguish them from other ursids. These include specific adaptations for herbivory including a pre-masseteric fossa—a profound depression of the mandible directly in front of the masseteric fossa—along with both a reduction in the superficial masseteric muscle and an increase in the mandibular-zygomatic masseteric muscle mass. They possess only 13 pairs of ribs instead of the 14 pairs present in all other bear species, and share with giant pandas *(Ailuropoda melanoleuca)* an epicondylar foramen in the distal humerus and a false thumb, which is an enlargement and posterior projection of the radial sesamoid.[4,5]

Feeding Ecology and Nutrition in Captivity

In the wild, almost 90% of these bears' diet includes leaves from plants in the Melastomataceae, Arecaceae, and Bromeliaceae families, along with fruits in the cactus, heath, myrtle, laurel, and mulberry families; rhizomes of Araceae, Heliconiaceae, Cyperaceae, Cyclanthaceae, and Orchidaceae; and cultivated plants such as bananas and custard apples.[4,7] The remaining 10% of their diet is protein-based and is obtained from hunting as well as frequent scavenging of rodents, deer, insects, and even domestic animals such as cattle, sheep, and horses.[5,7] Unlike social carnivores, these bears are solitary individuals in the wild and are usually not affected by conspecific competition for food. This enables them to eat maximally whenever feeding. In captivity, this natural pattern may lead to overeating and obesity,[8] particularly if they are provided diets poorly suited to their anatomy and digestive physiology such as high-sugar, high-starch processed fruits. Along with the giant panda Andean bears are the most naturally folivorous bear species,[8,9] but in the wild, they tend to choose higher nutritional content food when available, for example, fibrous green vegetation, but preferring wild fruits.[10] In captivity, this tendency results in bears ingesting more processed fruits and other feed high in energy and leads to weight gain.

Andean bear diets in zoological institutions in Latin America and in other parts of the world consist of mixtures of commercial dry omnivore or dog food, animal protein (horse, beef, rabbit, chicken, egg), fruits, and vegetables. No detailed assessment of these diets has been performed. Feeding schedules vary from one to three meals per day but the propensity to obesity depends on how much energy/nutrients are provided. With captive bears and other carnivores, overfeeding usually results from miscalculations of needed energy requirements (E Valdés, personal communication, March 10, 2017).

In one study, two Andean bears fed a mixed diet of produce and commercial dry feed showed dry matter digestibility coefficients of 60.5%. In the same study, the bears consumed 1.6% of body weight in dry matter daily. Apparent digestibility coefficients for neutral detergent fiber, crude protein, soluble sugars, and crude fat were 12.8%, 70.0%, 80.3%, and 64.5%, respectively. Acid detergent fiber showed zero digestibility, and the mean transit time of food through the gastrointestinal tract was between 8 and 24 hours. Results indicated that Andean bears have limited capabilities to digest higher fiber diets.[11]

Behavior and Related Issues

As in other bear species, stereotypies in captivity are common in Andean bears and require investigation to determine the underlying causes. In one facility, two captive Andean bears spent as much as 80%–85% of observed time engaging in stereotypical movements. The enclosure, husbandry, and medical issues were demonstrated to be associated with the stereotypies, and changing the habitat to a more naturalistic environment provided mental stimulation for the bears, and reduced the stereotypies significantly, improving chronic dental pathologies like alveolar injuries and canine fractures due to the chewing of the bars that have been described in other species.[12] Further investigation is needed with regard to how disease, perception and expression of pain, husbandry, and appropriate environmental stimulation can affect captive Andean bear welfare both negatively and positively.[13,14] Complementary therapies, such as Bach flower remedies in conjunction with an environmental enrichment program, were used at one facility and resulted in a decrease in abnormal, and an increase in natural, behaviors.[15]

Reproduction and Related Medical Issues

Captive Andean bears have been classified as polyestrous, facultative seasonal breeders,[5] showing follicular and luteal phases of 8 days and 22 days, respectively. Females usually have three to four ovarian cycles per year.[16] Some authors report a 7-month gestation period,[17] while others believe that embryonic diapause (delayed implantation) makes gestation length variable and difficult to calculate.[5,18] One to two cubs (and up to three cubs in North American zoos)[19] are born to females year round in zoos that lie within the bears' natural distribution range.[5] In facilities in the northern hemisphere—and thus outside the species' distribution—Andean bears have a tendency toward a winter birthing season.[5,17]

Sexual maturity in captivity ranges from 3 to 7 years with a mean range of 4 years for females and 5 years for males.[5,20] Reproductive life spans in captive individuals are around 15–17 years for females but almost double for males.[20] Assisted reproductive techniques in Andean bears, first attempted in the late 1990s, consisted mainly of electroejaculation and cryopreservation of semen.[21,22] More recently, semen collection via urethral catheterization has been performed (A Moresco, K Herrick, personal communication, February 28, 2017). Fecal monitoring of sex steroids, specifically estradiol and progesterone, has been carried out in six captive spectacled bear females in Peru.[22]

Contraception in Andean bears has included melengestrol acetate (MGA implants), hysterectomy, medroxyprogesterone injections (Depo-Provera), and deslorelin acetate (Suprelorin) (Association of Zoos & Aquariums RMC [Reproductive Management Center] at the Saint Louis Zoo). MGA implants are no longer recommended for carnivores generally, and in captive Andean bears its use has been reduced considerably since 2005 (M Agnew, personal communication, March 09, 2017). MGA implants have been used in Colombian Andean bears, however, without complications (F Nassar-Montoya, personal communication, August 10, 2016). Reproductive issues reported in Andean bears include polycystic ovaries,[23] recurrent vaginitis due to *Escherichia coli*,[17] and endometrial hyperplasia.[24] Recently, molecular sexing using polymerase chain reaction has been used on fecal samples found in the bear's natural environment to determine the animal's gender, a useful tool in monitoring populations.[25]

Handling, Restraint, and Chemical Immobilization

With cubs weighing less than 25 kg, manual physical restraint is possible using nets, heavy gloves, and blankets.[6,26] As with other bears, manual restraint should not be used with larger animals. Operant conditioning in captive Andean bears facilitates handling, training for direct drug administration, oral drug ingestion, dental health assessment, blood sampling, nail trimming, radiographs, endoscopy, and other procedures.[27] Nevertheless, even with training, protected contact should be used with Andean bears, and the benefits of squeeze cages and pole syringe should not be underestimated. The same safe practices and precautions used in other bear species should be utilized with Andean bears. Similarly, the complications of anesthesia that occur in other bear species can occur with this species as well, necessitating an appropriate anesthesia plan, whether in a zoological setting or in the field.

In Latin American zoos and in field research on Andean bears, the standard anesthetic protocols have included ketamine-xylazine (KX) and/or zolazepam-tiletamine (ZT). The choices of these drugs has been due primarily to poor or nonavailability of other alpha-2 agonists, benzodiazepines, and potent opioids in some range countries. ZT has been used in Andean bears at 3.2–11.1 mg/kg.[28] Combinations of ketamine (2.5–4 mg/kg), medetomidine (0.035–0.075 mg/kg), and midazolam (0.05–0.09 mg/kg) have also been successfully utilized for spectacled bears. An ongoing, prospective study on Andean bear medicine and anesthesia is investigating additional drugs and dosages in Andean bears using combination protocols with ketamine, midazolam, and dexmedetomidine or medetomidine.

TABLE 78.1 Anesthetic Protocols in Andean Bears *(Tremarctos ornatus)* used at Bioparque Wakatá Colombia

Drug	Dose (mg/kg), IM	Reversal Agent/Dose mg/kg	Comments	*n**
Ketamine Xylazine	3.6–9 0.5–1.5	Yohimbine 0.11 IV	Animals required redosing of ketamine	18
Ketamine Dexmedetomidine Midazolam (KDM)	5.6–7.3 0.02–0.035 0.27–1.01	Atipamezole 0.2 IV Flumazenil 0.01 IV	Large volume of dexmedetomidine could be problematic with darts. Animals needed redosing of KDM	5
Tiletamine-zolazepam Ketamine	6–6.5 2–6.2	Flumazenil 0.01 IV	Prolonged recovery	6
Ketamine Medetomidine Midazolam	4 0.04 0.1	Atipamezole 0.24 IV Flumazenil 0.01 IV	Initial effects within 2–5 minutes. No redosing needed	2

*n = Number of animals.

TABLE 78.2 Anesthetic Protocols Reported by ZIMS Medical for Andean Bears *(Tremarctos ornatus)*

Drug	Dose (mg/kg) IM
Ketamine	3.03–39.74
Xylazine	0.29–5.13
Ketamine	2.78–7.01
Medetomidine	0.023–0.07
Midazolam	0.036–0.23
Ketamine	2.08–10.14
Medetomidine	0.022–0.36
Medetomidine	0.013–2.77
Tiletamine-Zolazepam	1.00–7.87

No data reported for reversal agents.

This study is also assessing the effects of these drugs on basic physiologic parameters that will hopefully enable extrapolations for safer field anesthesia. Nevertheless, further information and additional protocols specifically for Andean bears are needed (Tables 78.1 and 78.2). Anecdotal reports of severe, life-threatening complications during anesthesia at poorly equipped Andean bear rescue centers and bear-holding zoos, including seizures in geriatric bears, and respiratory and cardiovascular complications in bears of all ages, highlight the importance of utilizing standard anesthetic techniques and monitoring with these animals. Pulse-oximetry, capnography, electrocardiogram, noninvasive blood pressure measurement, blood gases, venous access, fluid administration, availability of emergency drugs, and ultrasound are as essential and relevant for Andean bears as for any other ursid species (see also Chapter 29).

Almost no information exists on analgesia in spectacled bears except for a report describing the use of meloxicam[29] and piroxicam[30]; it seems likely that analgesics used with efficacy in other bears and carnivore species might be effective in Andean bears, although specific pharmacokinetic and pharmacodynamic studies are lacking in present time and appropriate precautions should be taken with regard to doses and dosing.

Clinical Techniques and Clinical Pathology

As with other bear species, training and positive reinforcement can enable awake venipuncture from Andean bears. Blood samples may be obtained from the superficial veins of the dorsal venous plexus and cephalic veins above the carpus joint area in awake animals and from the jugular, lateral saphenous, and femoral veins in anesthetized bears.[6,27] In anesthetized Andean bears (n = 13), we have found the femoral and medial saphenous veins to be suitable for the collection of larger volumes of blood. Tables 78.3 and 78.4 display hematologic and serum biochemistry values obtained from the Zoological Information Management System and from research from this chapter's authors in Colombia.

Diseases of Captive Andean Bears

Few peer-reviewed publications on Andean bear diseases exist. Many of the most frequently encountered medical issues are noninfectious acquired diseases primarily in geriatric and/or overweight/obese bears. These include spondylosis,[31] osteoarthritis, and degenerative joint disease confirmed with radiographs of the elbows, knees, and hips (Fig. 78.1 A and B). In some cases, available clinical histories indicate that affected bears received improper diets as youngsters following rescue or confiscation. Thus these degenerative lesions likely reflect the consequences of developmental

TABLE 78.3 Hematologic Reference Values for Andean Bears (*Tremarctos ornatus*)

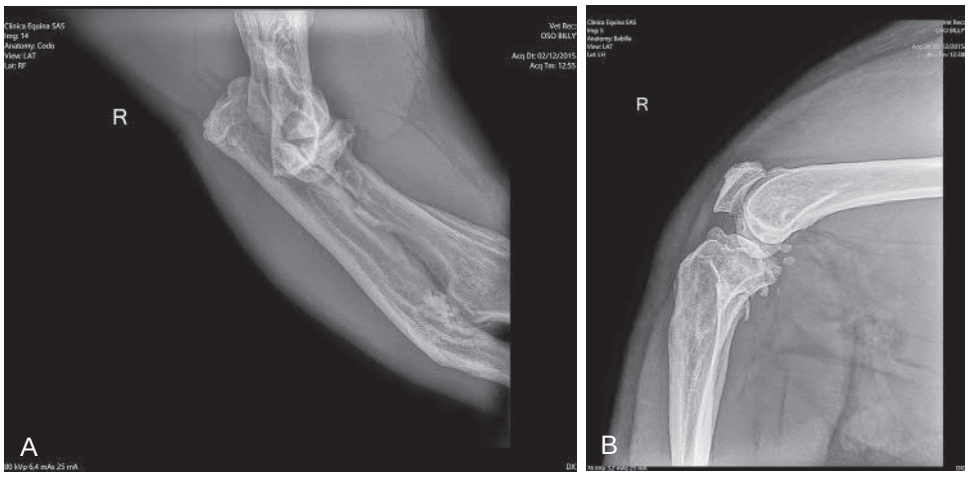
	Colombia*		ZIMS†	
Parameter	Mean	Range	Mean	Range
HCT (%)	40.74	(33.6–47.9)	41.0	(30.7–52.5)
Hgb (g/dL)	13.47	(10.8–16.2)	14.6	(11.2–18.1)
RBC (*10^6/μL)	8.55	(7.0–10.1)	8.46	(6.50–10.44)
WBC (*10^3/μL)	5.80	(3.0–8.6)	6.34	(3.59–10.34)
MCV (fL)	49.02	(37.6–60.4)	48.2	(36.0–57.2)
MCH (pg)	17.68	(14.4–21.9)	17.2	(14.4–19.4)
MCHC (g/L)	177.29	(7.5–347.1)		
Neutrophil count (*10^3 cells/μL)	3.93	(2.6–5.3)	4.36	(1.23–7.53)
Eosinophil count (*10^3 cells/μL)	0.18	(0.0–0.4)	398	(51–1220)
Basophil count (*10^3 cells/μL)	0.00	0.00	122	(0–303)
Lymphocyte count (*10^3 cells/μL)	1.52	(0.6–2.4)	1.27	(0.34–3.12)
Platelets (*10^3 cells/μL)	457.93	(154.1–761.8)	515	(166–840)
Monocyte count (*10^3 cells/μL)	0.03	(0.0–0.1)	184	(45–521)

*The data were obtained from 15 captive bears, from two different institutions: Bioparque Wakatá and Zoologico de Cali. Some of these data contribute to the ZIMS data base as well.
†Teare JA, editor: "Tremarctos ornatus No selection by gender All ages combined Standard International Units 2013 CD.html" in Species360 Physiological Reference Intervals for Captive Wildlife: A CD-ROM Resource, Species360, Bloomington, MN, 2013.

TABLE 78.4 Serum Chemistry Reference Values for Andean Bears *(Tremarctos ornatus)*

	Colombia*		ZIMS†	
Test	Mean	Range	Mean	Range
Alanine Aminotransferase IU/L	32.60	(21.8–44.12)	30	(8–70)
Aspartate Aminotransferase IU/L	43.04	(28.24–57.85)	36	(11–74)
Alkaline Phosphatase IU/L	62.83	(28.39–97.27)	40	(13–105)
Total protein (g/dL)	8.13	(7.21–9.05)	7.5	(6.0–8.7)
Albumin (g/dL)	3.67	(3.22–4.12)	4.1	(3.0–4.9)
Globulin (g/dL)	4.46	(3.65–5.27)	3.4	(2.3–4.5)
Creatinine (mg/dL)	2.06	(1.65–2.47)	1.7	(0.9–2.5)
Blood Urea nitrogen	10.33	(5.63–15.02)	13	(5–23)
BUN/Cr ratio	4.58	(2.59–6.57)	7.8	(3.3–15.9)
Sodium (mEq/L)	139.00	—	139	(131–151)
Potassium (mEq/L)	4.30	(3.94–4.66)	4.1	(3.2–5.0)
Chloride (mEq/L)	107.33	(106.18–108.49)	104	(96–112)

*The data was obtained from 9 captive bears, from the institution Bioparque Wakatá. Some of this data contributes to the ZIMS data base.
†Teare, J.A. (ed.): 2013, "Tremarctos ornatus No selection by gender All ages combined Standard International Units 2013 CD.html" in Species360 Physiological Reference Intervals for Captive Wildlife: A CD-ROM Resource., Species360, Bloomington, MN.



• **Figure 78.1** Osteoarthritis in (A) elbow and (B) knee of a captive Andean bear *(Tremarctos ornatus)* older than 20 years.

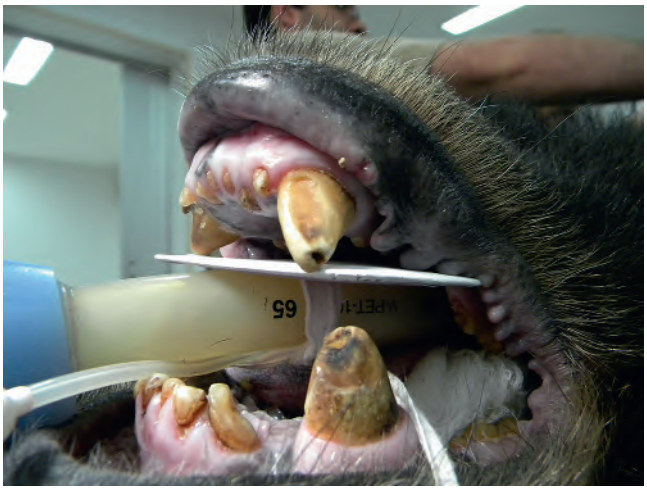
and nutritional deficiencies, pathologic fractures, and of inappropriate husbandry in small, concrete-floor enclosures without access to soft substrates.

Dental pathology, such as fractures (especially of canine teeth) and periodontal lesions[32] warranting endodontic and extraction procedures during annual overall health assessments under general anesthesia, are also frequently encountered in Andean bears (Fig. 78.2). Training bears via operant conditioning to open their mouths to allow intraoral visualization has been helpful in identifying these abnormalities so that they can be treated at an early stage (R Fecchio, personal communication, January 15, 2017).

A poorly understood "spectacled (Andean) bear alopecia syndrome" has been observed and reported, predominantly in females for the past 40 years.[6,33–37] This syndrome has been recognized in zoos worldwide, presenting initially as patches of alopecia on the dorsum and flanks, progressing to generalized intense pruritus, and development of alopecia on the face and extremities, then finally as generalized, full-body alopecia (Fig. 78.3).[33,36] In South America this syndrome has affected females in zoos in Peru, Colombia, and Ecuador. Andean bears in Europe, North America, and Japan have also been affected.[36]

Interestingly, in two captive males in Ecuador, a similar patchy and generalized alopecia pattern has been observed; the animal with generalized alopecia suffered from a concurrent pyogranulomatous lesion on the neck[34] due to a foreign body and results of the submitted biopsy including alopecic skin were "discrete to moderate lymphoplasmacytic perivascular dermatitis" (D Medina, personal communication, March 1, 2017).

The pathophysiology remains unknown. Hormonal studies comparing fecal cortisol, estrone sulfate, and progesterone levels of normal and affected bears have shown no differences that can explain the condition. Skin biopsies of affected bears in one study showed follicular atrophy and destruction of hair follicles and, in some cases, lymphocytic



• **Figure 78.2** Periodontal disease and dental fractures in Andean bear *(Tremarctos ornatus)*. (Courtesy Dr. Roberto Fecchio.)

• **Figure 78.3** Andean bear *(Tremarctos ornatus)* alopecia syndrome in a captive old female.

and giant cell infiltrates.[33,36] No clear conclusions regarding diagnosis or treatment are known either, although oclacitinib maleate, an inhibitor of pruritogenic, proinflammatory cytokines used to treat pruritic allergic dogs, gave promising results in three affected Andean bears.[36] Further information about the efficacy of this drug in treating alopecia syndrome in Andean bears is still needed, however.

In captive Andean bears, multiple types of neoplasia have been reported. These include lymphosarcoma,[38] gingival epidermoid carcinoma of the mandibular bone,[17] mesothelioma, thymoma, cardiac rhabdomyosarcoma, squamous cell carcinoma, pyloric leiomyoma, mammary adenoma, spindle cell thymoma, cholangiosarcoma, and transitional cell carcinoma of the urinary bladder.[29,30]

In studies on the parasites of free-ranging Andean bears in Peru, *Blastocystis* sp., *Giardia* sp.,[17,39] and *Cryptosporidium* sp. were found. All three of these parasites may reflect interactions between domestic animals, human settlements, and wildlife as well as the presence of polluted water sources. In one study, rhabditid and ascarid nematodes were also identified in wild Andean bears.[39] Preliminary data of a serologic screening survey on captive Andean bears in Colombia found 11 of 13 individuals (84.6%) positive for *Toxoplasma gondii* antibodies using the latex agglutination test.[38] Canine distemper has caused perinatal infection in captive Andean bears[29,40]; but whether other potential viral agents such as herpesvirus type-I and canine adenovirus type-I affect Andean bears remains unknown.[6,41]

In Colombia, the habitat of wild Andean bears overlaps with the territories of domestic and invasive species. Infectious diseases such as leptospirosis and rabies as well as parasites contracted from these species are a growing problem for the bears. In the wild, dental disease has been reported in juvenile bears, as well as decreased growth in females and even infanticide (JO Feliciano, personal communication, March 12, 2017).

Conclusions

Andean bear health both in captivity and in the wild remains a vast opportunity for research. Much of the information regarding the bears biological and medical aspects has been collected from zoos in the Northern hemisphere, outside of their natural range. Zoological collections, rescue centers, and wildlife stations within the Andean bears' geographic distribution are urged to report and publish their findings on this increasingly endangered animal; research on nutrition, anesthesia, infectious and noninfectious diseases, reproduction, clinical medicine, and pharmacology are clearly needed for Andean bears, both those living in captive environments and to help conserve the populations remaining in the wild.

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Canine Distemper Vaccination in Nondomestic Carnivores

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Canine distemper virus (CDV), a member of *Morbillivirus* genus in the family Paramyxoviridae, is a significant viral disease of both captive and free-ranging carnivores globally.[1] Morbidity and mortalities due to CDV infection have been described in nearly all extant families of Carnivora (Ailuridae, Canidae, Felidae, Hyaenidae, Mephitidae, Mustelidae, Otariidae, Phocidae, Procyonidae, Ursidae, and Viverridae)[1–7] including large-scale epidemics in multiple species.[6,8,9] Disease due to CDV continues to remain an issue in both captive and free-ranging populations due to CDV persistence through a wide range of carnivore reservoir hosts.[10–13]

CDV is an RNA virus that contains, similar to other paramyxoviruses, six structural proteins: nucleocapsid (N), phosphoprotein (P), large protein (L), single-envelope-associated or matrix protein (M), two glycoproteins—hemagglutinin (H) and fusion (F) protein, and two accessory nonstructural proteins (C and V).[14] CDV is primarily transmitted through respiratory secretions although transmission through other body excretions such as urine can result in infection in susceptible animals. CDV exhibits lympho-, neuro-, and epitheliotropism and disease most commonly involves the respiratory, enteric, integumentary, and central nervous system symptoms. Secondary infections reflecting virus-induced immunosuppression are commonly seen with CDV and may complicate clinical disease.[15,16] Clinical signs and the severity of disease are affected by various factors including host age and immune status, species sensitivity to virus, and viral strain virulence, as well as other environmental factors.[1]

There is currently no known effective treatment for clinical disease from CDV infection, and treatment is limited to supportive care and treatment of secondary infections. Disease from CDV is best prevented and controlled through vaccination. There are two major categories of commercially available vaccines for CDV: modified-live virus (MLV) vaccines and canarypox vectored recombinant vaccines. The introduction of MLV vaccines in the 1950s has helped to control disease; however, the use of MLV CDV vaccines has generally not been recommended in nondomestic carnivores because of the potential reversal of attenuation and vaccine-induced CDV (Table 79.1). Inactivated CDV whole-virus vaccines, however, do not produce sufficient immunity to prevent infection after virus challenge (sterile immunity) and are no longer available commercially in the United States.[12] Recombinant subunit CDV vaccines have become popular in zoological medicine because of their safety profile; however, availability and questions regarding the ability of some products to adequately elicit an immune response and provide adequate protection against CDV remain issues. This chapter summarizes the current knowledge on canine distemper vaccination with special interest in the application in nondomestic carnivores (see Chapter 44).

Recombinant Viral Vector Vaccines

Recombinant viral vector vaccines are genetically engineered vaccines produced through recombinant DNA technology that involve insertion of DNA encoding key antigens into a different viral vector (poxvirus, adenovirus, or alphavirus) for delivery. These vaccines have a similar safety profile to inactivated (killed) subunit vaccines but express antigens inside vector infected cells so that MHC class I and class II presentation can occur efficiently and stimulate both cell-mediated and humoral immune responses. The expression of only recombinant proteins allows the targeting of the immune response against a few antigens produced by the pathogen without including the entire pathogen, thereby significantly reducing or eliminating the risk of vaccine-induced disease.

Recombinant CDV (rCDV) vaccines have been created that have CDV genes for the H and F proteins inserted in a live canarypox viral vector. Adequate host immune response against the H protein is considered important

TABLE 79.1 Reports by Species of Vaccine-Induced Canine Distemper

	Species	Vaccine	Vaccine Type	Vaccine Strain	Tissue Culture Origin	Reference
Canidae						
African wild dog	*Lycaon pictus*	Paramune 5*	MLV combo (CDV, CAV, CPIV) + Lepto bacterin vaccine	n/a	n/a	17
		Candur SHLP†	MLV CDV + killed CPV, CAV-1 + Lepto bacterin vaccine	Rockborn	Canine kidney	18
Bush dog	*Speothos venaticus*	DA2MP‡	MLV combo vaccine (CDV, CAV-2, CPIV, CPV)	Snyder Hill	Canine kidney	19
Gray fox	*Urocyon cinereoargenteus*	Fromm-D§	MLV CDV vaccine	Onderstepoort	Chicken embryo	20
		Vanguard D¶	MLV CDV vaccine	Snyder Hill	Canine kidney	20
		Tissuevax**	MLV CDV vaccine	Rockborn	Canine kidney	20
Maned wolf	*Chrysocyon brachyurus*	n/a	MLV CDV vaccine	n/a	n/a	21
Ailuridae						
Red panda	*Ailurus fulgens*	Epivax-TC-plus††	MLV CDV vaccine	n/a	Chicken embryo	22
		Fervac-D‡‡	MLV CDV vaccine	Lederle	Chicken embryo	24
		Fromm-D§	MLV CDV vaccine	Onderstepoort	Chicken embryo	23
Procyonidae						
Kinkajou	*Potos flavus*	Vanguard¶	MLV combo vaccine (CDV, CAV-2)	Rockborn	Canine kidney	25
Mustelidae						
Black-footed ferret	*Mustela nigripes*	n/a	MLV CDV vaccine	n/a	Chicken embryo	26
European mink	*Mustela lutreola*	Galaxy 6-MPH-L§§	MLV combo (CDV, CAV-2, CPIV, and CPV) + Lepto bacterin vaccine	Onderstepoort	Primate Vero	27

*Dellen Laboratories, Omaha, Nebraska, USA.
†Behringwerke, Marburg, Germany.
‡Vanguard Smith and Kline, Isando, Republic of South Africa.
§Fromm Laboratories Inc., Grafton, Wisconsin, USA or Solvay Animal Health, Inc., Mendota Heights, Minnesota, USA.
¶Norden Laboratories Inc., Lincoln, Nebraska, USA.
**Pitman-Moore Inc., Washington Crossing, New Jersey, USA.
††Burroughs Wellcome & Company, London, England.
‡‡United Vaccines, Inc., Madison, Wisconsin, USA.
§§Solvay Animal Health, Inc.
CAV, Canine-adenovirus; *CDV,* canine distemper virus; *CPIV,* canine parainfluenza virus; *CPV,* canine parvovirus; *Lepto, Leptospira interrogans*; *MLV,* modified-live virus.

and may prevent subsequent CDV infection.[28] A vaccinia vectored vaccine using insertion of genes encoding the H and F proteins from measles virus, another morbillivirus, has been demonstrated to produce neutralizing antibodies to CDV to protect against viral challenge in domestic dogs.[29] The canarypox viral vector used in the CDV vaccine has the advantage that the canarypox vector is replication-incompetent in mammalian hosts so the vaccine is incapable of reversion to virulence and does not result in viral shedding.[30] Recombinant CDV vaccines have also been shown to be safe to administer during pregnancy.[31] Adverse reactions historically seen with MLV CDV vaccines such as postvaccinal encephalitis are not possible with rCDV vaccines.[30] Recombinant CDV vaccines have been shown to successfully immunize domestic ferrets and protect against subsequent viral challenge.[32]

A commercially available monovalent canarypox-vectored rCDV vaccine, PUREVAX ferret distemper

(Merial, Athens, Georgia, USA) approved for use in the United States in domestic ferrets, was first made available in 2001. This vaccine has been safely utilized via extra-label use in numerous species of nondomestic carnivores, and the humoral response to vaccination with this vaccine has been assessed in several species (Table 79.2). The ferret distemper rCDV vaccine has been advocated for use by many Association of Zoos & Aquariums (AZA) Species Survival Program veterinary advisors because of its safety profile and evidence of antibody response to vaccination in multiple species studied, and has been widely utilized for vaccination of nondomestic carnivores within North American zoos. This vaccine, however, has been frequently on manufacturer back order and unavailable for periods of up to several years at a time, forcing veterinarians to search for other rCDV vaccine options. A similar recombinant vaccine, RECOMBITEK canine distemper, is approved for use in domestic dogs and available in several combination preparations that include other MLV vaccine components for different canine infectious diseases depending on the preparation. This vaccine more recently became available in a monovalent version including only the rCDV vaccine (RECOMBITEK CDV). These vaccines involve the same live canarypox viral vector with H and F protein gene insertions as the ferret distemper vaccine; however, the amount of vaccine antigen load differs between the ferret distemper rCDV and canine rCDV vaccine series. The actual amounts are proprietary, but the ferret distemper rCDV vaccine dose is approximately eight times greater than the canine rCDV vaccine dose. This difference is based on the demonstrated protective doses that were established in licensure studies for ferrets and domestic dogs (J Maki, personal communication, March 29, 2017).

Recombinant CDV vaccines typically induce lower serum-neutralizing (SN) titers compared to MLV CDV vaccines in nondomestic carnivores although stimulation of cell-mediated immune response may also be an important factor in establishing protective immunity. Recombinant CDV vaccines appear to require minimally an initial vaccine plus booster to produce an SN antibody response in nondomestic carnivores,[37,49] although single vaccination may induce seroconversion in some species.[39] Recombinant CDV vaccines are superior to MLV CDV vaccines in inducing a neutralizing antibody response in the presence of maternal antibody; however, survival was only 14% following challenge in ferrets vaccinated parenterally with rCDV vaccine in the presence of maternal antibody.[32] It is therefore recommended to vaccinate multiple times for an initial series in naïve animals less than 16 weeks of age. The American Animal Hospital Association (AAHA) recommends a series of vaccinations every 3–4 weeks between the ages of 6 and 16 weeks followed by a booster at 12 months and then revaccination every ≥3 years with either rCDV or MLV CDV vaccines for domestic dogs.[50] Route of rCDV vaccine administration also appears significant. African wild dogs *(Lycaon pictus)* vaccinated orally with rCDV vaccine failed to seroconvert postvaccination.[36] Higher doses of vaccine (or increased concentration of antigen) appear necessary to elicit an adequate protective immune response when administering rCDV vaccine orally.[44] Because of this, parenteral administration of rCDV vaccines is recommended in captive nondomestic carnivores for routine preventative health programs.

The author is unaware of any reports of natural disease from CDV subsequent to vaccination in nondomestic carnivores vaccinated regularly (every 1–3 years) with the ferret rCDV distemper vaccine. Recombinant CDV vaccines appear very safe with very few adverse effects reported and no reports of vaccine-induced disease. There is one report of erythema multiforme in a spotted hyena *(Crocuta crocuta)* occurring after vaccination with ferret rCDV vaccine.[51] Following vaccination plus booster with the ferret rCDV vaccine, antibody levels considered protective (≥1:20 for vaccination response)[12] against CDV have been detected postvaccination in nondomestic Canidae, Herpestidae, Mustelidae, Phocidae, and Ursidae (see Table 79.2). Duration of humoral antibodies following an initial series of rCDV vaccinations followed by a booster at 1 year in domestic dogs has been shown to persist for at least 36 months.[30] Antibody persistence up to and beyond 1 year also appears to occur post-rCDV vaccination in some nondomestic species (see Table 79.2). In large felids (*Panthera* spp.) receiving two or more prior ferret rCDV vaccinations that showed evidence of prior seroconversion, 91% (21/23) of animals evaluated maintained SN titers at ≥1:24 for at least 24 months postvaccination.[52] Another report indicates antibodies may not persist as long following ferret distemper rCDV vaccination in other canid species.[36]

Results of canine rCDV vaccination in nondomestic species have been more varied. Several reports and additional data indicate some of the canine rCDV vaccines have successfully induced seroconversion in nondomestic canids,[37,39] raccoons *(Procyon lotor)* (E Ramsey, personal communication, August 3, 2014), and red pandas *(Ailurus fulgens)* (A Guthrie, personal communication, April 30, 2015). In a study in tigers, however, vaccination with one of the rCDV combination vaccines produced only low titers in 2/6 animals following initial vaccination plus booster.[33] Disease due to circulating wild-type CDV has also been documented in a snow leopard *(Panthera uncia)* despite prior vaccination with the monovalent canine rCDV vaccine 3 months prior.[33a] The difference in vaccine antigen loads may have had some impact on these results and highlights the need to determine species-specific vaccination recommendations. It may be possible to overcome some of these challenges by administering increased volume of vaccine (>1 mL), although this is unclear based on current information and further study is needed. There are also unanswered questions as to the safety of vaccinating nondomestic carnivores with polyvalent vaccines containing other MLV vaccine components (see Chapter 57 by Lamberski in *Fowler's Zoo and Wild Animal Medicine Current Therapy* [vol 7]).

TABLE 79.2 Summary of Canine Distemper Vaccine Studies in Nondomestic Carnivores

Species		Vaccine	# of Animals	Vaccination Details	Results	Reference
Felidae						
Tiger	*Panthera tigris*	RECOMBITEK C3*	*n* = 6	1 mL SC, boostered with 3 mL SC (mean) 39 days later	No tigers had detectable antibodies at day 26 postvaccination and 2 of 6 had low (1:16 and 1:32) antibody titers at day 66	33
		Nobivac Puppy-DPv† (Onderstepoort strain, primate Vero cell tissue culture)	*n* = 8	1 mL SC followed by 1 mL SC booster at day 171	7 of 8 tigers had titers of >1:128 (range 1:128–1:1024) at day 26 postvaccination; at 171 days, all animals still had detectable titers; *n* = 38 additional tigers received 1 mL vaccine SC or IM—no adverse effects observed in these animals	
African lion	*Panthera leo*	Nobivac D‡ (Onderstepoort strain, primate Vero cell tissue culture)	*n* = 4		All vaccinated lions responded within 3 week with high titers of antibody (≥1:1024) without any adverse clinical effects; in-contact lions remained seronegative throughout the study and showed no clinical effects, indicating no CDV had been transmitted from lion to lion	34
Herpestidae						
Slender-tailed meerkat	*Suricata suricatta*	PUREVAX ferret distemper*	*n* = 6	1 mL IM	4 of 6 animals had titers ≥1:32 at 1 year postvaccination; no adverse effects observed	35
Canidae						
African wild dog	*Lycaon pictus*	PUREVAX ferret distemper*	*n* = 21 (*n* = 8 PO, *n* = 13 IM)	Three 1 mL IM or PO at 1 month intervals	All postvaccination titers were negative for PO vaccinated animals at all sampling time points; 12 of 13 animals had titers by the end of the course of vaccination, but only ≥1:96 by 3rd vaccination but only 50% of animals sampled at 6.5 months postvaccination had titers ≥1:96; none of animals sampled at 21.5 months postvaccination had positive titers	36
Fennec fox	*Vulpes zerda*	PUREVAX ferret distemper*	*n* = 5	1 mL IM (*animals were previously vaccinated between 2 and 5 years prior to study with MLV vaccine)	4 of 5 animals had titers from 1:128–1:512 at 1 year postvaccination; 5th animal who received MLV booster 2 years prior to study had high titers throughout study and titer 1:4096 at 1 year; no adverse effects observed	35
Red fox	*Vulpes vulpes*	RECOMBITEK C6*	*n* = 17	1 mL SC as single vaccine, series of 3 vaccines, or booster in previously vaccinated animals	Only 2 foxes (2 of 4 naïve animals receiving series of 3 vaccines) had titers ≥1:100; no adverse effects observed	37

Channel Island fox	*Urocyon littoralis*	PUREVAX ferret distemper*	*n* = 16	2 mL PO (directly or via food) for 3 vaccinations	13 of 16 foxes showed measurable antibody response to vaccination (>1:6) at 1 month postvaccination; mean antibody titers were significantly higher in direct administration group	38
Gray fox	*Urocyon cinereoargenteus*	RECOMBITEK C3	*n* = 6 (1 control)	1 mL SC (total of 4 times)	5 of 5 fox kits seroconverted after the 1st vaccination; peak titer levels were not reached until after 3rd vaccination	39
Red wolf	*Canis rufus*	Fromm-D[§] (Onderstepoort strain, chicken embryo tissue culture)	*n* = 20	1 mL SC (*8 adults were previously vaccinated with different MLV combo vaccine as pups)	Mean postvaccination titers were at or above level normally measured in vaccinated domestic dogs; adult wolves had measurable titers more than 1 year postvaccination, but did not have significant titer increases after revaccination; no adverse effects observed	40
		Galaxy-D[¶] (Onderstepoort strain, primate Vero cell tissue culture)	*n* = 32	1 mL SC booster following previous juvenile series	100% of wolves developed and maintained a positive titer for 3 years; no adverse effects observed	41
Ursidae						
Giant panda	*Ailuropoda melanoleuca*	PUREVAX ferret distemper*	*n* = 2	1 mL IM or SC, multiple times (*animals had received vaccinations prior to study period)	Both pandas developed peak titers of 1:384–1:1538 by 7–14 days postvaccination and antibody levels slowly returned to a lower level (1:12–1:64) by 7–14 weeks postvaccination and remained at this level throughout the year until next booster vaccination	42
Procyonidae						
Raccoon	*Procyon lotor*	Galaxy-D[¶] (Onderstepoort strain, primate Vero cell tissue culture)	*n* = 39 (7 used as control)	1 mL SC once (different schedules) or three times in series	16 vaccinated raccoons were protected from clinical disease following experimental oronasal challenge 13–23 weeks postvaccination; 3 of 4 controls failed to mount any detectable antibody, euthanatized after developing CDV	43
Mustelidae						
Siberian polecat	*Mustela eversmanii dauricus*	rCDV (various antigen loads)	*n* = 36 (6 groups)	SC at $10^{39,3}$ $10^{5.0}$, and 10^{33} PFU/dose; PO at 10^{33} and $10^{8.0}$ PFU/dose	Survival rate following challenge in animals receiving 10^{220} PO was 83.3%; survival rate was 50.0% in the 10^{33} and 60.0% in the 10^{30} SC groups; all animals in the low-SC dose, low-PO dose, and control groups died after exposure	44

Continued

TABLE 79.2 Summary of Canine Distemper Vaccine Studies in Nondomestic Carnivores—cont'd

Species		Vaccine	# of Animals	Vaccination Details	Results	Reference
Fisher	*Martes pennanti*	Fervac-D** (Lederle strain, chicken embryo tissue culture)	*n* = 5	1 mL SC twice	Did not effectively stimulate development of a serologic antibody response (fishers did not seroconvert)	45
		Galaxy-D¶ (Onderstepoort strain, primate Vero cell tissue culture)	*n* = 6	1 mL SC twice	5 of 6 showed seroconversion after single vaccination; 100% of fishers had titers ≥1:196 at 26 days post-booster; superior to Fervac-D at stimulating humoral immunity in this species; no adverse effects observed	
Nearctic river otter	*Lontra canadensis*	Fervac-D** (Lederle strain, chicken embryo tissue culture)	*n* = 14	1 mL SC (1 dose, *n* = 9; 2 doses, *n* = 5)	Lower rate of seroconversion compared to Galaxy-D in species (6 of 14 showed increase in titer postvaccination); no adverse effects observed	46
		Galaxy-D¶ (Onderstepoort strain, primate Vero cell tissue culture)	*n* = 8	1 mL SC (1 dose, *n* = 4; 2 doses, *n* = 4)	6 of 7 otters exhibited increases in titers after single vaccination; 3 otters receiving booster vaccination saw additional titer increases 1:512–1:1024; no adverse effects observed	
Southern sea otter	*Enhydra lutra nereis*	PUREVAX ferret distemper*	*n* = 8	1 mL IM followed by 2–3 boosters	Most of otters demonstrated a 50–100-fold rise in antibody titer within 30 days of vaccination and a 100–500-fold rise in titer within 60 days of vaccination; titers considered protective were detectable for several years postvaccination in some animals	47
American badger	*Taxidea taxus*	Fromm-D§ (Onderstepoort strain, chicken embryo tissue culture)	*n* = 6	1 mL SC	5 of 6 seroconverted with titers ranging from 1:64–1:1024 by 63 days postvaccination; 6th animal did not seroconvert; no adverse effects observed	48
Phocidae						
Harbor seal	*Phoca vitulina*	PUREVAX ferret distemper*	*n* = 5	1 mL IM once or twice	3 seals vaccinated once did not seroconvert, but 2 seals vaccinated twice, 1 month apart, developed persistent titers (to 12 months); no adverse effects observed	49

*Merial, Athens, Georgia, USA.
†Merck Animal Health, Kenilworth, New Jersey, USA.
‡Intervet, Inc., Boxmeer, The Netherlands.
§Solvay Animal Health, Inc., Mendota Heights, Minnesota, USA.
¶Schering-Plough Animal Health Corp., Omaha, Nebraska, USA or Solvay Animal Health, Inc.
**United Vaccines, Inc., Madison, Wisconsin, USA.
CDV, Canine distemper virus; *MLV,* modified-live virus.

Modified-Live Virus Vaccines

MLV CDV vaccines have been used historically in nondomestic species, and several of these vaccines have been reevaluated in the face of rCDV vaccine shortages and questions regarding efficacy of some of the rCDV formulations. Vaccination with an MLV vaccine mimics natural infection stimulating both cell-mediated and humoral immune responses, and proper vaccination can provide long-lasting (at least 3–5 years) immunity. Where suitable safety can be established, MLV CDV vaccines may be advantageous in some situations, such as reintroduction projects, where repeat booster vaccinations of animals may be impractical.

Three major types of MLV CDV vaccines exist—the Onderstepoort and Lederle strains that are adapted to chicken embryo/cell culture or primate Vero cells and the Rockborn strain adapted through canine kidney cells. Snyder Hill strain is considered indistinguishable from the Rockborn strain. Onderstepoort and Lederle strains produce lower levels of humoral antibody and shorter duration of immunity compared to Rockborn/Snyder Hill strain; however, these vaccines do not cause postvaccinal encephalitis, which is occasionally seen with Rockborn strain vaccines.[12] In general, avian-cell cultured CDV vaccines are typically considered more attenuated in wildlife species compared to canine-cell cultured strains, but this is not always the case as reports of vaccine-induced CDV have occurred with all three types of vaccine strains listed (see Table 79.1). MLV CDV vaccines may be too virulent for some highly susceptible species such as red pandas, black-footed ferrets *(Mustela nigripes)*, and gray foxes *(Urocyon cinereoargenteus)*.[20,22,23,31]

Several MLV vaccines have been utilized successfully in some species from Canidae, Felidae, Mustelidae, and Procyonidae without adverse effects (see Table 79.2). The vaccines used in these studies all contained Onderstepoort or Lederle strains. MLV CDV vaccination in tigers resulted in significantly higher SN titers compared to titers determined following rCDV vaccination.[33] Lack of adverse vaccine effects may have been impacted by lower species' susceptibility to CDV and there may be more carnivore species for which these vaccines carry less risk. Clinicians will need to weigh the risk of exposure to CDV versus risk of vaccine-induced disease when considering MLV vaccine use. One strategy that may improve safety is a prime-boost vaccination protocol in which individuals receive an inactivated or recombinant vaccine followed by MLV vaccine booster. The initial vaccine aids in protecting against MLV vaccine-induced disease with loss of attenuation. This strategy has been employed successfully in African wild dogs (inactivated vaccine, boostered with Snyder Hill strain MLV vaccine)[53] and Siberian polecats *(Mustela eversmanii)* (rCDV vaccine, boostered with Onderstepoort strain MLV vaccine)[31] with no adverse effects.

Other Vaccines

DNA vaccines offer a similar safety profile to recombinant vaccines but can typically be produced at less cost compared to recombinant viral vectored vaccines. Historically, inefficient delivery systems have limited effectiveness of these vaccines; however, newer technologies have improved plasmid delivery to cells. A DNA vaccine containing H and N genes of wild-type and attenuated CDV has been demonstrated to successfully immunize American mink *(Neovison vison)* against CDV challenge.[54] This vaccine was also effective in the presence of maternal antibodies, differing from available vaccines.

Newer research has targeted the development of genetically engineered CDV vaccines by specifically introducing mutations to live virus genes to render them attenuated. Genetically engineered virus created by insertion into the L protein has been shown to produce CDV virus attenuation and immunize domestic ferrets against the virulent parental virus.[55] Variation between the H proteins of the CDV strains used in vaccines and those of the currently circulating wild-type strains may be a factor in some vaccine failures.[56] Specific targeted gene mutation may allow for development of newer safe vaccine strains specifically developed by modifying circulating wild-type CDV strains. Presently none of these vaccine technologies are commercially available but remain future directions for CDV vaccine research and production.

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SECTION 16

Great Apes

80

Infectious Diseases of Orangutans in their Home Ranges and in Zoos

JOOST PHILIPPA AND ROSALIE DENCH

Introduction

Wild, free-ranging orangutans are currently listed as endangered (*Pongo pygmaeus*, n = 55,000)[1] or critically endangered (*P. abelli*, n = 14,000),[2] and their fractured populations continue to decline due to loss of habitat, illegal capture, and trade. Infectious diseases may additionally play a role, although only limited data have been published on infectious diseases of orangutans in their home ranges of Indonesia and Malaysia.

From what we currently know, there are important differences in the prevalence of infectious diseases in orangutans (*Pongo* spp.) between home-range countries and zoos elsewhere (Table 80.1). Some of these differences are due to geographic and climatic factors, particularly for vector-borne pathogens (e.g., *Plasmodium* spp., arboviruses), which are common in the home range but rare in temperate zones, where most zoos are located.

Currently, there are 987 captive orangutans housed in 217 institutions worldwide.[42] Within the home ranges, more than 1000 animals are housed in (semicaptive) reintroduction centers, following confiscations and rescues from logging, mining, or oil palm sites. These wild-born orangutans potentially carry endemic pathogens from the forest into the human environment or are exposed to human pathogens once out of the forest.

Studies on infectious diseases in truly wild, free-ranging orangutans comprise only a limited number of invasive studies[4,43] and noninvasive fecal parasite studies.[4,33,35] The rest of our knowledge of home-range orangutan disease comes from rehabilitation centers. There are some factors related to captivity and human contact that apply to both zoo and rehabilitant populations, which do not affect wild orangutans. The solitary nature of orangutans in the wild may limit the spread of pathogens and may explain why there has never been a documented mass-mortality event due to an infectious disease in wild orangutans. It conversely means that they will be naïve to a plethora of pathogens encountered in the human environment. This susceptibility is exacerbated by numerous stress factors encountered in rehabilitation centers (change of diet, overcrowding, close contact with humans). Zoos face similar infectious disease threats but generally have a much smaller, stable population compared with the large influx of new arrivals at rehabilitation centers, which facilitates healthcare and biosecurity measures. Within rehabilitation centers, human contact is generally greater than in a zoo environment, increasing zoonotic risk. For this reason, proper use of personal protective equipment (PPE) by staff in contact with the apes is crucial, as well as closely monitoring the health of staff through regular testing for infectious disease and protocols to prevent staff working when unwell.

Although orangutans have their own endemic herpesviruses (Orangutan lymphocryptovirus),[9] they are highly susceptible to infection with human herpes simplex virus, Type 1 (HSV-1), with documented morbidity and mortality in zoo orangutans.[44] Confiscated orangutans with clinical signs similar to those seen in other nonhuman primates (NHPs) have tested positive serologically, although attempts at virus isolation were not successful.[45] No serologic evidence of HSV-1 has been seen in wild orangutans.[4]

The only confirmed case of rabies occurred in a confiscated orangutan in Indonesia.[17] It is highly likely that the infection occurred in the village where the orangutan was kept: the prevalence of rabies in Central Kalimantan is higher than anywhere else on Borneo.

Natural simian foamy virus infections have been isolated from wild orangutans and zoos out of the orangutan home range.[4,15,20] Transmission from numerous NHP species has been described in zookeepers, laboratory technicians, and hunters.[46,47] The viruses are generally species specific and cause persistent, nonpathogenic infections, even after cross-species transmission. Simian T-cell lymphotropic/leukemia virus type 1 (STLV-1) has also been isolated from wild-born and zoo orangutans without any clinical signs.[20]

Encephalomyocarditis virus (EMCV) has a rodent reservoir, and human infections are common but often not

Text continued on p. 570

TABLE 80.1 Important Infectious Diseases of Orangutans (*Pongo* spp.)

		Antibody/ Reaction	Antigen/ Pathogen Isolation	Wild*	Rehab†	Zoo‡	Mode of Transmission	References
Virus								
Adenoviridae	Adenovirus	+	+	+	+	+	Fecal-oral	4, 74
Papovaviridae	Polyomavirus	+	+	+	+	–	Fecal-oral	5
Herpesviridae	Herpes simplex viruses (1 and 2)	+	–	–	+	+	Body fluids, direct contact	6, 7
	Varicella zoster virus	+	–	–	–	+	Body fluids, direct contact, aerosol	6, 8
	Epstein-Barr virus/human herpesvirus 4	+	–	+	+	+	Body fluids, direct contact, bite	4, 6
	Orangutan lymphocryptus virus/Pongine herpes viruses/Epstein-Barr virus–like	+	+	–	–	+	Body fluids, direct contact, bite	9, 10
	Cytomegalovirus/chimp CMV	+	–	–	–	+	Body fluids, direct contact	6
Poxviridae	Monkeypox virus	+	+	–	+	+	Direct contact	11, 15
Hepadnaviridae	HBV	+	–	–	+	+	Body fluids, direct contact	6
	Orangutan hepadnavirus	+	+	+	+	–	Body fluids, direct contact	7
Picornaviridae	Coxsackie viruses	+	+	+	+	+	Fecal-oral	4, 12, 15
	Polio virus	+	+	–	+	+	Fecal-oral, direct contact	8
	HAV	+	–	–	+	+	Fecal-oral	6, 7
	Encephalomyocarditis virus	+	+	–	–	+	Fecal-oral, transplacental	13, 14
Togaviridae	Sindbis virus	+	–	+	–	+	Vector: *Culex* sp. and *Culiseta* sp.	4
	Rubella virus	+	–	–	–	+	Aerosol, direct contact	15
Flaviviridae	Dengue virus	+	–	+	+	–	Vector: *Aedes* spp.	4
	Japanese encephalitis virus	+	–	+	+	–	Vector: *Culex* spp.	4
	Tembusu virus	+	–	+	+	–	Vector: *Culex* spp.	4
	Langat virus	+	–	–	+	–	Vector: *Ixodes* sp.	4
	Zika virus	+	–	+	+	–	Vector: *Aedes* spp.	4
	Chikungunya virus	+	–	–	–	+	Vector: *Aedes* spp.	16
Reoviridae	Rotavirus SA11	+	–	+	+	–	Fecal-oral, direct contact	4
Orthomyxoviridae	Influenza A virus	+	–	–	+	+	Aerosol	3, 6
	Influenza B virus	+	–	–	+	+	Aerosol	3, 15

Paramyxoviridae	Parainfluenza viruses 1 and 2	+	–	–	–	+	Aerosol	6
	Parainfluenza virus 3	+	–	–	+	+	Aerosol	4, 6
	Measles virus	+	–	–	–	+	Aerosol	6
	Mumps virus	+	–	+	+	+	Body fluids, direct contact, aerosol	4, 15
	Respiratory syncytial virus	+	–	+	+	+	Aerosol	3, 4, 6
Pneumoviridae	Human metapneumovirus	+	–	–	+	–	Aerosol, direct contact	3
Rhabdoviridae	Rabies virus	–	+	–	+	–	Body fluids, direct contact, bite	17
Bunyaviridae	Hanta virus	+	–	–	–	+	Body fluids, direct contact	18
Filoviridae	Reston Ebola virus[§]	+	–	+	–	–	Body fluids, direct contact	19
Retroviridae	SFV	+	+	+	+	+	Body fluids, direct contact	4, 15, 20
	SRV	+	–	–	+	–	Body fluids, direct contact	7
	STLV-I	+	+	–	+	–	Body fluids, direct contact	20
	HTLV type I	+	–	–	+	–	Body fluids, direct contact	7
Bacteria								
Gram +	*Staphylococcus* sp.	–	+	+	–	+	Commensal overgrowth	8, 21
Gram +	*Streptococcus* sp.	–	+	+	–	+	Commensal overgrowth	21, 22
Gram +	*Dermatophilus congolensis*	+	–	–	–	+	Direct contact	23
Gram –	*Escherichia coli*	–	+	–	+	+	Body fluids, direct contact	4, 8, 22
Gram –	*Salmonella* sp.	–	+	–	+	+	Fecal-oral, direct contact, indirect	7, 24–26
Gram –	*Shigella* sp.	–	+	–	+	+	Fecal-oral, direct contact, indirect	25, 27
Gram –	*Campylobacter* sp.	–	+	–	–	+	Fecal-oral, direct contact, indirect	8, 27
Gram –	*Burkholderia pseudomallei*	–	+	–	+	+	Indirect	4, 28
Gram –	*Francisella tularensis*	–	+	–	–	+	Ingestion, aerosol, direct contact, vectors (multiple species)	29
Gram –	*Klebsiella* sp.	–	+	–	+	+	Direct contact, indirect	4, 8
Gram –	*Leptospira interrogans*[¶]	+	–	+	+	–	Urine, indirect	4
Gram –	*Helicobacter*	–	+	–	–	+	Saliva, fecal-oral	30
Misc	*Mycobacterium tuberculosis* complex	+	+	–	+	+	Aerosol	7, 8, 31, 32
Misc	*Mycobacterium avium*	–	+	–	+	–	Aerosol	4

Continued

TABLE 80.1 **Important Infectious Diseases of Orangutans (*Pongo* spp.)—cont'd**

		Antibody/ Reaction	Antigen/ Pathogen Isolation	Wild*	Rehab†	Zoo‡	Mode of Transmission	References
Endoparasite (GI)								
Amebae	*Entamoeba coli*	–	+	+	+	+	Fecal-oral	7, 33, 34
Amebae	*Entamoeba hartmani*	–	+	+	+	–	Fecal-oral	33, 34
Amebae	*Entamoeba histolytica*	–	+	+	+	+	Fecal-oral	33, 34
Amebae	*Entamoeba* sp.	–	+	+	+	+	Fecal-oral	34, 35
Amebae	*Endolimax nana*	–	+	+	+	+	Fecal-oral	33, 34
Amebae	*Iodamoeba buetschelii*	–	+	+	+	+	Fecal-oral	33, 34
Amebae	*Ballamuthia mandrillaris*	–	+	–	–	+	Fecal-oral	36
Amebae	*Blastocystis hominis*	–	+	–	–	+	Fecal-oral	33
Flagellates	*Giardia* sp.	–	+	+	+	+	Fecal-oral	7, 33–35
Flagellates	*Chilomastix mesnelii*	–	+	+	+	–	Fecal-oral	33
Flagellates	*Chilomastix* sp.	–	+	+	+	–	Fecal-oral	33, 35
Flagellates	*Dientamoeba fragilis*	–	+	+	–	–	Fecal-oral	33
Flagellates	*Trichomonas*	–	+	+	+	+	Fecal-oral	7, 8, 33
Ciliates	*Balantidium coli*	–	+	+	+	+	Fecal-oral	4, 7, 33–35
Coccidia	*Cryptosporidium* sp.	–	+	+	+	+	Fecal-oral	33, 37
Coccidia	*Cyclospora* sp.	–	+	–	+	–	Fecal-oral	7
Nematodes	*Ascaris* sp.	–	+	–	+	–	Fecal-oral	7, 34, 35
Nematodes	*Ancylostoma duodenale*	–	+	–	+	–	Fecal-oral	7, 33
Nematodes	*Baylisascaris procyonis*	–	+	–	–	+	Fecal-oral, direct contact	38
Nematodes	*Strongylida* sp.	–	+	+	+	+	Fecal-oral	33, 35
Nematodes	*Strongyloides stercoralis*	–	+	–	–	+	Fecal-oral, direct contact	33
Nematodes	*Strongyloides fuelleborni*	–	+	+	+	–	Fecal-oral	33
Nematodes	*Strongyloides* sp.	–	+	+	+	+	Fecal-oral	4, 33–35

Nematodes	*Trichostrongylus* sp.	–	+	+	+	–	Fecal-oral	33, 34
Nematodes	*Trichuris* spp.	–	+	+	+	+	Fecal-oral	4, 33–35
Nematodes	*Enterobius* sp.	–	+	+	+	+	Fecal-oral	4, 33–35
Nematodes	*Mammomonogamus laryngeus*	–	+	–	+	–	Fecal-oral	33
Nematodes	*Mammomonogamus* sp.	–	+	+	+	–	Fecal-oral	33, 35
Nematodes	*Oesophagostomum* sp.	–	+	+	+	+	Fecal-oral	7, 33
Nematodes	*Pongobius* spp. *(hugoti, foitovae)*	–	+	+	–	–	Fecal-oral	33
Nematodes	*Spirurida* sp.	–	+	+	+	–	Fecal-oral	33, 35
Cestodes	*Hymenolepis* sp.	–	+	+	–	–	Fecal-oral	34
Trematodes	*Dicrocoeliidae* sp.	–	+	+	+	–	Fecal-oral	33, 35
Trematodes	*Platynosomum fastotum*	–	+	+	+	–	Fecal-oral	7
Endoparasite (Blood)								
Sporozoa	*Plasmodium cynomolgi*	–	+	+	+	–	Vector: *Anopheles* spp.	39
Sporozoa	*Plasmodium pitheci*	–	+	+	+	–	Vector: *Anopheles* spp.	40, 41
Sporozoa	*Plasmodium silvaticum*	–	+	+	+	–	Vector: *Anopheles* spp.	40, 41
Sporozoa	*Plasmodium vivax*	–	+	+	+	–	Vector: *Anopheles* spp.	39
Sporozoa	*Plasmodium falciparum*	–	+	+	+	–	Vector: *Anopheles* spp.	41
Sporozoa	*Plasmodium* sp.	–	+	+	+	–	Vector: *Anopheles* spp.	4
Zoomastigophora	*Trypanosoma* sp.	–	+	–	–	+	Vector: Triatominae	8
Fungi and Yeast								
Yeast	*Candida* sp.	–	+	–	–	+	Direct contact, indirect	8
Fungus	*Microsporum gypseum*	–	+	–	–	+	Direct contact, indirect	8
Fungus	*Trichophyton* sp.	–	+	–	–	+	Direct contact, indirect	8

*"Wild" refers to free-ranging wild orangutans who have never been in captivity or come into close human contact.
†"Rehab" includes rehabilitation centers and zoos within the orangutan home range of Indonesia and Malaysia.
‡"Zoo" includes zoos, laboratories, and private collections outside of Indonesia and Malaysia.
§Letter of Concern raised against this study; see text.
¶Antibodies against the following *Leptospira* serovars were found: australis, autumnalis, ballum, bratislava, grippotyphosa, hardjo, icterohaemorrhagiae, pyrogenes, saxkoebing, serjoe, szwajizak, wolffi.
HAV, Hepatitis A virus; *HBV*, hepatitis B virus; *HTLV*, human T-lymphotropic virus; *SFV*, simian foamy virus; *SRV*, simian D-type retrovirus; *STLV*, simian T-lymphotropic virus.

recognized. Infections in zoo-based orangutans have caused fatal disease, and EMCV antigen or specific antibodies have been documented in zoos.[13,14]

The majority of our knowledge of infectious diseases of orangutans stems from serologic tests for antibodies. Most of these tests are validated for humans but not NHPs. Even in validated tests, there is known to be a certain level of cross reactivity with closely related[48] or unrelated antigens,[49] which may make accurate diagnosis challenging. A prime example of this was a study published on the serologic evidence of African strains of Ebola virus in orangutans in Indonesia,[19] the implications of which could have had a critical effect on release potential for orangutans in rehabilitation centers. Although it is possible that orangutans carry antibodies against Asian filoviruses such as Ebola Reston virus, it is highly unlikely that they have been in contact with African filoviruses. In addition, there were numerous factually wrong statements in the paper (origin of samples, sample collection methods), as well as questionable methodology, rendering the conclusions unfounded, which resulted in a published letter of concern.[50]

Unlike African great apes, there does not seem to be an orangutan-specific simian immunodeficiency virus (SIV) in the home range. Antibodies against SIV have previously been found by enzyme-linked immunosorbent assay in 2 of 19 orangutans in North American zoos, but confirmation tests (Western blot) were negative.[51]

Enteric parasites and protozoa (especially *Strongyloides*, hookworm, *Trichostrongyles*, *Balantidium coli*, and *Entamoeba* spp.) have a high prevalence in captive orangutans, in zoos as well as in home-range countries. *Balantidium* appear to thrive under stress, regardless of the orangutan's location. *Strongyloides* were reported to be the leading cause of death of orangutans younger than 15 years in zoos.[37] These enteric parasites have also been documented in wild orangutans.[4,33–35]

It is not in the scope of this chapter to go into detail for every pathogen reported in orangutans or the treatment; for such an overview, we refer the reader to Chapter 83.[52] Instead, we will highlight some of the biggest differences in infectious disease between orangutans in zoos and their home range, or those of greater importance with regard to zoonotic or release potential.

Air Sacculitis

Respiratory disease is the primary infectious health issue affecting zoo orangutans and is important in captive populations in home-range countries. A significant proportion of these cases are air sacculitis, where purulent material collects in the laryngeal air sacs,[53] which is frequently diagnosed both in zoos[8,54,55] and in rehabilitant populations.[56,57] Air sacculitis is not thought to be contagious[57]; therefore it is likely that husbandry factors in captivity contribute to the incidence of this condition.[53] There are no published reports of air sacculitis in wild orangutans, contrary to other free-ranging NHPs.[53,58,59]

TABLE 80.2 Bacteria Cultured from Air Sacculitis Cases in Orangutans (*Pongo* spp.)

Organism	Zoo*	Rehab.†
Aerobacter cloacae	—	1
Aeromonas hydrophilia	1	—
Alcaligenes fecalis	1	—
Bacterioides spp.	2	—
Enterobacter sp.	2	4
Enterococcus	1	—
Escherichia coli	20	2
Flavobacterium odoratus	1	—
Klebsiella oxytoca	—	1
Klebsiella pneumoniae	12	3
Micrococcus sp.	—	1
Morganella morganii	—	1
Proteus spp.	7	6
Pseudomonas aeruginosa	17	3
Pseudomonas spp.	2	6
Staphylococcus spp.	5	2
Streptococcus spp.	9	2
Yersinia sp.	—	2
Gram-negative bacilli	1	3

*Zoo cases (n = 33) from Wells et al. (1990, n = 7) and Zimmermann et al. (2011, n = 26).
†Rehabilitant cases (n = 25) from Lawson et al. (2006, n = 11) and Borneo Orangutan Survival Foundation unpublished data (n = 14).

Enteric bacteria are often cultured from infected air sacs (Table 80.2), suggesting that inhalation of fecal contaminants may initiate infection.[8,53,57] Infection risk from fecal aerosolization has been correlated with husbandry factors such as lower ventilation and smaller sleeping areas[56] and cage cleaning while the animals are present.[53] Additional causal factors could be chronic sinusitis[57,60] or respiratory irritants: severe smoke pollution in Indonesia and Malaysia from annual forest fires is linked to respiratory problems in human residents[61] and increased incidence of air sacculitis in rehabilitant orangutans.[56]

In zoos, adults represent the majority of published cases,[54,55] whereas the highest incidence in rehabilitation centers is in juveniles (2–8 years).[56,57] This may reflect the relative population structure in zoos, biased toward older animals, compared with rehabilitation centers with a younger population. A male sex bias has been found in air sacculitis cases in rehabilitant juvenile orangutans,[56] as in other NHPs.[62] In zoos, males are predisposed to respiratory infections, although not specifically air sacculitis.[55] There is marked sexual dimorphism in the air sac in adult

orangutans; it is possible that sex differences in air sac structure are present at a young age.

Vector-Borne Diseases

In the orangutan's home range, vector-borne infections are abundant, and malaria is the most common infectious disease in rehabilitation centers. Classically, two *Plasmodium* species have been described only in orangutans, *P. pitheci* and *P. sylvaticum*[40]; neither of these has been associated with clinical disease. Most *Plasmodium* species are considered to have a sylvatic cycle, where wild NHPs are often asymptomatic carriers. However, in rehabilitation centers, orangutans with clinical signs (high fever, lethargy) are commonly diagnosed with "human" *Plasmodium* species (e.g., *P. falciparum*, *P. vivax*) by microscopy and respond positively to treatment with artemisinin-based medications (authors' experience). The presence of the different *Plasmodium* species (including *P. knowlesi*) was confirmed by polymerase chain reaction (PCR) (unpublished) and published for another rehabilitation center,[41] although this was later disputed.[39] In centers, high primate population densities at ground level, combined with a higher density of mosquitoes (Culicidae) at ground level than in the canopy,[63] lead to an increased risk of *Plasmodium* infection.

Arboviruses are abundant in the home ranges and also have a sylvatic cycle, with nonclinical infections of NHPs. Antibodies against dengue and other arboviruses have been described in semicaptive and free-ranging orangutans.[4] At the Borneo Orangutan Survival Foundation (BOSF) rehabilitation center at Nyaru Menteng, febrile, lethargic, thrombocytopenic juvenile orangutans commonly tested positive in a Dengue-specific immunoglobulin M rapid test (designed for human use). We collected samples from these animals: all tested negative by reverse transcriptase PCR and for virus isolation. Serologic tests were negative for NS1 antigen and IgM antibodies, whereas several samples tested positive for immunoglobulin G (unpublished). The cross reactivity of antibodies between the different flaviviruses, as well as other pathogens, is well documented, warranting further research into natural flavivirus infections in orangutans.

Hepatitis B

Like most hepadnaviruses, hepatitis B virus (HBV) is fairly species specific. However, discovery of recombinant HBV of human and NHP origin has confirmed the potential for cross-species transmission,[64] which is a serious concern for release programs and potentially for zoos. The prevalence of human HBV infection is relatively high in orangutan home ranges, whereas vaccination has limited the prevalence in other countries. Orangutans have genetically very similar HBVs, which appear to be nonpathogenic, but antibodies cross-react with human HBVs.[65] In confiscated orangutans in the home range, a prevalence of up to 59% has previously been reported.[66] There have been very few cases of confirmed human HBV in confiscated orangutans, but the zoonotic risk is high. It is of the utmost importance to confirm the strain of hepadnavirus before release into the forest, where wild orangutans are naïve to the human strain. Preventative measures e.g., PPE are essential. People carrying a blood-borne zoonotic pathogen such as HBV should refrain from situations where the primate may bite the worker, which minimizes risk of transmission and is required in human health care.[67] Antiviral drug therapy that reduces viral load minimizes this risk further.

Tuberculosis

Tuberculosis (TB), caused by infection with *Mycobacterium tuberculosis* complex bacteria, can affect all mammalian species.[68] In addition to acute respiratory disease, infection can remain latent and undetected for many years, making it difficult to control[69] and resulting in preventative euthanasia in most infected captive NHPs. Cases of TB in orangutans have been reported from zoos and rehabilitation centers, but it has never been documented in wild orangutans.[8,70] In home-range countries, TB is endemic in the human population[69]; thus increased human contact increases the risk of naïve wild-born orangutans to exposure to pathogenic mycobacteria. Even where TB is not endemic, zoos must undertake regular TB testing to prevent entry and spread through their collections.[68]

Traditionally, the tuberculin skin test (TST) is used,[53] but orangutans seem to be more sensitive and often show a nonspecific T-cell response.[8,31] A comparative TST is recommended, using antigen from tuberculous and nontuberculous mycobacteria, to account for cross reactivity.[31,53,70] Using highly concentrated bovine purified protein derivative (100,000 IU/mL) and standard avian purified protein derivative (25,000 IU/mL) and reading the greatest response at either 48 or 72 hours provides the most reliable results in orangutans.[70] Positive diagnosis of active TB by culture or PCR of gastric or bronchoalveolar samples is definitive but has low sensitivity (approximately 60%–70% in human cases).[71] Trials have been done on several immunoassays as adjunctive diagnostics for TB,[32,68,72] although none appear to be conclusive for orangutans.[73] Further research into these tests is required.

Conclusion

Although there is some overlap in the infectious diseases of orangutans in zoos, rehabilitation centers, or in the wild, others are compounded by factors related to captivity and geography. The susceptibility of great apes to human pathogens—and vice versa—must be considered and preventative measures taken. Accurate detection of infectious disease in orangutans is vital, both for conserving the health of zoo populations and to minimize the risk of "human" pathogens being introduced to wildlife populations when rehabilitated orangutans are released to the wild. A good

understanding of the accuracy and limitations of the available diagnostic tests is key to achieving this.

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Medical Aspects of Chimpanzee Rehabilitation and Sanctuary Medicine

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Sanctuaries provide life-time care for animals that are not able to live in the wild. True sanctuaries are not open to the public, do not breed or purchase animals, and do not transfer them to other facilities for commercial or reproductive opportunities. They do not participate in the Species Survival Plan programs that accredited zoos use to manage populations and contribute to the conservation of the species.[1] The mission of sanctuaries is to allow animals not able to live in their natural world to thrive in a captive environment that facilitates species-specific behavior with the least amount of stress. There is a great variety in the type of sanctuaries that exist in the United States in terms of species, design, management, and oversight, but the underlying commonalty is ensuring the welfare of each individual animal. Sanctuary medicine follows the same principles by providing individuated medical care rather than population or herd management. Sanctuaries employ fulltime veterinarians or use consultants depending on size, budget, and availability of a skilled professional. Sanctuaries that do not have a knowledgeable veterinarian overseeing the care of their animals that includes frequent site visits and 24-hour emergency coverage are not true sanctuaries. Regulatory bodies exist to ensure the best quality of care for zoos through accreditation with the Association of Zoos & Aquariums (AZA) and the Institutional Animal Care and Use Committee (IACUC) for laboratories. The Global Federation of Animal Sanctuaries (GFAS) provides a comprehensive standard of excellence for sanctuaries. In the end, the veterinary oath "*I will practice my profession conscientiously, with dignity, and in keeping with the principles of veterinary ethics*" is what ultimately guides veterinarians to offer the highest quality of care possible in companion animal, zoological, research, sanctuary, or other fields of veterinary medicine.

Status of Chimpanzees at Sanctuaries

The use of chimpanzees *(Pan troglodytes)* in research has undergone significant changes over the past few years. In the United States the National Institutes of Health (NIH) commissioned the Institute of Medicine (IOM) in 2010 to review the use of chimpanzees in biomedical research. The report entitled, "Chimpanzees in Biomedical and Behavioral Research: Assessing the Necessity," stated that "while the chimpanzee has been a valuable animal model in past research, most current use of chimpanzee research is unnecessary."[2] Time was given for public comment from researchers, scientists, and other interested parties. After several years of spirited debate, the NIH announced that the decades-long use of chimpanzees in biomedical research would end and that all NIH-owned chimpanzees would be retired. Furthermore, the NIH would stop funding research on privately owned chimps. Then, in 2015, the United States Fish and Wildlife Service declared that captive chimpanzees would be reclassified from threatened to endangered, affording them the same protection as wild chimpanzees and severely restricting any research that does not show a clear benefit for the species in the wild. There is an emphasis to move chimps out of laboratories and into sanctuaries. As of 2017, a total of 330 federally owned chimpanzees had transferred to the NIH-supported national sanctuary, "Chimp Haven."[3] Hundreds of privately owned (i.e., not federally owned) chimpanzees are retired or projected to be retired from laboratories also, though there is a severe restriction of available space at this time. Research in sanctuaries, if allowed, is restricted to observational and noninvasive studies that do not disturb the animals and provide a purposeful, direct benefit to their quality of life.

Much of the following primate medicine information is from a private sanctuary that cares for 248 chimpanzees, many of whom came from a financially failing biomedical laboratory. Others arrived from roadside zoos, entertainment venues, and the pet trade. The 150-acre sanctuary houses 237 chimpanzees divided into 12 families made up of both males and females of varying ages. Each group has an indoor living space with six interconnected bedrooms attached to a 3- to 4-acre grass-covered island with trees, hills, climbing structures, and platforms. Eleven animals deemed psychologically or medically unfit for large social groups live with fewer chimps in a fission-fusion pattern in a building with separate large outdoor yards. Two of these chimpanzees are singly housed for psychological reasons but have close visual, auditory, and safe tactile contact with neighboring chimps. Attempts are ongoing to acclimate them with conspecifics.

Housing and Introductions

Captive chimpanzees have unique, complex personalities, diverse backgrounds, and abnormal rearing histories when raised for research, entertainment, or as pets, making it a challenge to fit them into cohesive hierarchal groups.[4] Human-rearing of chimpanzees and other nonhuman primates has undergone a significant shift in zoos and is now done only when deemed necessary and emphasizes an early return to conspecifics.[5,6] Social housing, defined by GFAS, requires that the chimpanzees be in a compatible group without physical or psychological stress and that there is adequate space to allow for more natural fission-fusion affiliations.[7] Chimpanzee introductions require knowledge of the individual's personality, a comprehensive understanding of chimpanzee behavior, and a building design that allows for quick and safe separation of chimpanzees if the introduction does not go as anticipated. Resocialization of socially deprived chimpanzees has been successful at multiple sanctuaries, zoos, and laboratories, and various techniques have been described elsewhere in the literature.[8–11] As noted, the number of chimpanzees arriving at sanctuaries is increasing due to the recent ban on their use in biomedical research. Many of them do not have the species' typical communication or social skills that wild chimpanzees learn from an extensive and long maternal childhood and supportive complex social communities.[12,13] Groups ranging in numbers from 12–27 chimpanzees are formed by dyadic introductions of all individuals, and mild aggression or tension often resolves spontaneously without intervention. If aggression is deemed moderate to severe from the outset or escalates, the individuals are separated for a period of 5–15 minutes. During this "time out" the chimps are maintained in close proximity separated by mesh. Reintroduction commences once the arousal dissipates and frequently results in affiliative interaction. If avoidance or aggressive behavior does not resolve, the introduction is discontinued for a period of time ranging from a day to months while other dyadic relationships commence. Chimpanzees appearing consistently anxious during the process may benefit from a partnership with a conspecific with excellent social skills. Once socially bonded, triadic and small group formations commence. Every relationship has its own time period and is deemed satisfactory if they fulfill behavioral benchmarks such as grooming, playing, and other affiliative behavior. However, the personality of the participants must be taken into consideration; otherwise, strictly defined criteria of a satisfactory pairing may impede an animal's successful entry into a social group. Finally, chimpanzees who do not exhibit species-appropriate behavior during initial introductions may learn the nuances of being a chimp during repetitive positive interactions that may lead to subsequent successful results. After each family member meets individually, different configurations of small subgroups are carefully combined to identify relationships that may be detrimental and need resolution prior to unity of the entire group.[8,11]

The behavioral and veterinary departments work collaboratively if psychopharmacologic treatment is deemed necessary for the health and well-being of particular animals. Definitive guidelines for behavioral drugs in chimpanzees are lacking, and therapy is based on human recommendations.[14,15] Short-term use of anxiolytic benzodiazepines has a rapid effect and good tolerability and helps chimpanzees that display inappropriate fear, anxiety, agitation, or mild forms of self-injury during introductions or in general. There is wide variability in dosing between animals, and response to therapy and lack of side effects dictate both the dose and treatment length. Diazepam at 5–30 mg administered once (SID) to twice (BID) daily appears to work better than lorazepam at 0.5–3 mg SID to BID. For introductions, diazepam may be stopped and started as needed. If used consistently, slow weaning is recommended to prevent acute withdrawal symptoms.[15]

Aggression, though fairly common in chimpanzee societies, may hamper an individual's opportunity to live in a large, complex community in captivity. Every effort should be made to help facilitate this process. Psychopharmacologic treatment from available veterinary and human literature is selected based on efficacy, taste, acceptance, and side effects. Drug selection must take into account the therapeutic index and hematological/biochemical effects of the medicine. Done judiciously, this will reduce the frequency and stress of venipuncture by positive reinforcement training or sedation. Blood drug levels, common in human medicine, may be more difficult to obtain in primates.

The central sympatholytic effects of the alpha-2 adrenergic receptor agonist, clonidine, is used to treat different forms of aggression in people. It appears to have similar effects in chimpanzees. An oral starting dose of 0.05–0.1 mg daily can be titrated up to 0.2 mg SID to BID depending on response and side effects. Guanfacine, a sister compound, may also be used but is less sedating.[18] A demonstrated change in temperament manifested by less piloerection, aggressive displays, and violence is considered

a positive response. The drug may be continued for months, but slow weaning must be done to avoid rebound hypertension.[17]

Risperidone is an atypical antipsychotic used for human psychiatric conditions including aggression. Controlled data is lacking for the use of this drug in chimpanzees but was tried on seven aggressive males that failed to assimilate into any large group after multiple attempts and configurations. The primates received oral risperidone, 0.02 mg/kg–0.07 mg/kg divided BID. All the chimpanzees were successfully integrated when treated with risperidone. The chimpanzees showed no side effects and were subsequently weaned at 6 months to one year, though one chimp was treated intermittently for 2 years. They have all remained in complex social environments.

Use of haloperidol, a conventional antipsychotic, was discontinued because oral doses of 5–10 mg/day produced bradykinesia in five male chimpanzees. Haloperidol has been used successfully in one female chimpanzee for generalized anxiety and intermittent self-mutilation at 2–4 mg/day.

Physical Examinations and Anesthesia in Sanctuaries

Clinical signs, age, history, risk-to-benefit ratio of anesthesia, and familiarity with the chimpanzee determine the frequency of physical examinations and the anesthetic protocol. Chimpanzees from laboratories are suspicious when separated from their companions, and sedation is best achieved in the morning after a 12–16-hour fast with no water restrictions. The chimpanzee can receive an oral dose of diazepam (10–40 mg) in juice 1–2 hours prior to anesthesia to decrease anxiety. Operant conditioning for acceptance of injections is useful, though time-consuming for a large population of chimpanzees.[22] Drugs and doses for great apes have been previously reported.[22–24] A positive relationship between the veterinarian and the primate may facilitate hand injections using a greeting or a small sip of juice as a distraction. Healthy animals may first be given the intramuscular (IM) sedative medetomidine (ZooPharm, Laramie, Wyoming, USA; 0.02–0.05 mg/kg, 10 mg/mL) in a 1 cc Luer Lock syringe and 23–25 g ⅝ inch needle held out of view or hidden inside a loose latex glove. The chimps are injected into any body area closest to the mesh directly or through the latex glove. Quiet reassurance from the veterinarians and/or care staff keeps the chimp close until complete recumbent sedation occurs within 3–15 minutes. An immobilization dose for ketamine or tiletamine-zolazepam is then administered IM by hand or pole syringe with no reaction from the chimpanzee.[22] Chimpanzees from research backgrounds generally require the higher dose range. Respiratory rate, the color of the mucous membranes, and heart rate are monitored during the induction period for an additional 10 minutes. Shaking or otherwise stimulating the chimp may not elicit a reaction, but a response to a light spray of water on the face will determine if supplemental drugs are necessary.

Oral transmucosal medetomidine, 0.1 mg/kg, has been administered in marshmallow crème or applesauce in chimpanzees in combination with tiletamine-zolazepam.[26] Attempts to use oral medetomidine prior to IM injection of an induction agent was not successful due to the inconsistency of the sedation, variable time to effect, and cyanotic mucous membranes.

Once anesthesia is induced, supplementation with ketamine at a dose of 2–5 mg/kg IM or gas anesthesia with isoflurane is selected based on the length of the procedure and depth of anesthesia required.[23] With the chimpanzee in dorsal recumbency, the vocal folds are easily visualized by placing a lighted laryngoscope with a long curved blade into the vallecula and gently pulling up. No laryngeal spasms or inability to intubate occurred in over 1000 intubations using a dry 6–8 cuffed endotracheal tube with a stylet. Once the tube is secured, the table is tilted to remove the pressure from the abdominal organs on the diaphragm. A surgical monitor measuring peripheral partial oxygen pressure (SpO_2), heart rate, end tidal carbon dioxide, electrocardiogram, and indirect blood pressure is recommended. The use of intravenous fluids, preemptive antibiotics, analgesics, local blocks, and temperature maintenance is determined by the patient and the procedure. In the event of an anesthesia complication, medetomidine can be quickly reversed by dividing the total dose of atipamezole (5 mg per 1 mg medetomidine) and administering one-fourth of the dose intravenously and the remainder IM.[23] Supplemental anesthesia should be immediately available to circumvent early arousal from reversal.[23] Chimpanzees recovering from noncomplicated anesthesia are placed in lateral recumbency with the upper arm positioned behind the back, and the head placed close to an area where extubating and suctioning of oral secretions is safely achieved behind a protective barrier. If atipamezole is used to reverse the medetomidine, waiting a minimum of 45 minutes after the last dose of ketamine prevents undesirable emergence reactions from the dissociative anesthetic and results in a smooth recovery within 7–15 minutes. Not reversing the alpha-2 adrenergic agonist also prevents emergence delirium and may be advantageous for postsurgical or painful cases. Tiletamine-zolazepam results in prolonged recoveries alone or after reversal of the medetomidine.[23]

Medetomidine is not recommended for suspected or confirmed cardiac disease, sick, elderly, or other high-risk patients due to potential changes in cardiovascular function.[24] Protocols using ketamine, ketamine/midazolam, or tiletamine–zolazepam are thoroughly outlined elsewhere.[22–24] Reconstitution of tiletamine-zolazepam to 200 mg/mL lowers the volume allowing for more successful hand injections. All high-risk patients should be intubated and receive oxygen alone or gas anesthesia and be monitored with a surgical monitor.

Sanctuary Preventative Healthcare

Preventative healthcare programs at sanctuaries follow similar guidelines as those at zoos.[25,27] Exceptions may include pretransport exams, length of quarantine time, immunoprophylaxis, and reproductive methods. Animals being transferred to sanctuaries may have minimal to no previous veterinary care. Lack of veterinarians, a seizure situation, or the volatile emotions of those relinquishing the primate may necessitate doing the physical examination, laboratory specimen collections, and intradermal tuberculin testing on the day of transport. A veterinarian should accompany the animal during transport to mitigate complications that could occur with an incomplete medical background. Quarantine is essential to prevent the risk of disease exposure to others, but the length is based on the source of the animals, test results, health status, and development of clinical symptoms. Similarly, all employees are tuberculin tested yearly and educated on both zoonotic and anthropozoonotic diseases.

Routine parasite screening includes bacterial fecal cultures and fecal examinations by direct wet mounts and centrifugal floatation with a commercial solution. Regular group samplings of freshly defecated stools and individual testing are recommended as a screening tool and for those with gastrointestinal symptoms. Plain fecal samples and feces in 2% formalin are intermittently submitted to a reference laboratory for confirmation. Fecal cultures on all animals at the sanctuary have been consistently negative for *Shigella*, *Salmonella*, *Yersinia*, *Campylobacter*, and *Escherichia coli*. Examination and sampling of the large and small intestines at necropsies may provide information on parasite burden. *Balantidium coli*, *Troglodytella* sp., *Entamoeba*, *Enterobius*, *Giardia*, and *Strongyloides* sp. have all been identified in captive great apes.[25,27,28] Proactive diagnostic and control strategies are mandatory as self-medication for parasites is severely limited in captivity.[29] Control of internal parasites has been successful with the use of fenbendazole (10 mg/kg PO) and ivermectin (200 mcg/kg PO [repeated again in 2 weeks]) alternated quarterly. *Balantidium* sp., an intestinal protozoa, does not generally cause intestinal symptoms. If no other causes of diarrhea can be identified and there is no response to dietary and nonpharmacologic therapy, treatment of Balantidium with doxycycline, 100 mg BID, for 10 days may reduce the number of cysts and trophozoites and resolve the symptoms. Metronidazole is highly effective, but acceptance is difficult even with compounded formulas.

Reproductive Management

Sanctuaries do not breed animals and there are different methods of prevention (see Chapter 22). Nonreversible male sterilization is the preferred method for permanent contraception at many primate sanctuaries. Vasectomies are performed through a prescrotal incision in chimpanzees. The vas deferens (VD) is isolated, and up to 2–3 cm is removed. Previous methodology involved placing the cautery unit flat on the ends of the VD for coagulation. This technique resulted in 86% regrowth and seven unwanted births. The surgery was modified to cauterize only the epithelium of the lumen by introducing a nonheated fine wire electrode (Jorgensen JO470DT5) into each end of the VD once a portion of it was removed. The electrocautery unit is turned to coagulation as the tip is withdrawn. Single portable battery thermocautery units may be used by pinching the tip with a sterile hemostat to facilitate introduction into the small lumen. After intraluminal cautery, recanalization is further prevented by fascial interposition of the VD ends, followed by subcutaneous and subcuticular suture closure.[30] The chimpanzees do not pick at the surgical site if chromic gut (3-0) is used for subcuticular sutures, but polydioxanone (PDS) caused significant postoperative self-grooming. Exploratory surgery 6 months to 2 years later confirmed no regrowth following this technique in novel animals and those that had a previous vasectomy. However, since histopathologic confirmation of VD was not done at the time of the initial surgery, it is unclear how much the vasectomy technique influenced the outcome.

Sanctuary Case Reports

Ocular Herpes

Alphaherpesviruses in great apes have been identified as human simplex virus groups 1 and 2 (HSV1 and HSV2).[31] Several young chimps developed acute white oral/pharyngeal ulcers that resolved without treatment. Viral culture and genome sequencing on the virus isolated from the oral lesions identified a new herpes virus, ChHV. This new virus is antigenically similar but not a variant of the human simplex viruses, and further studies determined that ChHV and HSV2 are more closely related to each other than either is to HSV1.[32,33] Serum was tested on 42 chimpanzees and 35% of these chimps had antibodies to the ChHV virus.

The most common clinical presentation of herpes at the sanctuary is acute ocular pain with or without corneal ulceration. The animals develop severe photophobia and are unable to keep their eyes open. Corneal scrapings on multiple animals for viral isolation were negative, yet a dramatic response occurred with antiviral medications. Valacyclovir, 1 g per os (PO) twice daily or acyclovir 400 mg PO every 6 hours significantly shortened the clinical signs from weeks to days. Having astute and well-informed caregivers able to recognize early warning signs shortened the course of the disease to 24 hours. The suspected mechanism is reactivation of the latent virus from the ophthalmic branch of the trigeminal nerve.[33]

Demyelinating Disease

An 11-year-old male chimpanzee previously used in hepatitis C biomedical research developed acute bilateral weakness of both legs and began to drool. The chimpanzee was anesthetized with tiletamine-zolazepam. The

examination was unremarkable, but the complete blood count (CBC) showed leukocytosis 18,200 µL, with neutrophilia (12,740 µL) and monocytosis (1820 µL).[34] The chimpanzee improved but 6 months later developed intermittent drooling, screaming, progressive leg weakness, and an obtunded level of consciousness. The blood chemistry, CBC, bile acids, fungal serology tests, and urinalysis were normal. Viral results were negative for antibodies to human immunodeficiency virus, simian immunodeficiency virus, HSV1 and 2, and the antigen for hepatitis C by polymerase chain reaction. The chimpanzee had antibodies to cytomegalovirus and the Epstein-Barr virus. Magnetic resonance imaging without and with gadolinium contrast-enhanced T1-weighted images showed abnormal signaling in the white matter of both frontal lobes extending into the genu of the corpus callosum and descending white matter tracts. The chimpanzee also had elevated very-long-chain fatty acids (VLCFA) consistent with a defect in peroxisomal fatty acid oxidation. He was euthanized 8 months later due to deteriorating symptoms, and postmortem histopathology confirmed a diagnosis of demyelination.

Behavioral Issues

Nonhuman primates in zoos, sanctuaries, and laboratories often display behavioral abnormalities such as anxiety, social isolation, stereotypies, displacement disorders, self-mutilation, and other aberrances. Facilities have different rates of these behaviors, and research is ongoing to improve captive care by understanding the causes of these deviations from the norm.[35–37] Assessing the psychological health of animals is challenging, and some experts suggest the use of the human Diagnostic and Statistical Manual of Mental Disorders, 4th Edition (DSM-IV) to categorize the psychological disorders of great apes, but others have made a valid argument against it.[14,35,36] The first step in dealing with acute or chronic behavior patterns that interfere with the quality of the sanctuary animal's life is to search for and treat medical issues that may be contributory.[15] Next, a comprehensive, individualized behavioral plan is designed based on symptoms, frequency, and severity. The use and timing of pharmacologic therapy depends on multiple factors. The genetic physiologic similarities between chimpanzees and humans and the lack of rigorous systemic research support extrapolation of treatment from human literature.[14,15,37] All drugs selected in consultation with psychiatrists, behaviorists, and literature review must be considered empirical at this time. Therefore the veterinarian must closely monitor for signs of efficacy, safety, and tolerability. Well-planned and scheduled attempts at weaning help to justify a drug's use.

Longer treatment options for anxiety, social isolation, aggression, or compulsive disorders are the selective serotonin reuptake inhibitors (SSRIs).[14–16,37,38] Consulting psychiatrists recommended fluoxetine due to its proven safety and the ability to discontinue the drug rapidly. Selection of different SSRIs for specific disorders may improve treatment success. Fluoxetine starting at 10 mg/day is increased to 20 mg after 1 week. If there is no therapeutic response in 2–3 months, titration to the maximum dose of 60–80 mg/day is attempted to determine effectiveness. Selective serotonin norepinephrine reuptake inhibitors (SNRIs) are a newer class of antidepressants showing promise for treatment of multiple disorders in humans.[15] SNRIs should be used judiciously due to their known withdrawal symptoms even with one to two missed doses and lack of compliance. However, one chimpanzee who severely self-mutilated his head for 7 years was responsive to the off-label use of the SNRI milnacipran with a dose of 50 mg BID, and attempts to wean the drug caused a resurgence of self-injury.[15,40] Intensive behavioral and husbandry management and multiple drug trials were less effective.[40]

Neuroleptics (antipsychotics) block dopamine D2 receptors in the brain.[14–16] The typical antipsychotic, haloperidol, produced extrapyramidal signs of dystonia and akathisia in all male chimpanzees within hours to days after administration of 5–20 mg/day. The second-generation atypical neuroleptics have different receptor affinities and produced no side effects at doses used.[15,16] Risperidone, 1.0–6.0 mg divided BID, appears to be successful for generalized anxiety as well as for aggression as discussed under Housing and Introductions earlier in the chapter.

Evidence-based science on psychopharmacology is essential as chimpanzees move from laboratories to sanctuaries, yet there is a lack of data. Not all behaviors warrant treatment or can be helped with medication. One male chimpanzee severely mutilated his arm for 17 years in the laboratory. Multiple sequential pharmacologic trials had minimal effects. We will never know if better husbandry, behavioral enrichment, relationships with conspecifics, human bonding, surgeries, the 72-hour negative pressure wound treatment with constant rate infusion (CRI) of ketamine, or the freedom to roam a 3-acre island helped him recover, but he has stopped. The key point is to keep trying multiple modalities.

End of Life and Euthanasia

As the population of captive chimpanzees age, geriatric medical issues develop that must be addressed to ensure optimal veterinary care.[40–43] Adding to the discussion is the diversity of the rearing history, nutrition, research, and previous veterinary care that may influence the type and progression of disease in older primates.[34,41,42] Ideally, the geriatric animal should stay with their social companions forever, provided there are husbandry, structural, and veterinary medicine modifications. Ramps, ladders, platforms, and straps facilitate mobility. Temporary separation from the group for feeding or resting ensures less stress, proper nutrition, and time for the administration of medication and evaluation by the veterinarian. Natural or planned formation of a smaller subgroup that facilitates daily access to the outside, opportunities for companionship, and a decreased risk of trauma is beneficial. Just as every

sanctuary animal has a specific medical and behavioral plan, end-of-life decisions are also individualized. Nutrition, appetite, weight, hydration, mobility, daily activity, and other objective criteria for assessing the quality of life are important but not definitive. For example, if an animal in renal failure responds to 20 mg megestrol acetate, an appetite stimulant, how should the end-of-life benchmarks be adjusted? Do chimpanzees that develop bilateral leg paresis from degenerative disc disease but are mobile with ramps and straps and interactive with family members be considered too compromised? How extensive and intensive should the veterinary care of geriatric animals be in zoos, laboratories, or sanctuaries? Electric cardioversion using an automated external defibrillator successfully revived three chimpanzees. One of the chimps, a geriatric male, died of a fatal arrhythmia 2 years later. The other two female chimpanzees are still alive and healthy.

Veterinarians should act as the patient's advocate and even more so as they decline. Some veterinarians suggest geriatric animals may need more frequent physical examinations, but there is also a valid argument against sedation due to a higher risk-to-benefit ratio.[43] The development of noninvasive diagnostic techniques might be more advantageous for managing the aging population.[44,45]

Veterinarians should remain respectful of the strong personal bonds and the considerable knowledge of dedicated caregivers and include them in the process when making end-of-life and euthanasia decisions. Finally, sanctuaries that care for chimpanzees should never underestimate the compassion, protection, and comfort the chimpanzee family provides for their failing companions (see also Chapter 15).

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Update on the Great Ape Heart Project

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Introduction

Cardiovascular disease (CVD) is a major cause of mortality in all four great ape taxa in managed care: western lowland gorillas *(Gorilla gorilla gorilla)*, orangutans (*Pongo pygmaeus*, *P. abelii*, and *P.* hybrids), chimpanzees *(Pan troglodytes)*, and bonobos *(Pan paniscus)*. CVD has been seen in wild living great apes, but there is limited information available on CVD prevalence and severity.[1–12]

The Great Ape Heart Project (GAHP), based at Zoo Atlanta (Atlanta, Georgia), is an innovative and coordinated program to investigate ape CVD and establish uniform strategies for the diagnosis, treatment, and prevention of great ape CVD. A focus of the GAHP has been to develop guidelines and recommendations that support zoological professionals in diagnosing and treating CVD in great apes. This has been accomplished by professional capacity building, cardiac examination reviews, clinical support to attending veterinarians, and easy access to subject matter experts (SMEs) such as cardiologists, pathologists, and species experts. The GAHP is led by a Project Director and a dedicated full-time Database Manager, assisted by an Executive Committee made up of cardiologists, sonographers, pathologists, and Species Survival Plan (SSP) veterinary, pathology, and nutrition advisors for all four great ape taxa.

What We Do

The GAHP functions like the hub of a wheel, facilitating communication and support among stakeholders and SMEs. This system aids zoos and stakeholders who are interested in CVD research, as well as great ape care and welfare. In essence, the GAHP creates connections to facilitate the highest level of cardiac care for these highly endangered apes (Fig. 82.1). A customized web-based database is used to trace ape relatedness and links clinical information to postmortem data, creating a foundation for CVD-based research and the establishment of taxon-specific echocardiographic reference parameters (Fig. 82.2). Facilitating access and clinical support between veterinarians, keepers, and dedicated SMEs has been the cornerstone to the success of the GAHP.

Great Ape Cardiovascular Disease

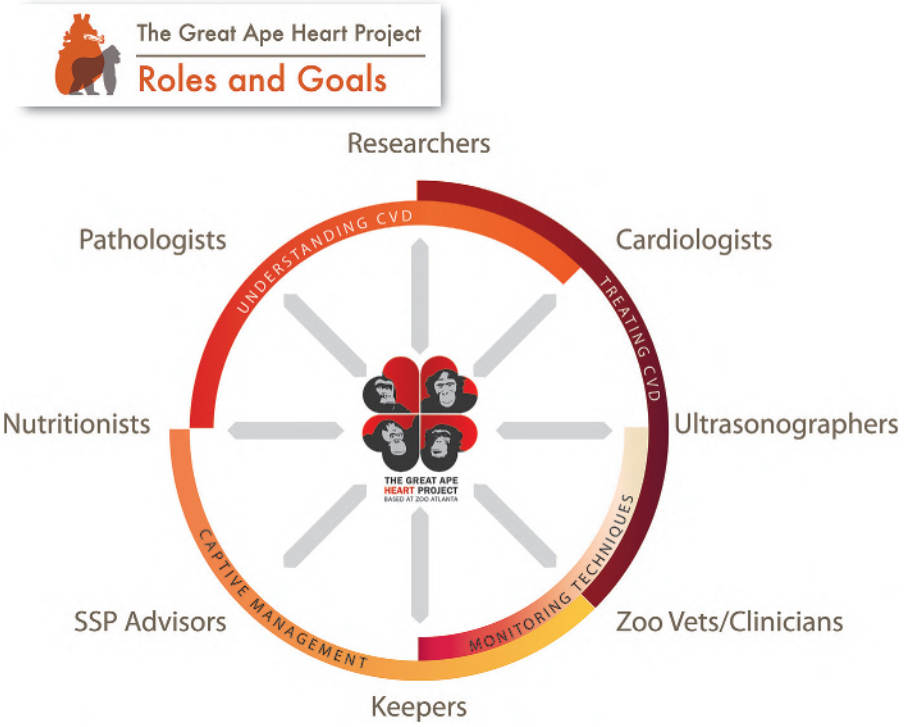
Types of great ape CVD reported include: myocardial fibrosis in the absence of coronary infarction, aortic dissections, atherosclerosis, arteriosclerosis, valvular degeneration, infectious myocarditis, and congestive heart failure.[3] Affected apes are typically adult to older adult individuals, and CVD predominately affects males. The most common finding at necropsy in affected apes is myocardial replacement fibrosis, often termed interstitial myocardial fibrosis or fibrosing cardiomyopathy.[10,13] Myocardial injuries, such as inflammation, ischemia, vasospasm, and hypertension, all may result in the formation of fibrosis of the myocardium. Regardless of the inciting process, myocardial fibrosis results in increased myocardial stiffness, loss of contractile ability, increase in arrhythmogenic potential, and eventual cardiac dysfunction or sudden cardiac death.

Clinical Signs

Subtle signs of CVD in apes may include lethargy, anorexia, changes in weight, avoidance of antagonistic or aggressive interactions with conspecifics, and loss of social ranking. As CVD progresses, animals may exhibit sudden death, especially during or immediately following respiratory illness or times of increased stress or activity. Progressive signs of CVD such as weight gain, peripheral edema, and progressive respiratory compromise may occur, and sudden death has been attributed to aortic dissections, arrhythmias secondary to extensive myocardial fibrosis, thromboembolic events, or decompensated congestive heart failure with multisystemic failure.[8,14]

Cardiovascular Changes

Expected cardiovascular systemic changes due to aging typically result in left ventricular (LV) wall thickening, increased myocyte size with decreased numbers, increased interstitial connective tissue, and loss of elasticity.[10] Histologic examination of the heart in great apes with CVD exhibits these changes, but the changes have typically been advanced, with fibrosis frequently seen around small intrinsic coronary arterioles, often with hyalinization (arteriosclerosis) and larger



• **Figure 82.1** By focusing on collaboration and information sharing, zoos and ape caregivers do not need to "reinvent the wheel" of establishing a network of subject matter experts, diagnostic approaches, and treatment methods in the care of their great apes.

areas of fibrosis extending through the cardiac chamber walls. Left ventricular hypertrophy (LVH) is a common finding and some apes will progress to have dilated and enlarged hearts in end-stage heart failure.[3] In humans, the association between sudden cardiac death due to myocardial fibrosis and fatal arrhythmias may be similar to what is seen in chimpanzees with interstitial myocardial fibrosis, cardiac arrhythmias, and sudden cardiac death.[13,15]

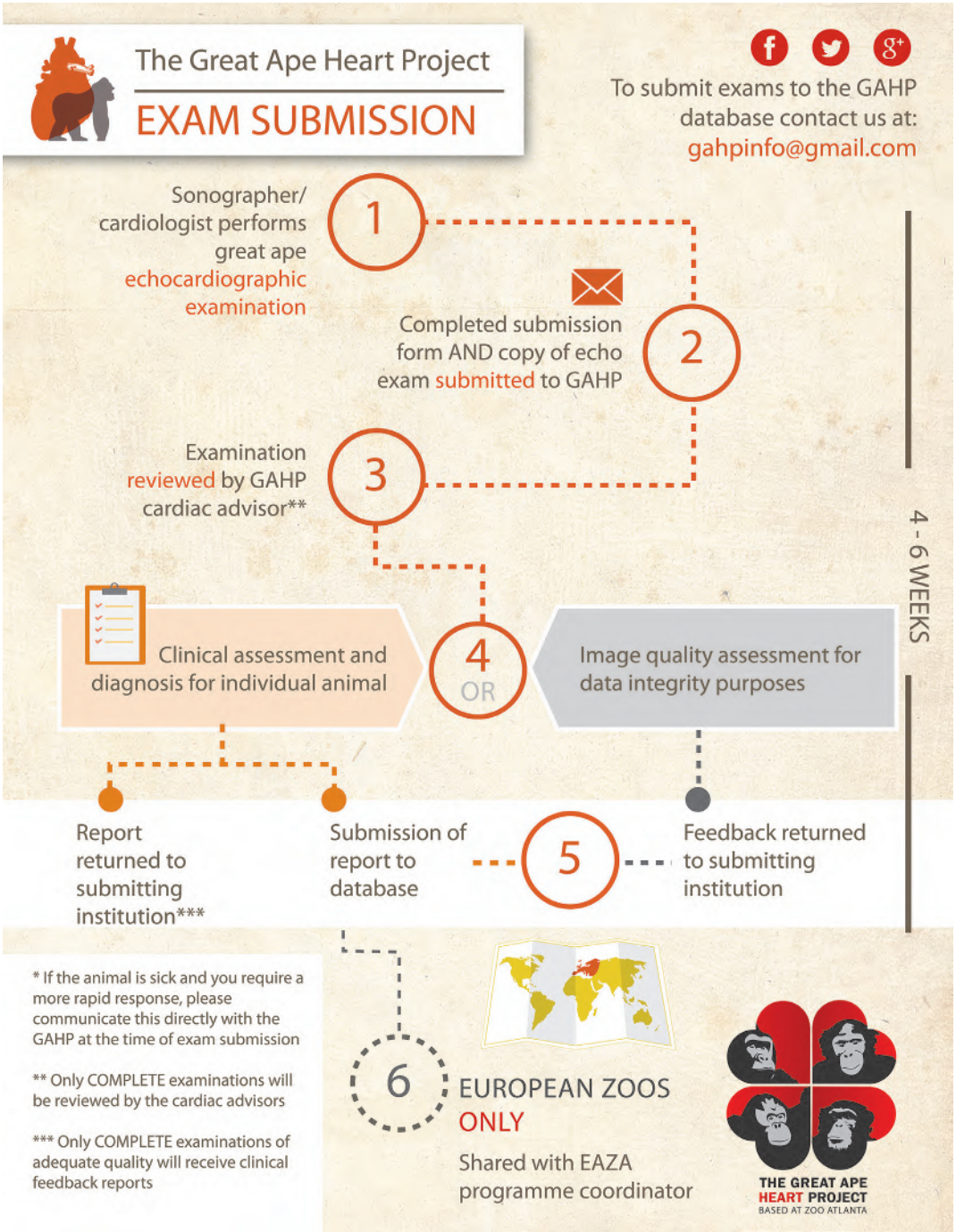
The GAHP has developed clinical assessment categories, using ejection fraction (EF) and assessment of functional capacity of the heart to determine disease severity. The majority of affected apes have been broadly categorized as follows: apes with LVH and intact systolic function, apes with LVH and systolic dysfunction, and apes with a dilated cardiomyopathy phenotype. Aortic dissections are the second leading cause of cardiovascular-related deaths in gorillas and bonobos and typically result in acute collapse and death.[10,14] Other significant findings (but not as frequently seen) have been aortic root dilation, left atrial enlargement, thromboembolism, right-sided enlargement, arrhythmogenic right ventricular dysplasia/cardiomyopathy, pulmonary hypertension, inflammatory heart disease, and pericardial effusions. Valvular regurgitation is not typically clinically significant. Etiologic factors contributing to ape CVD are yet unknown.

Blood Pressure and Hypertension

In humans, elevated blood pressure (BP) is a major risk factor for the development of heart failure, and long-term treatment of both systolic and diastolic hypertension has been shown to reduce the risk of heart failure.[16] Echocardiographic evidence of concentric LVH and systemic changes in affected apes, similar to those seen in humans with hypertension, have strongly implicated hypertension as a factor in great ape CVD.[10,17]

Defining BP reference ranges for adult, healthy great apes has been logistically challenging. Historically, BP measurements were attained only from anesthetized great apes and the effects of various anesthetic agents on BP have not been systematically analyzed. Attaining a BP reading as soon as possible after anesthetic induction and before commencing inhalant anesthetics, if not using alpha-2 agonists, may give the most accurate BP in an anesthetized ape. By human standards, consistent systolic readings of greater than 140 mm Hg or diastolic readings ≥90 mm Hg fit the definition of hypertension.[18] In chimpanzees, obesity has been shown to be a risk factor for the development of systolic hypertension in females, and increasing age is a risk factor for development of diastolic hypertension in both sexes.[19]

Attempts to define BP ranges in *nonanesthetized* great apes are underway. Generating individual "baselines" for BP by consistently attaining BP readings over time may help to provide an ancillary tool to monitor great apes for hypertension. This information can be used to detect changes in BP over time and during CVD treatment regimes. Until BP reference ranges can be developed, it would appear reasonable that animals with systolic BP consistently above 160 mm Hg be treated for hypertension.[18,19]



• **Figure 82.2** Great Ape Heart Project exam submission process.

Diet

Diet, lifestyle, body weight, metabolic syndrome (MS), and sodium intake have all been linked to hypertension and heart disease in humans.[16] Great apes have a predominantly vegetarian, low fat, high fiber, and very low cholesterol diet in the wild (see Chapter 83).[20] While some blood lipids, measured as HDL, LDL, and total cholesterol, change or increase with age in captive apes and may be well above the mean for the human population and for wild-living great apes, these levels do not appear to correlate to increased risk of ape CVD.[8,20–24] Increased sodium intake in humans is associated with increased systolic BP, cardiovascular events, and death in people with hypertension.[25] In one study done in chimpanzees, salt was progressively added to the diet over 20 months and caused a significant rise in body weight, as well as systolic, mean, and diastolic BP that was reversed after cessation of additional salt. Dietary sodium requirements for great apes are poorly understood, and the above study concluded that in chimpanzees, feeding a balanced diet with no more than 30–40 mmol of sodium per day would be advised.[26] The current recommended levels for nonhuman primates of 0.25%–0.65% dietary sodium is

potentially too high and, until more research can be done, it is prudent to monitor great ape dietary sodium intake closely.[27]

Metabolic Syndrome

In humans, MS is a risk factor associated with cardiomyopathy and is defined as the presence of at least three of the following: obesity, increased serum triglycerides, reduced HDL, hypertension, increased fasting glucose, and increased serum insulin levels.[21] More research into the role of MS in ape CVD is needed. In one study done on 16 geriatric female chimpanzees, 43.8% of the animals met the criteria for MS. Of these animals, 81.2% had some type of CVD.[21]

Cardiac Health Monitoring

Echocardiography provides the most practical, clinically relevant, and accurate assessment of cardiac functionality, valve anatomy, chamber sizes, and ventricular mass and is a critical tool used for diagnosing CVD in great apes.[28] Considerable expertise is needed in order to obtain a diagnostic echocardiogram. For this reason, the GAHP strongly recommends that institutions housing great apes establish relationships with local cardiologists and echocardiographers.

Performing Cardiac Examinations

Echocardiographic assessments should be done on great apes during every anesthetized examination once the ape reaches adulthood. At a minimum, adult echocardiograms should be done every 2–3 years on animals with normal cardiac health. Once an animal is determined to be affected by CVD, a risk analysis should be used to aid in determining anesthesia and examination frequency (Table 82.1).

TABLE 82.1 Great Ape Heart Project Recommendations for Frequency of Blood Pressure and Echocardiography Exam Based on Age and Health Status

Age/Health Status	Frequency of Exams
Neonate	Opportunistically if a neonate has to be removed from the dam for any reason, a neonatal exam should be done.
9 years	Baseline exam
10–20 years	Every 3–5 years
>20 years	Every 2–3 years
Animals with cardiac disease	Examination frequency should be determined on a case-by-case basis in order to monitor and manage treatment.

Echocardiograms

Standardization and accuracy of echocardiographic examinations done in great apes have been essential in detecting and monitoring great ape CVD, and the GAHP has developed standardized guidelines for great ape echocardiography. A great ape's size, positioning, and conformation may all affect imaging. It is generally recommended to perform echocardiography on anesthetized apes placed in left lateral recumbency, with the left arm extended cranially (Fig. 82.3).

Accurate and complete examinations require a skilled examiner and consist of a comprehensive, two-dimensional transthoracic echocardiogram with Doppler color flow study capabilities, and all GAHP recommended measurements stored as DICOM (Digital Imaging and Communications in Medicine).[29,30] While it is possible to review measurements saved as movie and jpg files, DICOM standard capabilities are the gold standard, especially if participation in the GAHP is to be maximized. This capability allows for postprocessing measurements and assessment of images, ensuring data validity and remote storage capability.

Echocardiograms on Nonanesthetized Great Apes

The GAHP has been able to utilize advances in animal training to encourage cardiovascular monitoring in great apes without the aid of anesthesia. The advantages of performing echocardiography and BP monitoring on nonanesthetized apes include less frequent anesthetic episodes, more frequent monitoring, and lack of anesthetic effects on the cardiovascular system. The disadvantages include training time and logistics, risk to the trainer and equipment, less thorough echocardiograms, and a missed opportunity to do a complete physical examination. Unfortunately, echocardiograms done on awake animals may take several sessions to obtain all the necessary measurements. Therefore, while not ideal, the GAHP recommends that measurements from training sessions obtained within a 30-day period be submitted as one examination.

• **Figure 82.3** An anesthetized gorilla *(Gorilla gorilla)* shown in left lateral recumbency.

Electrocardiogram

Whenever possible, an electrocardiogram (ECG) should be recorded. Male chimpanzees have been shown to develop increasing frequencies of cardiac arrhythmias as they age, with up to 75% of geriatric males showing some kind of ectopy, most commonly characterized by ventricular premature complexes.[15,30] To date most ECG information has been recorded from the great apes under general anesthesia. There have been several zoological and research institutions, however, that have successfully used implantable ECG loop recorders to monitor and detect for cardiac arrhythmias and heart rate variability in chimpanzees and gorillas, and this technology shows promise for use in animals at risk for or with cardiac dysrhythmias.[31–33]

Additional modalities for cardiac assessment include magnetic resonance imaging (MRI) and cardiac computed tomography (CT). Both of these modalities may provide additional information for cardiac evaluations such as detailed cardiac structural assessments, myocardial perfusion and viability information, and coronary artery assessment, and have been used extensively in human medicine.[16] Unfortunately, the availability, cost, and logistics involved in utilizing these advanced modalities have often been prohibitive in the great apes.

Anesthesia Considerations

Echocardiographic assessments performed while the animal is anesthetized offer the most diagnostic and accurate views of the heart, as well as providing opportunity for a complete physical examination and ancillary diagnostics. There is wide variation in anesthetic protocols used in great apes. The most commonly used protocols involve the use of either tiletamine hydrochloride (HCL) and zolazepam HCL (Telazol), ketamine HCL with or without additional sedatives, or ketamine HCL and medetomidine HCL combinations, all usually followed by inhalant anesthetic for maintenance of anesthesia.[34]

When choosing an anesthetic regime, in addition to drug safety, mode of delivery, efficacy, and reversibility, the effect of the drug on the cardiovascular system must be considered. There have been varying reports of the use of alpha-2 adrenergic agonists in great apes, with generally favorable reviews on safety and reversibility; however, in great apes with CVD, the cardiovascular effects of these agents deserve special consideration.[35,36] Alpha-2 agonists result in an increase in systemic vascular resistance and this intense vasoconstriction results in increased cardiac afterload and a reflex bradycardia. Decreased stroke volume and heart rate result in decreased LV blood flow and decreased cardiac output. As the peripheral vasoconstriction wears off, the central effects, primarily decreased sympathetic output, may result in hypotension.[37,38] Echocardiographic examinations of animals that received alpha-2 agonists are notable for the presence of an enlarged LV due to impaired LV outflow and slow heart rate. Increased LV pressure may also result in appearance of an alpha-2 agonist-induced mitral valve regurgitation, left atrial enlargement, and systolic dysfunction, potentially leading to an animal with a functionally normal heart being classified as abnormal.[39–41] When alpha-2 agonists are combined with inhalant anesthetics such as isoflurane, the combined decreases in mean arterial pressures due to vasodilation and decreased systemic vascular resistance may cause hypotension at a much lower inhalant concentration, exacerbating the cardiovascular effects of these drugs.[38]

Although there are not sufficient data to provide a clear contraindication on the use of alpha-2 agonists for anesthesia in great apes, the risk/benefit analysis of using these protocols needs to be considered. Due to these considerations, the GAHP considers echocardiograms done on apes anesthetized with alpha-2 agonists as nondiagnostic.

Biomarkers

Blood work may be a valuable ancillary assessment tool for great apes when evaluating their cardiac status, as well as general health. Ideally, biomarkers for CVD should have a high sensitivity and specificity, have good predictive values, be low-cost, and be validated for use in great apes.

B-type Natriuretic Peptide

B-type natriuretic peptide (BNP) is a cardiac neurohormone secreted from the cardiac ventricles, particularly the left ventricle, in response to ventricular volume expansion and increased pressures.[42] BNP is a recommended biomarker in human cases of LV fibrogenic remodeling, a classic finding in great ape CVD, making this particular biomarker of significant interest.[42]

In chimpanzees, BNP was found to be elevated in cases of cardiomyopathy and valve disease, and a preliminary reference interval for BNP was suggested as 23–163 pg/mL in healthy animals with BNP levels greater than 163 pg/mL having a specificity of 90.5% for CVD.[43]

C-Reactive Protein

C-reactive protein is a nonspecific indicator of inflammation and in humans has been associated with atherosclerosis and CVD. Studies performed in chimpanzees affected with CVD did not find that this was a useful or predictive biomarker.[43]

Troponins

Cardiac troponin T (cTnT) and troponin I (cTnI) are cardiac regulatory proteins that control the calcium mediated interaction between actin and myosin. The measurement of serum cTnI and cTnT is used to detect cardiac muscle damage, and increased cardiac troponin concentrations are standard biochemical markers used for the diagnosis of myocardial infarction in humans.[44] Cardiac troponins may also be increased in nonischemic myocardial disease, LV dysfunction, and hypertrophic cardiomyopathy, and as such are also of interest in the great apes.[44] In a study of

28 chimpanzees, cTnI levels were found to have a predictive value for CVD disease in cases of advanced/severe cardiac disease, but the levels were not predictive in cases of mild to moderate severity.[43] The authors of this study concluded that any observed value of cTnI above the detection threshold of the assay used (0.20 ng/mL) should be treated as an indicator of potential CVD in chimpanzees, although a limitation of this study was that the cTnI assay used had an analytic sensitivity above the threshold for humans and veterinary species; therefore some chimpanzees with heart disease may not have been detected.[43]

Biomarkers to detect extracellular matrix remodeling and fibrogenesis resulting in myocardial fibrosis formation and turnover in great apes have shown promise and are currently under investigation.[45]

Treating Cardiovascular Disease in Great Apes

Standard pharmacologic therapies for cardiomyopathies including LV dysfunction in humans include beta-adrenergic blockade, renin-angiotensin-aldosterone system antagonism, and diuretics as necessary for congestive heart failure.[16] To date, most of the GAHP advisory experience in the great apes has been with beta-blockade and angiotensin-converting enzyme (ACE) inhibition, with other pharmacologic agents used on a more sporadic basis.

It is worth emphasizing that no clinical studies have been performed on the efficacy, safety, or pharmacokinetics of any cardiovascular therapeutics in apes, so recommendations are based purely on experience with them in apes over the course of the GAHP studies, as well as hypothetical assumptions that apes would react in much the same way as humans do to these drugs. Attending veterinarians must use their best judgment when prescribing these drugs as "off-label" medications and should monitor animals very closely for any adverse side effects.

Thromboembolism and cerebral infarcts have been documented in the great apes.[21,46,47] In these cases, aspirin therapy has been recommended.[21]

Postmortem Cardiac Evaluations

The pathology group of the GAHP has collected and reviewed available necropsy and histopathology reports from captive apes. All information collected is reviewed and entered into the GAHP database and is analyzed for antemortem clinical correlations, inter and intra-specific taxon trends, and disease classifications. Novel postmortem cardiac tissue collection and evaluation techniques for great ape necropsies have been developed, and these protocols have established a new "best practices" approach to ape heart evaluation that is more closely aligned with techniques used in human cardiac autopsies.[48] These guidelines may be found at www.greatapeheartproject.org. Database inquiry coupled with simultaneous multipathologist review will allow the GAHP to establish more precise and clinically relevant diagnostic and treatment criteria for future evaluations.

Acknowledgments

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Great Ape Nutrition

DEBRA A. SCHMIDT AND MICHELLE E. SHAW

Wild Diets and Digestive Physiology

Diets of free-ranging great apes are largely plant-based. Relative inclusions of types of plants and animal material have resulted in their classification by feeding strategy. Captive diets have a tendency to reflect human-assigned feeding strategies rather than the capability each species has to digest the nutrient levels in their natural diet. Adaptations in the gastrointestinal (GI) tract of each species ascertain their relative ability to digest fiber and should be taken into account.

Gorillas (*Gorilla* spp.) are considered the most herbivorous of all great apes with a natural diet of plant leaves, stems, pith, bark, roots, flowers, and fruit.[1–3] Orangutans (*Pongo* spp.) have a very similar GI tract and therefore comparable digestive capabilities. Gorillas and orangutans have a lengthier small intestine and a more voluminous large intestine compared to other great apes indicative of their enhanced fermentative capabilities, which are more similar to those of a horse than a human (Fig. 83.1). Orangutans are often classified as frugivores, due to their preference for fruit when it is available, but they have been reported to eat a variety of foods including fruit, leaves, nonleafy vegetation, inner bark, flowers, and insects.[4–8] Gorillas and orangutans have been observed eating decaying wood and clay-rich soil respectively.[9,10] These may be maintaining a healthy gut environment by contributing to the gut microbiome or adsorbing toxins.

The GI tracts of chimpanzees *(Pan troglodytes)* (see Fig. 83.1) and bonobos *(Pan paniscus)* are very similar to each other with a relatively short small intestine and a longer large intestine and colon with comparatively lower fiber fermentative capacity than orangutans and gorillas, although they do have the ability to ferment fiber for energy production.[11] Chimpanzees consume a diet consisting primarily of fruit, but they also eat leaves, pith, seeds, flowers, insects, and meat leading to the classification of an omnivorous frugivore.[12] Bonobos consume a diet consisting mostly of plant material with the majority of it being fruit, but they also consume leaves, shoots, stems, flowers, and pith.[13–15]

Chimpanzees have been noted to eat bushbuck *(Tragelaphus scriptus)*, bushpig *(Potamochoerus larvatus)*, red colobus (*Procolobus* sp.), and animal parts and insects *(Dorylus rubellus*, *Oecophylla longinoda*, *Apis* spp.*)* are commonly found in fecal samples.[12,16–19] Bonobos have also been reported to consume animal matter including rodents (order Rodentia), duikers *(Cephalophus* sp.*)*, monkeys *(Cercopithecus wolfi, Cercopithecus ascanius schmidti)*, and invertebrates *(Macrotermes*, *Dorylus*, and *Apis)*.[20–24] However, the protein contribution is insignificant so the behavior may be primarily for social benefits.[25] There are also a few reports of carnivorous activity by orangutans, including the consumption of slow lorises (*Nycticebus* spp.), but animal matter is considered to comprise an even smaller portion of the diet than it does in chimpanzees.[26–28]

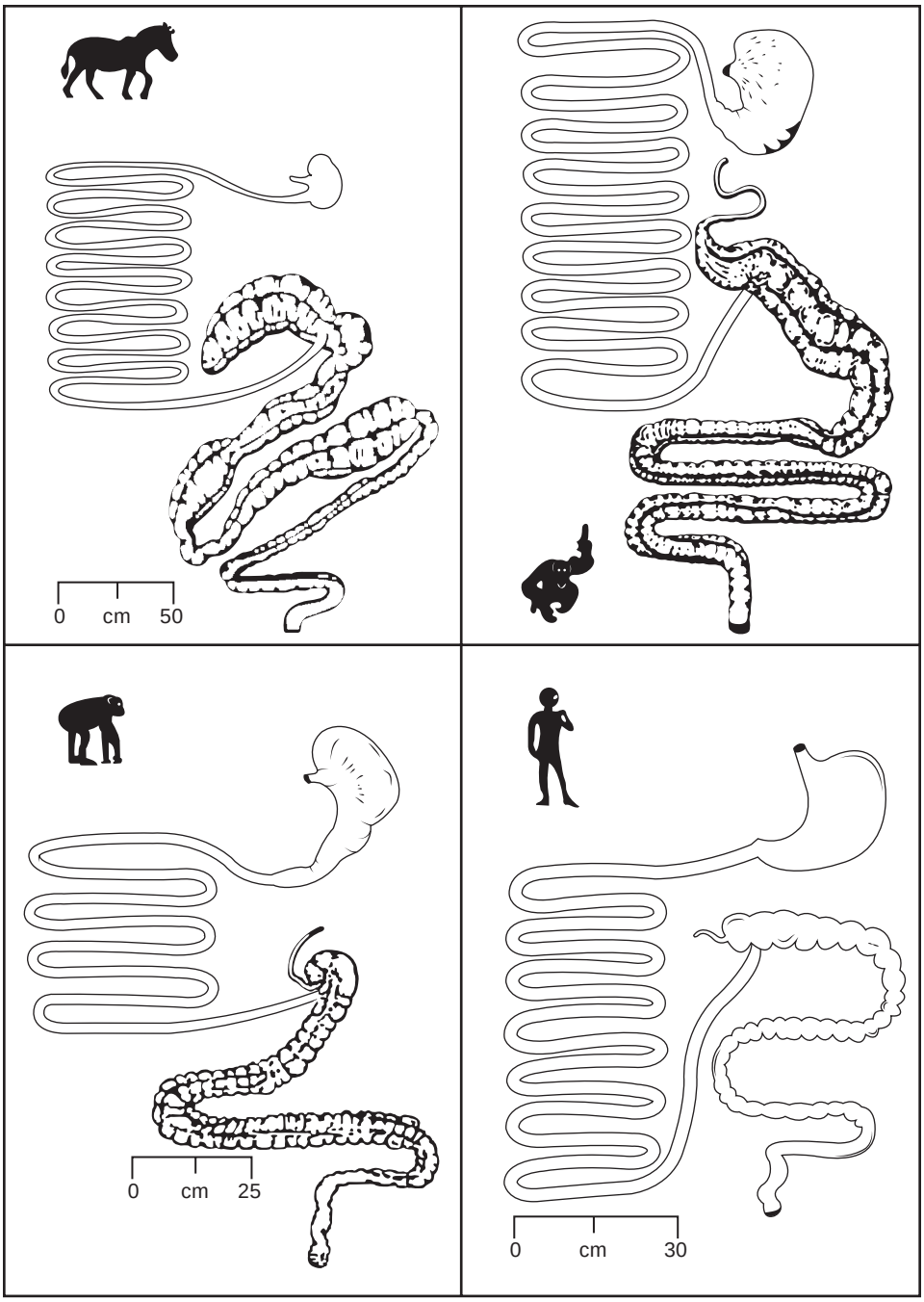
Body Weights

In captivity, all individuals should have a goal of weight loss, gain, or maintenance. Ideally, body condition scoring in conjunction with body weight assessments should be performed regularly to determine the optimal weight for each individual. Body weights for chimpanzees in the wild range from 49–80 kg for males and 40–68 kg for females.[29] Free-ranging bonobo weights are less available but are listed as 37–61 kg for males and 27–38 kg for females.[30] In the wild, male orangutans weigh 80–91 kg, while females weigh 33–45 kg; therefore it is not unusual for full-flanged males to be more than twice the size of females.[31] Free-ranging male and female gorillas have weight ranges of 130–218 and 68–74 kg respectively, although wild silverbacks were found to eat only 1.3 times the amount consumed by adult females.[32,33]

Recommended Diet Plan

Water

Water is the most important nutrient for any animal; an animal will die from dehydration before it will die from any other nutrient deficiency. Apes should have *unlimited* access to fresh, clean drinking water at all times. It is important to clean water containers or devices daily to prevent illness. Dehydrated animals are more likely to become constipated and have dry skin; in humans it can also lead to kidney



• **Figure 83.1** Comparative digestive anatomy of the zebra (top left), orangutan (top right), chimpanzee (bottom left) and human (bottom right). Stevens CE, Hume ID, editors: *Comparative physiology of the vertebrate digestive system*, ed 2, Cambridge, UK, 1995, Cambridge University Press.)

stones and liver, muscle, and joint damage, problems that may also affect great apes.

Kibble/Pellets

Some feed manufacturers (e.g., Mazuri or NutraZu, PMI Nutritional International, Brentwood, Missouri; Marion Zoological, Plymouth, Minnesota) produce a dry kibble (biscuit) specifically formulated for primates. The feed contains appropriate concentrations of vitamins, minerals, fats, and protein for primates. Using a product specifically formulated for use with primates as a basis for the diet is highly encouraged; however, a pellet formulated for horses may also be used and may be more economical and available (Shaw, personal communication). These products contain higher concentrations of fiber than produce items may provide and supplemental micronutrients that would otherwise be deficient. Although they may not be as

palatable to a primate as produce, they may be blended with more palatable items to provide the basis for a healthy, balanced great ape diet.

Browse

It is impossible to match the fiber concentrations consumed by wild apes using only produce commercially grown for human consumption. Leafy tree branches (nontoxic browse) and dried alfalfa (leafy material and stems) are excellent feed items to increase fiber in the diet. If these items are readily available and reliably consumed, then they should be offered as much as possible throughout the year. When browse is available, feeding time is increased and regurgitation and reingestion (R&R) are reduced.[34] Some animal care facilities have partnered with utility companies to use the tree branches that are trimmed away from utility lines as it is unlikely that trees along utility lines are treated with pesticides or herbicides. This partnership provides browse for the animals and offers a favorable image for the utility company as a result of helping feed endangered animals. If browse or alfalfa is not available all year, the amount of leafy green vegetables may be increased.

Vegetables

In a great ape diet, 85%–90% will be made up of browse and leafy green and low-starch vegetables. Large amounts of these vegetables will create a feeling of fullness and increase the amount of foraging time without adding a lot of calories to the diet. Offer vegetables with the peels and cores intact when possible as they provide potential sources of fiber and may provide a source of enrichment by requiring the ape to process food before consuming it. However, items that are potentially toxic, such as avocado pits and skins, or fibrous skins or seeds that may be a choking hazard in younger animals, should not be offered to apes; always err on the side of caution.

Cooked produce items, especially root vegetables, contain more easily digested sugars than uncooked items.[35] All produce should be offered raw to minimize the amount of easily digested sugars and only cooked when necessary. For example, animals with dental problems or older animals in poor body condition may benefit from having some items cooked to improve consumption.

Seeds, Nuts, Whole Grains, Pulses/Legumes

Locally available seeds and nuts can be used to balance and/or provide variety to a great ape diet. Whole grains (e.g., millet, barley, wheat, brown rice, and oats), legumes (e.g., alfalfa, peas, soybeans, and beans such as kidney, pinto, adzuki, mung, and broad beans), and lentils are a good source of protein, fiber, and minerals. High-fat seeds (e.g., sunflower), nuts, and high-fat legumes (e.g., peanuts), should be used in moderation, especially with overweight animals. On a gram-per-gram basis, fat has more than twice as many calories as protein or carbohydrates. Lower-fat seeds, such as pumpkin, hemp, and sesame seeds, are healthier and provide a good source of fiber, healthy fat, vitamins, and minerals.

Vitamin and Mineral Supplementation

Providing a variety of nutritionally appropriate foods will help reduce the risk of deficiencies. When apes receive and consume a well-balanced diet based on a formulated pellet, supplementation with vitamins and minerals is not necessary. For those animals that do not have access to primate kibble or a commercial horse pellet, a complete multivitamin formulated for adult humans is suggested to avoid deficiencies in micronutrients. It is possible to formulate a balanced diet without additional supplementation, but the complete nutrient profile of all available food items must be known to confirm that the overall diet is adequate. When an ape has been confirmed to be pregnant or is lactating, supplementing them with a prenatal vitamin may be considered.

Access to natural, unfiltered sunlight for at least 30 minutes daily is beneficial for all life stages of apes, but is most critical for young animals.[36,37] Because vitamin D_3 is not transported well through dam's milk, mother-reared infants who do not have routine access to natural (outdoor) unfiltered sunlight (not through glass, skylights or mesh) will benefit from an oral, supplemental dose of vitamin D_3 to support appropriate bone growth and development.[38] A vitamin D_3 supplement for human, breastfed infants works well with great apes.

Animal Products

Feeding apes animal products is not recommended as they are difficult to digest, promote obesity, and may increase the incidence of R&R, particularly in gorillas, orangutans, and chimps.[39] There are a few exceptions to this rule for hand-raised or compromised individuals. Dairy-based human infant formulas supplemented with omega fatty acids are recommended when hand-raising great apes as human milk is similar in composition to great ape milk.[40] Older animals or those with health concerns that are having difficulty maintaining weight may be supplemented with hardboiled eggs a few times per week to provide a high-quality protein source.

Fruit

High levels of fruit are reported in free-ranging ape diets; however, the fruits in the wild are significantly higher in fiber and lower in readily absorbable sugars than fruits and other produce cultivated for human consumption and should not be considered nutritionally equivalent.[41] The wild varieties are more nutritionally similar to the seed-bearing parts of plants, such as eggplant, zucchini, and capsicum, which are botanically defined as fruit and are more often considered

vegetables in culinary terms. Fruit is commonly used to define only the sugary seed-bearing parts of plants, such as bananas, grapes, apples, and pears, and its high sugar level causes a variety of health and behavioral issues. Apes have a high capacity for fiber fermentation, so it is recommended that fruit not be a typical part of ape diets because it could disrupt the gut microflora needed to ferment fiber resulting in acid production that can damage the digestive tract. Great apes in zoos are often maintained on diets high in fruit, causing a gradual increase in weight and increasing the likelihood of obesity and associated illnesses. Many wild populations of great apes maintain a fairly consistent amount of protein year-round, gaining weight during the lower-protein fruiting season and losing weight during the nonfruiting season.[8,16] Commercially available fruit often contributes to R&R and is potentially addictive in great apes, causing cravings that result in overeating sugary foods and overriding feedback mechanisms that signal satiation.[34,42]

Diet Proportions

The proportions listed in Table 83.1 are recommended when feeding apes and meet the recommendations established for the Nutrient Requirements of Nonhuman Primates (Table 83.2).[43] Though apes in the wild consume higher concentrations of fiber than those recommended, it is very difficult to mimic those high fiber concentrations in a zoo setting, and for this reason, all four great ape species are fed similar diets. Examples of amounts to offer when using biscuit/pellet/kibble, relative to animal body weight, are listed in Table 83.3. Examples of amounts to offer when the diet is based on tofu and seeds/nuts/legumes are listed in Table 83.4. Tofu may increase R&R in some animals. If this behavior occurs, replace tofu with a variety of seeds, nuts, and pulses. When feeding a diet based on tofu, seeds, nuts, whole grains, and pulses, a vitamin and mineral supplement tablet formulated for adult or juvenile humans is recommended, based on the age of the animal.

TABLE 83.1 Recommended Ape Diet Proportions by Weight (As-Fed Basis)

Diet Item	Kibble/Pellets (%)	Tofu and Seeds/Nuts/Legumes (%)
Kibble/Pellets	11	11
Leafy green vegetables	63	67
Vegetable	20	20
Root/starch	6	2

Feeding Guidelines—Diet Extrapolations

Diet amounts for weights not listed may be extrapolated from Tables 83.3 and 83.4. For example, an 85 kg animal would receive 568 g kibble/pellets. This is calculated using

TABLE 83.2 Proposed Nutrient Guidelines for Apes on a Dry Matter Basis

Nutrient	Proposed Nutrient Guidelines
Crude Protein, %	15–22
Neutral detergent fiber (NDF), %	10–30
Acid detergent fiber (ADF), %	5–15
Calcium, %	0.8
Phosphorus, %	0.6
Magnesium, %	0.08
Potassium, %	0.4
Sodium, %	0.2
Chloride, %	0.2
Iron, mg/kg	100
Copper, mg/kg	20
Manganese, mg/kg	20
Zinc, mg/kg	100
Iodine, mg/kg	0.35
Selenium, mg/kg	0.3
Vitamin A, IU/kg	8000
Vitamin D_3, IU/kg	2500
Vitamin E, mg/kg	100
Vitamin K, mg/kg	0.5
Thiamin (B_1), mg/kg	3.0
Riboflavin (B_2), mg/kg	4.0
Pantothenic acid, mg/kg	12.0
Niacin, mg/kg	25.0
Vitamin B_6, mg/kg	4.0
Biotin, mg/kg	0.2
Folacin, mg/kg	4.0
Vitamin B12, mg/kg	0.03
Vitamin C, mg/kg	200
Choline, mg/kg	750

From National Research Council: *Nutrient requirements of nonhuman primates*, ed 2, Washington, DC, 2003, National Academies Press.

TABLE 83.3 Kibble/Pellet and Vegetable Diet Amounts Based on Animal Weight (As-Fed Basis)

	Animal Weight					
Food Item (g)	50 kg	70 kg	90 kg	110 kg	130 kg	150 kg
Kibble/pellets	334	468	601	735	868	1,002
Leafy green vegetables	2,013	2,818	3,623	4,429	5,234	6,039
Low starch vegetables	644	902	1,159	1,417	1,674	1,932
Root/starch vegetables	191	268	344	420	496	573

TABLE 83.4 Tofu, Seeds/Nuts/Legume and Vegetable Diet Amounts Based on Animal Weight

	Animal Weight					
Food Item (g)	50 kg	70 kg	90 kg	110 kg	130 kg	150 kg
Tofu and seeds/nuts/legumes	660	925	1,190	1,450	1,715	1,980
Leafy green vegetables	3,960	5,545	7,130	8,710	10,300	11,880
Low starch vegetables	1,265	1,770	2,280	2,785	3,290	3,795
Root/starch vegetables	135	190	245	300	350	405

the following equation for each food category and uses the current weight of the animal:

$$85\ \text{kg} \div 70\ \text{kg} = 1.21 \times 468\ \text{g (kibble/70 kg animal)}$$
$$= 568\ \text{g kibble/pellets/85 kg animal}$$
$$122\ \text{kg} \div 110\ \text{kg}$$
$$= 1.11 \times 8710\ \text{g (leafy greens/110 kg animal)}$$
$$= 9660\ \text{g leafy greens/122 kg animal}$$

Feed Presentation

Group Feeding

When feeding animals in a group, the fastest or most dominant animal must be prevented from gathering and eating the most desired items. This can be overcome by target feeding the highly desired, high-calorie foods to animals individually; they may even be used as training rewards. The less calorically dense vegetables and leafy green vegetables may be group fed, ensuring a more balanced diet for all apes in the group.

If a variety of produce items are available, every opportunity should be taken to offer the apes an assortment of items throughout the week. More variety is attained when offering larger amounts of only four or five different items daily and rotating the selection throughout the week than to offer smaller amounts of the same 20 items daily. This will help to ensure that a few individuals do not always eat the more favored items every day. It will also allow seasonal items, which can be purchased at a lower cost, to be used and provide enrichment. Creating a weekly, documented schedule of the rotation may help caretakers maintain the variety and rotation.

Enrichment

Just providing the animal with additional food does not meet the definition of enrichment. All food may be classified as "enrichment" if it is provided in a stimulating manner that encourages the great ape to exhibit natural behaviors. It is also important to quantify and calculate the calories being offered in enrichment items daily. It is tempting to use higher-fat nuts and seeds as enrichment feeds due to their small size and potential processing requirements, but that could cause the apes to consume too many calories.

Enrichment and training may be done using the foods that are typically part of their diet. In the wild they spend a majority of their day searching for food; to mimic this, their produce can be cut into small pieces and scattered throughout the enclosure. It may also be mixed in hay or hidden in different areas of their habitat. If infant apes are present, consider the size of the foods so they do not create a choking hazard. Foods may also be kept whole and spiked around the exhibit or hung from ropes making them difficult to retrieve. Food items may be diced or pureed and placed in a tube or crafted concrete termite mound where the apes have to use long, thin sticks to "fish" for the items (like they would for termites). Puzzle feeders are another good way to make them think, expend energy,

and work for their food. It is always best to follow the diet plan and use only those food items and predetermined amounts as enrichment by providing them in novel and interesting ways.

Health

A recent review of reports published between 1990 and 2014 identified idiopathic and infectious diseases as well as cardiovascular, respiratory, and GI disorders as the leading causes of captive great ape morbidity and mortality.[44] Great apes are at serious risk for anthropozoonotic diseases. GI and upper respiratory tract disorders are most often reported and easily transmitted from caregivers through the preparation of ape diets. Washing hands frequently during diet preparation should be a top priority regardless of health status, and the use of facemasks and gloves is recommended in higher-risk situations.

Orangutan Air Sacculitis

Air sacculitis is the most frequently reported respiratory disorder in captive orangutans.[44] It is generally considered to be caused by environmental factors such as dust; however, there are several nutrition-related factors that may contribute to this condition. Anything that promotes aspiration of food particles into the air sacs will increase the risk of developing air sacculitis. Obese individuals and those that spend all their time on the ground without climbing or brachiating (which physically stretches out their bodies) may experience a physical constriction of the air sacs. Animals that habitually practice R&R are also at increased risk. This is one reason that any food that stimulates R&R behavior, such as sugary foods, dairy, and tofu, should be restricted and eliminated completely from the diet if R&R is observed.

Treatment of air sacculitis often involves administering antibiotics, which may disrupt the normal microbial population of the gut.[45] To improve gut function after antibiotics are administered, animals may be fed feces from a healthy individual to repopulate the microbiome. This can be achieved by adding the feces to oatmeal or vegetable purée. Additionally, group housed apes may naturally recolonize their GI tract through fecal exposure.

Obesity

Obesity in humans can lead to a multitude of health-related conditions, including death, high blood pressure, heart disease, cancer, degenerative arthritis, respiratory problems, diabetes, fatty liver disease, and lower fertility in women.[46,47] Obese apes may also be at risk for these problems.

Obesity—Weight Loss Diet

To begin a weight loss diet, it is important to first determine the animal's current weight and ideally take several photographs of the animal from various angles. Getting the animal accustomed to being weighed weekly or every other week is ideal. The next step would be to transition the animal to one of the above-mentioned diets (pellets/kibble or tofu/seeds/nuts/ grains if pellets/kibble are not available) in the appropriate proportions (see Tables 83.3 and 83.4). It is not as important for the animal to consume the listed amount of food that corresponds to their exact weight. Instead, it is important that the animal consume the majority of the diet offered and is not offered so much food that the animal may be selective and satiated on only the foods he or she chooses to eat. Finding the diet that most closely resembles the ape's current diet should be considered the starting point. Once the diet quantity has been determined and the animal has settled into this new diet, begin reducing all diet categories by 10%; do not be selective in which categories are reduced (i.e., only reducing the categories that the animal does not like). By reducing all diet categories, the items will remain in proportion with one another and the diet offered will remain balanced. Changing an individual's diet in group-housed apes can be particularly challenging.

It is also important not to move quickly with this process in an effort to encourage rapid weight loss. This is not a healthy way to lose weight; the animal's daily nutrient needs may not be met, and the metabolism could be altered, making it more difficult to achieve weight loss goals. Whenever diet changes are made, even to a healthier diet, digestive upset may occur. If an animal has gas or diarrhea for one day, this is perfectly normal, but if digestive upset is prolonged, no further diet changes should be made until fecal consistency improves. Never change an animal's diet by more than 5%–10% at a time to minimize risk of digestive upset.

Removing high-sugar items is particularly difficult. When removing sugary foods, the animal may exhibit signs of withdrawal (e.g., headaches, irritability, frustration, nausea). These symptoms are temporary and will usually disappear in a few weeks after sugar is removed. The removal of high-sugar items in primate diets has been found to reduce negative stereotypic behaviors such as begging and self-mutilation.[48] The overall improvements in psychological and physical health are worth a few weeks of symptoms associated with sugar withdrawal.

Weight changes should be achieved in a slow and controlled manner. Diet and activity levels are the two most critical components in maintaining animals at appropriate weights. Weighing animals frequently allows early detection of rapid weight changes. Enclosures should also provide opportunities for activities such as climbing, swinging, and hanging to increase energy expenditure and maintain muscle mass. Weight changes should not exceed 2% of body weight weekly. For example, a 100-kg orangutan, determined to be overweight, should have diet changes that result in a body weight loss of 1–2 kg weekly.

Obesity—Expending Energy

Consider using their typical dietary foods to encourage movement around the enclosure. Think of ways to offer

food that will require apes to climb ropes, move from platform to platform, hang upside down, move objects, or stretch high above their heads. Hanging food from ropes that keeps food just barely in reach will encourage these behaviors. Cutting vegetables in small pieces and scattering them around the enclosure requires the animal to move around the habitat to gather the food. Hiding food items so animals need to search for it will also stimulate more activity, which is helpful for weight loss. Creating challenges that require a greater effort to access food may have the added benefit of making less-favored items more desirable, improving the acceptance of a healthier diet.[49]

To reduce aggression with multiple apes sharing an enclosure, use the higher-value portion of the diet as a reward for training instead of scattering it within the enclosure. Providing food in several smaller meals throughout the day at various times will also encourage more foraging behavior.

Cardiovascular Disease

Although great apes are genetically similar to humans, they develop a very different type of heart disease. Apes do not get atherosclerosis with plaque buildup as seen in humans; instead, they are frequently diagnosed with fibrosing cardiomyopathy (i.e., scar tissue buildup in the heart) at necropsy.[50–52] Mortality reports cite cardiovascular disease as the primary cause of death in captive great apes and as a premature cause of death in young, captive apes (see Chapter 82).[53]

Sodium levels in the diet need to be considered in relation to heart health. A current theory exists that hypertension plays a role in great ape heart disease. High sodium intake is linked to high blood pressure in humans, and therefore monitoring and minimizing the amount of salt an animal consumes is important.

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SECTION 17

Marine Mammals

84

Marine Mammal Viruses

JIM WELLEHAN AND GALAXIA CORTES-HINOJOSA

Viral abundance in the ocean has been estimated at 10^{30} virions, one or two orders of magnitude greater than the estimated number of bacterial and eukaryotic cells, so viruses are the most abundant life in the oceans.[1,2] Marine mammals live in intimate contact with the ocean, and therefore a vast diversity of viruses. Advances in molecular techniques have allowed a rapid expansion of knowledge on viral diversity, enabling greater understanding of evolution, causes of mortalities, and pathogen interactions.

Clinical Implications of Viral Biology

Viruses are classified on the basis of their genomic material and key elements of structure and replication. A basic knowledge of these elements is also clinically useful for predicting disease risk and epidemiologic characteristics, and for establishing appropriate management protocols (Table 84.1). DNA viruses often replicate in the nucleus of the host cell, and tend to be more species specific. RNA viruses tend to replicate in the cytoplasm and are more prone to jumping between species. There are exceptions, such as poxviruses, asfarviruses, and iridoviruses, which are DNA viruses that replicate in the cytoplasm and have significantly lower host fidelity, and bornaviruses, which are RNA viruses with intranuclear replication.

Viral genome size is also important. Large viruses with many genes are better at evolving complex host interactions for things like latency. Smaller viruses are capable of more rapid evolution and therefore more capable of adapting to different hosts and tissues, resulting in host jumping and differing clinical presentations.

Genome organization is also clinically relevant; nonsegmented viruses that are not very capable of recombination, such as paramyxoviruses, largely change through mutation. Mutations are much more likely to be deleterious rather than beneficial, limiting rates of change. Segmented viruses, such as orthomyxoviruses or reoviruses, may swap segments, much like sexual reproduction in a eukaryote. This enables an organism to use homologous genes that have been functional in another conspecific, which is much more likely to be beneficial than random mutations. As a result, they may change much more rapidly, and this is why a segmented influenza virus changes to the extent where novel vaccines are needed every year or two, whereas vaccines for an otherwise biologically similar nonsegmented morbillivirus result in lifelong immunity, because the virus cannot change as rapidly.

Presence of an envelope is also clinically relevant. Viruses with envelopes tend to need them to infect target cells. This lipid layer is more susceptible to environmental conditions and disinfectants, consequently making cleaning easier and decreasing environmental persistence.

RNA Viruses

Astroviridae

Astroviruses are small (28–30 nm), spherical, nonenveloped, positive-sense, single-stranded viruses with intracytoplasmic replication. Astroviruses often cause diarrhea and have a high prevalence in children; most children over 5 years of age have antibodies to human astroviruses. There are also several astroviruses documented in association with encephalitis in nonmarine mammals. In the order *Carnivora*, astroviruses have been associated with diarrhea in mink *(Neovison vison)*, domestic dogs, cheetahs *(Acinonyx jubatus)*, and domestic cats.[3] In marine mammals, astroviruses have been reported in bottlenose dolphins *(Tursiops truncatus)*,[4] Steller sea lions *(Eumetopias jubatus)*,[4] minke whales *(Balaenoptera acutorostrata)*, and California sea lions *(Zalophus californianus)*.[4,5] Five different astroviruses have been found: one in bottlenose dolphins, one in Steller sea lions, and three in California sea lions.[4] Next-generation sequencing (NGS) has identified a total of eight additional astroviruses in California sea lions.[5] This study was done using fecal swabs of animals in rehabilitation facilities and found an astrovirus prevalence of 51%.[5] Bottlenose Dolphin Astrovirus 1 (BDAstV1) was found in 86% of stranded bottlenose dolphins and in 50% of healthy bottlenose dolphins from a managed collection. A subset of dolphins who were persistent shedders were identified. The clinical significance of astroviruses in marine mammals is likely to be greatest in young animals as a cause of diarrhea, as is seen in other species.

TABLE 84.1 Virus Taxa Associated With Clinical Presentations in Marine Mammals

Clinical Signs	Differentials
Mucocutaneous lesions	Poxvirus, herpesvirus, calicivirus, papillomavirus, picornavirus
Skin	Poxvirus, herpesvirus, calicivirus, papillomavirus
Respiratory	Influenza, morbillivirus, calicivirus, coronavirus, adenovirus
Enteritis	Adenovirus, astrovirus, coronavirus
Neurologic	Morbillivirus, herpesvirus
Neoplasia	Herpesvirus, retrovirus, polyomavirus, papillomavirus
Hepatitis	Adenovirus, coronavirus, herpesvirus
Ocular	Adenovirus, herpesvirus, calicivirus

Caliciviridae

San Miguel sea lion virus (SMSV) is a small (30–38 nm) icosahedral, nonenveloped, positive-sense, single-stranded virus with intracytoplasmic replication that belongs to the genus *Vesivirus* in the family *Caliciviridae*. This virus is genetically indistinguishable from vesicular exanthema of swine virus (VESV), which is a reportable foreign animal disease that has officially been considered eradicated in the United States since 1956. Perhaps most concerning, related vesiviruses have been found to rapidly evolve greater virulence where there are high host population densities.[6] Serologic studies suggest exposure to vesiviruses in cetaceans and pinnipeds.[7] In pinnipeds, SMSV clinical manifestations include vesicular lesions on the flippers and the mouth, as well as gastroenteritis in California sea lions.[8] Clinical presentations in premature pups include respiratory distress and locomotor impairment, with possible fatal consequences. Cutaneous lesions typically resolve without supportive care. SMSV has zoonotic potential, causing an influenza-like syndrome followed by blisters on the hands and feet.[9] SMSV has also been associated with hepatitis in humans.[10,11]

Coronaviridae

Coronaviruses are large (120–160 nm), round, toroidal or bacilliform, enveloped, positive-sense, single-stranded viruses with intracytoplasmic replication. In marine mammals, viruses in the genus *Gammacoronavirus* have been characterized in a captive beluga whale *(Delphinapterus leucas)*[12] and bottlenose dolphins,[13] and an *Alphacoronavirus* has been found in both captive and wild harbor seals *(Phoca vitulina)*.[14,15] Clinical signs in the beluga whale included generalized pulmonary disease and acute liver failure. For the captive harbor seals, the clinical signs included anorexia with abnormal behavior after the sudden death of two other harbor seals in the same pool. At clinical examination, the mucosa was purple and injected, and abnormal pulmonary sounds were auscultated; blood work abnormalities included leukocytosis, hypernatremia, and hyperchloremia. Postmortem findings included acute necrotizing enteritis and pulmonary edema. Confirmation of coronavirus was attempted on cell culture but was not possible at the time. In the epizootic event in wild harbor seals, the five carcasses in suitable condition had lymphocytic/histocytic necrotizing pneumonia diagnosed histologically. An *Alphacoronavirus* was diagnosed in lung tissue using consensus polymerase chain reaction (PCR) with product sequence identification.

Orthomyxoviridae (Influenza Virus)

Orthomyxoviruses are medium sized (80–120 nm), segmented, pleomorphic, enveloped, negative-sense, single-stranded RNA viruses with intranuclear and intracytoplasmic replication. Influenza viruses are further divided into the genera *Influenzavirus A*, *Influenzavirus B*, and *Influenzavirus C*. Influenza A viruses tend to be the most pathogenic and have been well known to cause pandemic outbreaks in humans since 1918/1919. In marine mammals, both Influenza A and Influenza B have been diagnosed in wild populations of cetaceans and pinnipeds since the late 1970s. Mass mortalities in association with several different strains of Influenza A have been reported in harbor seals since 1979. Influenza B has been reported in stranded harbor seals since 1999, but not in association with epidemics. In cetaceans, Influenza A has been detected in stranded long-finned pilot whales. Additionally, South American fur seals *(Arctocephalus australis)*,[16] Caspian seals *(Pusa caspica)*,[17] Baikal seals *(Phoca sibirica)* and ringed seals *(Phoca hispida)*,[18] Dall's porpoises *(Phocoenoides dalli)*,[19] beluga whales, and other species have been reported to be seropositive for influenza.[20] Clinical signs include epistaxis, conjunctivitis, subcutaneous emphysema, and general weakness. Diagnosis of influenza can be based on a combination of clinical signs, qPCR, serology, and postmortem findings (gross pathology, histopathology, and immunohistochemistry). Seal-to-human influenza transmission has been described. Direct contact on massive mortalities and one case of direct contact with respiratory secretion are associated with conjunctivitis in humans.[21] A comprehensive review of influenza in marine mammals is available.[20]

Paramyxoviridae

Morbillivirus

Paramyxoviruses are pleomorphic, enveloped, negative-sense, single-stranded viruses with intracytoplasmic replication. This family causes respiratory, neurologic, and multisystemic diseases in mammals.[22] In marine mammals, three viruses in the genus *Morbillivirus* have been described: canine distemper virus (CDV), phocine distemper virus (PDV), and cetacean morbillivirus (CeMV). CeMV has

significant diversity and may eventually be split into more than one species; morbilliviruses from the Northern Hemisphere [porpoise morbillivirus (PMV), dolphin morbillivirus (DMV), pilot whale morbillivirus (PWMV), and Longman's beaked whale morbillivirus (BWMV)] show significant divergence from CeMV from the Southern Hemisphere (from Swan River, Australia and from Brazil). Morbilliviruses have been recognized as causes of mass mortalities in pinnipeds and cetaceans since the early 1980s. Diagnosis in cetaceans is usually postmortem, but clinical signs include cachexia, abnormal mentation, and respiratory distress. In pinnipeds, clinical signs include respiratory distress, ocular and nasal discharge, pyrexia, and erratic swimming. Morbilliviruses infect via the signaling lymphocytic activation molecule (SLAM [CD150]) receptor, taking out memory T cells and leaving the host significantly immunosuppressed. Coinfection with other pathogens such as *Aspergillus fumigatus* or *Brucella* become more clinically significant.[23,24] Comprehensive reviews are available for both pinnipeds[22] and cetaceans.[23]

Respirovirus

The genus *Respirovirus* contains parainfluenza viruses infecting several mammal hosts. Parainfluenza virus was first identified in marine mammals in a bottlenose dolphin kept in an open water enclosure in 2008. Clinical signs included respiratory stridor, halitosis, and exudate from the blowhole.[25] Serologic data suggested that exposure may be common in wild and captive bottlenose dolphins.[26] This virus is closely related to *Bovine respirovirus virus 3* and *Human respirovirus virus 3*, and potential risks for host jumping of these viruses should be considered.

DNA Viruses

Adenoviridae

Adenoviruses are medium-sized (70–90 nm) icosahedral, nonenveloped, double-stranded DNA viruses with intranuclear replication. In marine mammals, adenoviruses have been reported in pinnipeds, mustelids, and cetaceans. Adenoviruses have been associated with death in yearling California sea lions in rehabilitation; clinical signs included photophobia, emaciation, blood-tinged diarrhea, leukopenia, and monocytosis.[27] In 2011 California sea lion adenovirus 1 (CSLAdV-1) was characterized as a cause of viral hepatitis in wild animals and has since been detected in several pinniped species in captivity, especially in elder animals.[28–30] California sea lion adenovirus 2 and phocid adenoviruses 1 and 2 have been identified in ocular lesions and in unaffected eyes of pinnipeds.[31] In cetaceans, adenoviruses have been associated with gastrointestinal (GI) symptoms in bottlenose dolphins and harbor porpoises from Europe[32,33] and the United States.[34] Recently, a novel adenovirus with unknown clinical significance was found in Southern sea otters.[35] Diagnosis of adenoviral disease may be made with a combination of clinical signs and use of PCR diagnostics and/or negative staining electron microscopy of feces from clinical cases, with intranuclear inclusions present on histologic examination in postmortem cases.

Herpesviridae

Herpesviruses are large (100–150 nm), icosahedral, enveloped, double-stranded viruses with intranuclear replication. Herpesviruses can be latent in different sites, with certain subfamily tendencies; alphaherpesviruses often establish latency in neurons, whereas betaherpesviruses and gammaherpesviruses establish latency in leukocytes. Herpesviruses infect a wide range of vertebrates. *Trichechid herpesvirus* 1 (TrHV-1) is a gammaherpesvirus reported in West Indian manatees *(Trichechus manatus)* and may be a biomarker for stress.[36]

Polar bears *(Ursus maritimus)* are susceptible to encephalitis caused by the alphaherpesvirus equine herpes virus 9 (EHV-9), which may be fatal (see Chapter 33).[37] This virus appears to be endemic in Grevy's zebras *(Equus grevyi)* and is associated with fatalities in diverse laurasiatherian mammals. Although it is unlikely to be significant in wild populations due to low contact rates with the endemic host, it is a more significant concern in zoological collections.

In pinnipeds, 11 herpesviruses have been reported to date.[38,39] Otarine herpesvirus 1 (OtHV-1) is a gammaherpesvirus associated with urogenital carcinoma in California sea lions. OtHV-1 was also detected in a captive South American fur seal *(Arctocephalus australis)* with urogenital carcinoma.[40] This virus has a high prevalence in wild adult California sea lions (46% in males and 22% in females). Rates of metastasis are high, with sublumbar lymph nodes and liver as common sites.[41,42] Otarine herpesvirus 4, found in clinically unaffected northern fur seals *(Callorhinus ursinus)*, is a very close relative of OtHV-1; investigation of this virus as a potential vaccine against OtHV-1 is merited.

Phocid herpesvirus 1 (PHV-1) is an alphaherpesvirus seen in fatal outbreaks in harbor seals and gray seals *(Halichoerus grypus)* in rehabilitation facilities, affecting mainly young and immunosuppressed individuals. This virus may target the respiratory system as well as the GI tract, adrenal glands, and liver. Clinical signs may include nasal and ocular discharge, coughing, inflammation of the oral mucosa, vomiting, diarrhea, lethargy, anorexia, and fever.[43–45]

In cetaceans, diverse alpha- and gammaherpesviruses have been reported. Delphinid herpesviruses 1 and 2, both alphaherpesviruses, have been associated with fatal necrosis of the spleen, thymus, and lymph nodes in bottlenose dolphins, with intranuclear inclusions present in affected tissues.[46] A harbor porpoise herpesviral encephalitis case was also likely to have been caused by an alphaherpesvirus. Delphinid herpesvirus 4, a gammaherpesvirus highly prevalent in bottlenose dolphins, is associated with plaque-like genital lesions, and gammaherpesviruses have been associated with similar lesions in other cetacean species.[38,47,48]

Papillomaviridae

Papillomaviruses are small (55 nm) icosahedral, nonenveloped, double-stranded viruses with intranuclear replication. In marine mammals, papillomaviruses have been found in manatees,[49] cetaceans,[50] and pinnipeds.[51] Clinical signs include warty lesions in lingual and genital mucosa, as well as cutaneous lesions. The possibility of progression to neoplasia should be considered. Treatments against papillomaviruses in marine mammals have not been studied, but imiquimod and cidofovir have shown efficacy in other mammal models. Diagnosis may be accomplished with a combination of histopathology and molecular diagnostics such as PCR or NGS.

Poxviridae

Poxviruses are large, brick- or ovoid-shaped, enveloped double-stranded viruses with intracytoplasmic replication. Viruses in the subfamily *Chordopoxvirinae* can be transmitted directly, indirectly, and by vectors. In general, viruses in this subfamily cause proliferative skin disease in vertebrates. Poxviruses in the genera *Orthopoxvirus* and *Parapoxvirus* have known zoonotic potential. In marine mammals, chordopoxviruses have been found in cetaceans,[52] pinnipeds,[52,53] and sea otters.[54] Clinical signs on cetaceans are typically called "tattoo skin lesions" and present as irregular gray, yellow, or black cutaneous lesions. These lesions usually do not present a risk for the animals and resolve in a few weeks. In pinnipeds, lesions include skin nodules and ulceration. Diagnosis can be accomplished with a combination of histopathology and molecular diagnostics such as PCR or NGS.

Overview of Viral Diagnostics

Virus diagnoses can be accomplished using different approaches, and it is important to be aware of the applications and limitations of each approach. Histopathology and electron microscopy are essential for diagnosis of viral disease, and provide clues to narrow down candidate viruses on the basis of affected tissues, replication site within the cell, structure, shape, and size. Although other techniques are useful for identifying virus presence or exposure, histopathology is usually critical for identification of viral disease.

Immune assays are used to determine whether the patient has been exposed to a particular agent, but it is important to be aware of limitations. The two branches of acquired immunity are humoral immunity and cellular immunity. Recognition of pathogens is done by antibodies in humoral immunity and by T-cell receptors in cellular immunity. Most commonly used assays look for antibodies and not for cellular immunity. The type of acquired immune response is dependent on how a pathogen is presented; humoral immunity is generally more effective for extracellular pathogens, and cellular immunity is more effective for intracellular pathogens such as viruses. Although both branches of acquired immunity may be stimulated, this is not always the case, and it is certainly possible to have a negative antibody titer in an exposed animal with an acquired cellular immune response; for some agents this is common. Antibodies may also cross react to related viruses that have significantly different clinical implications. This is especially important in host taxa whose viral diversity is not yet well known, such as marine mammals. Development of an acquired immune response also requires time; it is often a week or more after infection before a response is seen. Finally, there are limitations on available reagents; many techniques require host-specific reagents to detect antibody responses. Although there may be cross reaction when using reagents from closely related hosts, this needs to be validated for each host species.[55]

Methods for detection of virus rather than immune response include virus isolation, immunohistochemistry, and nucleic acid-based diagnostics. Virus isolation on cell cultures enables many other possible studies including experimental infections and construction of genetically modified viruses for vaccines or understanding of biology. However, culture conditions have not been determined for most marine mammal viruses. There are few available marine mammal cell lines, and when possible, culture is often comparatively very slow. Immunohistochemistry may detect viral proteins in tissue sections, but the availability of validated antibodies for specific detection of marine mammal viruses is very limited, and cross-reactivity concerns exist. In situ hybridization (ISH) is similar to immunohistochemistry but detects viral nucleic acids rather than viral proteins. The cost barriers to ISH assay development are significantly lower, and use of ISH is expanding. However, nucleic acids are much more badly damaged by formalin than proteins, and it is critical that tissues be processed into paraffin blocks rapidly and not remain in formalin for excessive periods of time.

Nucleic acid-based techniques are currently most broadly used for virus detection. PCR-based protocols are used for a wide range of viruses. PCR primers may be designed to amplify only specific viruses or clades of related taxa. It is critical that a PCR product is identified properly; the most rigorous methods are product sequencing and probe hybridization. Sequencing will provide absolute identification of the product amplified; any assay using broad-range primers to identify clades of viruses needs to have a product sequence ID. Probe hybridization uses a labeled complementary nucleic acid strand that will bind very specifically to the expected product under correct salt and temperature conditions. This is currently most commonly done in probe hybridization quantitative PCR (qPCR, a.k.a. real-time PCR) assays, such as TaqMan assays. These assays require less time, are less expensive, and provide quantitative information about the amount of virus present. However, a properly designed and validated probe hybridization qPCR will identify only known target viruses and is not useful for identification of novel agents. Note that methods other than probe hybridization are also

commonly called qPCR or real-time PCR, such as SYBR Green, which involves dye incorporation of dye into any amplified DNA and is less rigorous.

PCR-based methods are limited because they require a known conserved area of sequence for primer design. The detection of novel phylogenetically distant viruses may be challenging or even impossible. In the last few years, NGS technologies have developed. Rather than targeted sequencing of a single DNA template, NGS methods may sequence literally millions of different nucleic acid templates in a sample. Although NGS can obtain sequences from previously unknown and divergent pathogens, it must also recognize and sift through literally millions of nontarget sequences. This is a major bioinformatics challenge but has already resulted in significant advances in infectious disease. Costs for NGS and the time required for data analysis are still high but have dramatically decreased, and NGS technologies are already in use for marine mammal virus discovery.

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85

Mycobacterium pinnipedii

ALEXIS LÉCU

The *Mycobacterium tuberculosis* complex (MTBC) stands as a group of mycobacteria species of major concern, mostly because of their high zoonotic abilities. The constituent members of the MTBC are highly genetically related (0.03%) and can be split broadly between human and animal-adapted strains: the major human pathogens, where no obvious animal reservoir has been identified, are *Mycobacterium tuberculosis* and *M. africanum* (subtypes 1 and 2); the animal-adapted strains have been isolated from a range of wild and domesticated animals and are named after their host of initial/most frequent isolation,[1] including *M. bovis* in ruminants, *M. microti* in rodents, cats, and south American camelids, *M. caprae* in goats, *M. orygis* in oryx and other antelopes, *M. mungi* in banded mongoose *(Mungos)*, the Dassie bacillus in rock hyrax *(Procavia)* and *M. pinnipedii (M. p)* in seals and sea lions. Actually, recent phylogenic studies[2,3] are establishing an evolution route leading *M. pinnipedii* from original African pinniped host species to South American marine mammals through the ocean, and then to an adaptation to human beings in the South American continent, leading to a lineage of *M. tuberculosis* adapted to *Homo sapiens* more than 1000 years ago on this continent.

Etiology and Hosts

M. pinnipedii was formerly known as "seal bacillus" as it was first found in a colony of three different species of otariid seals in Western Australia between 1981 and 1986.[4] There were several papers between 1990 and 2000 where "tuberculosis outbreaks" in pinnipeds and other species were reported,[5–8] occasionally wrongly classified as *M. africanum* or *M. bovis* infections, and retrospectively attributable to *M. pinnipedii*. This mycobacterium was definitively classified as a standalone species of the MTBC in 2003, mainly based on its molecular[9] and genomic characteristics.[10]

Regarding phenotype, *M. pinnipedii* is a slow-growing mycobacterium, which means that on primary isolation, there is minimal visible growth during the first week of culture. Primary culture is obtained at 37°C on egg-based medium in 3–6 weeks on average, and antibiotic susceptibility could be determined after an additional 2–4 weeks. The isolates are usually susceptible to isoniazid, rifampicin, streptomycin, ethambutol, and para-aminosalicylic acid. Colonies are dysgonic, rough, and nonchromogenic.[11] However, biochemical and cultural characteristics do not clearly differentiate *M. pinnipedii* from *M. bovis*: genomic features are most often the key to differentiating *M. pinnipedii* from all other MTBC species.

All MTBC species share a similar 16S RNA gene sequence except *M. pinnipedii*, which has one single different nucleotide within this sequence. Phylogenetically, these species share the same GyrB sequence motif with *M. africanum* type I and *M. canetti*.[12] The genomic profile of MTBC species is very different from *M. bovis* and *M. caprae,* which underwent more deletions, but it has the same deleted regions (namely RD7, RD8, and RD10) as do *M. microti* and both *M. orygis* and the Dassie bacillus. The deletions that are specific to *M. pinnipedii* species are the sequences RD2seal and RDpin: the presence of these deletions is systematic in all strains and is independent from geographic origin or host type. It should also be noted that the RD2 sequence has been demonstrated as essential to the full virulence of MTBC,[13] so RD2seal deletion or mutation could lead to a reduction of pathology extension or organ burden in hosts, as for the Bacillus Calmette-Guérin (BCG) strain.[14] This could be linked to the suspected "latency" feature in some of the host species.

All *M. pinnipedii* isolates contain the sequences IS6110, IS1081, MPB70, MPB83, and MTP40 but fail to produce detectable MPB70 and MPB83 antigens[15]; therefore, use of serodiagnostic methods based on MPB70 and MPB83 antibody detection may be of questionable help in *M. pinnipedii* screening, and the lack of these antigen expressions may even be used as a key in distinguishing *M. pinnipedii* infection from other MTBC species.[16]

Genotyping, especially spoligotyping, is still the most efficient way to differentiate *M. pinnipedii* from other MTBC species. The spoligotyping method is based on polymerase chain reaction (PCR) amplification of a highly polymorphic direct repeat locus in the MTBC genome. Results can be obtained from a pure culture or also from a raw sample containing enough *M. pinnipedii* DNA, if obtained within one day. All isolated *M. pinnipedii* spoligotype patterns form a cluster that is clearly different from those of all

other members of the MTBC.[17] Thus, the clinical usefulness of spoligotyping is determined by its rapidity, both in detecting causative bacteria and in providing epidemiologic information on strain identities.

Hosts

M. pinnipedii is historically known as the "seal bacillus" and was initially discovered only in species from the Otariidae family, but was eventually determined to be able to infect other marine and terrestrial mammals, either naturally or experimentally (Box 85.1).

"Natural" wildlife hosts all originate from the southern hemisphere and outbreaks in wild pinniped colonies are mostly located on the Atlantic sides of the South American and African continents, and the Pacific Ocean around Australia and New Zealand.[18]

Epidemiology

The infection is likely to remain dormant for several years in pinnipeds, with no clinical signs detected. Among marine animals, the South American sea lion *(Otaria byronia)* seems to have the greatest risk of spreading infection, mainly because of sequential importation of infected wild animals in the 1980s and early 1990s from Chile and Uruguay to European zoos. The different outbreaks noticed in captivity since 1998 are often retrospectively found to share wild-born imported pinnipeds as an index case or as part of the affected group. Transmission mainly occurs via direct contact (within rookeries in the wild, common resting places in zoos or aquariums), water (oral route), and aerosols. Transmission to other species followed the same routes; for example, aerosols created by high-pressure cleaning of pinniped enclosures were found to create a contaminating fog in distant tapir or camel enclosures,[19,20] and close contact can be problematic, for example, between a pinniped and its human trainer.[7,21]

Contamination from pinnipeds to terrestrial mammals also occurred in the wild, with cattle infected while grazing on areas adjacent to seal beaches.[22,23] At necropsy, lesions in thoracic lymph nodes suggest aerosol transmission in those "non-zoo" species.

Postmortem Diagnosis

For every suspected tuberculosis case, and for any dead imported sea lion, all preventative measures and equipment should be used by the staff that performs the necropsy, because this procedure carries a higher risk for exposure and contamination of human participants, especially in large mammals. For pinnipeds, typical *M. pinnipedii* gross lesions consist of granulomas within lymph nodes (cervical, submandibular, tracheal, mediastinal, and mesenteric)[19,24] and/or organs such as lung, spleen, liver, uterus,[25] and bladder. In pregnant animals, lesions may be present in the placenta.[24] Granulomas may have a soft or thicker core and calcifications may be present in the center and felt while cutting tissue. Every suspected lymph node (either affected or draining a suspected area) and organ should be sent for cytology (either Ziehl-Neelsen or Auramine stains), PCR, and culture.

Gross lesions of *M. pinnipedii* infections could not be differentiated from those of other MTBC species (e.g., *M. bovis*)[26] or those of nontuberculous mycobacteria (NTM) that can also affect pinnipeds (e.g., *M. avium*).[26,27]

• BOX 85.1 Reported Hosts of *Mycobacterium pinnipedii*

Wildlife Hosts
Genus: Neophoca
(Australian sea lion)
Genus: Arctocephalus
(New Zealand fur seal, Australian fur seal, South American fur seal, Sub-Antarctic fur seal)
Genus: Otaria
(Southern sea lion)
Genus: Mirounga
(Southern elephant seal)
Genus: Tursiops
(Bottlenose Dolphin)

Captive Wild and Domestic Hosts
Genus: Tapirus
(Lowland tapir, Malayan tapir)
Genus: Hystrix
(Crested porcupine)
Genus: Camelus
(Bactrian camel)
Genus: Lama
(Llama)
Gorilla (Gorilla gorilla gorilla)
Genus: Panthera
(Snow leopard, Amur Leopard, Tiger)
Genus: Bos
(Domestic Cattle)
Genus: Homo
(Human being)

Experimental Hosts
Genus: Cavia
(Guinea Pigs)
Genus: Oryctolagus
(Rabbit)
Genus: Mus
(Mice)

From Bastida R, Guichon R, Quse V: Escenarios para el origen y dispersión de la tuberculosis en Patagonia Austral y Tierra del Fuego. Nuevos actores y líneas de evidencia. In: XVII Congreso Nacional de Arqueología, 11-15 de octubre de 2010, Argentina, Mendoza, pp 227–230.

Antemortem Diagnosis

Clinical Signs

The signs of a *M. pinnipedii* infection can range from no signs to signs suggesting various organ deficiencies, depending on the different organs affected by the mycobacteria infection. Even with extensive tissue involvement, clinical signs may remain absent for a long time before animals eventually decompensate. When present, the most frequent clinical signs are weight loss, amyotrophy,[16] enlarged lymph nodes (e.g., cervical in pinnipeds), coughing, and upper and lower respiratory discharge. Blood work (cell count and chemistry) are generally not specific with nonspecific anemia and/or transient neutrophilia (A. Lécu, personal communication) or simply mirror inflammation and organ necrosis.

Imaging

M. pinnipedii can induce calcified granulomas in hosts,[16,21,28] so this feature can be used for detection though radiographic examination. However, especially in large mammals like adult sea lions or hoofstock, thoracic radiography is likely not precise enough to reveal tiny calcified spots of a few millimeters, especially through thick layers such as sea lion blubber.[29] Thus, computed tomography (CT) remains the best antemortem imaging examination, with the possibility of three-dimensional inspection and localization of lesions.[29] Caveats are the size of the animal compared to the limits of the CT scan ring diameter/table weight support, and other pathology able to induce calcification (e.g., neoplasia).

Ultrasound can also be used to assess superficial lymph nodes (cervical and submandibular) and eventually biopsy them accurately.[30]

Direct Examination

M. pinnipedii organisms can be identified from relevant samples while infection is in the active stage. Collecting samples may be performed through immobilization, but in pinnipeds, medical training procedures may also be used to obtain sputum, swabs, urine, or even lavages from conscious animals with their cooperation. Considering the intermittent shedding of *M. pinnipedii*, training should be promoted because it is the best way to thoroughly monitor animals, but training staff safety should be maintained while performing these procedures to avoid contamination via proved[7] or speculated[21] means.

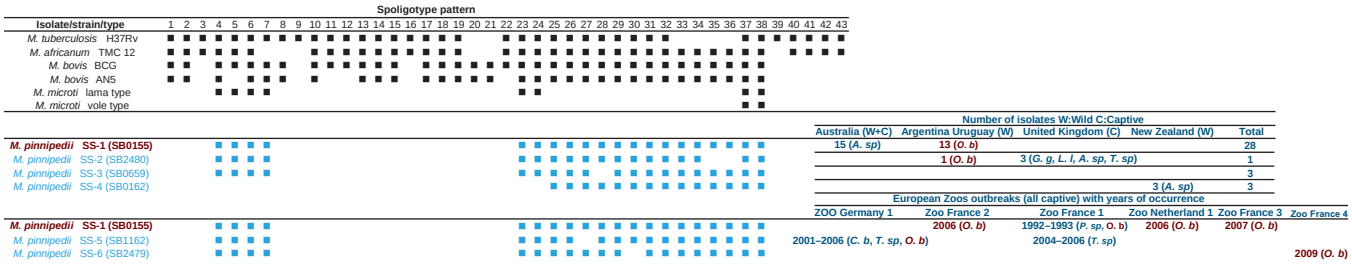
As for other MTBC species, fluids (e.g., lavages, urine) and granulomas (e.g., lymph nodes biopsies) may harbor *M. pinnipedii*, and can be detected with Ziehl-Neelsen or Auramine[9,31] stains. However, those samples may also contain very few mycobacteria, and the relevance of stain use depends on shedding and infection status, leading to overall low sensitivity (below 30% for sputum).[24] A negative result should always be analyzed considering detection limits of the examination: as an example, at least 1000–5000 organisms/mL are needed in a sputum sample to trigger a positive Ziehl-Neelsen stain, where only 10–100 organisms/mL (or even less than 10 mL) can result in a positive culture by PCR.[32] Existence of other acid-fast stain positive bacteria such as *Nocardia*[33] or *Gordonia*[24] should also be considered, because they can be found in pinniped organs and lesions, and mimic tuberculous granulomas.

Therefore, it is wise not to use stain alone for detection and to include PCR in any direct examination panel, because it is associated with a low detection limit and the ability to diagnose the species of mycobacteria by using appropriate probes.

Although good molecular genetic practices advise spoligotyping on a culture extract, some laboratories can perform this test from the primary sample if there is an adequate amount of DNA. This remains as a good method to quickly confirm or rule out *M. pinnipedii* by the presence of its unique spoligotype patterns but also allows comparison of strains by their spoligotypes and tracing of the origin of contamination (Fig. 85.1).[20]

Immunology

Host immune reaction to *M. pinnipedii* infection may be divided between humoral and cellular immunity, as for all other MTBC infections. However, onset of the appearance



• **Figure 85.1** Spoligotype patterns of several *Mycobacterium tuberculosis* complex species and those of *M. pinnipedii* strains gathered so far from the field (both in captive and natural settings). *Black* and *blue squares* represent the presence or the absence of an individual spacer sequence, respectively. Years Species affected in brackets for each outbreaks: *A. sp*, *Arctocephalus* spp.; *G. g*, *Gorilla*; *L. l*: *Lama*; *O. b*, *Otaria byronia*; *P. sp*, *Panthera* spp.; *T. sp*, *Tapirus* spp. Most represented spoligotype is the SS-1 (SB0155).

of a selected biomarker, either cellular or serologic, is always unpredictable and none of the current studies or reports were able to detect a theoretical profile of immune reaction sequence, as could be done for cattle.

Cellular Immunity Exploration

Cellular immunity may be assessed through intradermal testing; intradermal injection of tuberculin induces a type IV hypersensitivity within 24–72 hours, which can be measured by a local reaction. In cattle, *M. pinnipedii* infection was proven to induce a positive intradermal test performed with a purified protein derivative (PPD) dose of 5000 UI/0.1 mL.[22] In other terrestrial mammals, use of a comparative intradermal test with *M. avium* PPD as a comparison reagent was sometimes also applied to detect *M. pinnipedii* infection in tapirs,[28,34] but with an apparent lack of sensitivity and low negative predictive value. In pinnipeds, the skin test was also used with medium to poor success.[4,6,24,35] Although the recommended skin test sites may be the neck or flipper skin, the specific physiology of pinnipeds may explain this failure because skin test efficiency mostly relies on the population of T lymphocytes first, and then macrophages within the first skin layers: aquatic mammals such as pinnipeds have developed vasoconstriction and dilation abilities for diving and temperature regulation that may totally impair the hypersensitivity sequence, from none (apparent anergy) to overreaction (Koch reaction), which may induce flipper necrosis.

The use of interferon gamma release assays (IGRAs) could be considered in validated species like cattle because the MTBC antigens used in IGRAs (bovine PPD, ESAT-6, CFP10) are also shared by *M. pinnipedii* and could work as immunostimulants for lymphocytes. However, apart from cattle, this is not a currently available option, for example, for pinniped screening, mainly because there are no kits available with a pinniped-specific interferon enzyme-linked immunosorbent assay (ELISA). However, the concept of detecting mRNA sequence coding for interferon, instead of interferon itself,[36] could theoretically be applied to pinnipeds, because these sequences are very similar among carnivores.

Humoral Immunity Exploration

Pinnipeds and terrestrial mammals may produce detectable antibodies during the course of *M. pinnipedii* infection.[24,34] Although theoretical mycobacteria immune response predicts low antibody titers at the beginning of an infection course[37] and a rise in humoral response when mycobacteria are replicating, spreading, and invading organs (i.e., antigens become uncovered), serologic profiles may follow an unpredictable timeline pattern at the individual level. *M. pinnipedii* expresses the same immunostimulant antigens as other members of the MTBC, especially ESAT6, CFP10, and MPB83 (although the latter is not expressed in *M. pinnipedii*)[15]: there is currently no known antigen/antibody that is specific to *M. pinnipedii*. However, while the sequence does exist in its DNA, the absence of MPB70 antigen expression[9] in *M. pinnipedii* has already been used to differentiate it from all other MTBC species that produce this antigen (especially *M. bovis*). ELISA,[38] multiantigen print immunoassays (MAPIAs)[24,31] and lateral flow system hand kits (STAT PAK and Dual Path Platform [DPP], Chembio Diagnostic Systems, Inc., Medford, NY) have already been tested with various levels of success, but without consistent validated sensitivity and specificity. Regarding serologic assays, feedback from the field seems to show that DPP testing is more sensitive than STAT PAK,[25] for example, 58% for STAT PAK versus 87.5% for DPP on a sample of 10 cases.[24] However, none of these tests have been studied with a large enough sample comparison to indicate a gold standard, and therefore serologic results should be interpreted with caution. At the date of writing this chapter, only DPP remains available to the veterinary practitioner; MAPIA and STAT PAK have been discontinued.

The aquatic environment of pinnipeds is likely to expose them to many waterborne environmental mycobacteria and this may interfere with any indirect examination outcome, because gene coding for ESAT-6-like proteins and CFP-10-like proteins were demonstrated in several NTMs such as *M. kansasii*, *M. marinum*, *M. szulgai*, *M. flavescens*, *M. gastri*, and *M. smegmatis.*[39] However, the expression rate of these genes in those NTMs is questionable and homology of these proteins differs. Therefore, specificity of the humoral test will be based on the test technology and qualitative/quantitative approach to those MTBC proteins. Hence, cross-reaction should not be an immediate argument for antibody titers, whereas a single seropositivity with one serologic assay must not be associated with certain infection but should raise concerns and initiate deeper screening.

Interferences Between Cellular and Humoral Tests

When designing a diagnostic and examination plan, the veterinarian should know that application of an intradermal test may have an impact on subsequent humoral tests. Injection of antigens such as PPD could provoke a nonspecific immune reaction to several hundred antigens contained in the PPD and artificially induce antibody titers that are unrelated to the real infection by *M. pinnipedii* (A. Lécu and G. Lacave, personal communication, 2012), but may remain for months.

Injection of tuberculin has already been used as a "booster effect" in other animals[30,36] (including humans) to trigger an anamnestic rise of antibody in latent infected individuals with low preexisting immune response. However, considering the nonspecific humoral reaction that may happen with PPD injections and considering that the booster effect was not assessed in most zoo species that are already listed as hosts for *M. pinnipedii*, this procedure is not recommended because it is likely to increase false positive occurrence.[37]

Treatment

Depending on a country's animal health laws (*M. pinnipedii* is not considered in European Union Animal Health Law,

which focuses on *M. bovis*) and risk analysis, treatment could be an option for suspicion, contact (i.e., prevention), or even confirmed positive animals.

M. pinnipedii is usually sensitive to typical antituberculous drugs, at least in vitro,[9,40] but antibiograms for strains found circulating in zoos (see Fig. 85.1) were rarely performed. Reported treatment in pinnipeds relied on bitherapy[24] or tritherapy[41] with use of oral rifampicin (7.5 mg/kg), isoniazid (5 mg/kg), and addition of ethambutol (15 mg/kg). There are no reported pharmacokinetic studies of any antituberculous drugs in pinnipeds, and hence these dosages do not necessarily result in therapeutic plasma levels. Moreover, several major side effects were noted, such as anorexia (occurring 1–2 weeks after treatment began), abdominal discomfort, lethargy, and suspected hepatotoxicity.[41]

These side effects are likely to impair compliance and then to increase all risks associated with suboptimal antibiotic levels, such as persistence of shedding and resistance acquisition; persistence or relapse of infection have been noted in Patagonian sea lions 11 months after treatment initiation.[24] Therefore, a decision to treat should be appropriately balanced against euthanasia based on an exhaustive risk analysis including access to animals, medical training levels, ability to follow plasma levels, and all transmission opportunities (staff and other mammal species).

Prevention

Animals

Considering all antemortem diagnostic difficulties mentioned previously, the examination panel to be included in quarantine of any incoming pinniped should first relay the animal's history, especially a history of any contact with high-risk animals such as imported wild contact specimens. According to this history, serologic assays and PCR on bronchoalveolar lavage can be recommended, along with a CT scan and any other relevant clinical assessment in case of higher risk. Repetition of examination is highly recommended considering intermittent shedding and immunologic status variations.

Husbandry: Water

Little is known about *M. pinnipedii* survival rate in the environment,[42] but as a member of the MTBC, it can likely survive outside the host organism for a long time; this "compensates" for its limited ability to multiply outside the host organism. Even though they do not sporulate, MTBC species can usually still be cultured from a damp environment protected from direct sunlight after the lapse of several months or years.[42]

A life-support system (LSS) can be thought of as a primary defense against mycobacterium persistence and transmission within water. Mechanical and biological filtration are not efficient in removing MTBC organisms and may even create a hidden burden of mycobacteria, e.g., embedded in biofilms that coat pipes or filtration media (sand). Hence, a sterilization phase is required and the chosen disinfection usually includes one or a combination of the following items: ultraviolet (UV) light, chlorine, and ozone.

To decrease the MTBC load by 3–5 logs, UV devices should be set around 254 nm wavelength and should maintain power over 10–15 mJ/cm^2. Although these are the usual settings applied in zoo and aquarium LSSs, the age of the lamp, the turbidity of the water, and the actual percentage of whole filtered volume passing through UV devices are all factors that significantly decrease UV efficiency against MTBC species, thus leading to longer duration of mycobacteria in the water.

Chlorine is not considered effective in removing mycobacterium from water in which animals reside: in the concentrations of free chlorine that are compatible with pinniped health (below 0.7 ppm),[38] there is no bactericidal effect against mycobacteria, which require at least 1.0 ppm for more than 8 hours to decrease load by 5 logs.[43] For both UV and chlorine, organic aggregation that commonly occurred in marine mammal water actually impaired the disinfection level by harboring mycobacteria.[44]

Thus, ozone remains the most effective tool for eradicating *M. pinnipedii* because the contact time value (a product of the concentration and contact time) effective range for killing 99.9% of mycobacteria is within the range that is customarily applied in aquarium and marine mammal LSS contact chambers (0.06 mg/min/L).[45]

The destination of water waste from an LSS (filter backwash) should also be monitored because it may move *M. pinnipedii* farther in or out of zoo or aquarium enclosures.

Husbandry: Good Staff Practices

By default, pinnipeds should be considered at risk for *M. pinnipedii*, and thus management practices should be adjusted to avoid hazardous actions or procedures that may lead to transmission. Regarding staff and visitors, close-contact exercises, especially mixing aerosol production ("kiss," "blow," or even "bark to face") should be avoided as much as possible. Regarding animal management, high *Pinnipedii*-pressure hose use should be restricted to a minimum and the direction of the produced aerosol should be monitored when used. Any devices in contact with pinnipeds or water should be regularly cleaned and exposed to sunlight; especially, all tank cleaning and diving equipment should be disinfected because they are perfect support venues for biofilm persistence. Keepers and trainers should not care for other mammal species during their day, but if this is necessary, strict hygienic measures must be applied (foot bath, different clothes, and tools).

Finally, health monitoring of marine mammal keepers and trainers in zoos, aquariums, or rehabilitation centers should be promoted,[46] and attention must be focused on their preexisting status for tuberculosis. In case of a suspicious or confirmed case, the physician can recommend a skin test and/or IGRAs[21] to screen for exposure; knowledge

of initial staff status regarding these tests is important, especially in deciding whether to administer prophylactic antituberculous treatment.

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Lens Diseases and Anesthetic Considerations for Ophthalmologic Procedures in Pinnipeds

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Introduction

Lens diseases in pinnipeds are one of the two most common ophthalmologic diseases identified, the second being keratopathy (discussed later). Lens diseases include cataract and lens instability (i.e., subluxation and luxation). Thus far, numerous species of pinnipeds in managed populations have been examined, as well as some of the same species from wild stranded pinnipeds; individuals of these many species have been diagnosed with cataracts, lens subluxations, luxations, or both, in all age groups.[1,2] For purposes of this chapter, the term lens diseases will include cataracts and lens instability or luxation, unless otherwise specified.

Incidence of Lens Diseases in Pinnipeds

The incidence of lens diseases, including luxations, cataracts, or both, in pinnipeds under human care is 46.8%. Fifteen percent of animals had both lens luxations and cataracts, and 34% had cataracts alone.[1] When specific pinniped groups were assessed, the incidence in California sea lions *(Zalophus californianus)* was 44.5%, in harbor seals *(Phoca vitulina)* was 90%, and in walrus *(Odobenus rosmarus)* was 50%.[1]

Cataracts have also been diagnosed in stranded pinnipeds in many retrospective studies.[3–6] All age groups and sexes were affected. The most recent retrospective study[5] found that lens disease (i.e., cataracts and lens instability) had an overall prevalence of 0.6%. Of the 337 stranded animals with ophthalmic lesions, 31 animals had cataracts, specifically 25 California sea lions and 6 Northern elephant seals *(Mirounga angustirostris).* All age groups of California sea lions had affected animals, whereas only pups were affected in the Northern elephant seals. Seven animals were affected with lens luxations, specifically five California sea lions and two Northern elephant seals. These numbers were similar to the numbers previously published[4,7]; however, they were likely inaccurate because the stranded populations are only those who are fortunate enough to be found and attended to medically. It is impossible to determine the prevalence of lens diseases in the free-ranging population.

Risk Factors/Protective Factors

Numerous factors have been implicated to contribute to cataract formation in pinnipeds. In a cross-sectional study evaluating 111 pinnipeds, risk factors identified included aging, lack of sufficient access to shade, a history of fighting, and a history of nonspecific ocular disease.[1] Animals in this study ranged between 1 and 31 years of age, and, although the study did not have any affected animals between birth and 5 years of age, the author has diagnosed cataracts and lens luxations in animals in this young age group since that study was published.

Natural aging is a risk factor for cataracts in most species; therefore the finding of animals 15 years old or older being significantly affected, in more than 60% of animals, is not surprising; and all animals older than 26 years had some stage of cataracts. On occasion, some animals older than 25 years with adequate shade or who lived in latitudes further away from the equator may have signs only of nuclear sclerosis with incipient to early immature cataracts. However, these lenses may still progress to anterior luxation. In the author's experience, no eye from any pinniped older than 17 years has had normal zonular or ciliary body support to the lens at the time of surgery, making middle-aged to older animals predisposed to anterior lens luxations. The lenses are less likely to luxate posteriorly in otariids and phocids unless the vitreous is significantly degenerated. Walrus appear to be more likely to luxate their lenses posteriorly into the vitreous (Colitz, personal observation).

Exposure to ultraviolet (UV) light in the form of sunlight or other forms of radiation is a well-documented cause of cataracts in many species.[8–11] In a cross-sectional study, 100% of all species who had excessive exposure to sunlight had cataracts, and pinnipeds that did not have any access to shade were almost 10 times more likely to develop lens diseases.[1]

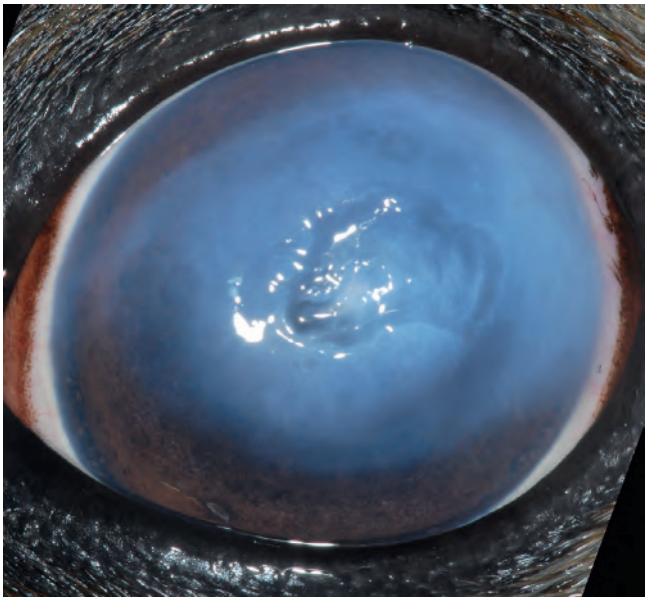
The risk factor regarding history of fighting would indicate a possible traumatic incident contributing to cataract formation. Therefore managing more aggressive animals properly is indicated. The risk factor regarding previous eye disease, most likely keratopathy, will be discussed later.

Because younger animals were also affected, there may be a genetic predisposition to cataracts in some lines of pinnipeds. Cataracts have been diagnosed in numerous generations of related animals in some facilities (Colitz, personal observation). With better data collection and genetic testing, there may be a means to evaluate this hypothesis in the future.

Concurrent Eye Problems

The most common concurrent eye problem in pinnipeds is keratopathy. Keratopathy had initially been described in otariids[12]; however, it occurs in all species evaluated to date and is better termed pinniped keratopathy (Fig. 86.1). Interestingly, it does not appear to occur in pinnipeds in the wild, although it is possible and may not yet be identified.

Corneal diseases, including keratopathy, ulceration, and abscess formation, may cause secondary inflammation or uveitis.[13] Because keratopathy does not appear to completely resolve, the chronicity of this disease likely causes the continuous subclinical uveitis contributing to cataract formation, lens instability, and eventual luxation.



• **Figure 86.1** The left eye of a California sea *lion (Zalophus californianus)* with stage 3 pinniped keratopathy. There is diffuse corneal edema, stromal loss, and temporal limbal hyperemia. Blepharospasm was alleviated using topical anesthesia for examination and photography.

It has been suspected that similar risk factors to those of lens diseases might be to blame for keratopathy. A cross-sectional study of 319 pinnipeds around the world found that UV Index less than 6, not having had eye diseases or trauma, being older than 10 years of age, and having been tested for Leptospirosis were risk factors for having pinniped keratopathy.[14] Most of these factors were similar to those affecting cataract incidence.

UV Index was not evaluated in the initial cataract study, although it would likely be significant because it was in the cornea study. The UV Index was established by the World Health Organization for human sun exposure, and the United States has created a scale that conforms to these guidelines.[15] Even a UV Index of 1 or 2, although "low," can cause sunburns in individuals who easily burn; therefore using broad-spectrum sunscreen and sunglasses are suggested. A UV Index of 3–5 is "moderate" in risk of harm from the sun. Even levels less than the number found to be risky to pinnipeds in this study (i.e., 6) can potentially cause harm to eyes if sun protection is not worn. The UV Index of 6–7 is in the high-risk range, and there are areas of the world with UV Indices higher than this. These animals, if without proper shade structure or other UV Index–lowering means, had chronic unrelenting keratopathy. Pinnipeds and all marine animals that do not have shade structures or means to lower the UV Index are at the highest risk for both keratopathy and lens diseases.

Providing shade structures may be complicated because the sun's movements throughout the day may cause reflection rather than protection, depending on time of day and season of the year. Other methods to lower the UV Index include using UV paint colors that absorb UV light and/or natural-appearing pool walls and bottoms. Some facilities have also allowed algae to grow on these surfaces as long as it is controlled and devoid of dangerous microorganisms.

Surgical Considerations

Regardless of the cause, visually impairing cataracts and painful anteriorly luxated lenses can be surgically removed. This allows the animal to regain his or her previous quality of life and relieves pain in those affected by uveitis and/or anterior lens luxation(s). Surgical considerations include overall health and wellness, although even elderly pinnipeds have undergone successful surgical lensectomy.

Surgical lensectomy for cataracts and/or lens luxation has become one of the most commonly performed surgical procedures in otariids and phocids under human care or at stranding facilities. There are two approaches for lens removal, extracapsular and intracapsular. Extracapsular cataract surgery is when the anterior lens capsule is opened (capsulotomy) and the lens cortex and nucleus are removed; this can be performed either via phacoemulsification or via manual extraction of the lens and nucleus. In the author's

experience, all pinnipeds with cataracts that were older than 2 years so far have had lenses so dense or unstable that phacoemulsification has not been practical or possible. After the lens cortex and nucleus are removed via a 160-degree limbal corneal incision, the remaining cortical material is flushed out of the capsule and posterior chamber and out of the eye; then the lens capsule is gently manipulated and most are removed completely. Yearling phocids and otariids have undergone phacoemulsification because their lenses are softer.[16] There is a report of a fur seal that underwent phacofragmentation,[17] and the author explained that the lens was so dense that he had to break it into quadrants and then manually remove the pieces via a larger incision; the lens material was too dense for traditional small incision phacoemulsification (J. S. Smith, personal communication). Many cataractous lenses are clinically luxated into the anterior chamber; these are removed via intracapsular lens extraction wherein the lens capsule is not opened and the entire lens (i.e., lens capsule, cortex, and nucleus) is removed.

Anesthesia of Pinniped for Ophthalmologic Procedures—an Overview

Here we cover concepts as they relate to anesthesia of pinnipeds for ophthalmologic procedures. The first step in the process is determining whether immobilization (general anesthesia) is necessary to perform the procedure. In general, anesthesia will be necessary for ophthalmologic surgical procedures of pinnipeds.

A voluntary preanesthetic health assessment provides a general overview of the animal's current health status prior to anesthesia in managed populations where medical behaviors can be cultivated. Where possible, this should include complete blood count, serum chemistry panel, and physical examination. Further information can be garnered from urinalysis, radiographs, ultrasound, and echocardiography, where appropriate. Unfortunately, changes in vision, as well as pain associated with many eye problems, may lead to loss of useful medical behaviors. Preoperative analgesia may be necessary, and trainers must be vigilant to maintain necessary medical behaviors. Given that ophthalmologic surgical procedures are often performed on geriatric patients in managed populations, the preanesthetic health assessment is imperative to identify other potential underlying disease states.

Anesthesia of pinnipeds requires a balance of behaviors, mechanical restraint, and chemical restraint. The practitioner must consider the patient species, size, age, demeanor, and training, as well as the working environment, to best tailor the anesthetic protocol. Darting an animal with ultrapotent anesthetic agents to cause immobilization (anesthesia) may result in severe negative physiologic effects. Desensitization training and voluntary medical behaviors contribute in no small part to positive outcomes. These voluntary medical behaviors may also reduce the need for mechanical restraint devices such as netting, slings, and squeeze cages. Unfortunately, excessive reliance on mechanical restraint may lead to physical injury to the patient or eye, hyperthermia, or exertional myopathic conditions.[18] With proper desensitization training and chemical sedation, mechanical devices may be used to improve the safety of anesthetic procedures for both the patient and the animal handlers, without undue added risk.

Although anesthesia of pinnipeds has been induced with inhalant anesthetic by mask under behavioral control,[19] a balanced anesthetic approach is more appropriate. Balanced anesthesia involves the use of a combination of drugs, with each drug delivered in an amount sufficient to produce the desired effect while keeping undesirable effects to a minimum. Inhalation anesthetics delivered alone will produce excessive cardiopulmonary depression and no analgesia. This does not imply that the practitioner cannot induce anesthesia by mask under behavioral control, but it would be wise to supplement the anesthetic event with analgesic agents, muscle relaxants, or other appropriate inhalation anesthetic–sparing drugs after the pinniped is intubated and monitored. The benzodiazepines, midazolam and diazepam, and/or the opioids, butorphanol and meperidine, have commonly been used for this purpose in pinnipeds. Alpha-2 agonists have also been used in pinnipeds but have proven problematic for geriatric ophthalmologic patients. Alpha-2 adrenergic agonists, such as medetomidine and dexmedetomidine, will provide profound sedation, analgesia, and muscle relaxation and are reversible. Unfortunately, they also cause bradycardia, interfere with contractility of the heart, and cause peripheral vasoconstriction leading to increased afterload. Unsurprisingly, alpha-2 agonists also reduce renal blood flow in healthy subjects under optimal conditions, even at low dosages. In addition, the alpha-2A adrenoceptor is the alpha-2 adrenoceptor subtype primarily involved in the regulation of blood glucose homeostasis. Alpha-2 agonists should be considered a poor choice in the patient with poor glycemic control. The reversibility of alpha-2 agonists is enticing; however, sudden reversal and reduction in afterload could lead to cardiovascular collapse in patients with limited cardiovascular reserve, such as the geriatric patient. Furthermore, a historically high negative outcome rate in ophthalmologic cases receiving medetomidine or dexmedetomidine has been observed by the authors, with the development of excessive perioperative intraocular hemorrhage leading to hyphema in pinnipeds in which alpha-2 adrenergic agonists (or the reversal agent atipamezole) have been used (Colitz, personal observation). For these reasons, the alpha-2 agonists are considered a poor choice in many geriatric pinnipeds undergoing ophthalmologic procedures, despite their reversibility. However, the alpha-2 adrenergic agonists are a tool of consideration for use in young, healthy pinniped subjects in which it is necessary to rely more on chemical restraint for the safety of human personnel. The practitioner will need to carefully weigh the risks and benefits of this choice.

Rapid sequence induction of anesthesia with intravenous (IV) agents is performed in cases in which venous access has proven feasible under mild sedation. Propofol IV, with or without additional midazolam IV, can be delivered in the extradural vein of phocids and odobenids. Both vascular access and behavioral control are often more complicated in otariids, and otariids are less likely than phocids to breath-hold during inhalation anesthetic delivery by mask, so mask induction is more often used for otariids. Both of the former methods of induction may become unnecessary when patients are darted with ultrapotent drug combinations to "immobilize" (anesthetize) the patient, but such combinations are not recommended here.

Vascular access is feasible in pinnipeds. Venipuncture of the extradural vein of phocids and odobenids can be converted to a small-diameter cannula using available epidural catheter kits. Venous access of the jugular vein of otariids is facilitated by ultrasound. Cannulation of pectoral flipper cephalic and brachial veins does not require ultrasound, but ultrasound is a useful tool to facilitate access of these veins, as well as the pelvic flipper medial saphenous veins, particularly in phocids. Standard over-the-needle catheters can be used to reduce cost, but modern microintroducer kits using a modified Seldinger method[20] simplify and expedite cannulation (4–5F × 10 cm Stiffen cannula, nitinol mandrel stainless steel tip wire, echogenic needle, Micro-Introducer Kit, Innovative Veterinary Medicine, Ponte Vedra, FL).

Monitoring is often noninvasive (electrocardiogram, pulse oximetry, capnography, temperature, and inspired/expired gases), but invasive blood pressure monitoring has become a standard of care for otariids, with limited success in phocids.[21] Doppler flow probes and ultrasound facilitate cannulation of the median artery of the pectoral flipper or saphenous artery of the pelvic flippers of pinnipeds. Hypotension cannot be detected if it is not measured. The purpose of monitoring blood pressure is to obtain an objective measure of systemic circulation. Hypotension may be defined as a mean arterial blood pressure less than 60 mm Hg. Where anesthesia is the sole cause of hypotension, the appropriate response would involve reducing the level of anesthesia where possible, balancing the anesthetic technique with less cardiodepressive drugs (e.g., opioids), volume loading where appropriate, inotropes such as dobutamine (0.2–2 μg/kg/min, IV; lower than terrestrial mammals) or ephedrine (0.05–0.1 mg/kg IV) and possibly vasopressors such as phenylephrine (1–3 μg/kg/min, IV) or norepinephrine (0.1–0.5 μg/kg/min, IV). Noninvasive methods of estimating blood pressure have yet to be validated, but the use of oscillometric cuffs on the pectoral flippers of phocids has been used to follow trends in blood pressure.

During anesthesia, body heat is lost through radiation, conduction, convection, and evaporation. Conductive and convective heat loss can be minimized by various methods of insulation. Evaporative heat loss is more difficult to control, such as losses to the breathing of anesthetic circuit dry gases, but dry-docking the patient prior to surgery or towel-drying after induction is helpful in limiting surface evaporative cooling. Warming of replacement fluids generally has an absolute minimal effect on patient warming; however, delivery of cold fluids is of no benefit. Several types of patient-warming devices are available. The most effective systems are likely the warm circulating air-blanket systems. The need for warming will depend on the working environment, but the majority of patients in an appropriate surgical environment will arrive normothermic and quickly become hypothermic after induction of anesthesia if the presurgical environment is temperature controlled to the needs of the surgeon and the presurgical process does not lead to exertion. It is important to remember that the dosage of inhalant anesthetic gas necessary to maintain anesthesia is reduced by hypothermia and recovery is prolonged by hypothermia. It is also important to know that cardiac contractility is reduced by hypothermia. Most patients do not benefit from hypothermia, and it should be avoided.

Neuromuscular blockage is often needed for ophthalmologic procedures of pinnipeds to properly centralize and immobilize the eye for surgery. Atracurium, which undergoes simple Hofmann degradation (elimination), has been successfully used by the authors for neuromuscular blockade in phocids, otariids, and odobenids. Even though easily eliminated from pinnipeds, any residual muscle weakness caused by atracurium can be antagonized by delivery of edrophonium. Delivery of edrophonium should be done slowly, while still monitoring to avoid or detect bradycardia that has been observed rarely in pinnipeds. The need for edrophonium may be assessed in part by use of nerve stimulators but is sufficiently imprecise to fully judge any residual paralysis. New true reversal agents, like sugammadex in combination with rocuronium, will likely replace the use of atracurium in pinnipeds in the near future.

Inhalation anesthetics depress the response of mammals to carbon dioxide (CO_2). This depression of the response to CO_2 varies with species but does appear to follow the trend: humans ≅ canid < equid < otariid ≅ odobenid < phocid. The shift in the response to CO_2 is such that moderately anesthetized otariids will not spontaneously ventilate until the arterial partial pressure of carbon dioxide ($PaCO_2$) is between 60 mm Hg and 80 mm Hg. However, it appears the $PaCO_2$ must rise very high—to the point of becoming an anesthetic itself ($PaCO_2 > 90$ mm Hg)—in phocids; phocids will be unlikely to spontaneously ventilate even when only moderately anesthetized. Permissive hypercapnia involves accepting hypercapnia and associated acidemia to avoid the potentially negative effects of mechanical ventilation. Application of permissive hypercapnia has been accepted by some in anesthesia of otariids, and its potentially beneficial cardiovascular effects have been described. Before using permissive hypercapnia, the effects of this therapy must be understood. High blood levels and alveolar concentrations of CO_2 could contribute to hypoxemia. Hypoxemia can be remedied by administering supplemental oxygen, which is commonplace. Hypercapnia may also cause tissue acidosis, resulting in decreased intracellular pH and altered

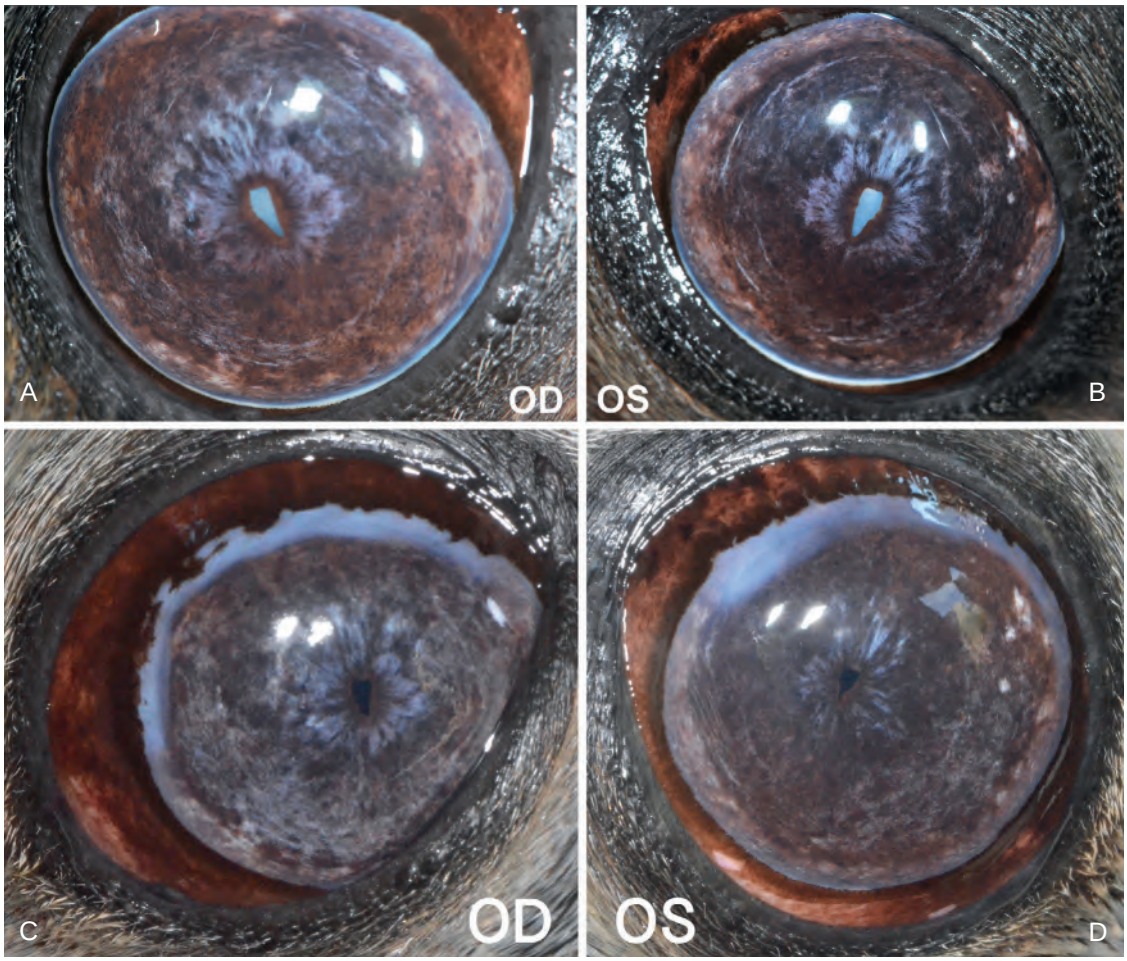
transmembrane electrolyte transport, glucose use, and structure-function relationships of amino acids. Neurologic sequelae of hypercapnia include sympathetic nervous stimulation with release of epinephrine and norepinephrine, significant increases in cerebral blood flow, and elevation of intracranial pressures. Hypercapnia also leads to increased cardiac output, decreased peripheral vascular resistance, and increased arterial blood pressure. However, as pH falls, left ventricular contractility is decreased (although cardiac output may still be maintained). In addition, decreasing pH causes a shift in the oxyhemoglobin dissociation curve to the right, promoting release of oxygen from hemoglobin. However, cardiac arrhythmias are reported in some species with $PaCO_2$ levels greater than 80 mm Hg. Type I hearts (canid, felid, human) tend to be more prone to such arrhythmias than type II hearts (equid, bovid, phocid, otariid, and odobenid). If permissive hypercapnia is used, the $PaCO_2$ should be maintained less than 80 mm Hg, the pH greater than 7.2, and the PaO_2 well above 60 mm Hg. A blood gas analyzer is mandatory for prompt, accurate decision making. Although one may rationalize a degree of benefit of elevated $PaCO_2$, recent work has suggested that high levels of CO_2 are not beneficial to pinnipeds and indicate a need for enhanced monitoring and support.[22] Given these facts—as well as the use of neuromuscular blocking agents—either manual or mechanical ventilation is mandatory for ophthalmologic procedures.

It is well known from pinniped dive studies that pinnipeds demonstrate a profound "dive reflex" when strapped to a board and forced to dive.[23] It is also known that the same animals, when allowed to dive voluntarily, do not demonstrate the same degree of dive reflex bradycardia as when forced into a dive.[24,25] As such, there appears to be a conscious stress component of forced dives (not knowing when the dive will end). In none of these instances did the subjects have a dive reflex leading to their death. There is no evidence to suggest an animal will die directly from the dive reflex, although it will die if not allowed to surface and breath. In addition, there is no evidence that the dive reflex remains intact during chemically induced unconsciousness of pinnipeds. On the contrary, there is evidence that anesthesia depresses or eliminates the dive reflex.[26] There is also clinical evidence that heart rates of marine mammals stabilize under general anesthesia when proper physiologic support is provided. Some reflexes, such as the laryngeal reflex and other vagally mediated reflexes, may well be present at light planes of general anesthesia—such as evidenced at induction and recovery—and vary with the anesthetic drugs used. These reflexes, or inadequate respiratory support of the anesthetized marine mammals, may well be confused with a "dive reflex." Indeed, severe hypoxemia will lead to myocardial hypoxemia and terminal bradycardia but is not mediated by a reflex.

Although a proper dive reflex may not be triggered during general anesthesia, the anesthetist should be aware of another related trigeminocardiac reflex—the oculocardiac reflex—that can still be triggered during anesthesia for eye surgery of marine mammals. This reflex is a well-known, albeit rare, cause of cardiac arrest during eye surgery of mammals. This reflex is observed during traction on extraocular muscles, is greatly exaggerated in the presence of hypoventilation, hypoxemia, and acidosis, but might be prevented by a retrobulbar local anesthetic block or administration of parasympatholytic drugs. The laryngeal/pharyngeal reflexes can lead to bradycardia as well. It is well established that general anesthetics modify the laryngeal reflex, but, at light planes of anesthesia, activation of laryngeal vagal reflexes can occur during manipulation of the airway. Adequate anesthesia prior to attempting intubation, topical local anesthetic sprays, and parasympatholytic drugs prevent this reflex.

Controlled mechanical ventilation (CMV) is the current mechanical ventilation method available on nearly all veterinary mechanical ventilators. This CMV mode of ventilation has often been treated as merely a convenience, taking the place of squeezing the anesthetic reservoir bag by hand to deliver a breath. However, the inconsistency of ventilation associated with hand ventilation over long periods makes CMV more of a necessity than a mere convenience in ophthalmologic surgeries. CMV begins the ventilator cycle at a baseline pressure and elevates airway pressure to deliver an appropriate tidal volume. Disadvantages to CMV include the application of relatively higher-peak inspiratory pressure and resultant higher mean intrathoracic pressure and reduced venous return and cardiac output, as well as poor distribution of inspired gas flow, with subsequent ventilation-perfusion mismatch. The apneustic plateau ventilation (APV) mode used in early dolphin ventilation was designed in an attempt to mimic conscious marine mammal breathing patterns but still allowed for development of atelectasis. Airway pressure release ventilation (APRV) was developed to improve ventilation of patients with low compliance, and apneustic anesthesia ventilation (AAV) was designed to best normalize respiratory mechanics of mammals under anesthesia. Both APRV and AAV also mechanically mimic the breathing patterns of conscious marine mammals. The objective of mechanical ventilation of marine mammals is not to merely mimic the normal conscious animal breathing pattern but to provide appropriate ventilation to unconscious marine mammals that are not spontaneously ventilating. The potentially beneficial modes of APRV and AAV ventilation for anesthetized pinnipeds are under development and will be available in the near future.[27]

Assigning someone to provide oversight of all activities related to the ophthalmologic surgical procedure—an "incident commander"—ensures personnel, policies, and procedures are optimized to improve emergency response, especially when media become involved in reporting the event. Quality staff should be informed and readily available to facilitate any potential emergency response. Assigning an individual to focus solely on the management of the anesthetized patient is critical to prevent anesthesia-related problems. Given the potential for anesthesia-related problems such as high CO_2 levels, apnea, hypoxemia,



• **Figure 86.2** (A and B) Right and left eyes of an Australian sea *lion (Neophoca cinera)* with bilateral mature cataracts. (C and D) Right and left eyes of the same Australian sea lion following bilateral lensectomies.

bradycardia, thermoregulatory problems, and prolonged recoveries, it is important to assign an individual to focus solely on the management of the anesthetic event who has a thorough understanding of intubation, mechanical ventilation, vascular access, and drug delivery methods for the species undergoing the procedure. Understanding the potential complications of anesthesia, appropriate vigilance monitoring and being prepared to manage complications improves the overall outcome of ophthalmologic anesthetic procedures of pinnipeds (see also Chapter 29).

Surgical Candidates: Poor, Good, Great

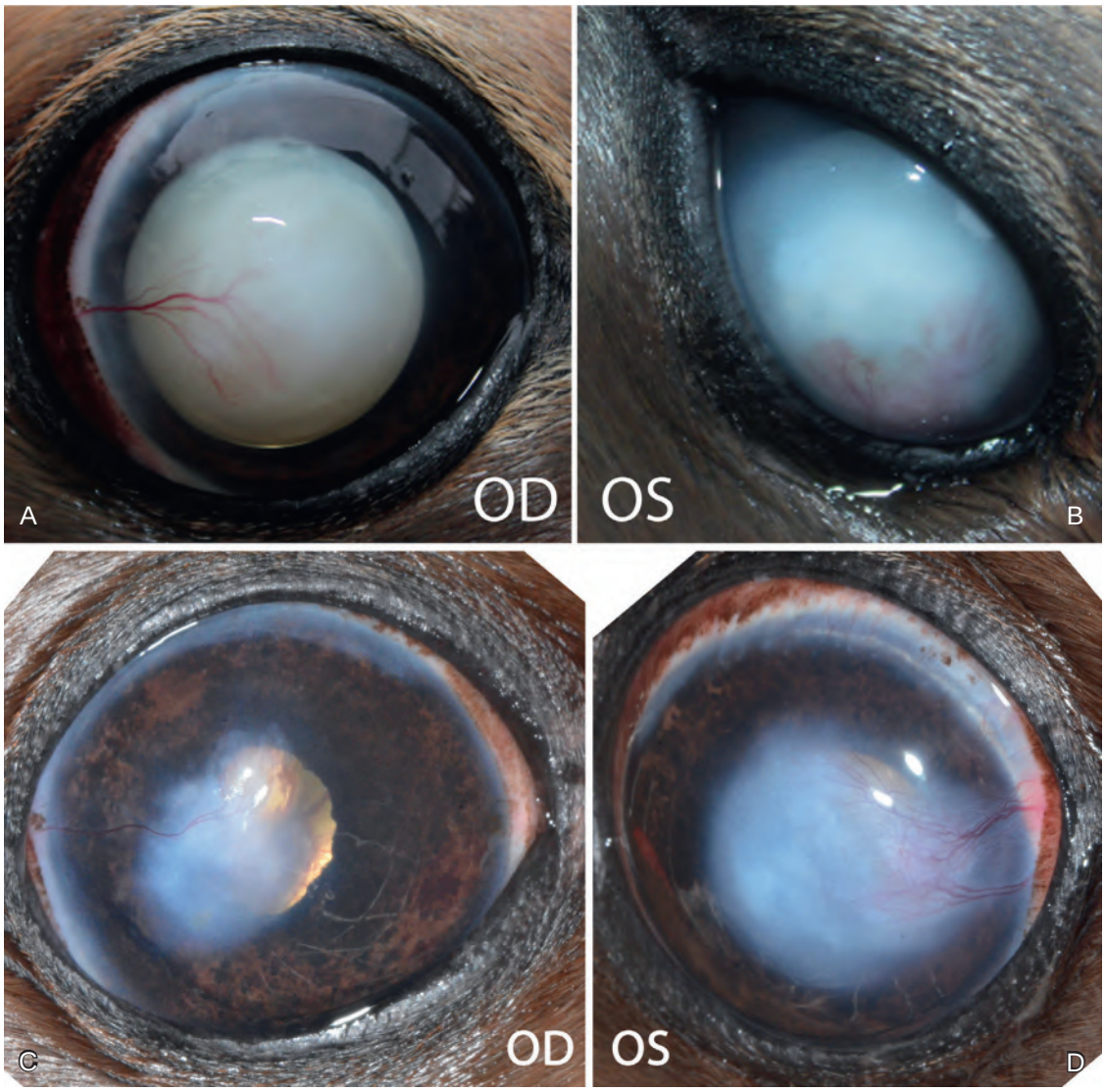
Aside from overall health and wellness, the status of the eyes is also important for predicting outcome. The best outcomes occur in eyes that have visually impairing cataracts that are not anteriorly luxated and that have not caused excessive chronic uveitis (Fig. 86.2). Following recovery, these eyes will typically only have a limbal opacity or scar and the rest of the cornea will be clear.

Eyes that have acute lens luxation can regain almost complete transparency or, in some cases, only have residual mild opacities (Fig. 86.3). Because lens luxations are severely painful in the acute stages, treatment with oral and topical nonsteroidal antiinflammatory medications is important, along with topical hypertonic saline solution, topical carbonic anhydrase inhibitors to control elevated intraocular pressure, and oral tramadol for control of pain. With time, the blepharospasm, epiphora, and photophobia improve and may appear resolved, although these signs may wax and wane. It is doubtful that the pinniped is pain free, and its stoic nature should not be cause for medications to be ceased.

In eyes with chronic anterior lens luxations, the cornea will be permanently opaque, although vision is improved in the majority of patients' eyes in this condition. In addition, the pain is vastly improved following surgical lens removal.

Outcomes and Sequelae

The ideal outcome is a pinniped patient that has resolution of pain and regains sight. The majority of eyes have resolution of pain; however, causes of continued blindness include severe keratopathy or retinal detachment.



• **Figure 86.3** (A and B) Right and left eyes of a California sea lion *(Zalophus californianus)* with bilateral anteriorly luxated mature cataracts. The corneas have vascularization, edema, and fibrosis secondary to the lens luxations. (C and D) Right and left eyes of the same California sea lion following bilateral lensectomies. The corneal fibrosis persists, but the animal's pain has resolved and useful vision is restored.

Retinal detachments are uncommon in pinnipeds following cataract surgery. Animals that appear at increased risk of retinal detachment are those that were stranded young who already had cataracts or developed them soon after being rescued. The vitreous in these animals is very liquefied, compared with the firm, clear, well-formed nature of normal pinniped vitreous material. Vitreal degeneration is a predisposing factor to retinal detachment.[28]

Preexisiting retinal detachments are not a reason to avoid surgical lensectomy in pinnipeds. The removal of the lens will alleviate pain if luxated, and it will remove the risk of luxation in the future in those eyes with cataracts. Lastly, cosmesis for the public may also be important in some collection animals. If cosmesis is not as important, then enucleation may be performed.

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SECTION 18

Ruminants

87

Giraffe Husbandry and Welfare

LAURIE J. GAGE

Introduction

Giraffe (*Giraffa* sp.) have specialized dietary, housing, and husbandry needs that must be met to ensure optimal welfare of these animals. A review of the captive giraffe mortalities, documented in the Zoological Information Management System (ZIMS) records, indicated many deaths were likely preventable.[1] The most common causes of death in the past 50 years were divided in approximate thirds between neonate-related deaths, infectious diseases, and trauma, with another 12% documented as restraint complications.[1] In recent years, trauma remains a significant problem, while there appear to be fewer neonate-related deaths and restraint complications. From 2012 through 2016, of the 761 reported giraffe deaths, 29% (222) were considered useable records, with 39% of those mortalities designated as trauma, 31% caused by noninfectious diseases, 14% neonate-related, and 6% related to restraint complications.[1] Because more than 70% of the reported causes of deaths for giraffe worldwide were not well-documented in the ZIMS records, these trends may only be extrapolated.

Cold Stress

Giraffe deaths related to hypothermia are still prevalent and are generally preventable.[2] In recent years, many cold weather–related giraffe deaths occurred during periods of unusually cold or damp weather in the more temperate regions of the southeastern and western United States.[2] Giraffe are intolerant of prolonged exposure to cold temperatures, and the resulting hypothermia may be exacerbated by damp or wet conditions. They lack the means to adapt to cold weather conditions and do not insulate themselves by growing a thick coat or depositing fat.[3] Most zoological institutions in the United States provide heated barns to giraffe whenever inclement weather occurs or when outdoor temperatures may consistently fall below 50°F (10°C).[4] To optimize their welfare and ability to survive colder weather conditions, giraffe should be fed a diet that meets their energy requirements and be provided shelter or housing that aids in their ability to maintain their core body temperature.[5] Even though giraffe fed an optimal diet may be more tolerant of spending time in suboptimal temperatures, they still must have access to a heated barn to enable them to maintain adequate core body temperature and to keep them comfortable. Giraffe barns should be heated to maintain an optimal temperature of 70°F (21°C) or higher.[4] Temperature monitoring devices within the giraffe barn placed at chest level of the giraffe will ensure the heat is distributed appropriately. While heaters located at the top of a barn will certainly warm a giraffe's head, they may not properly warm its body. Barns with heated floors may be preferable, as the heat will rise to warm the animal's legs and body.

Nutritional Disorders

Peracute mortality syndrome in giraffe has a suggested etiology of a negative energy balance caused by an inadequate diet[6] or the presence of dental disease,[7] where the event triggering death may be hypothermia or stress.[3,8,9] Urolithiasis is another disorder associated with nutrition, and while less common, it has been associated with a high dietary phosphorus content and a high level of nutrient concentrates in the daily feed.[8] Hoof disease and pancreatic disease may also be linked to nutritional imbalances in the diet. Many advances have been made in giraffe nutrition. Diets should be composed of a low-starch complete feed and a legume or legume-grass mixture of hay.[5,8] Crude protein levels of 10%–14%, fat 2%–5%, starch <5%, and a minimum of 25% acid detergent fiber meet current recommendations.[8] Woody browse, such as tree branches, shrubs, or woody vines, has been recommended to make up 10%–25% of the daily diet and is important for both nutrient supplementation and for behavioral enrichment.[5] Improving the nutrition for captive giraffe will help eliminate diet-related morbidity and mortality and increase the comfort and welfare of the animals. Ensure all feeding devices are constructed in such a way that giraffe cannot become entangled or entrapped. Cover ropes or chains used for suspending browse with sections of polyvinyl chloride pipe to prevent entanglement.

Hoof and Limb Disease

Overgrown hooves and lameness have been significant problems in captive giraffe. Modern husbandry and training

techniques as well as improved nutrition have been used to mitigate these problems (see also Chapter 88).[10]

Complications of Anesthesia

Giraffe tend to have more anesthetic-related complications and deaths than other Artiodactyla because of their unique anatomy and physiology, with most problems occurring during induction and recovery.[8] The use of a well-designed restraint device for induction, or a chute where a halter may be placed on a sedated giraffe to help control the head before induction, has helped prevent or mitigate injuries.[8] While anesthetic techniques have improved, applying training-based methods to obtain diagnostics such as radiographs of the feet and limbs,[10] blood collection, and maintenance procedures such as hoof trimming decreases the number of anesthetic events required to manage the animals and reduces the chances of complications of anesthesia.

Social Structure

Giraffe have a social structure, and individuals, especially females, may show a preference for certain other individuals within their herd.[11] For optimal welfare of the animals, these social preferences should be considered when selecting housing, planning movement between facilities, or planning how individual animals will be combined or housed within the same facility. A study in the United Kingdom of seven giraffe collections involving 40 individuals described bonds that are evident between animals, documenting that giraffe seek out preferred partners, and that partner preference is maintained over time.[11] The strength of these bonds must be considered when managing these animals. Overcrowding or separating compatible individual animals from each another may result in stress-related behavior. The movement of individual female giraffe between herds has a greater chance of being disruptive to these individuals than when moving the males, and this should be a consideration when determining the placement of animals for breeding purposes.[11]

Adult males may exhibit intolerance of other males within the same enclosure; however, serious altercations between males are rare. Adult bulls may be housed with one another or with younger males in the absence of females but should be offered ample space, especially in night quarters.[4]

Enrichment

Giraffe are inquisitive and benefit from a variety of enrichment items offered to them that will increase the amount of complexity and stimulation within their environment and provide opportunities for species-specific behavior.[12] Enrichment opportunities have improved the physiologic and psychologic well-being of captive animals. For giraffe, these may include puzzle feeders or elevated enrichment items that encourage the development of play and manipulation with their tongues. Giraffe that exhibit oral stereotypic behavior have responded favorably to the use of complex feeders that require the use of the tongue to access grain or hay.[13] The addition of slatted tops to hay feeders eliminated oral stereotypic behavior for some animals.[13] Providing means for giraffe to engage in more naturalistic foraging behavior and play behavior will improve their welfare. Each item introduced into the giraffe enclosure should be carefully scrutinized to ensure it is safe, because a number of giraffe have experienced morbidity and mortality due to unfortunate accidents involving entrapment or entanglement with enrichment items. Dangling enrichment, however safe it may seem, may best be offered only when staff members are available to respond in case of entrapment. Buckets or barrels with holes should be avoided or carefully evaluated to ensure giraffe cannot entrap an ossicone or other body part.

Trauma

Giraffe are prone to accidents that involve entrapment, entanglement, slips, and falls. Giraffe have died because their ossicones became entangled in hotwire, fencing, and enrichment devices. Openings in doors, fences, or furnishings within an enclosure that a giraffe may put its head or ossicone through are potential hazards. Giraffe have hung themselves in the crooks of trees, on pulley ropes designed to open stall doors, and in the triangular truss elements installed to support a shade structure (Fig. 87.1). Vertical

• **Figure 87.1** Giraffe (*Giraffa* sp.) shade structure with openings large enough for the head or ossicone; may cause a fatal entrapment.

posts spaced greater than 2 inches apart have proven deadly to giraffe, as they may slide their jaw or neck between the posts and become entrapped. Grass growing on the outside of a livestock wire fence may entice a giraffe, resulting in entrapment in the fence in its effort to gain access to the grass. Removing or mowing grass near fencing and covering any opening, including small keeper observation windows or ventilation doors in the wall of a giraffe barn with bars or mesh with spaces less than 2 inches, will help prevent giraffe head or ossicone entrapment. Materials on the outside of giraffe enclosures should be placed such that giraffe cannot reach them. One giraffe became entrapped in orange plastic safety construction netting on the outside of its exhibit and died. Icy, wet, or slippery footing, especially where there is a slant or grade to the exhibit, has proven fatal to giraffe. A faulty gate inside a giraffe barn allowed incompatible animals into the same stall, resulting in the death of another animal. Keepers and management staff should routinely inspect all giraffe areas, including areas outside of the enclosure that a giraffe might reach, and ensure there are no holes, cracks, or other spaces large enough for a giraffe to put its head or ossicone through. All ropes and wires within reach of a giraffe, including the keeper area, should be carefully evaluated to ensure entrapment is not possible. All heating elements should be checked routinely to ensure they are working, are clean and free of flammable materials nearby such as nests, and they must be installed and maintained properly.

Exhibit Design

Giraffe enclosures require thoughtful design to create an environment where there is adequate space for social interactions and where giraffe may carry out all of their natural behaviors. To prevent injury, ensure areas are free of obstructions and the footing is reasonably level and dry. Sloping exhibits may be hazardous. During cold and wet conditions, exhibits should be inspected to ensure the giraffe do not encounter areas with frozen or slippery footing. Flooring in barns should be constructed using a nonslip material to ensure traction. A giraffe restraint device is highly desirable, and those designed to allow the giraffe to pass thorough the device daily are optimal.[4]

Keeper Experience

Experienced keepers are key to ensuring the health and welfare of the animals. Giraffe do know their keepers, and trust may be built, which is very important when training techniques are applied. Knowledgeable keepers are aware of individual animal idiosyncrasies and will notice subtle changes in their behavior that will aid in early recognition of stressful or medical conditions. They will notice and eliminate problems before they could cause injury or death to the animals. They are able to evaluate the quality and condition of the diet and know what browse items are appropriate and which may be toxic or harmful to the animals. They will ensure the barn heaters are free of flammable material, such as bird nests, before the heaters are activated, and will know to check the exhibit when there is rainfall or freezing conditions to eliminate the chance a giraffe could slip or fall. Giraffe deaths have occurred from preventable barn fires and toxic oleander browse. Ensure new keepers are adequately trained and work together with experienced personnel to ensure optimal welfare.

Training

Using training techniques to address husbandry issues, such as the movement of animals within a facility, or for hoof maintenance, and to perform routine medical procedures, has been successful at many institutions housing giraffe. Training the animals not only helps limit the number of immobilizations necessary to conduct diagnostics or to treat issues, such as overgrown hooves, but it also provides a form of enrichment for the animals (see also Chapter 88).

Acknowledgments

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88

Lameness Diagnosis and Management in Zoo Giraffe

LIZA DADONE

Lameness is a common health problem of adult giraffe *(Giraffa spp.)* at many zoos. Up to 80% of giraffe immobilizations are done to address hoof overgrowth and limping,[1] indicating that lameness is an important health problem for this species. Giraffe anesthesia can have a 10% mortality rate,[1] and some giraffe have died during anesthesia for hoof work.[2] Due to risks associated with anesthesia, some cases are not diagnosed or treated until relatively late in the disease process. When lameness is severe or does not improve with treatment, giraffe are sometimes humanely euthanized.[3] This chapter describes diagnostics and treatments to help improve lameness management for zoo giraffe and highlights the need for early intervention and preventative care.

Etiology

Multiple abnormalities have been described in giraffe that may be associated with lameness. These include arthritis, bone cysts,[3,4] bruising, congenital deformities, dermatitis, fractures,[4–8] hoof overgrowth,[9–11] laminitis,[3,11–13] ligament injuries,[8,12] nutritional imbalances, osteitis,[5,10] osteochondrosis,[14] osteolysis,[4] osteomyelitis,[3] pigmented villonodular synovitis,[15] pododermatitis ("foot rot"),[3] sole foreign bodies,[10,14] tenosynovitis,[16] and trauma. While lameness is often related to pain, neurologic or mechanical dysfunction may also be an underlying cause.[17] When multiple lesions are present, identifying the most clinically significant cause of lameness can be challenging.

Hoof overgrowth is common in giraffe and may be associated with abnormal conformation,[13] hypothyroidism,[18] insufficient exercise, nutritional imbalances, inappropriate substrate, or trauma, among other causes.[17] Hoof overgrowth changes weight distribution in the foot, which can then lead to secondary pathologies such as arthritis or pedal osteitis.[5,10] While regular hoof trims are the standard of care for domestic horses, routine giraffe hoof trims have been relatively uncommon, except at zoos that train for voluntary hoof work.[10] For this reason, many zoos miss the opportunity to correct early changes of hoof shape before significant secondary pathology develops, leaving the giraffe with permanent hoof capsule distortions, which are challenging to correct once advanced (Fig. 88.1).

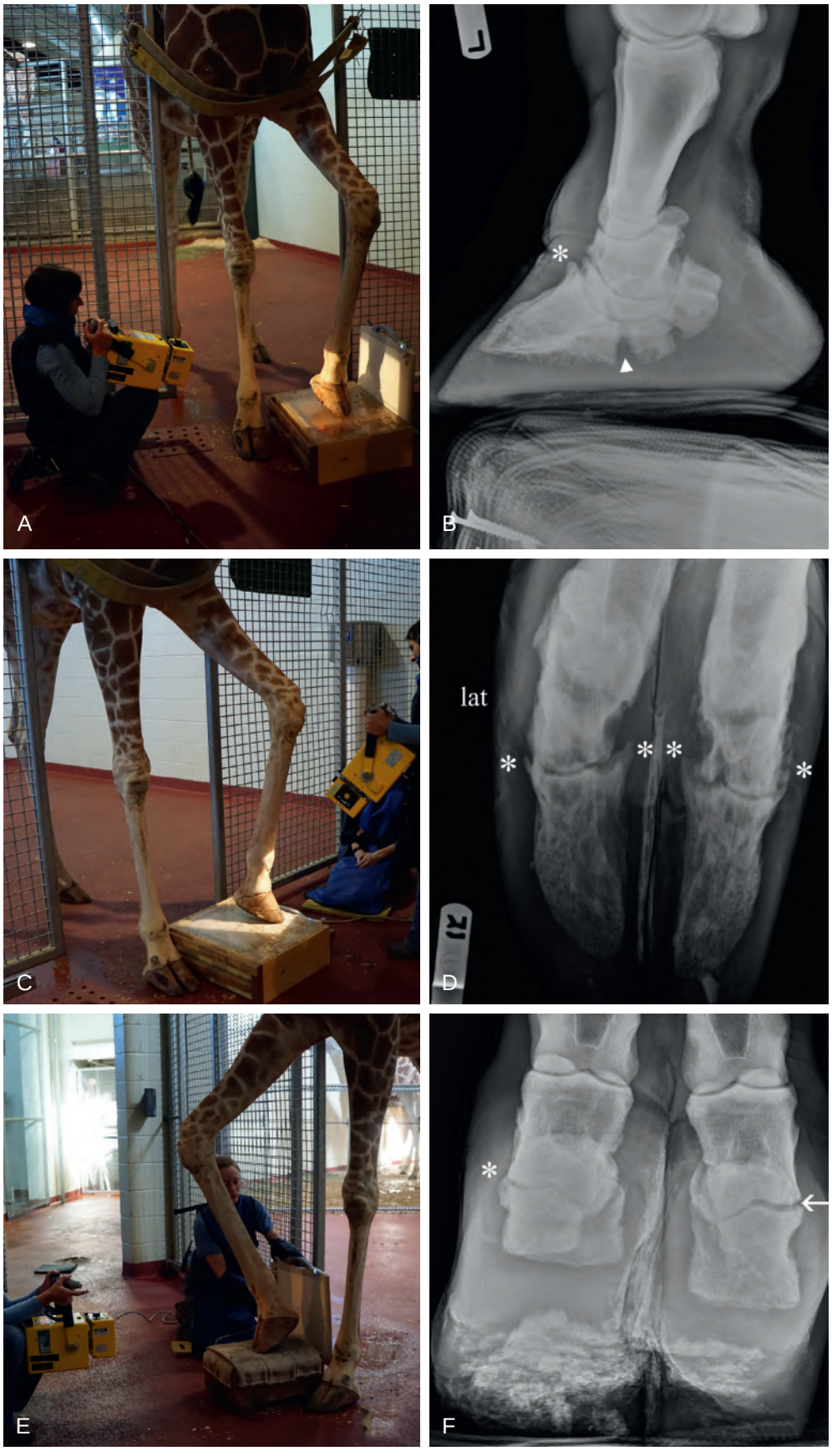
Arthropathies are frequently associated with giraffe lameness. A variety of etiologies have been reported, including mycoplasma-associated polyarthritis,[19] osteoarthritis,[5,19,20] osteochondrosis,[21] and septic and ulcerative arthritis.[16] In a survey of European zoos, joint problems were more commonly reported in front legs than in hind legs.[11] In a study of the front feet of all 22 giraffe at one zoo, all animals had radiographic evidence of osteoarthritis by age seven in the distal interphalangeal joint (coffin joint).[5] This seems to be a relatively early onset for a species with a maximum wild life span of 22 years for males and about 28 years for females,[22] but both age of arthritis onset and normal life span warrant further study.

Fractures have also been relatively commonly described in the distal phalangeal bone (pedal fractures) of the front foot.[5,6,10,23,24] Many of these cases are associated with severe pedal osteitis and hoof overgrowth, with fractures at the site of the deep digital flexor tendon insertion along the weight-bearing axis of the foot (Fig. 88.2).[5,10] Limb fractures at other sites are anecdotally reported and generally have a poor prognosis, except in relatively young animals.

Laminitis is likely underdiagnosed in zoo giraffe and may be either acute or chronic. On radiographs, laminitis present with a downward rotation of the digit, but upwards rotation of the distal phalangeal bone has also been described.[10,13] There are multiple possible etiologies for laminitis, but suboptimal diet for this specialized browser is likely a factor for some giraffe. In one survey, facilities that reported laminitis cases fed higher proportions of easily digestible feeds in the diet (bread, pure grains, fruits, vegetables) than facilities that did not report laminitis in their giraffe.[11] Further study is needed to better understand how rumen acidosis in giraffe, potentially caused by diets high in starch or low in fiber, may be related to some cases of laminitis.[13]

• **Figure 88.1** Photos comparing zoo and wild giraffe suggest that hoof distortions may begin in some zoo giraffe at a young age. Photos of the front foot of a 4-year-old female reticulated giraffe (*Giraffa camelopardalis reticulata*) in a zoo and, based on size approximation, front feet from a similarly aged young adult wild Nubian giraffe in Uganda. (A) In this zoo giraffe, the interdigital gap indicates interdigital overgrowth. (B) The wild giraffe has a thinner interdigital space, and the hooves are longer and smoother. (C) The sole of the zoo giraffe has asymmetric claw width, interdigital overgrowth, and a relatively hard, flat sole. (D) The wild giraffe has relatively symmetric toe width, a concave foot shape, a markedly more pliable sole surface, and longer toe tips.

• **Figure 88.2** Positioning for giraffe front foot radiographs using trained behaviors. (A) In a restricted contact setup, the giraffe is cued to position its foot on a hoof block, as close as possible to the radiograph plate. For a dorsomedial-palmarolateral oblique view (DMPLO), the radiograph generator is positioned at a 30° angle to the plate. (B) In the DMPLO radiograph image, this positioning partially separates the paired digits of the foot. Radiographic lesions may include upward rotation of both distal phalangeal bones, osteoarthritis of the distal interphalangeal joint (*), pedal osteitis of the solar margin, and an articular fracture of the medial phalangeal bone (Δ). In this case, the fractured piece is similar in size to the sesamoid bones and is at the site of the deep digital flexor tendon attachment. (C) Positioning for a Dorsoproximal-45°-Palmarodistal Oblique (DP-45-PDO) view. (D) In this DP-45-PDO view, pathologies include osteoarthritis of the distal interphalangeal joints (*), rotation of the lateral claw, and osteolysis of the second phalangeal bones near the joint surface. (E) Positioning for the Horizontal Dorsopalmar (HD-P) view. (F) In this HD-P view, lesions include marked hoof overgrowth with debris in the sole, osteoarthritis (*), and an asymmetric joint space associated with collateral ligament injuries (←).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Clinical Signs

The clinical appearance of lameness may vary based on the severity and location of the underlying problem. In severe cases, the giraffe may be non–weight-bearing or toe-touching. Moderate lameness could be associated with a head hike associated with each step, reduced activity, shifting weight repeatedly off a foot, or abnormal posture. More subtle signs may include behavioral changes such as reduced participation in trained behaviors, reduced shifting between exhibits, or adult giraffe spending time in recumbency during the day.

Diagnosis

A detailed visual examination is essential to localize the lameness and to determine if one or multiple limbs may be affected (Box 88.1). The clinician should also review the patient's history, husbandry, diet, exhibit, and holding area.

Thermography may help identify anatomy associated with some causes of lameness, without the need for restraint. Thermography is used to identify physiologically significant surface temperature asymmetries and has helped clinicians diagnose heat and inflammation associated with a distal phalangeal fracture[6] and with arthritis.[20] When possible, giraffe should be scanned indoors to help minimize possible imaging artifacts.

• BOX 88.1 The Giraffe Lameness Examination

Visual Assessment

- Lameness: Rate severity of lameness; identify if one or more limbs involved; assess for swelling, bleeding, skin lesions.
- Gait: Evaluate for head hike, joint laxity, proprioceptive deficits.

General Conformation

- Weight-bearing: Assess relative weight distribution for all four legs.
- Hoof assessment: Check for overgrowth (claw length asymmetries, coronary band steps, interdigital gaps), excessive wear (including toe tip scuffing), coronary band abnormalities.

Physical Examination

- Often limited or not possible without previous training or sedation.
- Hoof examination: Assess for hoof overgrowth, laminitis, cracks, bruises, pododermatitis, sole foreign bodies.

Diagnostics

- Radiographs: Multiple views of region of concern; when possible, also image contralateral leg.
- +/– Bloodwork: Screen for disease; check calcium:phosphorus ratio.
- +/– Ultrasound of ligaments
- +/– Thermography: May help localize abnormalities and quantify response to treatments.

With training or anesthesia, multiple other imaging modalities may be used to help identify pathologies associated with lameness. When possible, radiographs should be taken from multiple views (see Fig. 88.2) and may often be accomplished with training.[10] When imaging the foot, it is recommended to place the hoof on an elevated surface so the radiograph can include the palmar aspect of the distal phalangeal bone and some of the hoof sole. Depending on the case, additional diagnostics may include ligament ultrasound[12] or arthroscopy.[25]

Treatment

A combination of husbandry and medical management techniques can be used to treat many causes of giraffe lameness (Box 88.2, Table 88.1). Depending on the severity or chronicity, symptomatic treatment may be initially attempted. But if the lameness is severe or refractory to treatment, further diagnostics are warranted so the underlying cause can be identified and appropriately managed.

If lameness is attributed to a lower limb or if hoof overgrowth is seen, the foot should be closely evaluated. The sole should be brushed out and trimmed to address any possible abscess, sole foreign body, or wounds. As much as possible, hooves should be trimmed to remove overgrowth from both the hoof wall and sole; trimming only the toe tip does not correct hoof angle changes related to heel overgrowth and may predispose the foot to arthritis and pedal osteitis.[5,10] Depending on the case, chronic foot rot should be managed aggressively, as it can progress to life-threatening secondary osteomyelitis.[3]

In giraffe with distal phalangeal fractures, surgical management is generally not recommended. Treatment should focus on restoring normal weight-bearing to the foot by removing hoof overgrowth. In one case, a distal phalangeal fracture was successfully managed with hoof trims and a temporary wooden block applied to the sole.[22] In multiple

• BOX 88.2 Husbandry Changes for Giraffe Lameness Management

Management Changes

- Reduce activity: Separate from some or all of herd, stall rest. Based on patient temperament and severity of lameness, this could be for hours or multiple days.
- Change substrate: Sand or mats on floor; remove larger pebbles from outdoor exhibit.
- Evaluate and modify exhibit if warranted.
- +/– Diet change: If laminitis is suspected, consider short-term reduction or elimination of grain from diet. If diet change is made, monitor weight and for changes in lameness.

Hoof Care

- Remove hoof overgrowth from both toe tip and sole. This helps normalize weight distribution in the foot and removes sole foreign bodies.

TABLE 88.1 Formulary for Giraffe (*Giraffa spp.*) Lameness Management*

Category	Drug or Treatment	Dose	Route	Dosing	Possible Applications	Comments
NSAIDs	Firocoxib	0.1–0.3 mg/kg	p.o.	q24h	Arthritis P3 fracture Laminitis Swelling	
	Flunixin meglumine	1.1 mg/kg	s.c.	q24h for 1–3 days	Arthritis P3 fracture Laminitis Swelling	
	Meloxicam	0.2 mg/kg 0.3–0.5 mg/kg	s.c. p.o	once q24h	Arthritis P3 fracture Laminitis Swelling	
	Phenylbutazone	2–6 mg/kg 7–8 mg/kg	p.o. p.o.	q24h q48h	Arthritis P3 fracture Laminitis Swelling	Recommend washout period after 7–14 days of daily dosing
Opioids	Tramadol	0.5 mg/kg	p.o.	q24h	Arthritis P3 fracture Laminitis Swelling	
Joint Supplements	Adequan	0.75 mg/kg	i.m.	Loading dose: q5 days × 7 dose, then q30 days	Arthritis	
	Hyaluronan	Loading dose: 0.3–0.5 mg/kg Maintenance dose: 0.15–0.3 mg/kg	p.o.	Loading dose: q24h × 14 days, then maintenance dose q24h	Arthritis	
Topicals	Cold water hydrotherapy	5–10 min	Topical	q24h—b.i.d.	Laminitis	Based on individual giraffe temperament, consider irrigating with hose or a foot bath
	DMSO		Topical	q24h	Inflammation	Wear gloves when applying
	Epsom salts		Foot bath	q24h	Abscess Sole foreign body	
Antibiotics	Ceftiofur	6–9 mg/kg	i.m. or s.c.	q24h 3–14 days	Infection	*n* = 1 (calf)
	Doxycycline	4–5 mg/kg	p.o.	q24h	Infection	Sometimes used in combination with rifampin
	Enrofloxacin	4–8 mg/kg	p.o.	q24h × 5 days	Infection	
	Sulfadiazine trimethoprim	30–45 mg/kg	p.o.	q24h	Infection	For some individuals, oral dosing is easier with powdered instead of pill formulations
	Tulathromycin	2.5 mg/kg	s.c.	q72h	Infection	*n* = 1
	Rifampin	6–7 mg/kg	p.o.	q24h	Infection	Sometimes used in combination with doxycycline

Continued

TABLE 88.1 Formulary for Giraffe (*Giraffa spp.*) Lameness Management—cont'd

Category	Drug or Treatment	Dose	Route	Dosing	Possible Applications	Comments
Others	Chiropractic				Abnormal conformation Chronic lameness	May help correct changes to spine or other limbs associated with abnormal weight distribution
	Gabapentin	1–5 mg/kg	s.i.d.	q24h—b.i.d.	Suspect spinal injury Chronic lameness	Consider starting at lower doses and tapering up as needed. Has been given with NSAIDs and tramadol
	Methocarbamol	12–25 mg/kg	p.o.	q24h	Spinal injury Abnormal body posture	
	Therapy laser	3000 J/digit 8000 J to lateral hock	Topical Topical	q72h for 1 mo, then 1× weekly q72h for 1 mo	Arthritis Cellulitis	Thermography may help identify inflammation and quantify if treatment is helping. If treating digit, target from coronary band to proximal to fetlock joint (avoid hoof wall). Consider using noncontact laser on PVC extension

*Listed doses are anecdotal and based on the author's clinical experience, as no pharmacokinetic data are currently available for giraffe. Clinicians are advised to assess whether any of these doses are appropriate to an individual case and should be especially cautious when giving oral medications to ruminants. Results from an analgesia survey[27] or from the Species360 (2017), zims.Species360.org Global Drug Usage reports may also provide additional medicating options.

other cases, clinical improvement has been seen with just corrective hoof trims (Dadone, unpublished results).

A distal metatarsal fracture was successfully managed in a 15-month-old giraffe by using external coaptation with a cast, prolonged stall rest, and pain management.[26] In this case, serial radiographs were used to monitor callus formation and bone remodeling, and the final cast was removed 5 months after the initial injury.

Surgical management has been reported to be effective in a small number of cases, generally involving young animals. In a 4-month-old giraffe, arthroscopy was used to remove an avulsion fracture along the lateral trochlear ridge of the femur.[8] Arthroscopy was also successful for diagnosing and surgically repairing an osteochondral fragment in the metacarpophalangeal joint of an 8-month-old giraffe.[25]

Operant Training

Trained medical behaviors using positive reinforcement strategies are key for improving giraffe care.[10] This increases patient access for medical workup and repeat treatments. Written safety protocols and restricted contact setups are strongly recommended to help ensure both human and patient safety.[10] The author recommends that target training and routine hoof care start for giraffe by about 1 year of age, similar to what is recommended for domestic horses.

Prevention

Further study is needed to identify best practices to prevent many causes of lameness in giraffe. Like other

megavertebrates, increased attention to foot health, substrate, diet, and routine foot care will likely reduce morbidity and early mortality in this species. Opportunistic complete necropsies that include the joints and feet will help improve our understanding of causes of lameness in giraffe. For the giraffe in our care, training may provide opportunities to develop new, more effective treatments for lameness.

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89

Mass Mortality Events Affecting Saiga Antelope of Central Asia

RICHARD ANTHONY KOCK AND SARAH ROBINSON

Saiga *(Saiga tatarica)* are a unique Central Asian antelope, composed of two subspecies (*S. t. tatarica* and *S. t. mongolica*), relatively unchanged in form since the mid to late quaternary period based on fossil remains.[1] It is a species highly adapted to the semiarid desert steppes of Russia, Kazakhstan, and Mongolia with more than 90% of the global population in the Uralsk and Betpak-Dala regions of Kazakhstan. They are ruminants, exploiting a rich diversity of plants for their nutrition[2–6] in highly saline, often mineral (e.g., copper) deficient pasture and sometimes soil toxic conditions with high boron that few species can tolerate.[7] Temperatures plummet to −16°C on average in January, with severe conditions reducing this further to −40°C at times, and temperatures rise to higher than 30°C in summer. To cope with these harsh conditions, saiga have a compact body mass and grow a highly insulated thick hair coat, shed in spring. They possess unusual nasal anatomy with an extended proboscis, which reduces the risks associated with breathing the freezing and dusty air and may help drying of exhaled air to help conserve precious moisture that is unavailable over much of the year. Key to survival in these semidesert conditions is annual migration[1,2] and careful calving site selection to optimize survival.[8] The antelope runs at remarkable speeds for prolonged periods and covers huge distances over a year or even a day, avoiding contact with humans consistent with historic poaching pressure. With few other prey species in these vast lands, predators seek out saiga, but none other than humans in 4 × 4 vehicles can keep up. Predators benefit from chance encounters and two periods of the year when the saiga must stop for calving and rutting. These periods are highly synchronized and tuned into the vegetation cycles to ensure a rising plane of nutrition in spring for calving and lactation and optimal body condition in the autumn for the rut to ensure maximum fertility. The population of females and some younger males cluster around May in herds of a few to tens of thousands of animals. They form dense birthing aggregations over a week or so and produce one to three calves each, with the majority of females reproducing in their first year of life and having the highest relative fetal biomass of any mammal.[9] This extraordinary lifestyle and reproduction is not an accident. These harsh lands have developed a hard bargain with this species; annual losses will be naturally high, with sometimes catastrophic declines, from which they can and do rapidly recover.

Their biggest challenge in recent times has come from humans, an even more daunting challenge than the environment and from which they can only run. Poaching with high velocity rifles has been the single most serious cause of premature death over recent decades, with numbers at the end of the 20th century declining dramatically from more than a million to a few tens of thousands of individuals.[10] As with so many other species, this toxic mix of human persecution and increasing environmental challenges such as habitat fragmentation threatens this species with extinction.[11]

The major causes of mass mortality events (MMEs), other than humans, are heavy snowfall or freezing rain (known as *dzhut* in Kazakh) and disease-related events (Table 89.1). The majority of diseases were attributed to *Pasteurella* species by contemporary observers and described under the umbrella term of "pasteurellosis." The largest of these occurred in 1981,[12,13] 1984,[14,15] 1988,[16–18] and 2015,[19] causing the death of tens to hundreds of thousands of animals (Table 89.1; Fig. 89.1). Some smaller disease events have also been attributed to *Pasteurella* in 1974,[20] 2011,[21,22] 2012,[23] and 2013.[24] Both large and small events were inadequately documented for diagnosis, with the exception of 2015, and it was notable in 1974 that the saiga had been chased off feed crops over a number of days prior to their death. The majority of *Pasteurella*-related die-offs occurred around calving but not exclusively, and a number of the largest events were clustered in the northern part of the Betpak-Dala population range (Fig. 89.2).

TABLE 89.1 A Summary of Mass Mortality Events in Saiga *(Saiga tatarica)* Affecting More Than 1000 Animals

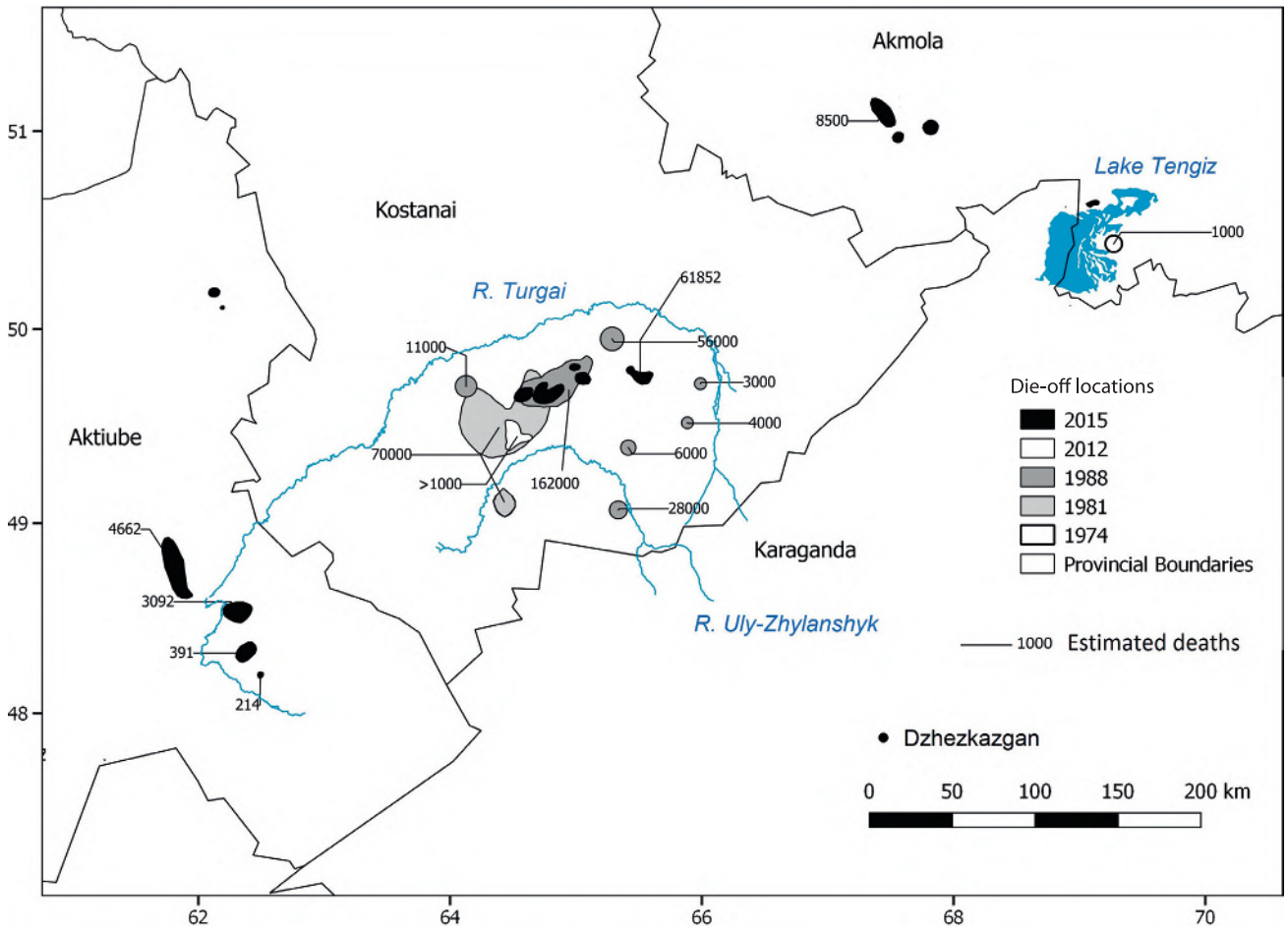
Syndrome	Year	Population	Deaths	Mortality (%)*	Source
Peste des petits ruminants	2017	Altai	5400	54	OIE FAO CMC[31]; WWF[32]
Hemorrhagic septicemia	2015	Betpak-Dala	~210,000	~88	IUCN/SSC Antelope Specialist Group & Saiga Conservation Alliance[11]
"Pasteurellosis"	2012	Betpak-Dala	>1000	<1	Zuther[23]; Dieterich[41]
"Pasteurellosis" or Fog fever/bloat	2010	Ural	12,000	75	Kock et al.[21]; Kock[42]
"Pasteurellosis"	1988	Betpak-Dala	270,000	75	Institute of Zoology & Betpak-Dala State Hunting Organisation[16,17]; Turgai Regional Executive Committee[18]
"Pasteurellosis"	1984	Ural	110,000	73	Institute of Zoology and Department of Reserves and Hunting[14,43]; Aikimbaev et al.[15]
"Pasteurellosis"	1981	Betpak-Dala	70,000	15	Institute of Zoology and Department of Reserves and Hunting[12,13]
Foot and mouth disease	1957	Kalmykia	40,000	9	Bannikov et al.[36]
Foot and mouth disease	1967	Betpak-Dala	50,000	—	Fadeev and Sludskii[2]

*As % of regional population (Betpak-Dala, Ural, Altai, Kalmykia).

Of the largest MMEs, in 1981 "pasteurellosis" was given by the cited sources as the cause of death, but the specific identity of the organism and detailed gross pathology information that might have allowed confirmation of the syndrome were not available. *P. multocida* was isolated from dead saiga in 1988, and there is reasonable supporting evidence for hemorrhagic septicemia (HS), but animals had suffered from severe *dhzut* during the previous winter. In 1984, an organism described as *P. haemolytica* was isolated, and the die-off was possibly related to a pneumonic form of pasteurellosis. In the 2010 die-off in Ural, *Pasteurella multicoda* was isolated, but the symptoms resembled those of a pasture-related disease (e.g., bloat or fog-fever), more than those of *Pasteurella*-related syndromes. A small die-off of 400 animals among survivors in 2011, again with isolation of the same organism, occurred at exactly the same location as the previous year's die-off, while animals grazing elsewhere remained healthy, suggesting location and perhaps pasture as the primary factor.[25–27]

For the 2015 event, the identity of the principal pathogen, *P. multocida* serotype B, and syndrome, HS, is of little doubt.[19] Moreover, climatic conditions during this event and the two other likely large HS die-offs in Betpak-Dala (1981, 1988) were found to be on average warmer and more humid compared with control calving areas in which die-offs did not occur.[19] Such patterns have also been associated with HS prevalence in domestic livestock.[28,29]

Die-off is not unique to Kazakh populations. In late 2016, the small Mongolian population in the Gobi Altai region of Western Mongolia became infected with peste des petits ruminants virus (PPRV), as a result of spillover from livestock during the first epidemic of this disease to be recorded in the country.[30] The saiga proved to be highly susceptible to the virus, perhaps indicative of their close phylogeny to the family Caprinae (the main host to this disease), in which they used to be classified, while only more recently being incorporated into the Antilopini on a basis of modern genetic analysis. In the Altai the saiga expressed a virulent form of the disease with mortality reaching more than 5000 animals or 54% of the population within a matter of weeks,[31,32] and the epidemic continued up until June 2017, affecting the entire population and other wildlife species. In addition, much was written on foot and mouth disease (FMD) in saiga following outbreaks in the 1950s and 1960s, but these tended to cause mortality mainly in calves, and only on two occasions were many thousands of animals affected.[2] Besides these reported infections, and a number of other MMEs with uncertain diagnoses and etiologies, saiga die from expected mortality in calves around time of birth, from sudden weather shifts, from low birth weight and weakness where there is failure to suckle, from predation during the aggregation, and over the first few months of life. In adults, mortality occurs



• **Figure 89.1** Locations of Saiga *(Saiga tatarica)* die-off events in Betpak-Dala 1974–2015. *Notes to Fig. 89.1: 2015 sites*: Sources of estimated deaths: authors' observations; Zhubaniyz.[40] *2012 site*: Zuther.[23] *1988 sites*: The figures given here, originally based on total deaths of over 400,000, which are believed to have been an overestimate, have been re-scaled proportionally to give the eventual estimated total of 270,000.[16] They are thus indicative only and meant to give an impression of *relative* numbers dying at each site. *1981 site*: Institute of Zoology and Department of Reserves and Hunting.[12]

• **Figure 89.2** Mass mortality of more than 60,000 saiga *(Saiga tatarica)* in Amangeldy, Turgai River basin, Kazakh steppe in May 2015, showing all the adults in the picture dead and calves wandering lost, apparently unaffected by this catastrophe.

from starvation or exhaustion associated with living on a knife-edge of nutrition and high metabolic demand in an uncertain environment, as well as from predation and calving-associated mortality such as dystocia.[33–35]

Discussion

Saiga antelope remain a keystone species of the Central Asian steppe which, following extinctions of the wild horse and ass, lacks significant competition for the dry desert-like grasslands other than with livestock and rodents. Their populations have been sorely depleted, due to displacement by agriculture and settlement in parts of their range, and more recently from disease causing MMEs and poaching. Older literature is remarkably silent about MME other than from *dzhut* and FMD events in the 1950s and 1960s, which affected up to 50,000 calves.[2,36] Pasteurellaceae are prominent as a feature in recent events, but this may reflect modern diagnostic

methods rather than emergence. Commensal parasites, part of the natural microbiome (e.g., *Pasteurella* spp. and other organisms like *Clostridium* spp.) are frequently isolated from carcasses and may be postmortem invaders. For example, *C. perfringens* was detected by polymerase chain reaction in ~40% saiga carcasses sampled during the HS mortality in 2015, but the use of immunohistochemical diagnostic techniques showed these to be late invaders with *Pasteurella* as the primary disease factor. In contrast, *P. multocida* was isolated from carcasses in Mongolia where PPRV was the primary factor (State Central Veterinary Laboratory [SCVL], personal communication, 2017). The nature of disease may be complicated, and the isolation of an organism may be a convenient descriptor while not always enlightening the underlying causal factors. A broader view of causes of death is critical to modern approaches to epidemiology and disease understanding. Whether these reported infections in saiga are a result of the conditions saiga experience in a human-influenced landscape is not certain, but there is evidence to suggest human factors may underpin some of these mortalities. There is no conclusive evidence that weather events associated with saiga mortality are a result of climate change, but trends over the last two decades are of warmer wetter spring weather in the region. This is supported by the lack of *Pasteurella*-related mortality events recorded in the Ustiurt population, or to our knowledge in the Mongolian populations of saiga where conditions are drier. There is some clustering of cases between years in the same general geographic zones, and hence a possible locational/environmental component to saiga pasteurellosis. Historic FMD and current PPRV are spillover infections from livestock with no indication of historic maintenance of these diseases in saiga populations, again implicating human influence. Some death is the inevitable cost of a high reproductive rate, with high calf mortalities during inclement weather or from dystocia where nutrition is very good and in the absence of historic competitors and with high fetal growth rates. Much still needs to be learned about MMEs in saiga, the most significant question being the clustering of the MMEs reported in the Kazakh Betpak-Dala population. This might be coincidence, a reflection of optimal calving zones and vegetation, or perhaps there are topographical, soil, mineral, hydrologic, or other environmental characteristics such as the association with humidity, which have some role to play in triggering these events. A connection between microelement levels and MMEs in steppe species has been suggested by some workers[37,38]; suspected marginal tissue copper levels, based on domestic animal baselines, have been recorded in samples taken from saiga carcasses by Berkinbaev[39] and in 2015.[19] What this or other elements might play in the susceptibility of saiga to infections remains to be determined.

Whatever nature has to throw at the saiga, the future of this remarkable and resilient species is truly in the hands of man.

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90

Musk Ox Sedation and Anesthesia

CARSTEN GRØNDAHL

Musk oxen *(Ovibos moschatus)* are some of the toughest ruminants—their resilience and tolerance to environmental challenges in the Arctic are unsurpassed. They are examples of extreme adaptation. Musk oxen are true ruminants and, therefore, when sedated or anesthetized, must remain in a sternal position to allow for saliva to drain and for rumen gases to be eructated. This minimizes the workload of ventilation and maximizes gas exchange. Their lungs are small, and supplemental oxygen is almost always mandatory, as the arterial oxygenation drops rapidly when oxygen is not supplemented in most anesthetic combinations.[1]

Biology

The musk ox is thought to have experienced significant genetic bottlenecks. Despite these bottlenecks, two subspecies of musk ox, *O. m. wardi* (white faced musk ox or Greenlandic musk ox) and *O. m. moschatus* (Barren Ground musk ox), have been commonly accepted based on their morphologic differences and geographic separation. However, when control-region sequences of mitochondrial DNA were compared among 37 musk oxen, there was little variation found among the musk oxen sampled. These results do not support musk oxen subspecies.[2]

Distribution, Habitat, Size, and Weight

Musk oxen primarily live in the Canadian Arctic and Greenland, with small introduced populations in Sweden, Siberia, Norway, and Alaska. The size varies but is often overestimated due to the thick fur. The Canadian variant is often bigger (weighing 286 kg on average and up to 410 kg[3]) than the Greenlandic type, the latter having free-ranging cows weighing 160–210 kg and free-ranging bulls weighing 270–320 kg.[4]

Environmental Considerations

Calves are born on snow-covered ground in May and should not be on gravel substrate, as they will often eat the gravel and develop geosediment-related disease. They do not have water-shedding fur. It is important to note that there is no precipitation in the arctic in May, and calves may develop pneumonia and die if they become hypothermic from a soaking rain. The calves are also very sensitive to overheating. Great care should be taken on sunny days during the first months of life, as calves may fatally overheat, even when environmental temperatures are not elevated. Ensuring shade and monitoring the cow and calf to ensure the use of shade provided is crucial. It is important not to put calves in with other musk ox cows other than their dams, as cows will not tolerate other calves and will often show aggression toward the unfamiliar calf.

Restraint/Sedation/Immobilization

Reasons for restraint include identification and marking of calves, treatment of calf diarrhea, transport, health surveillance, hoof trimming, treatment of trauma from other musk ox, dystocia, and blunting of horn tips with epoxy.

A special concern regarding restraint/anesthesia is that musk oxen are very heat sensitive. Do not restrain/sedate or immobilize musk oxen of any age on warm days, and continuously monitor temperature with a rectal probe.

Physical and Chemical Restraint

In the first 3 months, physical restraint may be performed with experienced "cowboys." We often use a bale of straw as a treatment table, and if physical restraint is performed frequently, the calves may become tame (Fig. 90.1).

If chemical restraint is needed for these young calves (such as for transport to a medical facility, radiography, ultrasound, or other procedure), sedation using an alpha-2 agonist opioid, and benzodiazepine combination is usually adequate. For example, a 3.5-month-old calf (weighing 22 kg) was sedated for transport and radiology using 1 mg detomidine, 2 mg butorphanol, and 1 mg midazolam. The calf was calm and recumbent but awake during transport and radiography.

Larger calves older than 3 months up to 1 year can be sedated with an alpha-2 agonist, butorphanol/methadone, and midazolam, which may be supplemented with low dose ketamine.

For animals over 1 year, sedation is recommended only in very tame or debilitated animals, as sedation of healthy

• **Figure 90.1** Manually restraining a young musk ox *(Ovibos moschatus)* calf on a bale of straw.

animals often provides unsatisfactory results. Animals exhausted due to dystocia or traumatized animals with major injuries (e.g., pneumothorax) may be sedated for corrective surgery or obstetric maneuvers with less cardiovascular and respiratory compromise than during general anesthesia. In these cases, combining an alpha-2 agonist, butorphanol, midazolam, and occasionally low dose ketamine has worked well.

Immobilization

Many drug combinations have been used in musk ox including xylazine-ketamine, ketamine-medetomidine, tiletamine-zolazepam-medetomidine, or ketamine-medetomidine-butorphanol. Combinations with potent opioids include carfentanil-xylazine, carfentanil-xylazine-ketamine, etorphine-xylazine, or etorphine-xylazine-midazolam-ketamine. See Table 90.1 for Zoological Information Management System (ZIMS) (Species360 [2017], zims.Species360.org) anesthesia summary reports doses used (primarily North American use). Other combinations like BAM (butorphanol-azaperone-medetomidine) or tiletamine-zolazepam combinations have been used by colleagues, but published evaluations are not available (Kimberlee Beckmen, personal communication).

Long needles of adequate length to penetrate the thick hair coat should always be used. Snow and ice can coat the hair making intramuscular (IM) darting challenging. Lubricate the needle with sterile silicone oil (not spray). If there is not an adequate effect after 12–16 minutes, dart again with 50%–100% of initial dosing depending on the efficacy of the first dart. An animal not recumbent would receive a full dose, a heavily sedated animal that is recumbent but able to get up when approached would receive half the initial dose, and a recumbent animal that cannot get up when approached, but who is able to lift the head and look around, would receive 20%–30% of initial dosing often by hand injection, ensuring that a good IM injection is made.

TABLE 90.1 Musk Ox *(Ovibos moschatus)* Anesthesia Summary Reports

	Mean	Median	Range
Carfentanil citrate	0.005	0.004	0.003–0.016
Xylazine	0.19	0.19	0.08–0.44
Etorphine	0.014	0.012	0.007–0.032
Xylazine	0.17	0.16	0.09–0.33
Ketamine	2.21	1.90	0.43–5.33
Medetomidine	0.046	0.047	0.028–0.07
Butorphanol	0.11	0.09	0.048–0.28
Ketamine	2.72	2.80	1.0–3.92
Medetomidine	0.047	0.044	0.015–0.12
Carfentanil citrate	0.006	0.006	0.003–0.13
Ketamine	1.21	0.75	0.36–4.17
Xylazine	0.10	0.07	0.045–0.3

Data from General Zoological Information Management System (ZIMS) database 2017.
NOTE: All doses are mg/kg and are compiled of 455 anesthesia events on 108 musk oxen.

• **Figure 90.2** Mask inducing a young musk ox *(Ovibos moschatus)* calf with sevoflurane in oxygen.

If walking into a transport crate is the desired procedure, administer 75%–80% of a normal dose, and after approximately 6–8 minutes, blindfold the animal and guide it into the crate.

Gas Anesthesia

For prolonged procedures, gas anesthesia is an option, and both sevoflurane and isoflurane work well. When mask inducing smaller calves or sedated juveniles, sevoflurane gives less mucosal membrane stimulation and a faster and easier induction of anesthesia (Fig. 90.2). Intubating musk ox is as challenging as for all small- to medium-sized ruminants. The largest animals may be intubated manually with a guide tube through the endotracheal tube and by ensuring the guide tube placement is between the plica vocalis. Most

TABLE 90.2 Author's Recommended Doses for Sedation and Anesthesia in Musk Ox *(Ovibos moschatus)*

Musk Ox Sedation	
Age	**Drug Combination (Total mg/animal)**
0–3 months	Manual restraint; may be supplemented with 0.5–1 mg detomidine + 1–2 mg butorphanol + 0.5–1 mg midazolam
4–6 months	1.5–3 mg detomidine + 3–6 mg butorphanol + 1.5–4 mg midazolam
7–9 months	2.5–3.5 mg detomidine + 4–6 mg butorphanol + 2–4 mg midazolam
10–12 months	3–5 mg detomidine + 5–8 mg butorphanol + 3–5 mg midazolam
Adult musk ox sedation (only recommended for very tame or debilitated animals)	17 mg detomidine + 10 mg butorphanol + 10 mg methadone (used successfully on a 200 kg adult cow with pneumothorax)
Musk Ox Anesthesia	
Age	Drug combination (total mg/animal) or per 100 kg bodyweight (bwt)
Yearling	0.6–0.8 mg etorphine + 6–8 mg xylazine + 8–10 ketamine + 1 mg midazolam
Subadult 14–18 months	0.8–1.0 mg etorphine + 8–10 mg xylazine + 8–10 mg ketamine + 1–2 mg midazolam
2 years old	1–1.2 mg etorphine + 10–12 mg xylazine + 10–12 mg ketamine + 1–2 mg midazolam
Adult cows (per 100 kg bwt)	0.8–1 mg etorphine + 8–10 mg xylazine + 10 mg ketamine + 1 mg midazolam
Adult bulls (per 100 kg bwt)	0.6–1 mg etorphine + 6–10 mg xylazine + 10 mg ketamine + 1 mg midazolam
Bulls usually need less per kg bodyweight than cows: several times bulls (300 kg) have been anesthetized with a dose calculated for 200 kg cow	1.8 mg etorphine + 18 mg xylazine + 10 mg ketamine (used successfully on a docile 315 kg bull for a hood trim lasting 45 min)

animals will require a long straight laryngoscope with a good light source for direct visualization of the larynx and placement of the tube. Use preferably high-volume low-pressure cuffs. If using a low volume high-pressure cuff, take special care not to overinflate the cuff, which can lead to tracheal mucosa ischemia and serious complications. See Table 90.2 for recommended doses of sedation and anesthesia.

Free-ranging musk ox immobilization (e.g., satellite collar) involves slightly higher doses; Greenlandic musk oxen cows in October,[5] body weight around 200 kg, have successfully been immobilized with 1–1.2 mg etorphine/100 kg + 15 mg xylazine/100 kg + 20 mg ketamine /100 kg + 0.15 mg medetomidine /100 kg.

It is the author's experience that combining two different alpha-2 agonists (xylazine and medetomidine) and two opioids (butorphanol and methadone) gives a synergic effect while not increasing the side effects. This is maybe due to different subtype affinity for the four alpha-2 receptors and the mu and kappa opioid subtype receptors.

Use of Long-Acting Tranquilizers

Long-acting tranquilizers such as zuclopenthixol-acetate Cisordinol-Acutard 50 mg/mL and zuclopenthixol decanoate Cisordinol depot 200 mg/mL (Lundbeck Pharma, Valby, Denmark) are effective at a dose of 1 mL/80–100 kg for the introduction of new herd members, introduction to a new enclosure, or transport. Both the short-acting (72 hours) and depot (21 days) formulations are very effective. Older reports[6] indicate a good effect and negligible side effects using perphenazine (Trilafon Dekanoat, Orion Pharma, Denmark) at a dose of 0.51 mg/kg.

Perianesthesia

If possible prior to anesthesia, food and water are withheld overnight for adults; however, water is not withheld from juvenile animals.

During the anesthetic procedure, always keep recumbent animals in a sternal position or as close as possible to sternal. Point the nose downward so saliva may dribble out.

Supplement oxygen by placing a nasal insufflation catheter deep into the nasal cavity (1–2 L O_2/100 kg) and monitor arterial saturation using pulse oximetry.

When working with adult animals, secure the workspace. Have a designated person responsible for the head and apply rubber stoppers on the horn tips (Fig. 90.3). The person at the head should also monitor eye and ear reflexes together with ventilation and be able to manually restrain the head

• **Figure 90.3** Protecting people by placing rubber stoppers on the musk ox *(Ovibos moschatus)* horn tips. A short tube is inserted in one nostril for end tidal CO_2 measurements.

• **Figure 90.4** Drawing blood from the metacarpal vein of a musk ox *(Ovibos moschatus)*. The head is to the right.

by holding the horns as needed. Strapping the legs together with soft lifting straps with a person holding the end of the loop is a very effective restraining tool.

When performing a hoof trim with power tools, protect the eyes of the musk ox, as well as the eyes of the people around the musk ox.

During anesthesia, it is possible to deepen or lighten the depth of anesthesia of the musk ox when using the potentiated opioids combined with alpha-2 agonists.

If the animal is too deep, characterized by respiratory depression and low blood pressures, partially reverse some of the depression induced by the alpha-2 agonist and stimulate respiration, by giving a microdose of atipamezole together with doxapram IM (e.g., 200 kg cow receives 1–2 mg atipamezole and 10–15 mg doxapram total dose).

If the animal is too light (i.e., able to hold the head up and move the head around, but not able to get up when approached), hand inject 25%–50% of the initial dose. If the animal starts to wake up during a procedure, supplement with ketamine 0.5–1 mg /kg IM.

Monitoring During Anesthesia

Basic: Depth of anesthesia (reflexes) and ventilation (frequency and depth), saturation (pulse oximetry), and continuous rectal temperature.

Medium: Basic monitoring supplemented with noninvasive blood pressure measurements (carpal or tarsal cuff placement) and end tidal CO_2 ($ETCO_2$, nasal tube connected to a sidestream or mainstream capnography (EMMA). I cut a normal polyvinyl chloride single use endotracheal tube in half (internal diameter 8 mm for an adult cow) and insert it into one nostril (only about 3–4 cm depth) and connect it to the capnography (see Fig. 90.3).

Advanced: Basic and medium monitoring supplemented with arterial blood samples and gas analysis (e.g., I-stat) and direct arterial blood pressure measurements. Arterial access is possible by cannulating the auricular artery (Lian et al.[1]), and tissue perfusion assessment may be done using near infrared spectrophotometry (NIRS) on clipped skin (inside hind leg) and/or venous blood gas measurements (e.g., I-stat).[7]

Use eye drops (clear type, not petroleum-based, as the latter will impair vision and cause unnecessary distress during the recovery phase).

Blood collection is possible from the tarsal vein (when the animal has a summer coat) just above the tarsal-metatarsal joint or the metacarpal vein (Fig. 90.4). Both locations are more easily accessed by using a tourniquet made from thick rubber hoses or similar. The jugular vein can also be used if a larger quantity of blood is needed. The use of extension tubing on the needle is advantageous, especially when a colleague is available to draw the blood from the tube end.

Weighing the animal is possible even in remote areas by using a tarpaulin and 4–6 ordinary suitcase scales. At a given time point, the scale weights are added and then the procedure is repeated—after subtracting the weight of the tarpaulin, the weight of the musk ox often differs less than 1% between the measurements.[5]

There are different opinions regarding the routine use of prophylactic antibiotic treatment after immobilization in nondomestic ruminants. My policy is not to use antibiotics when performing a hoof trim or other scheduled tasks but to use a broad spectrum long-acting antibiotic when immobilization for transport (to prevent shipping fever) and when working on nonfasted animals to minimize the pulmonary reactions if a few drops of ruminal fluid or saliva are accidently inhaled.

When reversing potent opioids in animals expected to have moderate or severe pain after the procedure, buprenorphine 0.01–0.02 mg/kg intravenous (IV) or IM can be used as the reversing agent. The onset of action is approximately 20 minutes for full opioid reversal, but significant respiratory improvement is seen after about 5 minutes. There is residual analgesia for probably 6–8 hours after reversal, in contrast to animals receiving naltrexone. Naltrexone completely antagonizes the administered opioid, including

analgesia effects of the endorphins for several hours after reversal, which is not desired.

Total reversal is usually done by reversing the potent opioid, naltrexone, at 20:1 to 50:1 ratio for etorphine, 50:1 to 100:1 ratio when reversing carfentanil, and 0.5–1:1 ratio when reversing fentanyl. Total reversal when no pain is expected after recovery is better with naltrexone, occasionally combined with atipamezole if a more alert animal is wanted—care should be taken to not overdose with atipamezole—as overstimulated and vocalizing agitated animals may result. The normal dose of atipamezole at the Copenhagen Zoo is 1–3 mg total dose for adult animals. Care should be taken when introducing the musk ox to the rest of the herd. Animals should be isolated until fully recovered, usually 6–8 hours when reversing with buprenorphine and much faster when using naltrexone. We have not seen renarcotization using naltrexone as the reversing agent for etorphine in musk ox, but this occurs quite frequently when reversing etorphine with diprenorphine.

Postoperative pain relief is often used, and nonsteroidal anti-inflammatory drugs are every effective in maintaining mobility and appetite. Ketoprofen at a dose of 1–3 mg kg IM or IV is most potent and recommended for late-term pregnant animals. For longer treatments, we usually use meloxicam, as compliance in accepting the honey-flavored syrup registered for use in horses is quite high. We have used meloxicam for up to a week with daily administration of 0.4 mg/kg without observing any side effects.

When treating very young calves for more than an hour, it is recommended to sedate the mother to minimize the risk of maternal neglect afterward.

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Capripoxviruses in Nondomestic Hoofstock

WOUTER PIETERS

Capripoxviruses (CaPVs) are the cause of diseases in domestic ruminants, having significant economic impact on the livelihood of farmers in endemic areas. The diseases they cause are characterized by fever and nodular lesions on the skin and internal organs. The viruses are expanding their historical territory and have the potential of becoming emerging disease threats because of global climate change and increasing trade in animals and animal products. A number of wildlife species have been implicated in the disease epidemiology, but clinical symptoms have hardly been reported. This chapter addresses the rising concern for disease in nondomestic ruminants after a severe outbreak with high mortality in captive wild animals in Qatar in 2015.

Etiology

The genus *Capripoxvirus* is classified in the subfamily Chordopoxvirinae of the family Poxviridae. It is composed of three virus species—namely sheeppox virus (SPPV), goatpox virus (GTPV), and lumpy skin disease virus (LSDV).[1,2] CaPVs are brick-shaped, enveloped, double-stranded DNA viruses with genomes approximately 150 kbp in size. They are among the largest viruses, measuring 170–260 nm by 300–450 nm. The three viruses in the genus are closely related antigenically and therefore cannot be distinguished serologically. They are also difficult to distinguish morphologically from orthopoxviruses.[3] All CaPV outbreaks are categorized as notifiable diseases in the World Organisation for Animal Health (OIE) guidelines.

Epidemiology

Highly contagious SPPV and GTPV may cause very high morbidity rates (70%–90%) in domestic animals. Mortality may be up to 50% and as high as 100% in young or naive animals. For LSDV infections, morbidity rates may vary from 5%–45% and sometimes be up to 100%. Mortality usually remains below 10%, although mortality rates over 75% have been recorded. The virulence of different CaPV may vary, but the severity of clinical disease often depends on the infected species, age, and immune status.[3–5] Morbidity and mortality numbers in wild animals are poorly recorded and mainly anecdotal. Mortality rates as high as 100% were reported in 16 captive ruminant species in a GTPV outbreak event in 2015 (Table 91.1).[6]

Range and Host-Specificity

There are distinct differences between the host species and the geographic distribution of SPPV, GTPV, and LSDV. CaPV are considered highly host specific, but exceptions are recorded sporadically. Historically they infect only domestic ruminant species and have no zoonotic potential.[7] They are expanding their range and have the potential of becoming emerging disease threats.[3] The geographic range of SPPV and GTPV extends from Africa north of the Equator, across the Middle East and Turkey, to the Indian subcontinent and Asia, including parts of Russia and China. In recent years the range has extended with disease outbreaks occurring in Vietnam (2005 and 2008), Mongolia (2006 and 2007), and Greece (2008).[3,5] Most SPPV and GTPV strains are host specific and cause clinical disease in either domestic sheep or goats, while some strains have equal virulence in both species.[3] In 2015 an outbreak of GTPV in a wildlife collection in Qatar caused clinical disease and high mortality in 16 species of caprinae, antilopinae, and hippotraginea (see Table 91.1).[6] This was the first documented case of GTPV in wild animals, although similar outbreaks have been witnessed in the region (T. Cavero, personal communication, January 16, 2017).

Originally LSDV was restricted to sub-Saharan Africa, but today it occurs in most African countries (including Madagascar). Since 1990 outbreaks have been reported in the Middle East and between 2013 and 2015 in Iraq, Iran, Turkey, Cyprus, and Greece.[3,5,8] In domestic cattle, LSDV causes clinical disease, but susceptibility of wildlife is seen sporadically (Table 91.2). Natural infections have been reported in Asian water buffalo *(Bubalus bubalis)*, springbok *(Antidorcas marsupialis)*, and an Arabian oryx

TABLE 91.1 Wild Ruminants Affected in Goatpox Outbreak, AWWP, Qatar, 2015

Affected Species	No. of Individuals at Risk	No. of Deaths	Mortality Rate (%)*
Laristan mouflon *(Ovis orientalis laristanica)*	48	25	52.08
Iranian wild goat *(Capra aegagrus)*	39	37	94.87
Nubian ibex *(Capra ibex nubiana)*	2	2	100.00
Addax *(Addax nasomaculatus)*	47	38	80.85
Arabian oryx *(Oryx leucoryx)*	43	6	13.95
Gerenuk *(Litocranius walleri)*	28	10	35.71
Beira antelope *(Dorcatragus megalotis)*	2	2	100.00
Soemmering's gazelle *(Gazella soemmeringi)*	112	67	59.82
Idmi gazelle *(Gazella gazella)*	93	8	8.60
Yemeni gazelle *(Gazella gazella cora)*	40	6	15.00
Erlanger gazelle *(Gazella gazella erlangeri)*	7	7	100.00
Chinkara *(Gazella bennettii)*	24	4	16.67
Dama gazelle *(Gazella dama ruficollis)*	47	3	6.38
Speke gazelle *(Gazella spekei)*	59	10	16.95
Persian goitered gazelle *(Gazella s. subgutturosa)*	172	3	1.74
Arabian goitered gazelle *(Gazella s. marica)*	97	4	4.12
Red-fronted gazelle *(Gazella rufifrons)*	51	1	1.96
Dorcas gazelle *(Gazella dorcas isabella)*	77	1	1.30
Pelzeln gazelle *(Gazella pelzelni)*	41	4	9.76

AWWP, Al Wabra Wildlife Preservation.
*Mortality rate = No. of deaths/No. of individuals at risk.

(Oryx leucoryx), while clinical signs have been demonstrated in impala *(Aepyceros melampus)* and giraffe *(Giraffa camelopardalis)* after experimental inoculation with LSDV.[9–13] However, so far there have not been any confirmed LSDV outbreaks in any wildlife species. Antibodies against LSDV have been detected in a range of African game species. Nonetheless, serologic positivity does not necessarily mean that the virus is being replicated and excreted.[11,14–17] The presence of antibodies in an animal, however, does indicate its susceptibility to CaPV and its potential involvement in the epidemiology of the disease. Nevertheless, the role of wildlife in the epidemiology of LSDV is currently not well understood.[16]

Transmission

SPPV and GTPV are highly contagious and may remain viable in crusts and scabs in the environment for several months. Shedding occurs via ulcerated papules on the mucous membranes and nasal, oral, and conjunctival secretions. Transmission is usually through aerosols and close contact with infected animals or by indirect contamination of cuts and skin abrasions. The amount of viral shedding correlates with the severity of clinical disease, and chronically infected carriers do not occur. Due to high virus concentrations in the skin, indirect transmission via insect vectors has been suggested.[18]

In contrast to SPPV and GTPV, the main path of transmission of LSDV is mechanical via biting insects, and direct contact is considered a minor source of infection.[19,20] The virus seems to be capable of spreading over long distances, depending on factors benefiting the arthropod vectors.[5,21]

Clinical Signs and Pathology

Clinical symptoms may vary from mild to severe and even lead to death, depending on susceptibility of the host and virulence of the strain. Following infection, rectal temperature rises to above 40°C after an incubation period of 8–14 days. Skin nodules of 1–5 cm in diameter, involving all layers of the skin, develop concurrently with the fever and may cover over 50% of the skin surface (Fig. 91.1). Draining lymph nodes are often enlarged, especially the prescapular lymph nodes. Animals show depression, loss of appetite,

TABLE 91.2 Lumpy Skin Disease Virus Detected in Exotic Ruminants

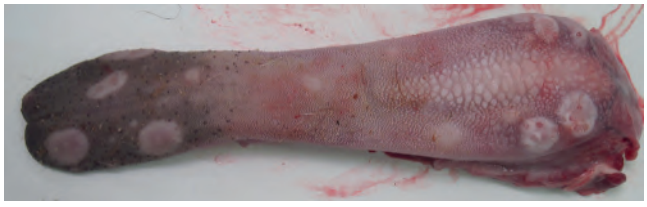
Study (Year)	Species	Geographic Origin	No. of Animals Affected	Diagnosis
Young et al. (1970)	Impala *(Aepyceros melampus)*	South Africa	1	Clinical signs after experimental inoculation
Young et al. (1970)	Giraffe *(Giraffa camelopardalis)*	South Africa	1	Clinical signs after experimental inoculation
Davies (1982)	African buffalo *(Syncerus caffer)*	Kenya	150	Ab detected, virus neutralization test
Hedger and Hamblin (1983)	Kudu *(Tragelaphus strepsiceros)*	Zimbabwe	2	Ab detected, virus neutralization test
Hedger and Hamblin (1983)	Ellipsen waterbuck *(Kobus ellipsiprymnus)*	Zambia	3	Ab detected, virus neutralization test
Hedger and Hamblin (1983)	Defassa waterbuck *(Kobus ellipsiprymnus)*	Chad	1	Ab detected, virus neutralization test
Hedger and Hamblin (1983)	Reedbuck *(Redunca arundinum)*	Zimbabwe	1	Ab detected, virus neutralization test
Hedger and Hamblin (1983)	Springbok *(Antidorcas marsupialis)*	Botswana	1	Ab detected, virus neutralization test
Hedger and Hamblin (1983)	Giraffe *(Giraffa camelopardalis)*	Zimbabwe	1	Ab detected, virus neutralization test
Hedger and Hamblin (1983)	Impala *(Aepyceros melampus)*	Zimbabwe, Zambia, Kenya, Botswana	14	Ab detected, virus neutralization test
Ali et al. (1990)	Asian water buffalo *(Bubalus bubalis)*	Egypt	5	Natural infection
Greta et al. (1992)	Arabian oryx *(Oryx leucoryx)*	Saudi Arabia	2	Natural infection, virus neutralization test
Barnard (1997)	Blue wildebeest *(Connochaetes taurinus)*	South Africa	4	Ab detected, virus neutralization test
Barnard (1997)	Black wildebeest (Connochaetes *gnu)*	South Africa	3	Ab detected, virus neutralization test
Barnard (1997)	Impala *(Aepyceros melampus)*	South Africa	5	Ab detected, virus neutralization test
Barnard (1997)	Springbok *(Antidorcas marsupialis)*	South Africa	12	Ab detected, virus neutralization test
Barnard (1997)	Eland *(Taurotragus oryx)*	South Africa	1	Ab detected, virus neutralization test
Le Goff et al. (2009)	Springbok *(Antidorcas marsupialis)*	South Africa	2	Viral nucleic acid extracted from skin lesion
El-Nahas et al. (2011)	Asian water buffalo *(Bubalus bubalis)*	Egypt	nk	Natural infection, PCR
Fagbo et al. (2014)	African buffalo *(Syncerus caffer)*	South Africa	70	Ab detected, ELISA test

Ab, Antibody; *nk,* not known; *PCR,* polymerase chain reaction.

and are reluctant to move. Ulcerative lesions on mucous membranes of the eyes, nose, mouth, pharynx, and tongue result in excessive lacrimation, salivation, mucopurulent discharge, and labored breathing (Fig. 91.2). Diarrhea is possible when gastrointestinal mucosae are affected. Secondary bacterial infections and pneumonia are common, and death may occur at any stage of the disease. Lesions observed on postmortem exam include necrotic and ulcerated mucous membranes of the eyes, nose, and oropharynx (Fig. 91.3), and tracheal congestion with blood-tinged foam. The lungs

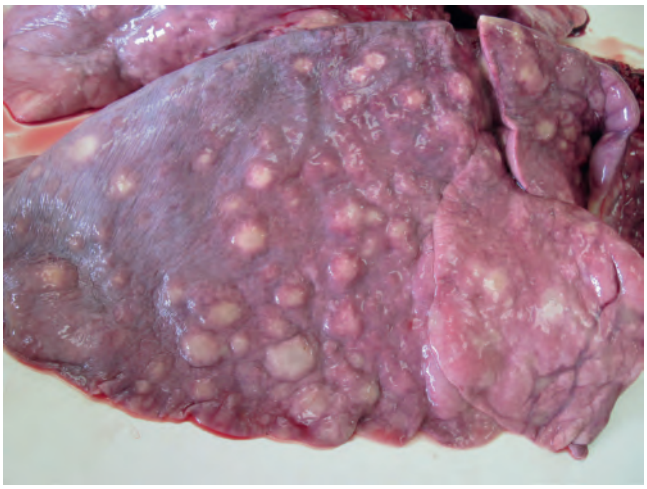
• **Figure 91.1** Sedated male addax with severe cutaneous goatpox lesions being euthanized.

• **Figure 91.2** Juvenile Laristan mouflon with conjunctivitis, ulcerative mucosal goatpox lesions of the mouth and nose, excessive lacrimation, and mucopurulent nasal discharge.



• **Figure 91.3** Multifocal goatpox nodules on the tongue of a wild goat.

often show extensive and coalescing pox nodules (Fig. 91.4) with congestion, edema, red hepatization, and focal areas of consolidation and necrosis. The mediastinal and other lymph nodes are generally swollen, edematous, and hemorrhagic. Pox lesions may also be found throughout the digestive tract but mainly on the mucosae of rumen and abomasum.[3,4,8,22,23] Microscopically, the pox lesions are characterized by a massive cellular infiltrate, vasculitis, and edema. The infiltration consists of macrophages, neutrophils, lymphocytes, plasma cells, and occasionally eosinophils.



• **Figure 91.4** Extensive multifocal goatpox nodules in the lungs of an Arabian oryx.

Typical of CaPV infections are the large intracytoplasmatic eosinophilic inclusion bodies and vacuolated nuclei in macrophages in the dermis, lymph nodes, and lesions in internal organs. The vasculitis is accompanied by thrombosis and infarction, causing edema and necrosis. Epidermal changes include acanthosis, parakeratosis, and hyperkeratosis.[24] The clinical signs of a severe infection with SPPV, GTPV, and LSDV are highly characteristic, but milder forms may be confused with parapoxviruses causing bovine papular stomatitis, pseudocowpox, and contagious ecthyma or the orthopoxvirus causing cowpox. The differential diagnosis should also include insect bites, bluetongue virus, peste des petits ruminants virus, and pseudo-LSDV, a bovine herpesvirus.[22,23]

Diagnosis

The typical pox lesions caused by CaPV infections are strongly indicative, and a presumptive diagnosis could be made based on clinical signs, gross pathology, and typical pox virions on histopathology. However, virus differentiation in atypical hosts like wild animals is difficult, and mild disease may be difficult to diagnose, prompting laboratory confirmation of a definitive diagnosis. A correct diagnosis relies on identifying the agent in biopsy samples from skin nodules, lung lesions, or lymph nodes. Polymerase chain reaction (PCR) is a rapid and sensitive diagnostic technique for CaPV genome detection. The virus strains may then be identified by sequencing and phylogenetic analysis.[11] Several research groups have reported using conventional PCR or real-time PCR for detecting CaPV genetic material.[3,8] Other CaPV identification methods like virus isolation and transmission electron microscope fail to differentiate among SPPV, GTPV, and LSDV.[3] Neither may electron microscopy distinguish CaPV from orthopoxviruses except by the application of specific immunologic staining.[3] The classical serologic tests are unreliable methods for identifying CaPV species. All the viruses in the CaPV genus

share a common major antigen for neutralizing antibodies, and it is thus not possible to distinguish SPPV, GTPV, or LSDV antibodies from one another.[4] Immunity to CaPV infection is mainly cell mediated, and infected animals may produce only low levels of neutralizing antibodies. A virus neutralization test is insufficiently sensitive to detect these levels.[8] Other serologic tests show cross-reaction with other poxviruses or are simply too difficult and expensive to perform.[22,23] Several enzyme-linked immunosorbent assay (ELISA) tests have been reported, but currently no validated ELISA for detecting antibodies against CaPV is available.[4,8]

Treatment, Prevention, and Control

Other than supportive care, there is no specific treatment available for CaPV infection. The clinical management of infected animals includes symptomatic treatment and strong antibiotic therapy to limit and control secondary bacterial infections. Potentially susceptible animals showing typical signs of infection should be separated from others or isolated if possible. Only prophylactic vaccination might protect susceptible wild hoofstock.

Due to poor treatment options, prevention of virus introduction into hoofstock herds is crucial. Capripox-free countries may maintain their disease-free status by respecting strict import restrictions on livestock and animal products from affected areas. In countries where viruses are enzootic, sanitary prophylactic measurements should be respected to avoid infection. Due to the highly infectious character of SPPV and GTPV, contact between exotic ruminants and domestic sheep and goats should be limited, and a strict quarantine policy should be respected. Arthropod vector control measures may minimize the risk of LSDV infection.

In case of an outbreak, successful control relies on early detection, a strict stamping-out and movement control policy, and quarantine.[5] However, the culling of rare species valuable for conservation might be controversial, and this measure needs to be evaluated on a case-by-case basis. Proper disposal and destruction of dead animals is crucial, as is thorough cleaning and disinfection of the premises and materials. The viruses are sensitive to phenol (2%), ether (20%), chloroform, formalin (1%), Virkon 2%, iodine compounds (1:33 dilution), sodium hypochlorite (2%–3%), sodium dodecyl sulfate, and quaternary ammonium compounds (0.5%).[22,23] In addition, vector control management is recommended for the prevention of LSDV transmission.

Vaccination is the most effective way to control the spread of CaPV in domestic ruminants. However, very little is known about the use and effectiveness of vaccines in wildlife or their immune response. There are currently no vaccines registered for animals other than sheep, goats, and cattle. Only live attenuated vaccines are available, and none of these are authorized for use in nonendemic countries.

Live and inactivated SPPV and GTPV vaccines have been reported, using different strains or isolates of the viruses. Live vaccines, which have been attenuated by multiple passages in cell culture, are favored over killed vaccines, as the latter do not provide adequate and long-lasting immunity.[25] Several locally produced SPPV and GTPV vaccines are available using virus strains from Russia, Yugoslavia, Romania, and countries in Africa and the Indian subcontinent.[5,21] In general, SPPV and GTPV are considered very host-specific, but a number of strains have been known to infect both sheep and goats. Some naturally occurring recombinant strains are used for vaccines to protect both sheep and goats.[26]

There are currently three LSDV vaccines produced in South Africa. Two of the vaccines contain strains of the original LSDV Neethling strain. The third vaccine uses an attenuated South African LSDV field isolate.[5] Due to antigenic homology and cross-protection between CaPV, SPPV and GTPV vaccines are used against LSDV only in countries where the three CaPV overlap. A dose higher than originally indicated is commonly used, but incomplete vaccine protection has been reported.[5,26] In case of an emergency scenario in a CaPV-free country, killed vaccines are recommended and safe to use.[5] Currently, no Differentiating Infected from Vaccinated Animals (DIVA) vaccines are commercially available against CaPV. Therefore, these need to be developed for use in nonendemic countries.[5]

To date, there are no data published on the use of CaPV vaccines in nondomestic ruminants. The empiric use of Kenyavac, a live attenuated GTPV and SPPV vaccine containing the KSGP 0240 strain, in wild ruminants by the author proved effective in Laristan mouflon *(Ovis orientalis laristanica)*. Nonetheless, its efficiency in other species is unclear (unpublished data). Others in the Arabian Gulf region have used vaccines in wild ruminants with variable success (T. Cavero, personal communication, January 16, 2017).

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Babesiosis in Cervidae

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Introduction

Babesiosis is an emerging infectious disease of North American and European cervids. It is caused by infection with any of a group of vector-borne, protozoal hemoparasites of the phylum Apicomplexa (order Piroplasmida, family Babesiidae, genus *Babesia*). There are more than 100 described *Babesia* spp. worldwide, identified primarily from mammalian host species, but also described in several avian hosts.[1]

Life Cycle and Epidemiology

Different *Babesia* spp. have distinct geographic distributions based on the presence of competent vertebrate hosts and invertebrate vectors. Table 92.1 summarizes the *Babesia* spp. identified globally in wild and captive cervids. The main *Babesia* spp. reported to cause clinical disease in cervids are *Babesia capreoli (B. capreoli)* and *Babesia venatorum* (*B. venatorum*; previously *Babesia* sp. EU1) in Europe, and *B. odocoilei* in North America.[2–4]

The primary vector of babesiosis worldwide is the Ixodid tick, with some tick vectors demonstrated to carry more than one *Babesia* spp. For example, in Europe, *Ixodes ricinus* is the vector for both *B. venatorum* and *B. capreoli*. In North America, the known vector for *Babesia odocoilei (B. odocoilei)* is the black-legged tick, *I. scapularis*; however, *Dermacentor* spp. ticks have been implicated in babesiosis transmission in some cases.[4,5]

Transmission of *B. odocoilei* to a vertebrate host occurs during blood feeding by the tick vector, at which time tick saliva containing the parasite is introduced into the host's bloodstream. The parasites are engulfed by host erythrocytes and undergo asexual replication (merogony) within the cell. Asexual replication occurs within infected erythrocytes, with cyclical development of trophozoites and merozoites. The parasites induce lysis of the erythrocytes, releasing more infectious life stages into the blood and invading uninfected erythrocytes. In addition to direct red blood cell lysis by the parasite, both intra- and extravascular immune-mediated hemolysis contribute to anemia. Thrombocytopenia may also occur as the result of immune-mediated platelet destruction or disseminated intravascular coagulopathy.[6] The life cycle of all *Babesia* spp. is completed when ticks consume a blood meal from an infected host. At this point the parasite undergoes sexual reproduction within the epithelium of the tick's gut and travels from the gut through the hemolymph to the salivary glands where it undergoes asexual reproduction to produce large numbers of infectious life stages. Infection rates vary among herds and species, but serosurveillance for *B. odocoilei* antibody activity has shown prevalence rates of up to 100% in some elk herds.[7] *Babesia* spp. are also capable of being transmitted via blood transfusion.[8]

North America

The single identified agent of clinical babesiosis in North American cervids is *B. odocoilei*. *B. odocoilei* was first identified in the southeastern United States in free-ranging white-tailed deer *(Odocoileus virginianus)*, the presumptive natural host and reservoir of the disease. Clinical disease is rarely reported in this species, although it may be induced experimentally by immunosuppression of carrier animals.[4,9]

Clinically silent *Babesia* spp. infections have been described in a wide range of endemic and exotic cervid and bovid species in North America, including desert bighorn sheep *(Ovis canadensis nelsoni)*, markhor *(Capra falconeri)*, muntjac *(Muntiacus reevesi)*, and yak *(Bos grunniens)*.[4,6,7,10]

Overt hemolytic disease resulting from infection with *B. odocoilei* has been reported in reindeer *(Rangifer tarandus tarandus)*, caribou *(Rangifer tarandus caribou)*, American elk *(Cervus elaphus canadensis)*, musk oxen *(Ovibos moschatus)*, and immunosuppressed white-tailed deer.[5–7,9,11] *B. odocoilei*-associated hemolytic anemia in captive cervids was first reported in the United States in 1993 but has only recently emerged in Canada, with cases reported since 2012.[5,11]

Europe

Free-ranging roe deer *(Capreolus capreolus)* are the reservoir hosts for *B. venatorum*, a zoonotic pathogen that is the cause of fatal babesiosis in zoo reindeer in the Netherlands, Germany, and Switzerland.[3,12,13] Roe deer are also asymptomatic hosts of *B. capreoli*, the causative agent of fatal hemolytic anemia in zoo reindeer in the Netherlands and in free-ranging chamois *(Rupicapra rupicapra rupicapra)*

TABLE 92.1 ***Babesia* spp. Identified Globally in Cervids**

Host Species	*Babesia* spp.*	Geographic Location	Wild or Captive Animal	Clinical Signs (Present/ Absent)	Diagnostic Method	Reference
European reindeer *(Rangifer tarandus tarandus)*	*B. odocoilei*	Ontario, Canada	Captive	Present	PCR and sequencing	Pastor et al. (2016)[27]
		Quebec, Canada	Captive	Present	PCR and sequencing	Benoit et al. (2014)[28]
		Manitoba, Canada	Captive	Present	PCR and sequencing	Mathieu et al. (2018)[59]
		Pennsylvania, USA	Captive	Present	PCR and sequencing	Schoelkopf et al. (2005)[7]
		New York, USA	Captive	Present	PCR and sequencing	Schoelkopf et al. (2005)[7] Bartlett et al. (2009)[6]
		Wisconsin, USA	Captive	Present	PCR and sequencing	Holman et al. (2003)[10]
	B. odocoilei-like	Germany	Captive	Absent	PCR and sequencing	Wiegmann et al. (2015)[13]
	B. divergens†	Scotland, UK	Captive	Present	PCR and sequencing	Langton et al. (2003)[25]
		Germany	Captive	Absent	PCR and sequencing	Wiegmann et al. (2015)[13]
	B. venatorum	Netherlands	Captive	Present	PCR and sequencing	Kik et al. (2011)[3]
		Germany	Captive	Absent	PCR and sequencing	Wiegmann et al. (2015)[13]
		Switzerland	Captive	Present	PCR and sequencing	Robert et al. (2008)[12]
	B. capreoli	Netherlands	Captive	Present	PCR and sequencing	Bos et al. (2016)[2]
		Germany	Captive	Absent	PCR and sequencing	Wiegmann et al. (2015)[13]
	B. divergens	Germany	Captive	Absent	PCR and sequencing	Wiegmann et al. (2015)[13]
	Babesia sp.	California, USA	Captive	Absent	In vitro culture PCR and sequencing IFA	Holman et al. (2002)[29] Kjemtrup et al. (2000)[30]
	B. jakimovi	Russia	Semi-domesticated	Present	Parasite morphology	Nikol'skii et al. (1997)[31] Holman et al. (2002)[29]
Woodland caribou *(Rangifer tarandus caribou)*	*B. odocoilei*	Minnesota, USA	Captive	Present	Protozoal culture PCR and sequencing	Holman (1994)[32] Holman et al. (2000)[4] Petrini et al. (1995)[11]
American elk *(Cervus elaphus canadensis)*	*B. odocoilei*	Ontario, Canada	Captive	Present	PCR and sequencing	Pastor et al. (2016)[27]
		Saskatchewan, Canada	Captive	Present	PCR and sequencing	Pattullo et al. (2013)[5]
		New York, USA	Captive	Present	PCR and sequencing	Ameri et al. (2012)[33]
		New Hampshire, USA	Captive	Present	PCR and sequencing	Schoelkopf et al. (2005)[7]
		Quebec, Canada	Captive	Present	PCR	Benoit et al. (2014)[28]
		Indiana, USA	Captive	Present	Protozoal culture IFA PCR and sequencing	Gallatin et al. (2003)[23]
		Texas, USA	Captive	Present	Protozoal culture PCR and sequencing	Holman et al. (1994)[34] Holman et al. (2000)[4]
		Wisconsin, USA	Captive	Absent	PCR and sequencing	Holman et al. (2003)[10]

White-tailed deer *(Odocoileus virginianus)*	*B. odocoilei*	New Mexico, USA	Wild	Present (rare)	Parasite morphology	Spindler (1958)[35]
		Texas, USA	Wild	Absent	Parasite morphology IFA PCR and sequencing	Emerson and Wright (1968, 1970)[36,37] Waldrup et al. (1992)[38] Ramos et al. (2010)[39]
		Tennessee, USA	Wild	Absent	PCR and sequencing	Fritzen et al. (2014)[40]
		Oklahoma, USA	Wild	Absent	IFA	Waldrup et al. (1989)[22]
		Virginia, USA	Wild	Absent	Parasite morphology	Perry et al. (1985)[9]
		Minnesota, USA	Captive	Absent	Protozoal culture IFA PCR and sequencing	Holman et al. (2000)[4]
	B. bigemina	Texas, USA	Wild	Absent	PCR and sequencing	Holman et al. (2011)[41]
		Mexico	Wild	Absent	PCR and sequencing	Cantu et al. (2007)[42]
	B. cf. bovis	Texas, USA	Wild	Absent	PCR and sequencing	Ramos et al. (2010)[39]
		Mexico	Wild	Absent	PCR and sequencing	Cantu et al. (2007)[42]
Roe deer *(Capreolus capreolus)*	*B. capreoli*	Italy	Wild	Absent	PCR and sequencing	Zanet et al. (2014)[43]
		Netherlands	Captive	Present	Parasite morphology	Dorrestein et al. (1996)[18]
		Germany	Wild	Absent	PCR and sequencing	Overzier et al. (2013)[44]
		Poland	Wild	Absent	PCR and sequencing	Welc-Falęciak et al. (2013)[45]
		Switzerland	Wild	Absent	PCR and sequencing	Hoby et al. (2009)[15] Michel et al. (2014)[46]
		France	Wild	Absent	PCR and sequencing	Bastian et al. (2012)[47]
	B. venatorum	France	Wild	Absent	Protozoal culture PCR and sequencing	Bonnet (2007)[48] Bastian et al. (2012)[47]
		Italy	Wild	Absent	PCR and sequencing	Zanet et al. (2014)[43]
		Germany	Wild	Absent	PCR and sequencing	Overzier et al. (2013)[44]
		Poland	Wild	Absent	PCR and sequencing	Welc-Falęciak et al. (2013)[45]
		Switzerland	Wild	Absent	PCR and sequencing	Michel et al. (2014)[46]
		Slovenia	Wild	Absent	PCR and sequencing	Duh (2005)[49]
	B. divergens†	Slovenia	Wild	Absent	PCR and sequencing	Duh (2005)[49]
		Spain	Wild	Absent	PCR and sequencing	Garcia-Sanmartin et al. (2007)[50]
	B. bigemina	Italy	Wild	Absent	PCR and sequencing	Zanet et al. (2014)[43]
Red deer *(Cervus elaphus elaphus)*	*B. divergens*†	Slovenia	Wild	Absent	PCR and sequencing	Duh (2005)[49]
		Ireland	Wild	Absent	PCR and sequencing	Zintl et al. (2011)[51]
		Switzerland	Wild	Absent	PCR and sequencing	Michel et al. (2014)[46]
	B. capreoli	Italy	Wild	Absent	PCR and sequencing	Zanet et al. (2014)[43]
		Switzerland	Wild	Absent	PCR and sequencing	Hoby et al. (2009)[15]
		Scotland	Wild	NA	Parasite morphology	Gray et al. (1990)[52]
	B. pecorum	Spain	Captive	Absent	PCR and sequencing	Jouglin et al. (2014)[53]
	B. bigemina	Italy	Wild	Absent	PCR and sequencing	Zanet et al. (2014)[43]
	B. divergens	Austria	Wild	Absent	PCR and sequencing	Silaghi et al. (2011)[54]

Continued

TABLE 92.1 ***Babesia* spp. Identified Globally in Cervids—cont'd**

Host Species	*Babesia* spp.*	Geographic Location	Wild or Captive Animal	Clinical Signs (Present/ Absent)	Diagnostic Method	Reference
Moose *(Alces alces)*	*B. capreoli*	Norway	Wild	Absent	PCR and sequencing	Pūraitė et al. (2016)[16]
	B. odocoilei†	Norway	Wild	Absent	PCR and sequencing	Pūraitė et al. (2016)[16]
Fallow deer *(Dama dama)*	*B. capreoli*	Austria	Wild	Absent	PCR and sequencing	Rehbein et al. (2014)[17]
	B. bigemina	Mexico	Captive	Absent	PCR and sequencing	García-Vásquez et al. (2015)[55]
	B. bovis	Mexico	Captive	Absent	PCR and sequencing	García-Vásquez et al. (2015)[55]
	Babesia sp.	California, USA	Captive	NA	In vitro culture PCR and sequencing	Kjemtrup et al. (2000)[30]
Pampas deer *(Ozotocerus bezoarticus)*	*B. bigemina*	Brazil	Wild	NA	PCR and sequencing	Silveira et al. (2013)[56]
	B. bovis	Brazil	Wild	NA	PCR and sequencing	Silveira et al. (2013)[56]
Brown brocket deer *(Mazama gouazoubira)*	*B. bigemina*	Brazil	Wild	Absent	PCR and sequencing	da Silveira et al. (2011)[57]
Marsh deer *(Blastocerus dichotomus)*	*B. bovis*	Brazil	Captive	Absent	PCR and sequencing	da Silveira et al. (2011)[57]
Mule deer *(Odocoileus hemionus)*	*Babesia* sp.	California, USA	Wild	Absent	Parasite morphology In vitro culture PCR and sequencing	Thomford et al. (1993)[58] Kjemtrup et al. (2000)[30]

*_Babesia_ spp. as identified by authors.
†This isolate is now thought to be *B. capreoli* (Malandrin et al., 2010).

in Switzerland.[2,14] Various *Babesia* spp. are found in European red deer *(Cervus elaphus elaphus)*, moose *(Alces alces)*, fallow deer *(Dama dama)*, and also in roe deer (see Table 92.1).[15–17] Clinical disease has not been reported in these species, with the exception of a single report of clinical hemolytic disease in a captive roe deer in the Netherlands attributed to *B. capreoli* based on parasite morphology; no molecular diagnostics were performed in this case.[18] In northern Europe, sporadic cases of clinical babesiosis are reported predominantly in captive reindeer and are associated with *B. venatorum* and *B. capreoli* infection. However, a German polymerase chain reaction (PCR) survey found that 23.6% of clinically healthy zoo reindeer were hosts to five different *Babesia* spp., suggesting that subclinical infections also occur in this species.[13]

Factors Involved in Disease Emergence

Climate change is an important driver of tick population dynamics and may aid the range expansion of vector-borne pathogens into habitats that were previously too cold to support the vectors.[19] The large-scale seasonal movements of migratory birds provide opportunities for bird-associated ectoparasites and their pathogens to disperse rapidly over thousands of kilometers.[19] This combination of factors, along with the presence of suitable local host species, has resulted in the establishment of ticks and the diseases they carry in new geographic regions (see also Chapter 36).

Zoonotic Potential

Only one of the *Babesia* spp. reported to cause clinical babesiosis in cervids, *B. venatorum*, is considered zoonotic. At least three clinical cases of *B. venatorum* infection have been reported in immunocompromised human patients in Europe.[20] Clinical manifestation of the disease in humans was a moderately severe malaria-like syndrome.[21]

Clinical Signs

Sporadic cases, epizootics, and clinically silent infections have all been described in captive cervids. Clinical presentation of cervid babesiosis has been described as consisting of four syndromes: peracute, acute, chronic, and subclinical. Peracute presentation is characterized by sudden death with no prodromal signs. Acute cases may manifest with any of the following: hemolysis, hemoglobinuria, hemorrhage, icterus, lethargy, anorexia, or respiratory distress. At the Toronto Zoo, separation of individuals from the herd was noted as an early clinical sign in several reindeer and one elk (Pastor, unpublished). Chronic cases have been identified with any or all of the following clinical signs: pyrexia, emaciation, anemia, and low-level parasitemia of erythrocytes.[22] Subclinical cases may experience transient anemia, as reported in experimentally infected white-tailed deer, but notably, clinical signs do not always occur upon first exposure to the parasite; rather, subclinical or persistent infection may progress to clinical disease in the face of concurrent stressors.[9] High population density, comorbid disease, recent transport, reproductive status (rut, calving), and poor nutrition have been identified as potential risk factors in the development of clinical disease.[23]

Pathology

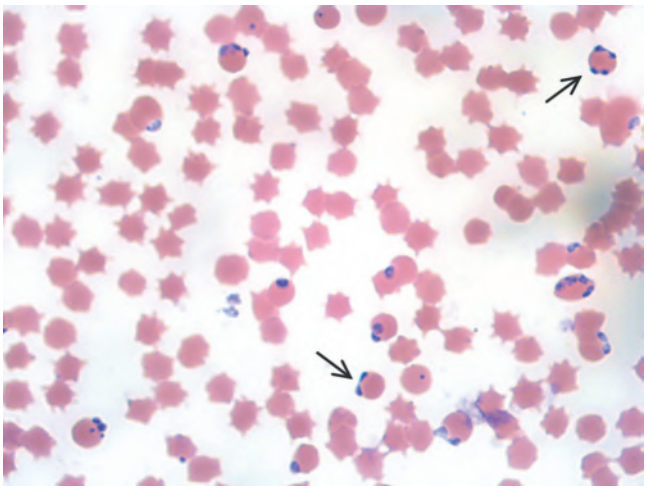
Clinical Pathology

Romanowsky-type staining of a peripheral thin blood smear may not consistently identify intraerythrocytic life stages. When present, *Babesia* appear as either single or paired piriform and ring-shaped organisms, often at the periphery of the erythrocyte (accolé position; Fig. 92.1) or in Maltese cross arrangements. The number of parasites visible in blood smears is extremely variable due to the removal of infected erythrocytes: parasitemia of up to 80% of red blood cells may be observed in acute babesiosis, but parasitemia is not always visible on a blood smear. In addition, intraerythrocytic *Babesia* are sometimes identified on routine blood smears of clinically normal animals.[13]

Hematology of animals with clinical babesiosis typically shows a normocytic, normochromic hemolytic anemia with low red blood cell and hemoglobin values. An inflammatory leukogram may also be present. Serum is often hemolyzed or icteric, which may interfere with biochemistry results. No biochemistry changes are characteristic of *Babesia* infection, but hyperbilirubinemia and bilirubinuria are often seen secondary to extravascular hemolysis, and in severe disease azotemia occurs secondary to hemoglobinuric nephropathy. Metabolic acidosis may result from increased lactate generation secondary to tissue hypoxia.

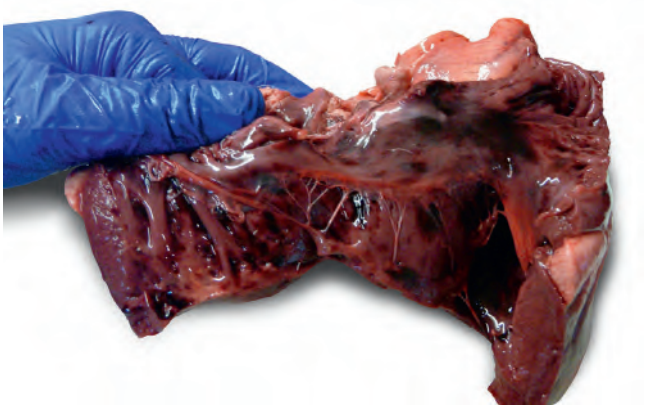
Gross Pathology

Gross postmortem findings are not specific for babesiosis and may include hepatomegaly; splenomegaly; hemoglobinuria



• **Figure 92.1** *Babesia odocoilei* infected erythrocytes on peripheral blood smear from a European reindeer *(Rangifer tarandus)*. *Arrow* indicates organisms in the accolé position. (Courtesy Adriana R. Pastor.)

• **Figure 92.2** Hemoglobinuria in the bladder of a European reindeer *(Rangifer tarandus)* with clinical *Babesia odocoilei* infection. (Courtesy Adriana R. Pastor.)

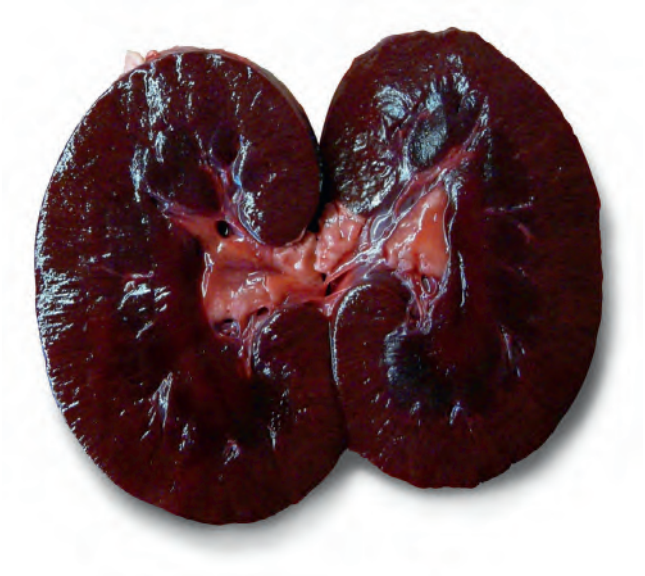


• **Figure 92.3** Endocardial hemorrhage in the heart of a European reindeer *(Rangifer tarandus)* with clinical *Babesia odocoilei* infection. (Courtesy Adriana R. Pastor.)

(Fig. 92.2); icterus; petechial hemorrhages, particularly of the endocardium (Fig. 92.3) and adrenal glands; and diffuse dark red to black discoloration of the kidneys (pigmentary nephrosis; Fig. 92.4).

Histopathology

Histologic tissue changes are variable and are generally consistent with hemolytic disease. Impression smears of parenchymatous organs may reveal intraerythrocytic life stages. Hepatic centrilobular vacuolar degeneration and necrosis may occur due to decreased hepatic perfusion and oxygenation secondary to hemolytic anemia. Splenic hemosiderosis and erythrophagocytosis and generalized lymph node erythrophagocytosis reflect extravascular hemolysis. In the kidney, hemoglobinuric nephropathy (pigmentary nephrosis with acute tubular degeneration) and dilated renal tubules containing granular hemoglobin casts may



• **Figure 92.4** Pigmentary nephrosis (diffuse dark red to black discoloration of the kidneys) in the kidney of a European reindeer *(Rangifer tarandus)* with fatal *Babesia odocoilei* infection. (Courtesy Adriana R. Pastor.)

be observed in advanced cases. Cardiac and adrenocortical hemorrhage are commonly reported.

Diagnosis

Antemortem diagnosis is based on the presentation of typical clinical signs, hematology and biochemistry changes consistent with hemolysis, the presence of intraerythrocytic parasites on peripheral blood smear, or conventional PCR on whole blood. However, intraerythrocytic *Babesia* may not be identified on peripheral blood smear, even in clinical cases, complicating diagnosis. Postmortem diagnosis is based on the presence of hemolysis, extensive hemorrhage, hemoglobinuria, and icterus. PCR of DNA extracted from fresh-frozen spleen samples has been used to successfully diagnose the disease in deceased elk, reindeer, and white-tailed deer, including tissues that have been stored at –20°C for up to 6 years (Pastor, unpublished). The use of PCR on routine blood samples or banked frozen spleen samples could potentially aid in disease surveillance efforts and detection of subclinically infected wild and captive cervids. Recently, sequencing of the mitochondrial COI region has been used to identify multiple strains of *B. odocoilei* in affected cervids at the Toronto Zoo (Pastor, unpublished), and similar techniques may prove useful in determining the epidemiology of the disease in affected areas.

Prior to the development of molecular characterization of protozoal organisms, identification of piroplasms was based primarily on morphologic and morphometric characteristics of the different protozoal life stages. Serology and indirect fluorescent antibody assay have also been used to identify *Babesia* spp. infection. Due to the great degree of similarity between piroplasms in size and appearance, and the variable specificity of antibody binding, it is likely that

some *Babesia* spp. historically reported in cervids were misidentified. Molecular diagnostics have allowed for improved identification of different *Babesia* spp.[24] For example, a *Babesia* sp. isolated from an outbreak in a Scottish reindeer herd, identified by the authors as *B. divergens*, is now thought more likely to have been caused by *B. capreoli*.[24,25] Submission of *Babesia* isolates from cervid babesiosis cases for molecular diagnostics is strongly encouraged, to advance species identification and further elucidate the epidemiology of the disease.

Treatment

A number of chemical compounds have been reported to be effective against *Babesia* spp. in domestic animals, including diminazene diaceturate, imidocarb dipropionate, and amicarbalide.[8] These drugs are not available worldwide, and attempts to obtain antiprotozoal drugs in an emergency may be unsuccessful. Dose rates and frequency of administration have not been established for most drugs when used in cervids; therefore therapeutic regimens are based on the manufacturer's recommendations for cattle. Imidocarb dipropionate at 2.2–3.0 mg/kg body weight (BW) by intramuscular injection has been used successfully in captive elk and reindeer to treat clinical disease and eliminate subclinical *Babesia* infections.[6,25] Following treatment with babesiacidal compounds, anemia may worsen for up to 7 days due to the continued removal of parasitized erythrocytes from circulation.[6]

Successful treatment depends on early diagnosis and prompt administration of babesiacides. The prognosis is grave in animals that are debilitated by acute disease.[8] Aggressive supportive treatment is required in animals undergoing hemolytic crisis, and should include anti-inflammatory drugs and fluid therapy to minimize the secondary renal effects of hemolysis. Based on clinical experience with domestic ungulates, blood transfusions may be life-saving in very anemic animals, although this technique has not yet been reported as part of the clinical management of babesiosis in cervids. Donor animals will ideally be well-conditioned, and the packed cell volume of the donor should be checked before collecting blood. No published information on known blood groups in cervids could be found at the time of writing, but use of related donor animals may be advisable.

Prevention and Control

Complete eradication of the tick vector from the environment is rarely feasible, and consideration should be given to maintaining enzootic stability in endemic regions. In a captive setting, tick control programs should be designed to reduce the risk of tick populations developing resistance to acaricides. Wild deer should be excluded from outdoor zoo exhibits. A strategic tick control program should integrate pasture management (removal of bushes and long grass) with application of acaricides. Both topical amitraz and permethrin at the recommended label dose for cattle have been used safely and effectively in zoo cervids in order to control ticks; ivermectin at 0.4 mg/kg BW (double the cattle dose) may be administered subcutaneously or orally.[23,26]

The use of babesiacidal compounds, in combination with acaricide treatment, is recommended when translocating cervids to and from *Babesia*-endemic areas. Cervid translocations should be designed to avoid widening the geographic range of this parasite, and to protect naïve populations from *Babesia* infection. Stress has been shown to precipitate acute hemolytic disease in animals harboring subclinical infections, so efforts should be made to reduce stress where possible. Strategic treatment of subclinically or potentially infected animals with babesiacidal compounds may be a useful prophylactic measure when stress is anticipated.

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SECTION 19

Elephants and Rhinoceroses

93

Management of Dental Disease in Elephants

GERHARD STEENKAMP

Introduction

Elephants belong to the order Proboscidia with one family (Elephantidae) and two genera (*Loxodonta* and *Elephas*). The African elephant has recently been divided into two distinct species, namely *L. africana* (savannah elephant) and *L. cyclotis* (forest elephant). The latter was based on research showing no nuclear gene flow existed between the two species.[1] Currently this division is not accepted by the International Union for Conservation of Nature (IUCN), and further research is required. The Asian elephant has only one species, *Elephas maximus*, whose closest relative appears to be the extinct woolly mammoth *(Mammuthus primigenius)*.[1] Three subspecies of Asian elephant have been recognized by the IUCN—namely, *Elephas. m. indicus* (Mainland Asia), *Elephas m. maximus* (Sri Lanka), and *Elephas m. sumatranus* (Sumatra).

Diet

Elephants are categorized as herbivore generalists that consume both grasses and browse.[2] Their natural diet includes grasses, tree roots, bark, branches, leaves, and fruits. Stable isotope analysis in African elephants has shown that elephants may change their diet according to seasonal environmental changes.[3,4] In captivity it is important to try to mimic the natural diet of these megaherbivores. Roughage gained from browse is essential, not only for its nutritional value to the animal, but also for abrasion of the teeth and ultimately the expelling of the molars (particularly in captive Asian elephants). Feeding is also a key component of elephant environmental enrichment.[5]

Management

Management of elephants in captivity may contribute to the high incidence of dental disease seen in captivity.[6] The practices that may contribute to dental disease include diet (see above), enclosure design, herd structure, or the daily management routine.

Concrete and steel often predominate in construction of traditional elephant enclosures. Concrete floors, with a smooth surface, allow for ease of cleaning. This is often done with high pressure hoses washing away feces and bedding material. These wet floors, however, create a slippery surface, and elephants may slip and fall, leading to potential tusk fractures. The caustic alkaline nature of poorly cured cement floors on the feet of the elephants is an additional concern. Cement walls separating herd members may also be detrimental if the elephants may rest their tusks on them. It is advisable that all cement walls should be covered with sectioned logs in order to prevent direct contact between tusks and cement at any time, and that flooring be sand, dirt, or other natural materials.

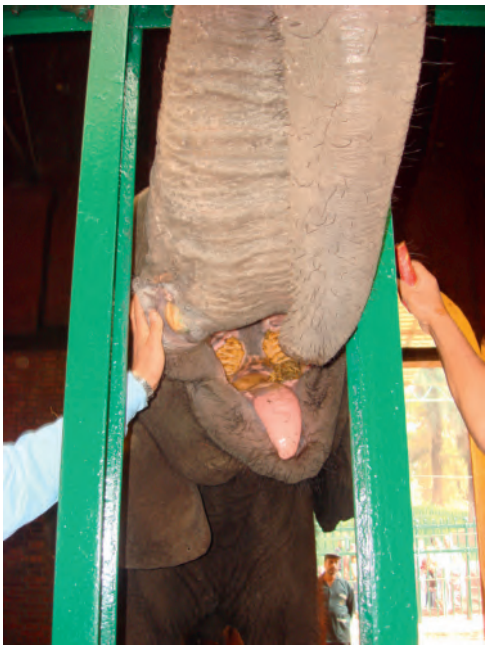
Large iron barriers may cause tusk trauma in several ways. In protected contact situations, elephants are often required to reach between the bars for treats (Fig. 93.1). In doing so, they may damage the tusks against the bars on either side of the trunk. Iron bars with acute angles may cause tusk abrasions as the elephants slide their tusks from side to side over them (Fig. 93.2). Lastly, tusks may fracture as elephants try to force steel gates open.

Tusks may also be fractured when elephants fight. Herds with a skewed sex ratio favoring bulls may be problematic, especially when they come into musth. This will increase conflict between bulls (depending on enclosure size and ages of the bulls) and thus increase the risk for tusk fractures.

Some management strategies include housing elephants individually at night. Because elephants are social animals, they have the need to communicate and touch each other. By separating the individuals, the risk is increased that they may reach over dividing walls, through separating bars, or interfere with their foot chains (if these are used to secure them) in an attempt to make contact with each other.

Dental Anatomy

Elephants have a dentition that consists of incisors, premolars (discussed later), and molars. Their incisors, which occur only in the maxilla, consist of the second incisor



• **Figure 93.1** An adult African elephant *(Loxodonta africana)* cow reaching through metal railway tracks (used for its strength) for a treat. This behavior may cause abrasion of the tusks and may lead to tusk pulp exposure.

• **Figure 93.2** An adult African elephant *(Loxodonta africana)* cow in an outdoor enclosure reinforced with railway tracks positioned on their side. The sharp sidewalls of these tracks are very abrasive for the tusks, as may be seen on the ventral surface of the left tusk.

teeth, and these are the only teeth that have a primary precursor. The primary incisors are commonly referred to as "tushes" and the secondary incisor as the tusks.[7,8] The function of the tushes is unclear, but it is my hypothesis that the eruption and ultimate loss of these tushes at around 1 year of age may delay the eruption of the secondary tusk. This will give adequate time for the calf to suckle without injuring the cow with potentially long tusks.

The denomination of the cheek teeth varies in the literature, with some authors referring to the first three cheek teeth as premolars or deciduous molars and the last three as molars.[9,10] There are, however, others who refer to all six of these cheek teeth as molars.[9,11] Using this denomination, the molars are numbered 1–6 in the order in which they erupt,[11] and in this chapter I will follow this convention.

• **Figure 93.3** The conically shaped pulp and the tusk it originated from. This is a healthy tusk from an adult African elephant *(Loxodonta africana)* bull. There is a clear distinction between the white ivory (that was in the alveolus) and the brownish ivory of the exposed tusk.

African Elephant Compared With Asian Elephant

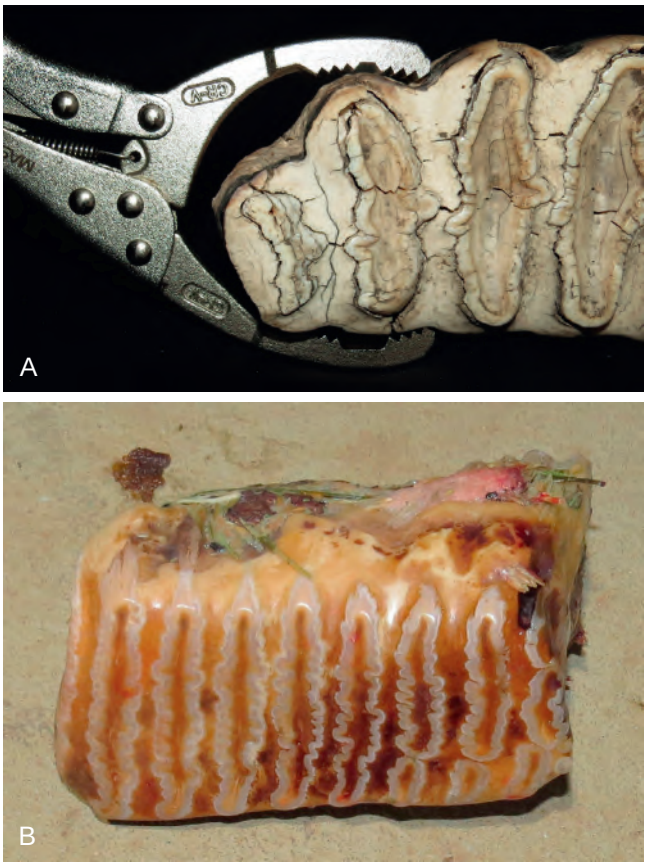
In African elephants, tusks are usually present in both bulls and cows. The prevalence of tusklessness in African elephant cows is variable in different wild populations and dependent on the size (and tusk-bearing potential) of the founder population, as well as on the poaching pressure exerted on it. In areas with small founder populations (e.g., Addo Elephant Park, South Africa), the prevalence of tusklessness is high (91%). In areas with high levels of poaching, it is lower (21%–26%), and in populations free of any disturbances, it is relatively low (0%–4%).[6] In contrast, tusklessness in African bull elephants is rare.[6]

In the Asian elephants, tusks may be present in both bulls and cows. The prevalence of tusklessness, however, is also more common in cows. Tuskless bulls are commonly referred to as a "makna." Asian elephant cows may have small tusks, which are also often referred to as "tushes."[12,13] Because this term is also used to describe the deciduous tusks in elephants, I would suggest the term is only used to describe the latter.[7,8] The fact that Asian cows have smaller tusks does not warrant the use of a different term. If they indeed are secondary incisors, they should be called tusks.[13] In the literature I found no record of Asian elephant cows possessing only tushes without the growth of secondary tusks.

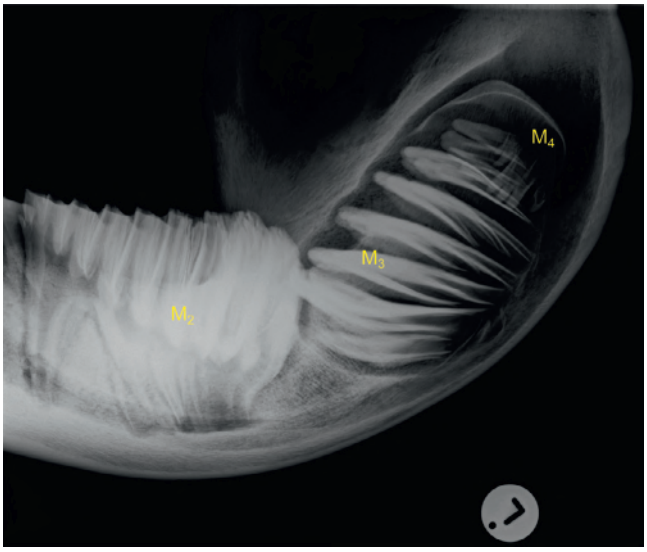
Dental Morphology

Tusk

The tusk consists of dentine covered by an enamel coronal cap at eruption. Without this covering, the ectoderm-mesenchymal interaction, necessary for tooth development, would not be possible. Dental pulp in the elephant tusk (like other mammalian teeth) consists of neurovascular structures within loose connective tissue.[14] The innervation of the pulp was initially questioned.[15] However, clear innervation was demonstrated in two independent studies.[14,16] The pulp is conically shaped with a large base at the apex (Fig. 93.3) and is lined by dentin producing odontoblasts. As soon as



• **Figure 93.4** The lophodont dentition of the African *(Loxodonta africana)* and Asian *(Elephas maximus)* elephants differ significantly. African elephants (A) have much coarser laminae in comparison with the smaller narrower laminae of the Asian elephant (B).



• **Figure 93.5** A lateral radiograph of a young African elephant *(Loxodonta africana)* shows the molar 2 *(M_2)* in wear, molar 3 *(M_3)* developing caudal to it in the angle of the mandible, and molar 4 *(M_4)* developing perpendicular to M_3.

the enamel cap is worn away, the unprotected dentin is referred to as ivory.

Molar

Elephant molars have a unique ridged pattern referred to as lophodont dentition. The term "lophodont" refers to the ridges (laminae) that are formed perpendicular to the long axis of the jawbones. The shape of these ridges differs between African (Fig. 93.4A) and Asian elephants (Fig. 93.4B). Each ridge (lamina) contains enamel, dentin, and pulp, which is bound to similar laminae by cementum. The six molar teeth progressively increase in size and number of ridges (laminae). Mandibular molars develop in the angle of the mandible and maxillary molars caudal to the molar in occlusion. It is interesting to note that there may be two developing teeth present in the angle of the mandible at the same time (Fig. 93.5).

Eruption Sequence

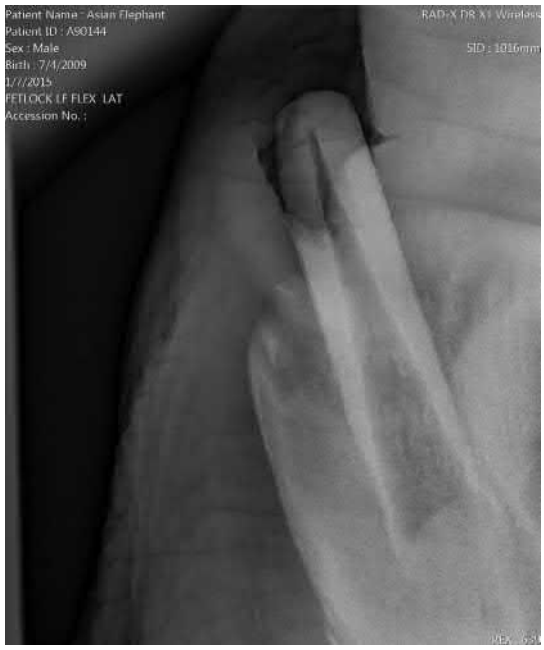
The eruption sequence of African elephants was initially described by Laws.[10] Since then, the eruption sequence has been adapted and improved by several authors. The age groups described by Laws are still a good indication of early and middle-aged eruption and replacement of the mandibular molars. Recently, the older age categories were revised using data from known-age individuals.[11] A novel technique of aging elephant mandibles shows promise; however, the age reference point and age reference line described will be difficult to obtain in a living elephant.[17] The age categories of African elephants based on the eruption and replacement of the mandibular molars as described by Laws and improved by Lee do have better clinical relevance and should be consulted.[10,11] Similar published data on Asian elephants have proved difficult to find.

Diagnosing Dental Disease in Elephants

Working with elephants remains a privilege that, if not performed with the utmost care, may have serious consequences for the veterinary team or the animal.[18,19] Cursory evaluations of the oral cavity or tusks will be improved considerably if elephant keepers train the animals to allow conscious evaluation of the mouth. In one example, an Asian elephant was trained well enough for a veterinarian to perform a tusk restoration without sedation.[20] However, if you suspect pathology that may be painful or if the behavior of the animal is not predictable, a standing sedation or general anesthesia is required to make a definitive diagnosis.

Making a definitive diagnosis of dental disease in elephants may be uncomplicated when the tooth in question is a tusk and the pathology is visible (Fig. 93.6). This presentation does indeed appear to be the exception rather than the rule. Tusk pathology may be very subtle, and the veterinarian may be required to do further investigations

• **Figure 93.6** A long-standing complicated crown fracture (pulp exposed) in a 17-year-old African elephant *(Loxodonta africana)* bull.



• **Figure 93.7** A rostro-caudal oblique radiograph of a 4-year-old Asian elephant *(Elephas maximus)* bull. In these smaller individuals, radiography may assist in making a definitive diagnosis. Radiograph reproduced with the kind permission of the Taronga Conservation Society, Australia.

in order to understand the pathology present and whether or not the pulp is exposed. In tusk fractures or when tusks are amputated, it is important for the veterinarian to know the extent to which the pulp extends into the coronal tusk (that part of the tusk visible beyond the skinfold covering the alveolus), as the exposure of the pulp will require immediate intervention. Initially the extent of the pulp into the coronal part of the tusk was speculated to be the same as length from the lip (skinfold) covering the tusk to the eye.[21] Many African elephants have tusks that are not even as long as this lip-eye length; therefore a study to quantify the length of the pulp in the coronal tusk was done. There was no relationship of pulp length into the coronal part of the tusk and age, and due to this, there was no correlation between the pulp length with the circumference or height of the tusk at the lip.[22] Physical evaluation of the tusk with a dental explorer still gives the most reliable results.

Intraoral evaluations may be challenging, and the use of an adjustable speculum greatly improves the visibility and accessibility of the molar teeth. The usefulness of a quality light emitting diode (LED) headlamp to illuminate the oral cavity cannot be overstated. Periodontal probes and explorers developed for horses are suitable for use in elephants and are long enough to reach to the caudal extent of the molars.

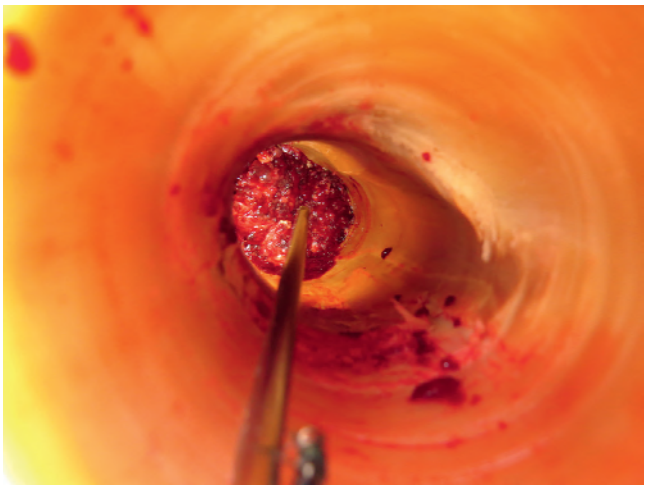
Radiography of teeth in elephants has limited value. The thick alveolar bone that covers the tusks and the dense cortical bone of the mandible are not easily penetrated by even the most powerful radiographic equipment. It is also difficult to get the correct radiographic angles that provide useful diagnostic images. Oblique rostro-caudal views of the tusks may be achieved in young animals either standing (depending on the demeanor of the animal) or in lateral recumbency (Fig. 93.7). In older individuals, lateral radiographs of the rostral tusks that extend beyond the alveolus are possible.

Endoscopic evaluation of the tusk pulp (pulposcopy) is possible in elephants. Direct access to the pulp cavity is gained via a coronal tusk fracture if the tusk was cracked, or after tusk amputation. Direct visualization of the pulp cavity would allow the health of the pulp to be assessed. Furthermore, treatment of the pulp deep in the pulp cavity is possible under direct visualization. With chronically affected tusks where pulp necrosis caused the destruction of most of the pulp, it is possible to visualize the entire pulp cavity up to the apex (Fig. 93.8). Endoscopic removal of fractured tusk fragments in a chronically draining alveolus post extraction is also possible.

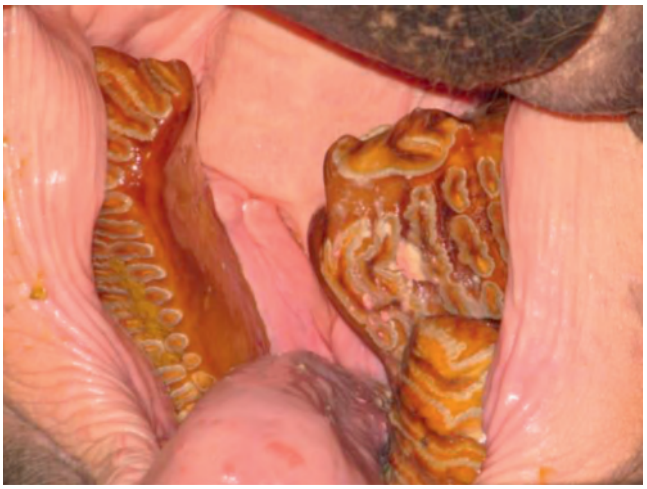
Diseases/Anomalies Affecting the Molars

Delayed Shedding

Delayed shedding of molar fragments is a condition unique to Asian elephants.[23,24] It is thought that dietary factors such as the lack of hard fibrous food, particularly from browse, may contribute to this problem. These animals will present with clinical signs ranging from painful mastication and halitosis to anorexia, weight loss, and colic.[24] A thorough oral evaluation is essential to make the diagnosis. These



• **Figure 93.8** Endoscopic evaluation of the pulp cavity (pulposcopy) is another imaging technique that is valuable to help the clinician make a diagnosis. In this pulp cavity, only approximately 10 mm of pulp (thickness) was left after chronic pulpitis. This individual responded poorly to a partial pulpectomy, and the tusk was extracted 1 month later.



• **Figure 93.9** The left maxillary molar 4 in this adult Asian elephant *(Elephas maximus)* cow has rotated through 90 degrees and is now blocking the eruption of the molar behind it. Molar extraction is indicated in these cases. (Photo reproduced with the kind permission of Dr. Chris Visser, Phoenix, AZ.)

teeth or tooth fragments are often loose. After extraction of the molar fragment, the opposing molar should be evaluated and trimmed if necessary.

Supernumerary Molars

Supernumerary or seventh molars have been described from mandibles of African elephants.[25] In one skull I observed, the seventh molar was present in the mandible but had not erupted.

Rotation

Due to delayed shedding in Asian elephants, the piece of molar that is still present in the mouth may rotate by up to 90° and cause retention of the molar fragment and delay the eruption of the next molar in sequence (Fig. 93.9).[23,24] These impacted molars may present with similar clinical signs, as mentioned in delayed shedding, in addition to impeding the eruption of the caudal molar.

Perforations/Cavities

Molar perforations/cavities have been described. It is speculated that a diet high in silica may predispose elephants to this condition.[23]

Tumors

Tumors of the dentition of the elephants or indeed of the oral cavity per se are rare. One case of an unknown tumor is described causing abnormal maxillary molar eruption in an African elephant bull.[25]

Diseases/Anomalies Affecting the Tusks

Absent Tusks

Elephants without visible tusks, especially individuals with unknown histories, should be evaluated for tusks or tusk fragments. The causes of absent tusks may be due to lack of tusk development (e.g., in Asian elephant cows), odontogenic tumor formation, or premature loss of the tusk. I am aware of two different reports, one in Addo Elephant Park (Dr. Markus Hofmeyr, personal communication, 2012) and the other in the Johannesburg Zoo (Mr. Phillip Cronje, personal communication, 2006), where individuals lost a complete tusk. The cause of the tusk loss in the case of Addo bull is unclear, while the zoo elephant fell into the enclosure moat and suffered a complete avulsion of one of its tusks.

Supernumerary Tusks

This condition was first described by Sir Frank Colyer in the previous century.[26,27] To my knowledge, no clinical case has ever been published.

Abnormal Structure

Because tusks are elodont (continuously growing teeth with no root) teeth, the potential to produce new dentin is retained, and tusks increase in length and circumference throughout life. This unique feature may sometimes be detrimental to the health of the tusk, as the pulp may in times of chronic infection be stimulated to produce excessive dentin (called ivory pearls, as discussed later), which may complicate pulp treatment or extractions.

Tusklets

Due to trauma, small tusks may be formed in the same alveolus as the original tusk. These smaller tusk-like structures are called tusklets.[27] Each of these small tusklets contain its own pulp tissue and will grow as a normal tusk does. Extraction of these tusklets is indicated when the health of the original tusk is compromised.

Dilaceration

Dilaceration is the term used when a tooth crown or root undergoes an abrupt change of direction commonly thought to be the result of trauma.[28] The author saw one case as an incidental finding in an African elephant bull.

Ivory Pearls

The pulp is lined by specialized odontoblasts that produce dentin (dentinoblasts). With trauma to the pulp and especially chronic inflammation, these dentinoblasts are stimulated to produce haphazard dentinal structures called ivory pearls.[23,25,27] These pearls are tertiary dentin, alternatively known as "reparative dentin," and resemble pearl-like structures or a bunch of grapes. They may be of little consequence if the causative agent may be isolated. The presence of it in the pulp cavity significantly increases the challenge of treating the pulp.

Abrasion

The abnormal wearing of tooth structure (abrasion) is often associated with enclosure design. Elephants reaching over concrete walls, large boulders, or metal bars will cause increased wear of their tusks (see Fig. 93.2). This may either wear the tusks extremely short without pulp exposure or weaken the tusks, causing them to fracture and potentially expose the pulp.

Fractures

Tusk fractures may be classified as uncomplicated (where there is loss of ivory, but the pulp is not exposed) or complicated (loss of ivory and pulp exposure; see Fig. 93.6). In captivity, tusks may fracture due to excessive abrasion, fighting, slipping on wet concrete floors, or elephants using their tusks to force gates open.

Pericoronitis

Pericoronitis is infection and inflammation of the pericorium, which is the soft tissue covering the tusk entrance into the alveolus. (For intraoral teeth, this would be the equivalent of the gingiva.) Elephants often react to these wounds by packing them with mud, dust, or feces, increasing the risk for infection at the entrance to the alveolus. Flies and fly larvae, attracted to the site of infection, could also create further irritation around the tusks.

Treatment

Tusks

Fractured tusks may be treated through partial pulpectomy (when the pulp is still viable) or extracted if the pulp is no longer viable.[25] Fig. 93.10 provides a guide to the most appropriate treatments for tusk injuries. The success rate of partial pulpectomies on tusks after amputations (n = 18) and fractures (n = 22) is 95% (Steenkamp, unpublished data; Fig. 93.11). It is important for clinicians to act immediately when a complicated tusk fracture has been diagnosed. With chronic inflammation or infection, the pulp will become compromised and extraction may be required. Tusk extractions are extremely challenging procedures to perform, requiring specialized equipment and expertise. After extraction, the alveolus should be cleaned and no tusk fragments should still be attached to the periodontal ligament. The alveolus is left open to drain after extraction and may take several months to close, depending on the size of the extracted tusk.

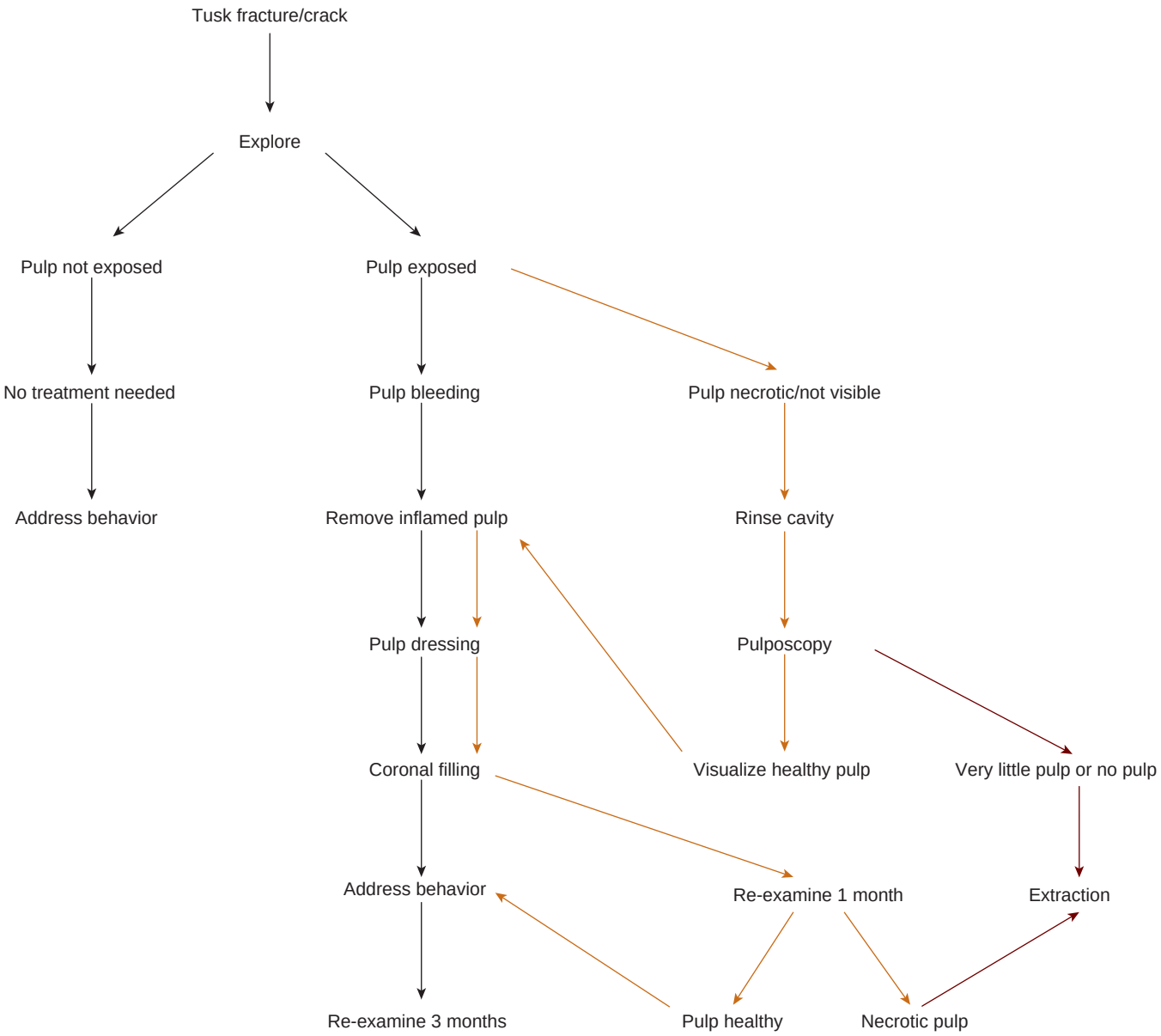
Complications include maxillary bone fractures, draining sinuses due to incomplete extraction of the ivory, and alveolo-nasal fistula formation.

Several institutions perform regular "tipping" (removal of up to about 5 cm of tusk tip) of all of their animals' tusks. This practice does not appear to have a sound scientific basis and, because it is not possible to predict where the pulp will be in the coronal part of the tusk, the practice should be avoided. Tusk amputations have been successfully performed in order to prevent African elephant bulls from using them to break electrified perimeter wires.[29] Care should be taken when these procedures are done, and all equipment/consumables should be at hand in order to perform partial pulpectomies should the pulp be exposed.

Molars

Molar extraction is required when there is delayed exfoliation of such a tooth. These teeth may be loose due to increased periodontal disease caused by food impaction; however, this may not always be the case. Sectioning the tooth may be required, depending on the size of molar fragment retained. In the absence of advanced periodontal disease, large elevators are required to destroy the periodontal ligament attachment before successful extraction may be performed.

Cleaning and filling cavities on elephant molar is possible.[23] Careful and meticulous cleaning of the cavities, as well as restoration thereof, is essential to prevent pulp necrosis and eventual abscess formation. The use of an adjustable speculum will improve the access to the molar surface that needs to be restored.



• **Figure 93.10** When confronted with a fractured or cracked tusk, the clinician needs to follow a logical process to determine the correct treatment protocol to be used. This diagram represents the author's decision-making process when assessing fractured or cracked tusks.

• **Figure 93.11** This 7-year-old African elephant *(Loxodonta africana)* bull fractured his left tusk at the level of the skinfold after trying to open a steel gate. The author performed a partial pulpectomy within 7 days of the accident, and this picture, taken 2 years later, shows healthy tusk growth on the treated tusk.

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Elephant Mycobacteriosis: New Diagnostics and Management

KAY A. BACKUES AND ELLEN WIEDNER

Infection with *Mycobacterium tuberculosis* (Mtb) is now widely recognized as an important primarily respiratory tract disease of Asian elephants, *Elephas maximus*, in human care, but is rarely reported in captive African elephants, *Loxodonta africana*.[1] *Mycobacterium bovis* also occurs in elephants, but this chapter focuses on *M. tuberculosis*.

Between 1997 and 2011, the point prevalence of Mtb in North America was 5.1% in Asian elephants but zero in African elephants.[1] The close working partnership between humans and Asian elephants likely explains many of the Asian elephant exposures to this human disease. However, there have been two reported cases of confirmed Mtb in free-ranging Asian elephants and one unconfirmed case in an African elephant.[2–4] Needless to say, the introduction of Mtb into free-ranging populations of Asian elephants could pose yet another significant threat to this highly endangered species. Extensive knowledge about Mtb's behavior, diagnosis, and treatment in its primary reservoir, humans, has been used as a loose model for the disease in Asian elephants.[5] Such assumptions may provide a starting point, but they require the caveat that our understanding of Mtb in elephants is still in its infancy, and many of these assumptions may not be correct.

Transmission

As occurs with transmission of Mtb between humans, transmission of Mtb between elephants and between elephants and humans requires close, prolonged aerosol contact with another infected individual.[6–9] At one facility individuals that only had brief casual contact with an infected elephant did not respond to tuberculin intradermal testing.[9] At another facility, numerous human intradermal test conversions occurred following use of a high-pressure washer near a poorly placed intake vent for an office inside an elephant barn. The barn housed an Mtb-infected and shedding elephant.[7] The strong correlation between close, prolonged aerosol contact and disease transmission highlights the necessity of stopping active shedding by infected elephants as soon as possible via antibiotic treatment. Aerosolized material from infected animals appears to be the most common source of transmission. Fomites and manure, significant sources for the transmission of *M. bovis*, do not appear to be of much importance for the transmission of Mtb.

Diagnosis

Screening for Mtb should be included in all preventative medicine programs for captive elephants. This entails monitoring individual elephants as well as overall herd health. Keeper health, zoo-wide medical concerns, and general husbandry procedures also need to be considered.[5] Diagnostic screening tests routinely used in humans and other species—such as thoracic radiographs and lung and gastric washes—are precluded in elephants by their great size and anatomy and poorly studied immune systems. Intradermal testing with tuberculin causes nonspecific reactions and is contraindicated in elephants.[10] Multiple diagnostic tests are often necessary to screen for Mtb in living elephants, and definitive antemortem confirmation of disease presence or absence may be elusive.

Direct tests such as culture and real-time polymerase chain reaction (qPCR) confirm the actual presence of Mtb complex organisms. Culture is the gold standard test for Mtb diagnosis in all species and the only test that confirms active infection. For living elephants, a trunk wash (TW) is commonly submitted for Mtb culture because it contains material from the lower respiratory tract. A properly performed TW necessitates training the elephant to permit the instillation of 60 mL of saline into its trunk and then exhaling into a sterile collection container.[11]

Indirect tests such as serology, interferon gamma (IFN-γ) tests, and cytokine stimulation assays demonstrate exposure to Mtb complex organisms.[12] Serologic tests identify antibodies to specific mycobacterial antigens, whereas cytokine stimulation assays and IFN-γ tests detect specific components of cell-mediated immunity (CMI) triggered by Mtb.[13–16] However, indirect tests cannot confirm active infection. Most of the CMI tests are experimental, minimally

validated, and not available commercially.[12] See Table 94.1 for details of Mtb diagnostic tests.

If an elephant is positive on an indirect test, confirmatory direct tests are recommended, such as increased frequency of TW cultures and qPCR (if available). If a positive TW culture is obtained, confirm results either by submitting a duplicate retained specimen or by submitting new TW for cultures. Multiple TWs performed over weeks to months may be necessary to confirm a positive result due to low bacterial numbers or intermittent shedding. Herdmates should also undergo increased surveillance, and regulatory agencies should be contacted.[5]

Positive cultures should be tested for their antibiotic susceptibility and the isolate sequenced for molecular typing. Complete DNA sequencing of isolates can be performed upon request and can help to elucidate the epidemiology of the infection. Molecular probes can identify genes associated with drug resistance in elephant Mtb samples. If the animal is treated but then relapses, these tests should be repeated to see if the susceptibility pattern has changed or if a different Mtb isolate is involved.[17]

As with many other aspects of elephant health, screening over significant periods of time appears essential to truly understanding a given herd's Mtb status as opposed to using a single time point's test results. All elephants that die in a collection should have a complete necropsy performed to confirm their Mtb infection status. All necropsies should be undertaken as if an elephant may be positive for Mtb, and for elephants known to have confirmed Mtb infection at death, a risk-benefit analysis of human safety should be evaluated before necropsy. In addition to infrequent shedding and equivocal test results, long periods of latency appear likely in Mtb-infected elephants, further challenging diagnosis.

Treatment

In treating an elephant for Mtb infection, several issues must be considered. The first is prevention of shedding of infectious organisms into the environment, which is best accomplished using a combination of first-line antitubercular medications with consistent dosing at sufficient levels. The second is avoidance of serious drug-related adverse events. The third is achieving and maintaining adequate serum drugs levels throughout treatment.

Treatment should be started as soon as an Mtb-infected elephant is identified by culture, even before antibiotic susceptibilities are completed. Therapy may be altered as needed once susceptibilities are known. The primary drugs used in elephants are isoniazid (INH), rifampin (RIF), pyrazinamide (PZA), ethambutol (ETH), and levofloxacin (LEVO). Another common veterinary fluoroquinolone, enrofloxacin (ENRO) has been used to treat TB in elephants, but its effectiveness may be questionable based on research showing that Mtb organisms develop rapid resistance to its active metabolite, ciprofloxacin.[18] Four concurrently used drugs are recommended, although in some cases, three drugs will suffice. INH and RIF are recommended in all elephant Mtb treatment regimes. INH causes early rapid killing of actively replicating Mtb organisms within a few days of starting treatment and has been documented to stop trunk shedding if compliance is good, the isolate is INH-susceptible, and the dosing is sufficient.[19] INH should not, however, be used as monotherapy because of the risk of developing Mtb resistance.[20] The importance of RIF stems from its ability to resolve cavitary lesions and activity against latent Mtb organisms. PZA and ETH are synergistic with the other drugs and important in preventing resistance, but neither should be used in place of INH or RIF. LEVO can be substituted for PZA, ETH, or RIF but not for INH. There is evidence from human treatment regimens that fluoroquinolones may be useful in situations involving resistance to first-line antibiotics or nonreplicating (latent) Mtb organisms.[21–23] Fluoroquinolones can be delivered orally or rectally, unlike RIF, which requires oral administration. See Table 94.2 for drug dosing information.

The appropriate drug doses and concentrations needed for cure in elephants are unknown; only the dosages needed to achieve specified blood concentrations.[24–28] Individual elephants may show significant variation in drug pharmacokinetics.[28,29] Although the plasma/serum drug concentrations used in humans are starting points, they may not always be satisfactory for elephants and may even result in toxicity. Documented adverse events include depression, colic, inappetance, and black fetid manure.[30,31] Blepharospasm, ocular tearing, and lethargy are also reported. Nevertheless, the highest doses of antitubercular drugs should be given that do not cause toxicity in order to maximize drug exposure at the infection site and to minimize development of resistance.[32–34]

Culture-positive elephants may be treated with a short intensive phase (or initiation phase) and a longer continuation phase. During the intensive phase, high and frequent doses of at least three to four drugs are given to cause a rapid decrease in the number of infectious organisms and to avoid development of resistance. This phase may last 8–10 weeks if tolerated by the elephant. Efficacy may be monitored via frequent TW culture to document cessation of mycobacterial shedding. In the continuation phase, antibiotics may be used at lower frequency but not necessarily lower doses over many months to kill remaining viable organisms. Other treatment regimens exist, and several facilities have used the same dose and frequency throughout an elephant's treatment regimen. Treatment regimens vary, but all should strive for doses and frequency of medications that are well tolerated, TW monitoring during treatment that continues to show no detectable Mtb shedding and drug levels that exceed the minimum inhibitory concentration (MIC) of the cultured organism. Drug levels should be confirmed at 6-month intervals or whenever there are changes in drug selection or dosage (see Table 94.3).

Treatment failures in elephants have occurred in a few cases. Some of these were associated with individual elephant intolerance to medications and development of resistance. Ongoing research into additional diagnostic techniques, safer drug regimes, and Mtb epidemiology in elephants will hopefully improve our ability to manage this disease.

TABLE 94.1 Elephant *Mycobacterium tuberculosis* Diagnostic Tests

Test	Type of Test	Samples Needed	Interpretation of Results	Test Advantages	Test Disadvantages	Availability	Comments
Culture	Direct	Trunk washes most common Other body fluids may be submitted for culture (semen, vaginal secretion, ocular secretion, mucus, lung lavage samples)[35] Postmortem samples of either fresh or frozen tissue, including suspicious lesions such as granulomas or caseated lymph nodes, plus lung, trachea, thoracic lymph nodes, salivary gland, and lower esophagus sections. Body fluids as described above	A positive result from a living elephant indicates active infection and shedding at the time of sampling. A positive result from a necropsy sample indicates infection at the time of death, but not whether the animal was shedding or if the infection was latent A negative result from a living elephant indicates the elephant was not shedding at the time of sampling. A negative result from a necropsy lesion suggests either that the infection was resolved (only dead organisms present) or that the submitted lesion was not Mtb. qPCR should be performed to confirm presence of Mtb organisms	The only test that confirms active infection. Test is 100% specific TW are noninvasive, inexpensive	False negatives in live animals due to technique, intermittent shedding, lesion size and location, and activity. Test has poor sensitivity Slow turnaround for results (10 days to 8 weeks) TW require elephant and staff to be trained for sampling Lung lavage requires extremely specialized endoscopic equipment plus sedation	Available from laboratories approved for mycobacterial testing and ideally with experience culturing elephant samples because TW are often heavily contaminated and special techniques are needed for processing	Culture is the gold standard for diagnosis of Mtb in elephants Trunk wash cultures may also grow NTM Although two cases of pulmonary disease in elephants have been associated with M. szulgai,[36] NTM grown in a trunk wash do not raise zoonotic concerns or necessitate treatment
qPCR	Direct	Trunk washes from living elephant, other body fluids and mucus Postmortem samples as described above	A positive result indicates Mtb organisms within the sample, but does not prove active infection because qPCR only confirms DNA presence of an organism, but not its viability A negative result indicates no Mtb DNA was present in the samples tested	Potentially highly specific Can be done using same samples sent for culture Can be performed on formalin-fixed tissue	Testing still in progress, thus sensitivity and specificity are not yet known For TW, elephant and staff must be trained for sampling	Only one laboratory available in the US (NVSL) Cost is less than $50 US No charge if TW is submitted	Potentially high specificity and sensitivity when done in combination with TW culture, particularly with new techniques being developed to enhance the sensitivity of PCR for TW

Continued

TABLE 94.1 **Elephant *Mycobacterium tuberculosis* Diagnostic Tests—cont'd**

Test	Type of Test	Samples Needed	Interpretation of Results	Test Advantages	Test Disadvantages	Availability	Comments
Acid-fast staining	Direct	Fluid or mucus smear or histopathology	A positive result indicates the presence of organisms with cell walls that resist decolorization with acid or alcohols after Ziehl-Neelsen staining A negative result indicates that no acid fast organism was present in that particular sample	Very rapid time for results Inexpensive	Very low sensitivity and specificity. Many non-Mtb organisms are acid fast Many false negatives	Readily available. May be done in house or by any clinical pathology laboratory	
Serology (e.g., DPP, dual plate pathway; MAPIA, multiple antigen print immunoassay)	Indirect	Serum	A positive result indicates exposure to Mtb complex but does not confirm shedding or active infection. Positive results also have occurred with some tests with *M szulgai*, an NTM A treated animal may remain positive for unspecified amount of time after treatment A negative result indicates either no exposure to Mtb complex or that exposure was too recent for seroconversion, or that elephant did not seroconvert despite exposure (due to low dose of organisms, route of exposure, etc.)	Has potential use as a screening test Rapid turnaround	Elephant must be trained for blood draw Incompletely documented sensitivity and specificity and not validated in living elephant populations Mtb does not stimulate a strong antibody response Cross-reactivity with atypical mycobacteria Poor repeatability with some tests Not recommended for regulatory purposes for reasons listed here, but often used for this purpose	Availability of some tests has been problematic Costs highly variable, ranging from less than $20–500 US per test In other species, animal age and health and sample quality affect results,[12] which appears to be the case with elephants as well	Tests use a chromogenic reaction to identify serum antibodies to specific mycobacterial antigens The degree of color change cannot be correlated with antibody titers at this time Time course of Mtb antibody production and duration in elephants is unknown

Cytokine stimulation assays	Indirect	Whole blood	A positive result indicates exposure to Mtb and a functioning innate immune system, specifically CMI A negative result indicates no exposure to Mtb or a nonfunctioning immune system	CMI response is likely more relevant for Mtb diagnosis than evaluating the acquired immune response, thus potentially more sensitive and specific than serology	Validation still under way	Not available. Experimental only	
Tuberculin skin test	Indirect	N/A	Does not work in elephants		Results do not correlate with TB status[10]		Should not be used in elephant
Elephant gamma interferon (IFN-γ) test	Indirect	Whole blood	A positive result indicates exposure to Mtb and a functioning immune system A negative result indicates no exposure to Mtb or a nonfunctioning immune system		Validation still under way	Minimal availability	

CMI, Cell-mediated immunity; *IFN-γ,* interferon gamma; *Mtb*, *Mycobacterium tuberculosis*; *NTM,* nontubercular mycobacteria; *TB,* tuberculosis; *TW,* trunk wash, *qPCR,* real-time polymerase chain reaction.

TABLE 94.2 Suggested Drug Doses for the Treatment of Elephants With *Mycobacterium tuberculosis*[24–29]

Drug	Route	Dose (mg/kg)
Isoniazid (INH)	Oral or rectal	2–7*
Rifampin (RIF)	Oral	10
Ethambutol (ETH)	Oral	15
Pyrazinamide (PZA)	Oral or rectal	20
Levofloxacin (LEVO)	Oral Rectal	5† 15

*INH has been associated with adverse effects in elephants and higher doses may need to be lowered. Determine dose using MIC of organism and elephant tolerance. Some elephants are completely intolerant of INH. ENRO has been used in treatment of TB in elephants but may not be effective due to potential for development of rapid resistance to ciprofloxacin.
†No pharmacokinetic data are available for oral or rectal levofloxacin in elephants but have been published for oral enrofloxacin.[37] Enrofloxacin metabolite, ciprofloxacin is ineffective in killing Mtb organisms by development of rapid resistance and is not used in human TB treatment regimens.[18,38]

TABLE 94.3 Example of a *Mycobacterium tuberculosis* Treatment Protocol for an Asian Elephant *(Elephas maximus)

Treatment Phase	Drug	Frequency	Notes
Intensive†	INH 2–5 mg/kg rectally plus PZA 20 mg/kg rectally or ETH 15 mg/kg orally plus RIF‡ 10 mg/kg orally Plus/or LEVO, 15 mg/kg rectally	5 days/week	INH should be started as soon as possible and even before other drugs Combination of 3 drugs should continue for minimum of 8–10 weeks
Continuation	INH 5–7 mg/kg rectally plus RIF, 10 mg/kg orally Plus/or LEVO, 15 mg/kg rectally	3 days/week	44 weeks

*Sample protocol only. Elephants have been treated with these doses and intervals but that does not imply success or tolerance of these medications and doses in other elephants. This table's treatment schedules have been used in elephants and are adapted from those recommended for the treatment of *Mycobacterium tuberculosis* (Mtb) in humans.[38]
†Elephant should receive weekly trunk wash (TW) testing by combined culture and real-time polymerase chain reaction (qPCR) for first 8–10 weeks of therapy. It is recommended that detectable shedding of Mtb should cease during initial phase before proceeding to continuation phase.
‡A fluoroquinolone may be used instead of or in conjunction with rifampin (RIF) based on susceptibilities and elephant's compliance with taking oral medications.
ETH, Ethambutol; *INH*, isoniazid; *LEVO*, levofloxacin; *PZA*, pyrazinamide; *RIF*, rifampin.

Occupational and Public Health Considerations

Mtb is an important zoonotic disease with regulatory responsibilities for the veterinarian and elephant holding institution. Once a diagnosis is made, it is important that the proper authorities be notified in a timely manner. Decisions about human contact exposure and use of proper personal protective equipment should be made with the recommendations and oversight of local public health authorities. Meticulous health record keeping is strongly advised to assist in documentation of the disease and assisting public health authorities.[5]

Conclusions

Mtb infection is an important disease of the Asian elephant in human care, with animal and human health considerations. Much remains to be learned about the disease. However, with appropriate treatment, management of Mtb-infected elephants can be accomplished while mitigating the risks to human health.

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Elephant Endotheliotropic Herpesvirus

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Elephant endotheliotropic herpesvirus (EEHV) can cause acute, often fatal, hemorrhagic disease (EEHV hemorrhagic disease [EEHV-HD]) in young Asian elephants *(Elephas maximus)*, most commonly between 1 and 8 years of age. For zoo veterinarians, this disease may be one of the most challenging and daunting aspects of caring for a breeding elephant herd at their institution. The zoo clinicians' best tools for managing EEHV are preparedness, vigilance, and an early, aggressive approach to treatment. Details on the therapeutic approach to an elephant ill from EEHV-HD have been published, and this chapter is meant to augment, not replace, that important information.[1]

Elephant Endotheliotropic Herpesvirus Impact and Epidemiology

Healthy Asian and African *(Loxodonta africana)* elephants have been shown to shed one or multiple types of their species specific (or species-related) EEHV virus as part of a natural infection cycle that has evolved over millions of years.[2–6] Based on current research, all adult elephants appear to carry and shed one or more EEHV strains and/or species intermittently and asymptomatically. Applying known herpesvirus biology to EEHV, this tells us that all adult elephants survived an episode of EEHV exposure and viremia at some point in their lives and are now latent carriers of the virus.

Viruses endemic within Asian and African elephant populations are listed in Table 95.1. The complete genome sequences have been determined for all the major EEHV species endemic for Asian elephants (EEHVs 1, 4, and 5).[7–10] This achievement has led to significant advances in the understanding of the evolution of EEHV and development of tools with which to diagnose, treat, and develop future vaccines to EEHV.[11] Although exposure to EEHV appears to be a natural process, Asian elephants between 1 and 8 years of age are at high risk of developing EEHV-HD associated with EEHV infection, most commonly due to EEHV1.[1] Older Asian elephants and African elephants are also susceptible to EEHV-HD but with less documented frequency thus far. Asian elephants under human care in North America, Europe, and several Asian range countries have succumbed to EEHV-HD, and deaths have also been documented in wild Asian elephants.[12–15] EEHV is a global issue that we are also fighting at a very local level.

Asian Elephants

Based on May 2017 population numbers, EEHV-HD was the cause of 53% of deaths in all Asian elephants born in North America since 1980, making it the single greatest cause of death in this cohort. The year 1980 is selected as a cutoff in North America because of the increased reliability of records, and the beginning of captive births around that time period. As of May 2017, there have been 35 cases of EEHV-HD confirmed in Asian elephants born in North America since 1980 (of 141 elephants born), indicating that 25%, or one in four, elephants in this cohort have developed EEHV-HD. Eleven of these elephants survived infection and 24 succumbed, leading to an overall mortality rate of 68% in North America.

In Europe, of more than 200 Asian elephants born since 1995, 43 have died, and 60% of those deaths (26) were due to EEHV-HD, making it the largest cause of death in Asian elephants born in Europe, as well.

The first published, polymerase chain reaction (PCR)-confirmed case of EEHV in Asia was reported in Cambodia in 2006.[16] More recently, publications have documented a fatal EEHV1 infection in Laos, fatal EEHV1 and EEHV4 infections in Thailand, and nine fatal EEHV1 infections in both free-ranging ($n = 4$) and captive elephants ($n = 5$) in India.[12–14] Healthy Asian elephants under human care in India were shown to shed EEHV1, EEHV4, and EEHV5 from their trunks in 2014, a finding very similar to what is seen in Asian elephant herds in North America and Europe.[4,3,17] Much remains to be learned about the impact of EEHV on the estimated 15,000 captive Asian elephants and greater than 40,000 wild elephants across Asia. During the First and Second Asian EEHV Strategy Meetings in 2015 and 2016, more than 80 cases of EEHV-HD were identified among most Asian elephant range countries (Thailand, India, Cambodia, Indonesia, Myanmar, Nepal, Sabah, Borneo, Laos, and Peninsular Malaysia), with less than 5 elephants surviving EEHV-HD and 12 of the deaths identified in free-ranging elephants in India. Wildlife

TABLE 95.1 Endemic Elephant Endotheliotropic Herpesviruses

	Fatalities Worldwide	Year of Discovery
Asian Elephants *(Elephas Maximus)*		
EEHV1A	38	1999
EEHV1B	4	2001
EEHV4	2	2007
EEHV5	1	2008
African Elephants *(Loxodonta Africana)*		
EEHV2	2	1999
EEHV3	1	2007
EEHV6	1	2009
EEHV7	0	2010

G.S. Hayward, personal communication, May 2017.

veterinarians in some countries suspect much higher losses due to EEHV but are limited in their ability to confirm cases due to lack of diagnostic laboratories.

African Elephants

EEHV has caused disease in African elephants as well, and more research is needed to better understand the epidemiology in this species. The impact of EEHV on African elephants, both on free-ranging populations and on those under human care, remains largely unknown. Multiple EEHVs have been identified from pulmonary and skin nodules of asymptomatic, free-ranging African elephants, as well as African elephants under human care.[18,19] Very sporadic reports of African elephant fatalities from EEHV exist, including EEHV2 in a 11-month-old male and a 13-year-old female.[20] Two African elephants, one 15-month-old calf and one 5 years old, have been treated for EEHV-HD and survived EEHV6 and EEHV3B infections, respectively.[21–24] A 10-year-old African elephant housed in a zoo in Thailand succumbed to HD associated with EEHV6 infection.[25] Preliminary surveys evaluating trunk wash shedding in two North American African elephant herds have demonstrated asymptomatic shedding of EEHV6 and EEHV3 sporadically in trunk washes.[26]

Elephant Endotheliotropic Herpesvirus Preparedness

All institutions housing elephants should establish an EEHV plan that outlines monitoring for viremia, treatment of ill animals, and necropsy guidelines and highlights the supplies needed for each. Drug acquisition should be thought out ahead of time; famciclovir (FCV) is the antiviral drug most often used to treat EEHV, and the amount required to treat an elephant is not readily available on short notice. All protocols should be developed jointly with veterinary and elephant care teams, with support of key zoo administrators. Decision-making strategies and communication tactics should be discussed ahead of time so that critical time is not wasted on long meetings when an elephant is ill. Preparation for EEHV may be a costly and time-consuming process and requires the full cooperation of all stakeholders.

A potentially overlooked hallmark of EEHV preparedness is to cultivate and maintain open lines of communication between the veterinarians and the elephant care team and to establish institutional support from administrators and public relations personnel. This is critical to allow for bilateral flow of important information, as well to streamline the process of discussions and decisions that will be part of any EEHV-HD case.

Elephant Endotheliotropic Herpesvirus Vigilance

Until more is known about the virus, the recommendations listed later represent the EEHV community's best attempts at increasing young elephant survival in face of the constant threat of EEHV. Knowledge on EEHV is constantly growing; it is likely that some of the information in this chapter will be outdated by the time it is in print. The zoo clinician's most up-to-date resource for EEHV information on epidemiology, treatment, necropsy, sample protocols, and the EEHV Advisory Group current recommendations is our website: www.eehvinfo.org.

Historically we have seen that elephants die of EEHV-HD rapidly, often within 24 hours of showing clinical signs of illness.[1,15] By the time the virus has caused enough internal damage for illness to be perceptible in these stoic, frequently inaccessible patients, the damage is often irreversible, even with treatment. Research has shown that elephants ill from EEHV-HD can be viremic up to 2 weeks *prior* to the onset of clinical signs.[22] Clinical experience has shown that elephants with EEHV-HD often demonstrate changes in their hemograms early in viremia, also prior to the onset of clinical signs.[27–29] The changes include mild to moderate leukopenia, particularly monocytopenia, and thrombocytopenia. These subtle changes are most notable when compared with the individual elephant's own complete blood count (CBC) ranges and may be overlooked when compared with more general elephant reference values. Although monitoring for anemia has been recommended in previous references, the authors have found that hematocrit fluctuates with hydration status and is not as prognostically helpful as overall leukocyte count, monocyte count, and thrombocyte count.

Regular measurement of fecal bolus temperature, body weight, noninvasive blood pressure, heart rate, respiratory rate, oral mucosa coloration, and normal sleeping patterns are important steps in establishing normal value ranges for

• BOX 95.1 Clinical Findings Associated With Elephant Endotheliotropic Herpesvirus Hemorrhagic Disease in Pre, Early, and Peak Elephant Endotheliotropic Herpesvirus Viremia in Asian Elephants *(Elephas maximus)*

Preclinical EEHV Viremia: Hematologic Findings	Early Clinical EEHV Viremia: Clinical Findings	Peak (Late) Clinical EEHV Viremia: Clinical Findings
Leukopenia: mild to moderate	Changes in sleep patterns: too much or not enough	Tachycardia
Monocytopenia: moderate to severe	Swelling of temporal gland	Bruising, hemorrhages
Thrombocytopenia: mild to moderate	Changes in feces: diarrhea or constipation	Cyanosis, primarily of the tongue
	Scleral injection	Edema, primarily of the head and forelegs
	Colic	Ascites
	Lethargy	Pericardial fluid (detected via ultrasonography)
	Decreased participation in training behaviors	
	Changes in intake: decreased appetite for food or water	
	Lameness, stiffness	

EEHV, Elephant endotheliotropic herpesvirus.

each individual elephant, which will allow for identification of subtle changes that may be a first clue to a more serious illness. Clinical findings in pre, early, and peak EEHV-HD viremia are listed in Box 95.1. Detailed hematologic and clinical findings in EEHV-HD are listed in Box 95.2.

The recommendation of the EEHV Advisory Group is to monitor at-risk elephants (1–8-year-old Asian elephants) weekly for EEHV viremia via whole blood quantitative PCR (qPCR). This is the best way to detect emerging EEHV-HD early and allow for early, aggressive treatment. In addition, measurement of CBC of at-risk elephants weekly helps to establish individual reference ranges for key parameters (white blood cell count, monocytes, and platelets) and helps us identify subtle decreases that may be associated with early viremia. If at-risk elephants show any signs of abnormal behavior, including decreased appetite, changes in sleep patterns, lameness, or changes in mentation or training, blood should be collected immediately for EEHV qPCR and CBC, even if this requires standing sedation to accomplish.

Early, Aggressive Treatment for Elephant Endotheliotropic Herpesvirus

All ill young elephants should be considered as possible EEHV-HD cases until proven otherwise by the results of whole blood qPCR testing. Treatment should be initiated rapidly and often before confirmation of EEHV qPCR results is possible. FCV is the antiviral most often used in North America and in Europe to treat EEHV-HD, and acyclovir and ganciclovir have also been used. The efficacy of FCV against EEHV has not been proven. However, to date, there are no peer-reviewed data available to establish that FCV does not have effect against EEHV. Until proven otherwise, it remains best practice to treat EEHV-HD cases with FCV. Pharmacokinetic data of FCV in healthy Asian elephants and limited data from clinically ill animals indicate that potentially therapeutic blood levels of penciclovir may be achieved.[28,30–32] Antivirals are only one aspect of treatment, and it is becoming apparent that supportive care is just as important, if not more so, in the management of an EEHV-HD case.[1,28,29] Rectal fluids should be initiated immediately, and they have a striking ability to improve an elephant's hydration and demeanor. Rectal fluids should be administered through a soft-tipped hose and may be administered as frequently as every 2 hours, at a minimum of 3–4 times daily in dehydrated animals. Fresh and frozen-thawed elephant plasma, along with crystalloids, may be administered via intravenous boluses under standing sedation, every 1–3 days as indicated by clinical condition and CBC status. Antibiotics for secondary infections, antiinflammatories (at low doses in well-hydrated animals), and opioids have also been given to elephants with EEHV-HD. A small number of Asian elephants ill from EEHV-HD have been treated with corticosteroids, when evidence of disseminated intravascular coagulation is observed. This treatment option has not been evaluated thoroughly enough to be strongly recommended, although clinicians should consider its use. Table 95.2 describes dosages and frequencies recommended for EEHV therapy.

When to treat an elephant with EEHV-HD is as important as how to treat one. With weekly monitoring of CBCs and EEHV qPCR in at-risk elephants, the zoo clinician

• BOX 95.2 Detailed Clinical, Laboratory, and Physical Examination Findings by Group in Disease Associated With Elephant Endotheliotropic Herpesvirus Hemorrhagic Disease in Asian Elephants *(Elephas maximus)*

Clinical Observations Associated With EEHV-HD

Group 1: Changes in Activity/Sleeping Pattern
Sleeping more
Sleeping less

Group 2: Signs of Discomfort or Acute Pain
Abdominal pain: stretching, rolling, colic behavior
Musculoskeletal pain: lameness or stiffness, may shift between legs

Group 3: Changes in Water or Food Intake, or in Fecal Output
Decreased appetite
Decreased water consumption
Diarrhea or hard stools
Decreased stool production, constipation

Group 4: Mentation Change
Subdued, lethargic
Appears confused, any neurologic signs
Decreased participation in training with keepers

Group 5: Oral Lesions
Hyperemic oral mucosa
Petechiae/ecchymoses

Group 6: Ocular Abnormalities
Scleral injection
Icterus of sclera
Retinal hemorrhage

Group 7: Edema
Swelling or fluid accumulation visible grossly, particularly head, trunk, and neck
Excluding presence of ventral or dependent edema (nonspecific finding in elephants)

Group 8: Cyanosis
Present on tongue or other mucous membranes

Laboratory and Physical Examination Findings Associated With EEHV-HD

Group 1: Presence of EEHV Viremia
Confirmed via whole blood PCR at a validated EEHV testing laboratory

Group 2: Clinicopathologic Abnormalities (20%) Above or Below Normal Values
>2% neutrophil bands present
Changes in WBC: leukopenia (early viremia) or leukocytosis (later)
Changes in monocytes: monocytopenia (early viremia) or monocytosis (later)
Changes in platelets: thrombocytopenia (early viremia) or thrombocytosis (later)
Changes in RBC: Anemia
Elevations in acute phase proteins

Group 3: Cardiac Abnormalities Noted on Physical Examination
Tachycardia
Arrhythmia
Heart murmur
Changes in blood pressure (must know individual baseline)
Pulse oximetry <95%

Group 4: Alteration in Body Temperature (Must Know Individual Baseline)
Fecal bolus temperature: Above or below normal

Group 5: Evidence of Fluid Accumulation
Abdominal fluid
Pericardial fluid

EEHV, Elephant endotheliotropic herpesvirus; *EEHV-HD*, elephant endotheliotropic herpesvirus hemorrhagic disease; *PCR*, polymerase chain reaction; *RBC*, red blood cell; *WBC*, white blood cell.

may identify a case of EEHV-HD early, often prior to the onset of any visible clinical signs of illness. Alternatively, regular monitoring of blood via qPCR may also pick up low-level, subclinical viremia that is likely a normal occurrence in young elephants. It may be a challenge to predict if viremia will remain subclinical or will climb and cause clinical disease, and repeated blood testing, up to daily, may be necessary. Fig. 95.1 shows criteria for starting treatment for EEHV-HD. Box 95.3 lists criteria for discontinuing EEHV-HD treatment. It is important to note that most of the data collected thus far are based on experience with EEHV1A and EEHV1B in Asian elephants, whereas interpretation of viral loads for EEHV4, EEHV3, and EEHV5 in Asian elephants and any viral loads in African elephants are not as well established.

Even conscientious monitoring and timely treatment cannot guarantee a successful outcome. Necropsy of an EEHV-HD case is an important opportunity to gain more information on this devastating disease. Full necropsy guidelines are available at www.eehvinfo.org.

Future Directions

Although our understanding of EEHV has grown astronomically in the past 5 years, there is still very much we do not know about this virus. Current research efforts are focused on antibody measurement and T-cell assays to identify key immunogenic viral proteins as a basis for future vaccine development and tools to evaluate response to such vaccines, as well as fine tuning EEHV-HD treatment recommendations. Some headway is being made into understanding the elephant immune response, although there is much more to learn.[33–35] An ultimate goal of the EEHV community is to develop an EEHV vaccine to decrease the severity of clinical illness, if not eliminate illness altogether, and there is still much work to be done before

TABLE 95.2 Treatment Recommendations for Elephant Endotheliotropic Herpesvirus Hemorrhagic Disease

	Recommendation	Comments/Source
Fluid Therapy		
Rectal fluids	Bolus of 10–20 mL/kg TID to QID, up to every 2 h	Warm water, hose or tap water, administered with soft-tipped hose
Crystalloids	IV bolus 0.3–4 mL/kg	Should always be followed by rectal fluids*
Elephant plasma	IV bolus 0.5–2 mL/kg†	
Antiviral Therapy		
Famciclovir	15 mg/kg PO or rectally TID‡	Elephant PK[30]
Ganciclovir	5 mg/kg IV BID, each dose given slowly diluted in 1 L of NaCl	Used in 2 elephants in United States with no apparent adverse effects
Aciclovir	15 mg/kg BID orally, rectally or IV	Used in 2 elephants in Thailand and 1 in Sweden (survived EEHV-HD)
Antibacterial therapy	Broad spectrum recommended	See literature[1]
Adjunctive Therapy		
Butorphanol	0.008–0.014 mg/kg IM q4h	For signs of discomfort/colic
Omeprazole	0.7–1.4 mg/kg PO SID	Equine dose[36]
Flunixin meglumine	0.25–0.5 mg/kg IM SID	Equine antiendotoxemia dose,[36] only in well-hydrated animals
Furosemide	1 mg/kg IM	Equine dose[36]
Triamcinolone	0.067 mg/kg IV	Suggested dose, use only if signs of DIC are seen
Dexamethasone	0.05–0.1 mg/kg IV or IM	Suggested dose, use only if signs of DIC are seen
Standing Sedation		
Asian Calves		
Butorphanol	0.045–0.075 mg/kg IM	Houston Zoo EEHV Protocol
Detomidine	0.011–0.022 mg/kg IM	Houston Zoo EEHV Protocol
Atipamezole	5 times detomidine dose	Houston Zoo EEHV Protocol
Naltrexone	2.5–5 times butorphanol dose	Houston Zoo EEHV Protocol
Light/Calming Sedation		
Adult Asian Cows		
Butorphanol	20 mg (0.006 mg/kg)	Houston Zoo EEHV Protocol
Detomidine	10 mg (0.0026 mg/kg)	Houston Zoo EEHV Protocol
Atipamezole	5 times detomidine dose§	Houston Zoo EEHV Protocol
Naltrexone	2.5–5 times butorphanol dose§	Houston Zoo EEHV Protocol

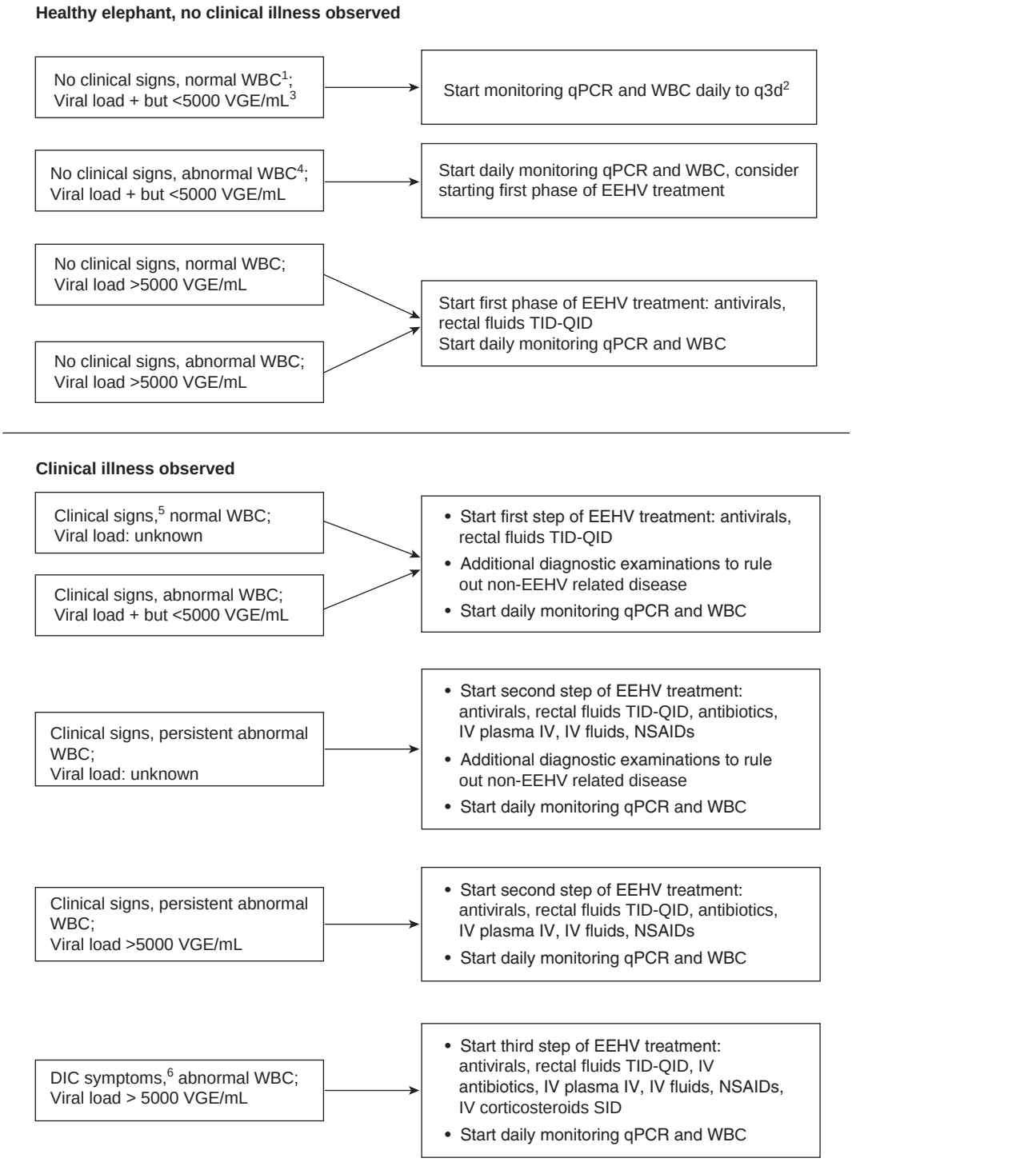
*Asian elephants *(Elephas maximus)* have very low serum osmolarity and are hyponatremic and hypochloremic compared with other species. The normal serum osmolarity range of Asian elephants is 252–270 mOsm/L. Commercial crystalloids should be considered hypertonic for elephants and should always be followed by rectal fluid administration. (E. Wiedner, personal communication, May 2015.)

†Plasma should be polymerase chain reaction (PCR) tested when collected from the donor elephant, and cross matching should be performed to reduce the chance of transfusion reactions. More information on plasma transfusion and cross matching may be found on www.eehvinfo.org.

‡Once viral load peaks and starts to decline, the author has decreased frequency to BID.

§Reversal not always needed; cows usually recover smoothly on own.

DIC, Disseminated intravascular coagulation; *EEHV*, elephant endotheliotropic herpesvirus; *EEHV-HD,* elephant endotheliotropic herpesvirus hemorrhagic disease; *IM,* intramuscular; *IV,* intravenous.



• **Figure 95.1** Elephant endotheliotropic herpesvirus treatment in Asian elephants between 1 and 8 years of age. *CBC*, Complete blood count; *DIC*, disseminated intravascular coagulation; *EEHV*, elephant endotheliotropic herpesvirus; *EEHV-HD*, elephant endotheliotropic herpesvirus hemorrhagic disease; *IV*, intravenous; *qPCR*, quantitative polymerase chain reaction; *WBC*, white blood cell.

• BOX 95.3 Discontinuation of Elephant Endotheliotropic Herpesvirus Hemorrhagic Disease Treatment in Asian Elephants *(Elephas maximus)* 1–8 Years Old

Consider decreasing frequency of rectal fluids/famciclovir to BID when:
- Viral load begins to decrease consistently (may bounce up and down first)
- WBC/monocytes/platelets are normal or elevated
- Elephant appears clinically normal

Consider discontinuing treatment with rectal fluids/famciclovir when:
- Viral load is <5000 vge/mL
- WBC/monocytes/platelets are normal or elevated
- Elephant appears clinically normal

WBC, White blood cell.

that is accomplished. In addition, a better evaluation of the epidemiology and distribution of EEHV in North America, Europe, and Asian range countries is important to better understand the impact of this disease on wild and captive elephant populations. Capacity building, in the form of EEHV diagnostic laboratories throughout Asia, is the first step in this process. Finally, education of zoo professionals, as well as the lay public, by sharing our success and advances with EEHV, is critical to establishing public support for elephant institutions and EEHV-related research.

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96

Elephant Pregnancy and Parturition: Normal and Abnormal

IMKE LÜEDERS

Although elephant births occur with some regularity in selected zoological facilities, globally, we still remain a long way from having a self-sustaining captive population. Although the birthing process and postnatal period are critical periods for mother and calf, the increased number of captive elephant births during the past 20 years has led to a greater understanding of elephant reproductive physiology as well as to significant improvements in elephant birth management. The aim of this chapter is to provide a general overview of elephant pregnancy, parturition, and peripartum complications and to make recommendations regarding appropriate veterinary care for both the elephant cow and calf when complications arise. Although Asian *(Elephas maximus)* and African elephant *(Loxodonta africana)* reproductive physiology is similar during gestation and parturition, a few differences exist, which are described. In addition, more data have been generated on Asian elephants, because the numbers of captive births in African elephants remain much lower than in Asian elephants, as is similarly reflected in the ensuing text.

The Normal Pregnancy

The mean gestation length (GL) of African and Asian elephants in European facilities is 642 and 655 days, respectively.[1] Similar mean gestation periods of 640 and 658 days for African and Asian elephants, respectively, have been recorded in a study including gestations from North America and Japan.[2] Thus Asian elephants carry longer than African elephants. No difference was seen between GL with respect to calf gender, but individual variation in GL is high (Table 96.1), and GL appears to increase with parity (A. Oerke, personal communication, November 3, 2016).

The elephant pregnancy is likely maintained only by progestagen secretion of multiple corpora lutea, because the placenta is steroidogenically inert.[3] Based on ultrasonographic findings, implantation does not occur earlier than 45 days postovulation, after which luteal rescue occurs.[3,4]

Pregnancy is best confirmed by weekly serum hormone monitoring or noninvasively by urine or fecal progesterone metabolites. The normal luteal phase is between 8 and 10 weeks; thus prolonged elevation of progestagens beyond 16 weeks is indicative of pregnancy. It should be noted, though, that a prolonged luteal life span has been observed in nonpregnant Asian elephants as well as very low progesterone values in pregnant elephants (unpublished data). However, the typical progestagen profile of a pregnancy shows an increase within a few hours up to 3 days postovulation, after which levels rise and remain elevated for about 6–8 weeks. This is usually followed by a transient decline from weeks 8–10, before progestagens increase to pregnancy levels. African and Asian elephants show slight differences in their pregnancy hormone patterns.[5]

Pregnant and cycling elephants show multiple corpora lutea on both ovaries, despite being uniparous although with rare twin births. A single corpus luteum that forms after ovulation develops along with additional, up to 10, accessory corpora lutea (CL).[3,6] These accessory CL are derived from luteinized follicles that appear after the first of two luteinizing hormone peaks and prior to ovulation.[6] The main steroid produced by elephant luteal cells is not progesterone itself but its 5α-reduced metabolites.[7] Thus commercial progesterone assays must be tested and validated for sufficient cross-reactivity. Serum prolactin (PRL) measurements may provide another endocrine verification of pregnancy. Prolactin values increase by more than 100 times after months 4–6 of gestation and remain high until after parturition.[8] Therefore a single elevated serum PRL measurement 6 months after ovulation is indicative of pregnancy. Again, elephant PRL detection warrants special endocrine assays. Mating observations are not always reliable indicators because some bulls will still copulate with a pregnant female.

Pregnancy confirmation is also possible by transrectal ultrasound, using a 2- to 7-MHz convex ultrasound probe. The earliest observations of an embryonic vesicle—a small, anechoic, 5–8 mm round structure within the uterine horn lumen—may be possible by 45–50 days postovulation, but an inexperienced investigator should wait 70–100 days to confirm a true embryo.[9,10] Transrectal ultrasound may be

TABLE 96.1 Data Documenting Normal Elephant Pregnancies and Parturition Resulting in Live Births

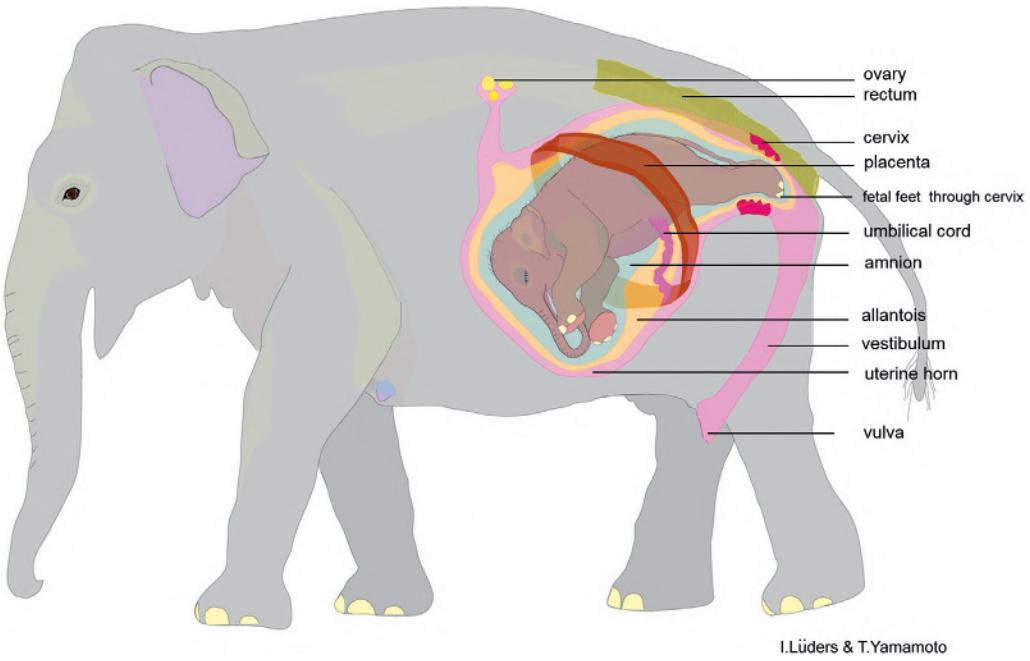
Variable	Asian Elephant *(Elephas maximus) (n)*	African Elephant *(Loxodonta africana) (n)*
General Pregnancy		
GL total (range)	622–693 (28)[1] 617–690 (21)[2]	624–660 (18)[1] 625–667 (28)[2]
GL female calf (mean)	655 (10)[1] 645 (11)[2]	646 (10)[1] 654 (12)[2]
GL male calf (mean)	657 (18)[1] 635 (10)[2]	638 (8)[1] 661 (16)[2]
Beginning of breast development	~80% in 2nd or 3rd trimester (*14*)[11]	
Beginning of milk secretion (prev. nonlactating females)	~60% a few days prior parturition, 30% at 2–4 weeks prior parturition, seldom cases of several months prior birth (10)[11]	
Labor		
Time from serum progesterone drop until parturition	<24 h up to 7 days, usually within 1–3 days (personal observation), up to 14 days reported[15]	
Time from expulsion of mucus plug until visible labor	~ 60% within 12 h, up to 72 h recorded (14)[11]	
Time from expulsion of mucus plug until parturition		median: 15 h, range: 4–99 h (8)[28]
Time from first active labor until calf expulsion	~70% within 6 h, up to 24 h recorded (*25*)[11]	
Time from bulge under tail until parturition		median 13 min, range: 3 min–2.5 h (10)[28]
Parturition		
Fetal position during birth: Posterior presentation	65% (37)[11]–71% (35)[20]	
Fetal position during birth: Anterior presentation	29% (35)[20]–35 % (37)[11]	
Time from rupture of allantois until birth	90% within min to 6 h, one case of 55 h (32)[11]	
Afterbirth expelled	85% within 6 h (41)[11]	median: 3 h, range: 72 min–17 h (12)[28]

Because more captive births of Asian elephants have been recorded, most data refer to this species. It should be noted that large variation exists between individual elephants. Thus deviations from these values may still result in healthy calves being born.
n, Number of subjects contributing; *GL*, gestation length.

inconclusive beyond 6 months of gestation, because the fetus is then large enough to sink into the abdominal cavity, removing it from ultrasound visibility when the elephant is standing. Yet another indicator of pregnancy may be visualization of a mucus plug sealing the cervix and vagina and appearing as an anechoic mass within the vagina. Usually, from months 10–12 onward, the fetus is visible by transabdominal ultrasound. The elephant skin should be soaked in water for several minutes before the probe is applied. Both flanks should be screened by slowly moving the probe dorsally to ventrally. As the pregnancy advances, the fetus may be found by scanning the ventral abdomen.

Normal Parturition Process

The majority of elephant births occur at night. The maternal and/or fetal trigger for onset of labor is unknown. Due to space limitations caused by the increased body size of the fetus, which restricts its mobility in utero, the position in which an elephant calf enters the birth channel is likely determined several weeks prior to parturition. An elephant calf may be born in either anterior or posterior position. However, approximately 70% of elephant calves are born hindlimbs first, and this position appears advantageous for successful delivery (Fig. 96.1).[11,12]



• **Figure 96.1** Schematic diagram of a pregnant Asian elephant giving birth to a fetus in posterior position. Usually the allantoic sac breaks, releasing fluids, and the calf slides fully enveloped within the amniotic membranes through the vagina and vaginal vestibulum.

The normal progress of events during parturition may be described as follows: (1) Onset of increased discomfort 7 days to 48 hours prior to birth; (2) restlessness and first abdominal contractions representing the first stage of labor; (3) loss of the mucus plug that sealed the vagina during pregnancy; (4) second stage of labor with increased signs of pain, stronger abdominal contractions, straining, agitation; (5) rupture of allantois, although water may break later; (6) appearance and disappearance of a bulge under the tail below the anus and advancement of the fetal feet (see Fig. 96.1); (7) third stage of labor with strong contractions and final entrance of the fetus into the vestibulum; (8) continued visibility of the bulge as soon as the calf's body enters the female's pelvic cavity (and the hymen breaks in nulliparous cows); (9) movement of the calf through the vaginal vestibulum and expulsion from the mother's body, usually still covered in the bluish amnion; (10) delivery of the afterbirth (girdle placenta and allantoic membranes) 45 minutes to a few hours later.

The stages are not always clearly discernible from each other, and large variation in time spans from what is described here may still result in a successful birth (see Table 96.1). The entire process may be finished within less than an hour but may take up to 48 hours.

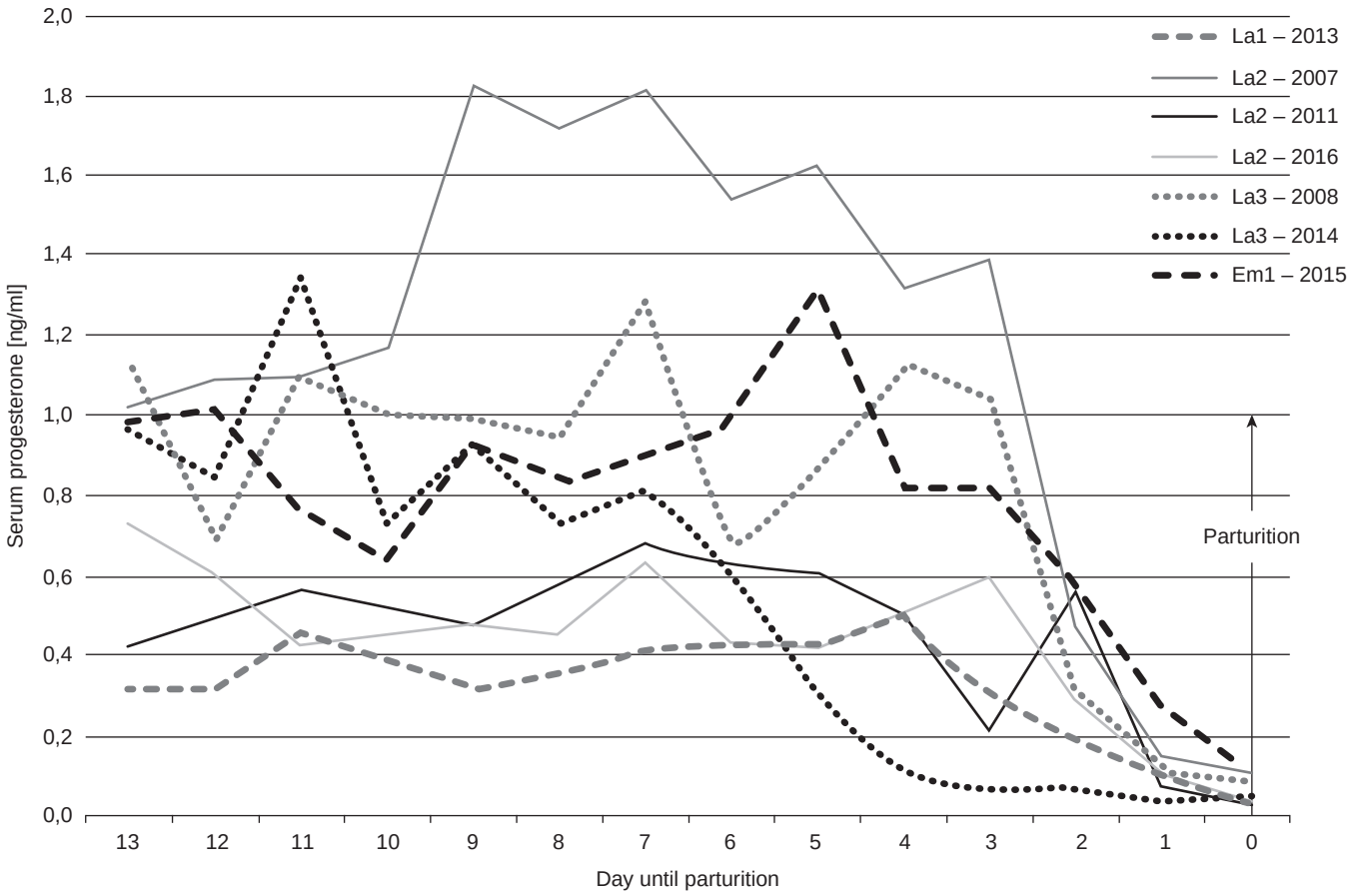
Prediction of Parturition

The most likely time of parturition can be calculated as 620–660 days from the day of last observed mating or measured rise in progestagens. As described earlier, gestational length in elephants resulting in live birth can vary by up to 3 months (see Table 96.1). Prediction of parturition is possible on an individual basis only by observation of physiologic or behavioral changes or, preferred, daily serum progesterone determinations, because progestagen levels usually drop at least below two-thirds of the mean pregnancy value a few days prior to parturition (Fig. 96.2).[13,14]

Physical changes predictive of birth may not always be as obvious in elephants as in other species, especially if the pregnant female has a nursing calf with her, which precludes use of breast development and milk secretion as indicators. Ventral abdominal or vulval edema may become visible early in gestation and may cause discomfort, although edema has not been observed to cause any difficulties during parturition. Regular exercise and hydrotherapy with cold and warm water may be helpful to increase circulation and avoid the development of necrotizing tissue.

One indication of upcoming birth is loss of the mucus plug from the vaginal vestibulum, which usually occurs a few hours prior to birth, although up to several days prior or even partial mucus plug loss in the middle of gestation have also been described (see Table 96.1).[14,15] The plug can be white, yellowish to brown, sticky and mucoid, or bloody. Other signs may include frequent defecation and urination, small fecal ball size, reduced appetite, restlessness, nervousness, beating the vulva with the tail, and finally labor. Onset of the labor is characterized by "freezing" intermittently (standing still with no movement), spread legs, moving flanks, tail flagging, straining, kneeling, lateral recumbency, and repeatedly lying down and then standing back up.

The most reliable and exact measurable indicator for imminent parturition is through the daily measurement of



• **Figure 96.2** Examples of hospital-measured serum progesterone courses at the end of pregnancy in three different African and one Asian elephant with live-born calves (La1: one pregnancy, La2: three pregnancies, La3: two pregnancies; Em1: one pregnancy). Daily and later twice-daily serum samples should be measured to detect imminent parturition in elephants. Hormone values start decreasing significantly up to 5 days prior to parturition (day 0), but as late as 1 day prior to giving birth in these examples. (Data credit: Dr. Arne Lawrenz, Wuppertal Zoo, Germany.)

serum progesterone. Usually, a sudden greater than 50% drop in measured pregnancy progestagen levels around the due date indicates that birth may occur soon (see Fig. 96.2). From this point, blood should be taken daily and immediately checked for its progesterone concentration. A good idea is to ask a hospital nearby to analyze the blood/serum for progesterone on a daily basis.

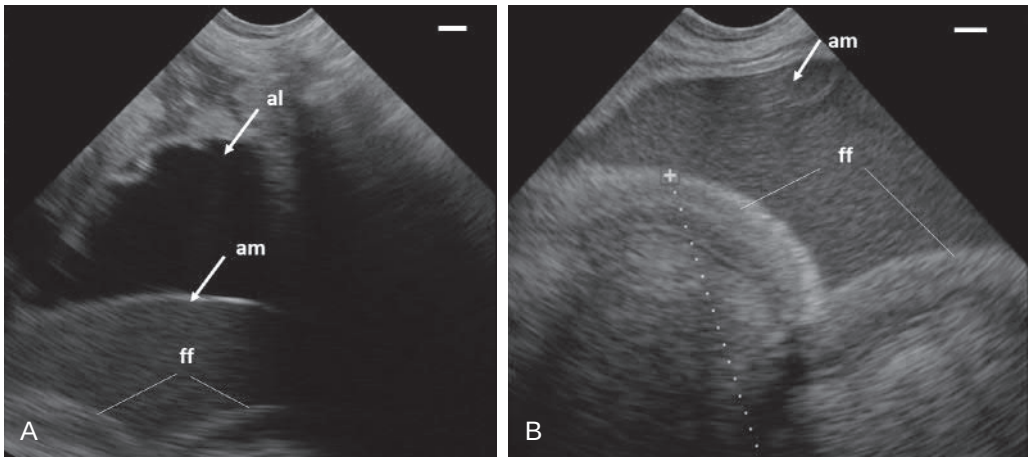
Another invaluable tool for the monitoring of the pregnant female is transrectal ultrasound. Tolerance for rectal examination and manipulation should be trained prior to birth and become a routine occurrence. The ultrasound is important to assess the progress of the birth and degree of the opening of the cervix. Fluid and fetal feet pushing up toward the cervix may become visible up to 32 hours prior to birth, even before visible signs of labor or discomfort occur. Once the cervix is opened and is visible by ultrasonography and both placental membranes and fetal limbs are identified (Fig. 96.3), the calf's birth may be expected within a few hours. Thus having an ultrasonographic look at the cervix may provide information regarding the timing of parturition and the position of the fetus and will also help to justify use of labor-inducing medication if needed.

Abnormal Pregnancy

A variety of factors may be associated with unsuccessful pregnancy outcomes. Early embryonic loss or resorption may remain unnoticed. We have encountered one case of embryonic loss suspected to be due to implantation next to a uterine leiomyoma.[10] Uterine pathologies may cause elephants not only to fail to conceive but also to fail in sustaining the growing conceptus after implantation.

As in other species, infectious diseases may cause abortion in elephants. Both poxvirus[16] and *Salmonella* infection have caused fetal loss.[17] Although elephant endotheliotropic herpesvirus (EEHV) is often suggested to be a cause of abortion or stillbirth, to date no proof exists that EEHV infection can cause fetal death (see Chapter 95).

Anecdotal reports exist of abdominal trauma causing abortion. Full-term fetal retention has also been described, which may be due to insufficient contractions resulting from malposition or malformation of the fetus (discussed later).[18] Death of the fetus, whether premature or full term, may result in the long-term retention of the conceptus without any obvious problem for the dam. The retained



• **Figure 96.3** Asian elephant ultrasound images of the cervix and uterine body during parturition. The images were obtained when labor stopped for more than 2 hours. (A) When allantoic *(al)* and amniotic membranes *(am)* were seen pushing into the opened cervix and (B) during labor, fetal feet *(ff)* were observed, the decision was made to administer 40 IU of oxytocin intramuscularly, and the calf was born 30 minutes later. (Image credit: Dr. Arne Lawrenz, Wuppertal Zoo, Germany.)

fetus may remain within the uterus or be naturally expelled days to years after death.[15,18,19]

Complications During Parturition

Problems during parturition in captive elephants occur relatively often and may be fatal for mother and/or calf. Recent statistics from the European population indicate that 12% of calves (Asian and African) are stillborn.[20] However, Asian timber elephants in Myanmar reportedly experience only 4% stillbirths.[12]

Overall, Asian elephants seem to be more prone to dystocia compared with the African species.[19] In both species, dystocia is significantly associated with stillbirth, resulting in a 50% chance in African elephants and 86% chance in Asian elephants of a dead-delivered calf.[20] Dystocia in Asian elephants was more often reported in historic data from Europe, with an incidence rate of 36%,[11] compared with only 16.4% of Asian elephants in a more recent study.[20]

Reasons for Dystocia

A number of factors may lead to dystocia and subsequent stillbirth or sometimes retention of the fetus. These are discussed in the following paragraphs.

Oversized Fetus

More problems during parturition seem to occur in the Asian elephant species compared with African elephants. The reason may be that Asian elephant calves exceed Africans for mean birth height (93.7 vs. 89.9 cm) and birth weight (116.5 vs. 103.5 kg).[2] Stillborn Asian elephant calves are heavier compared with live-born calves.[2,20]

The calf's body weight may be related to pregnancy length and the body condition of the female. A relationship between obesity in zoo elephants and dystocia thus appears likely.[15]

Malposition/Malformation of Fetus

Dystocia has been linked to both breech position and anterior presentation.[15,18,20] In a recent study, 60% of calves born head first experienced dystocia, compared with 16% born hindlimb first.[20] The same study found that 40% of the calves born head first were stillborn compared with only 12% of those born hindlimbs first. Dystocia is reportedly associated with deformities that include ankylosis, encephalomeningocoele, hydrocephalus, spina bifida, cleft palate, missing maxilla and trunk, and tetralogy of Fallot, or with malpositions such as head tilt, twisted legs, and entanglement with the umbilical cord.[15,22]

Weak Labor/Uterine Inertia

Dystocia has furthermore been linked to lack of physical fitness,[2] calcium deficiency,[23] and insufficient labor due to external stressors.[11,15,19] Studies suggest that calcium levels may actually be too low in general, and the authors have reported a dystocic Asian cow whose calcium was at baseline.[23] Normal plasma concentrations of total calcium should be around 3.6 mmol/L, and normal plasma concentrations of ionized calcium should be around 1.25 mmol/L. Elephants should be fed calcium- and cholecalciferol-rich diets at all times, particularly when close to parturition. Additionally, serum calcium levels should be monitored closely prior to parturition.[23]

It is believed that external stress factors may lead to a delay in the birthing process. Some authors suggest that elephants may actively interrupt labor, especially when humans are present.[11,15,19] However, this is difficult to confirm. Labor is a time of psychophysiologic stress. In humans, it is well established that cortisol levels increase throughout

pregnancy and continue to increase at term with advancing labor.[24] These physiologic changes are important and may be a necessity for maintaining maternal/fetal well-being and for promoting normal labor progression.[24]

For wild elephants, allomothering and herd-supported births are the norm. However, many captive elephant herds still consist of unrelated individuals. A subdominant female with no relationship to the rest of the herd may be stressed by feeling vulnerable and intimidated by other elephants. Thus being separated from the group might relieve stress, and tethering might be beneficial for an inexperienced female. However, in another elephant unused to restraint, tethering and separation might increase stress. Survey data suggest separation from other elephants and chaining as high-risk factors for dystocia.[12] Regardless, preplanning, preparation, and habituation to the envisaged birthing situation are essential to reducing external stressors for an expectant elephant.

Difficulties in Passage Through the Birth Canal

Inadequate dilation of the cervix, poor softening of the vagina and the vaginal vestibulum in older, primiparous females; fibrosis of the hymen; lower reproductive tract pathologies; and obesity may all pose obstacles during parturition.[2,15] Although older survey data suggest that nulliparous cows more than 30 years of age experience a higher incidence of stillbirth and dystocia,[25] more recent surveys do not support an association between age and dystocia but rather with primiparity.[20] Thus parity is a primary factor for passage problems.

Fitness and physical condition may play a role in the successful expulsion of a calf as heavy as 100–140 kg. This may explain why there is only a 4% stillbirth risk in working Myanmar timber elephants compared with a 12% risk in zoo elephants.[21] Exercise does help with weight loss and body shape and thus may increase the elephant's overall physical fitness.

In Myanmar timber elephants, stillbirth probability dropped from 11.3% for first-born calves to only 1.8% for later-born calves. Thus first-born calves were 6.83 times more likely to be stillborn than subsequent calves from the same mother.[21] This has also been confirmed for captive Asian elephants in Europe: of 41 first-born calves, 11 were stillborn (26.8%); but of 116 later-born calves, only 6 were stillborn (5.2%).[11]

The hymen-like structure that "seals" the entrance from the vaginal vestibulum into the vagina breaks only during first parturition (not during mating). The hymen may become increasingly fibrotic with age and thus present another obstacle during birth in older first-time mothers.[15]

Obstetrics

Veterinary intervention options are limited in elephants due to their size and special reproductive anatomy. Nevertheless, in accessible elephants with a high level of training, several valuable tools are available for assessing the situation and taking appropriate actions. Use of rectal palpation and, even better, transrectal ultrasonography are the first steps to judge the situation. The clinician should determine the position of the fetus and whether any body parts have entered the birth canal. This requires an elephant cow, whether in restricted or nonrestricted contact, to be trained to allow these procedures on a routine basis. Remote camera monitoring may help to generate information on behavior and crucial events such as time of onset of labor or allantoic rupture. For elephants managed in a captive breeding program, it is furthermore advised to contact the Taxon Advisory Group veterinary advisors or colleagues experienced with elephant birth management.

Conservative Approaches for Fetal Delivery

If the fetus is not delivered despite an open birth canal due to weak labor or if the birthing process stops, the following steps may be taken.

Triggering the Ferguson Reflex

Before any medical stimulation of labor and ripening of the cervix, manual massage of the rectal floor may be attempted to stimulate the underlying vagina and cervix. Again, training the elephant to allow rectal palpation and manipulation is essential. Both arms up to the elbows may be inserted into the elephant's rectum. By repeatedly pulling one's knuckles along the bottom of the rectal wall toward the anus with some force, stimulation of underlying vaginocervical receptors will trigger release of endogenous oxytocin (the Ferguson reflex), helping relax the cervix and induce uterine smooth muscle contractions.[15]

Calcium

If weak labor is observed and the normal passage through the birth canal seems possible, serum calcium should be measured. If total and ionized calcium is below 2.5 and 1.2 mmol/L, respectively, oral or a slow intravenous (IV) infusion of calcium should be administered.[15,23] Intravenous administration of Ca-gluconate (23%) at a dose of 400–2000 mL has been reported in a dystocic Asian elephant.[26]

Oxytocin

The elephant uterus appears to be very sensitive to oxytocin. Before this drug is administered, the degree of cervical dilation must be assessed. If the cow is multiparous, the cervix is open, parts of the fetus have entered the vagina, and the birthing process has stopped for at least an hour, oxytocin administration may be considered.[15] Uterine rupture has occurred in some cases when these preconditions were not met. The dose recommendations range from 20–60 IU intramuscularly (IM) every 2 hours (personal experience).[15] Note that it may take as long as 20 minutes from IM injection until the onset of full effect. If available, carbetocin (e.g., Depotocin, 70 µg/mL, Veyx-Pharma

GmbH, Germany) a longer- and smoother-acting metabolite, may be considered alternatively at a single dose of 200–400 µg.

Denaverine

If the cervix is not dilated and possibly only one of the calf's legs has entered the vagina, besides manual stimulation of the Ferguson reflex, denaverine hydrochloride (Sensiblex, 40 mg/mL Veyx-Pharma GmbH, Germany) where available may provide an option. This antispasmodic drug is used in domestic cattle and dogs and acts to help relax the cervix and the rest of the birth canal while not affecting labor. There is not much experience with its use in elephants, but it may be worth considering in nulliparous females with suspected inadequate opening of the cervix. This drug is administered IM at a dose of 800–1600 mg, which may be repeated once an hour later. It should be noted that this drug has no effect on early cervical dilation.

Estradiol and Prostaglandin E

Other drugs recommended in elephants to induce dilation of the cervix are transrectal or transcutaneous application of estradiol or prostaglandin E.[13] Estradiol gel (600–800 mg) may be applied by transrectal massage and may be repeated at a dose of 300–400 mg every 3–4 hours.[15] Both prostaglandin E1 (PGE1) and prostaglandin E2 (PGE2) are used in humans and mares for cervical ripening and induction of labor and may be helpful in elephants. Misoprostol, a synthetic PGE1, has been administered to elephants to dilate the cervix and induce uterine contractions at empiric doses of approximately 1000 mg orally or 500 mg transrectally every 12 hours for a 4000-kg elephant.[15] Administration of dinoprostone, a PGE2 analogue, used at a dose of 1.5–2.5 mg transrectally above the cervical region, was suggested to be effective.[15]

Surgical Approaches for Fetal Delivery

Surgical management of dystocia also warrants thorough assessment of the situation first. Only if the calf has entered the birth canal and the membranes have ruptured and use of conservative measures has failed to progress parturition should surgery be considered. Surgery is typically regarded as the last resort to save the dam's life. Cesarean section is, however, not an option in elephants. To date, six cesarean sections have been carried out in elephants, all of which resulted in the death of both mother and calf.[15] This was due to the weight of the intestines and the large size of the incision needed to extract an elephant calf, in combination with mechanical forces and the slow healing of elephant skin.

Episiotomy/Vaginal Vestibulotomy and Fetotomy

Given the nature of the elephant reproductive tract's anatomy, with the vulval opening between the hind legs and the long canal of the vaginal vestibulum, no direct access to the vagina and cervix is possible (Fig. 96.4). Thus, to reach and remove a dead fetus lying within the cervix and vagina, direct access into the vaginal vestibulum must be established by a means of a vertical incision of about 15 cm length, 8 cm ventral to the anus (see Fig. 96.4).[15,22] Extraction forces should be applied physiologically (i.e., by pulling ropes or chains attached to the fetal legs or trunk in the direction of the ground, e.g., by guiding the ropes through metal rings fixed at the floor) and not horizontally. Although use of local anesthesia rather than standing sedation has been recommended to enable the cow's straining to assist in fetal expulsion,[15] this may not always be feasible, as some females will require chemical restraint to tolerate the procedures. In cases of fetal oversize, malposition, or malformation, fetotomy is a last option; but it is needed because leaving a fetus within the birth canal will result in fatal ascending infection.[22] Fetotomy remains a delicate procedure in elephants and requires modified cattle equipment.[22] So far, several fetotomy attempts have resulted in death of the dam due to uterine trauma and infection.[15] However, one successful description in an Asian elephant exists.[22] During this 9-hour procedure, all large fetal parts were extracted through the normal birth canal using ropes passed through the vaginal vestibulotomy incision.[22]

A common postsurgical complication that has been seen following episiotomy is a persistent vestibular fistula.[22] This unnatural opening carries with it the risk of ascending infection due to fecal contamination. Therefore trauma of the tissue surrounding the incision should be avoided. Furthermore, second-intention healing of the skin while only suturing the mucous membranes and the muscular layer of the vaginal vestibulum are mandatory in order to prevent this problem.

• **Figure 96.4** Episiotomy in a dystocic Asian elephant with subsequent fetotomy. Chains have been attached to the fetal feet through the incision. After successful attachment of the chains to a part of the dead fetus, the chain was guided through the distal vaginal vestibulum and the dissected parts of the fetus were removed, following the natural route to avoid tissue trauma at the incision site. (Photo credit: Dr. Willem Schaftenaar, Rotterdam Zoo, The Netherlands.)

Fetal Retention

Another relatively common scenario is the onset of obvious labor around the expected time followed by its cessation long before the calf is born. If the fetus never enters the birth canal, no membranes rupture, and the cervix remains closed, no further interventions should be done. Expulsion of the calf at a later date, sometimes years later, may occur, and females with a history of retained fetus have been observed to cycle and become pregnant again after fetal expulsion.[15,18] This option is the safest for the dam under these circumstances.

Postpartum Complications

After delivery, the placenta is usually expelled within a few hours. The afterbirth consists of the zonary placenta (a brownish placental band, which may be segmented, see Fig. 96.1), the larger section of the umbilical cord, and fetal membranes (mostly allantois). It weighs typically around 20–25 kg in total. If there has been no afterbirth expulsion observed 24 hours after parturition, oxytocin may be administered at low doses (20–40 IU IM). Case reports exist on placental retention warranting medical attention[27] or surgical access (vestibulotomy) to flush the uterine cavity. Intermitted bleeding from the vaginal vestibulum and discharge (no odor, small amounts) may occur up until 2 months postpartum. Rupture of the inner mucosa of the vaginal vestibulum and subsequent infection may occur during the delivery process, which may require systemic antibiotic treatment. Uterine prolapse in association with parturition has been observed, resulting in the death of the newborn African elephant 2 days postdelivery. Such a prolapse was successfully repositioned in an Asian elephant dam by manual massages several months postpartum (personal observation).

Conclusion

Although elephant parturition remains a very vulnerable moment for female elephants, several practical options are available for veterinarians and elephant caretakers for managing a birth successfully. Advance preparation—including appropriate barn modifications, planning of the birth scenario, and exposing the female early enough to the probable environment she will experience at the time of parturition—are important components. Additionally, training the female for rectal access, having relevant pharmaceutical options available, and consulting with experienced colleagues on the upcoming event may lead to a successful outcome even if problems arise.

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97

Elephant Care in Southeast Asia

GERARDO MARTINEZ AND JOHN ROBERTS

History

To find evidence of elephants being kept for the "modern" purpose of working with and for humans, we have to look at the early Kingdoms of the Indian subcontinent and, of course, to the Asian elephant. Two thousand years ago, the Arthashastra,[1] an Indian essay on Kingship, included chapters on and detailed instructions for keeping of captive elephants: from salaries, fines, and penalties for mahouts/keepers in charge of protecting elephants and their habitat (including capital punishment for the killing of a wild elephant), to how essential it was to any kingdom to keep a stable of war elephants, and the worth of aggressive males that could be bought into musth.[1] While wild herds have ranged from northern China[2] to the Indonesian islands since ancient times, the earliest evidence of elephants being captured to work alongside and for humans dates back to the 1st century A.D. in the Kingdom of Funan, along the border of current day Vietnam and Cambodia.[3] For the majority of the 4000+ years of elephants and humans working together because of their usefulness in war and labor, even as some nations or realms enforced a monopoly on elephant owning, very little is seen of civilian use of elephants until the British annexation of upper Burma.

The long history of elephants in captivity provided several capture and training techniques designed to take the most aggressive specimens of the planet's strongest land mammal and convert it to a willing tool of humans in as short a time as possible. These techniques were—by any standard—brutal. The Myanmar Timber Enterprises historical record puts capture and training mortality as high as 30%[4] for certain methods, while even the lowest (anecdotal) estimate is still 10%–20%.

Private wild capture of elephants in Southeast Asia has been progressively outlawed, beginning in the 1950s in Thailand and ending in the 1980s in Myanmar. However, government capture continues under certain circumstances. The majority of these captures utilize chemical immobilization.

Such high mortality rates are unconscionable, as well as unsustainable due to limited captive breeding and limitation on replacement of captive animals by capture of wild elephants; moreover, such brutality is unnecessary for an elephant that has grown up with human company.

Captivity and Handling

Elephants in Asia are maintained in captivity for a variety of purposes, depending on country laws, job opportunities, proximity of resources, and individual physical ability. They may be involved in logging, tourism, cultural and religious activities, and transportation. Furthermore, the way to teach and to perform these tasks has always been a subject of discussion due to the questionable methods that are frequently used when human and elephant interaction needs to happen.

Currently, in many Asian territories, the misconception still exists that in order to handle and control an elephant, it is necessary to impose a certain level of firmness, intimidation, and curtness directed toward it. After all, the mahouts who care for and handle the elephants have ancient training knowledge and are following common practice and the teachings and skills that have been passed down from one generation to another. Among other things, they have learned that aggressiveness and intimidation can be used as an effective tool to tame both the wild and captive-born elephants in order to control them. It is surprising that this practice has not changed throughout the years, despite the long list of injuries and fatalities of mahouts, passersby, and elephants alike, arguably attributed directly to the use of these methods.

Target Training Project

With the Convention on International Trade in Endangered Species (CITES) officially reporting 10,862[5] captive elephants in Southeast Asia (including China), and stated intent to maintain these numbers through captive breeding, it is clear that an effort to further introduce scientific positive reinforcement training techniques will be a powerful tool in improving captive elephant welfare throughout the region.

Therefore there is an urgent need in Southeast Asian countries for practical guidelines and clear recommendations

on how to effectively manage captive elephants in such a way that good health, reproduction, and welfare are equally addressed and ensured at all times. In 2011, Africam Safari Park and The Golden Triangle Asian Elephant Foundation (GTAEF) founded the "Target Training Project" in the region. This project provides an alternative nontraditional method to replace the conventional training method and is raising awareness across the region of these issues. All this is done by giving local, national, and international workshops, in which mahouts, veterinarians, and camp managers may learn and practice the skills that international professional instructors have effectively developed by working in zoos with high standards of elephant care and welfare, but without the intention to criticize or undervalue their current management system or impose a different one. Positive reinforcement is a well-known operant conditioning technique worldwide that has demonstrated its effectiveness in managing wild and domestic animals. It has been proven that it is effective for captive elephants, and thus it can help avoid punishment and the infliction of pain, by convincing and giving the elephants the choice to cooperate voluntarily through motivation and communication. This enables a mahout to manipulate the elephant's behavior safely, in a protected contact environment, by providing simple and practical ways to handle, train, and offer medical treatment without the need to cause physical or psychological harm.

A high turnover among mahouts makes elephant-oriented tourism and elephant-utilized activities potentially hazardous. High turnover is not only bad for camp owners, because it requires additional resources for hiring and training new personnel, but it is also bad for elephants because mahouts with insufficient skills and experience think they must resort to rough methods in order to control an elephant that in turn has not had sufficient time to bond with the mahout. Mahouts unfamiliar with an elephant are often unable to notice when things are amiss with an elephant. This lack of familiarity may lead to the elephant suddenly attacking the mahout or nearby tourists. The use of a training wall that works as a barrier and as a protected contact system is highly recommended for many important reasons, and it may be very valuable even to extremely experienced mahouts when uncomfortable but necessary medical procedures need to be performed on aggressive, unpredictable, and hormone-influenced (musth) elephants (Fig. 97.1).

Among different countries, the basic elephant management deceptively may seem very similar, but they can have some important variants; what the mahouts conceive as proper in one region may be dramatically different in another, from the age that the elephant has to have to begin the training process, to the feeding pattern, the kind of restraint during the day and night, and the manner to "break and crush" the elephants' spirit to make them submissive to humans. Without diminishing these differences, the "Target Training Project" immerses itself in great variety of scenarios to enact quality training lessons to produce highly qualified mahouts, tailored to address the occupations in which the elephants are involved in Fig. 97.2.

• **Figure 97.1** Foot care by using protected contact. (Photo by Rodrigo Salas.)

• **Figure 97.2** Dr. Khyne U. Mar hosting attendees from eight nationalities at the 2016 workshop in Myanmar.

GTAEF facilities in Thailand have served as a school camp, where people from several Asian countries have joined various sponsored workshops imparted by nine international instructors who have given practical and theoretical lessons about training, management, medicine, behavior, enrichment, and basic foot care. A mutual agreement with many of the attendees in the months following the workshops allows some of the instructors to visit the students' homeland and workplaces, which may be from a well-established camp in Thailand to a remote forest in the wilderness of Myanmar, and there they can teach the mahouts how to train their elephants, design and set up a training wall, or help with many of the other challenges associated with caring for captive elephants (Fig. 97.3). One of the most important difficulties has been to convince the extremely talented mahouts, whether young or experienced, to modify the ancestral practices they have been taught by their own relatives, used their whole life, and followed for many centuries; therefore, changing their mind-set to use

• **Figure 97.3** Improvised settings for classes at elephant camps inside the forest. (Courtesy Rodrigo Salas.)

• **Figure 97.4** Mahout working through a training wall in Thailand. (Courtesy Gerardo Martinez.)

• BOX 97.1 Organizations Involved in the Project

39 Organizations	Participating Countries
Elephant Camps	Thailand, Myanmar, Cambodia, Laos, Vietnam, China, Indonesia, and Sri Lanka
Conservation Centers	
Rescue Centers	
Natural Parks	
Zoological Parks	

another way to do, solve, or plan their daily work, would be impossible if there is no proof that what is being offered will benefit both their elephants and their community in some way. Thus, given the circumstances, even with a previous friendship and mutual confidence, the first practical demonstrations have to be quick, convincing, and effective, which is a challenge in itself. This may be complex when there is no option but to choose the novice elephants that the instructors have to face, but even more prevalent and daring when having to work on the most problematic, aggressive, edgy, or ailing ones. And while all that may be explained inside the classrooms, the truth is that only when the mahouts themselves see the rapid progress, with immediate benefits and fewer complications, will many of them embrace this new idea.

In a few years, the impact of the project so far has reached 166 elephant handlers and veterinarians, representing 39 different organizations from eight countries across Southeast Asia with beneficial results (Box 97.1). Both the official Myanmar and Thai government organizations responsible for training young elephants are embedding this technique into their initial training methods for young elephants, boosting the implementation of a management option that is based on gentler tendencies and benefits the health and welfare of captive elephants. This offers the elephant caretakers an opportunity to improve the health and life quality of their most valuable possession, and it is our hope that the mahouts themselves spread these skills throughout the region and teach them to future generations (Fig. 97.4).

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98

Updates in African Rhinoceros Field Immobilization and Translocation

PETE MORKEL AND PIERRE NEL

Drugs for Field Immobilization

White Rhinoceros

In general, choose a dose that provides the lightest plane of anesthesia that is safe for completing the procedure. There are essentially two options for immobilizing white rhinoceros *(Ceratotherium simum)*.

1. **Potent Opioid + Sedative + Mixed Opioid Agonist-Antagonist** (Table 98.1)

   Intramuscular (IM) etorphine is combined with IM azaperone or midazolam, and the respiratory depressant effects of etorphine[1–3] are partially reversed with IM or intravenous (IV) diprenorphine or butorphanol.[4–9] This option is currently used for most white rhinoceros field immobilizations, particularly if animals are in good condition and the terrain is challenging. Butorphanol is routinely administered at 10–20 mg per mg etorphine IV once the rhinoceros is recumbent. This improves blood gas values and thereby indirectly reduces muscle tremors.[9–11] Tracheal or intranasal oxygen supplementation in combination with butorphanol may improve hypoxemia.[12] When butorphanol is administered in the dart, the animal may stop but remain standing; this medication should be used only with experienced staff. Diprenorphine at 0.1–0.2 mg per mg etorphine IV[8] may be given as an alternative to butorphanol after the animal is recumbent.
2. **Mixed Opioid Agonist-Antagonist + Sedative** (Table 98.2)

   Combining IM butorphanol with either medetomidine or azaperone[6,13] is a good alternative when immobilizing a compromised white rhinoceros. For adult white rhinoceros, 160 mg butorphanol + 10 mg medetomidine gives standing sedation in 6–8 minutes and recumbency at 12–15 minutes. The large volume of drugs may be a limiting factor with this combination. Standing animals can be pulled down with ropes, or 200–400 mg ketamine can be administered IV to induce recumbency. Reversal is achieved with administration of IV naltrexone and atipamezole.[6] If the rhinoceros needs to be "walked" (see explanation later), give 1 mg atipamezole per mg medetomidine IV. This combination with oxygen supplementation at 10 L/min usually results in good blood oxygen levels. For a deeper level of anesthesia, IV ketamine may be given.[6]

   Newborn or very small calves may be adequately restrained with approximately 20 mg butorphanol IM. For older calves, use 0.1 mg etorphine IM for every month of age up to 1 year, combined with IM butorphanol at 10–20 times the etorphine dosage in milligrams. When darting cows and calves, the cow is darted first and the calf 2–3 minutes later, or once the cow is recumbent.

Black Rhinoceros

Etorphine is still the drug of choice at approximately 4 mg IM for an adult male or female black rhinoceros *(Diceros bicornis)* in good condition.[14] Adults of the subspecies *D. bicornis bicornis* may need 5 or even 6 mg of IM etorphine.[14,15] Younger animals are given a scaled-down dose according to their size, and, for animals in poor condition, be prepared to reduce the dose by 25% or more.

Thiafentanil is being used as an alternative in a 50:50 combination with etorphine IM or by itself.[14] The dose is the same as etorphine, induction is slightly faster, and the animals are less inclined to stay on their feet. Muscle relaxation is generally good, but respiratory depression may be more profound.

Approximately 60 mg of IM azaperone is usually added to etorphine or thiafentanil for immobilizing an adult black rhinoceros, although doses as high as 200 mg may be used. Lower doses are preferred if the rhinoceros is to be translocated. IM midazolam is gaining favor as an alternative to azaperone at 20–40 mg for an adult (D. Grobler, personal communication, 2017). Alternatively, 100 mg xylazine IM for an adult is still being used.

Hyaluronidase (2500–7500 IU) added to the immobilizing mixture reduces the induction time by a minute or two, and this significantly reduces the risk in rugged terrain.[14,15]

TABLE 98.1 Suggested Intramuscular Dosages When Using Etorphine and Butorphanol in Combination With a Sedative in White Rhinoceros *(Ceratotherium simum)*

Age	Etorphine (mg)*	Butorphanol (mg) (20× Etorphine Dose)
1 month	—	20
3 month	0.2	4
6 month	0.5	10
1 year	1	20
2 year	2	40
3 year	3	60
4 year	4	80
Adult	4.5	90
Large Adult	5–6	100

**Use etorphine and butorphanol (butorphanol either in dart or intravenously once the animal is down) in combination with one of the sedatives listed below (midazolam or azaperone preferred):*

Age	Midazolam (mg) (10 × Etorphine Dose)	Azaperone (mg)	Detomidine (mg)	Medetomidine (mg)
1 month	—	—	—	—
3 month	2	8	0.6	0.5
6 month	5	15	1.25	1
1 year	10	20	2.5	2
2 year	20	40	5	4
3 year	30	50	7	6
4 year	40	60	8	8
Adult	45	60	10	9
Large Adult	50	60	12	10

TABLE 98.2 Suggested Intramuscular Dosages When Using Butorphanol in Combination With a Sedative in White Rhinoceros *(Ceratotherium simum)*

Age	Butorphanol (mg)*	Medetomidine (mg) OR	Midazolam (mg) OR	Azaperone (mg)
1 year	35	2	10	20
2 years	70	4	20	30
3 years	110	6	30	40
4 years	140	8	40	50
Adult	160	10	45	60
Large adult	180	12	50	80

**Use butorphanol and medetomidine, midazolam OR azaperone. Medetomidine is the sedative of choice because it may be easily reversed.*

Managing an Immobilized Rhinoceros

Time Use and Priorities

The first priority is to stabilize an immobilized rhinoceros quickly, and the first 5 minutes after induction are of particular importance. The most important considerations are ensuring there is a patent airway, the animal is breathing and blood oxygenation is adequate, there is adequate blood flow to the muscles of the legs, and the body temperature is not excessively elevated (Table 98.3).[2]

TABLE 98.3 Cardiopulmonary Parameters in Resting Non-Immobized, Captive Healthy White Rhinoceros *(Ceratotherium simum)*

Parameter	Mean ± SD	Range
Heart rate (beats/min)	39 ± 0.8	32–42
Respiration rate (breaths/min)	19 ± 0.6	16–23
Rectal temperature (°C)	36.8 ± 0.1	36.6–37.2
Corrected Indirect systolic pressure (mm Hg)	160 ± 2.9	146–183
Corrected Indirect diastolic pressure (mm Hg)	104 ± 0.7	88–117
Corrected Indirect mean pressure (mm Hg)	124 ± 2.2	108–135
S_aO_2 (%)	97.2 ± 0.1	96.6–98
Arterial pH	7.391 ± 0.007	7.346–7.431
$ETCO_2$ (mm Hg)	45.1 ± 0.7	41.7–48
P_aO_2 (mm Hg)	98.2 ± 1.4	90.2–108.6
P_aCO_2 (mm Hg)	49 ± 0.9	44.4–53.7
Base excess (mmol/L)	3.5 ± 0.4	1.9–5.9
HCO_3^- (mmol/L)	29.3 ± 0.4	27.3–32.2

From Citino SB, Bush M: Reference cardiopulmonary physiologic parameters for standing, unrestrained white rhinoceros. *J Zoo Wildl Med* 38(3), 375–379, 2007.

Body Position and Blood Circulation to the Limbs

A rhinoceros in sternal position has better blood oxygenation,[10] but the lateral position supports improved circulation to the leg muscles, is better for cooling, gives access to the udder, penis, and prepuce, and allows oral examination. Whatever the position, make sure both nostrils are patent and any fluid can freely run out of the mouth and nose. Depending on the circumstances, decide the best position for the animal and be prepared to pump the animal's legs every 15 minutes to assist circulation, especially before walking a rhinoceros into a crate.[14]

Monitoring Breathing and Response to Hypoxemia and Apnea

Respiratory depression is the biggest challenge and should be the main focus of patient monitoring.[10,12,16] A rate of 6–12 breaths/min should be the aim, but slower breathing (4–6 per minute) if deep and regular may be sufficient. Oxygen saturation (SpO_2) levels should be monitored. A pulse oximeter[17] is an essential tool for rhinoceros immobilization; an SpO_2 greater than 80% is usually adequate for short procedures, with levels greater than 90% ideal. The value of a pulse oximeter lies in observing trends, and readings must be evaluated in conjunction with the rate and depth of breathing, arterial blood color, heart rate, and blood pressure. Clip-on or reflectance sensors can be applied to the ear (after both sides have been scraped with a blade), anus, vulva, prepuce, nasal mucosa, eyelid, nictitating membrane, buccal mucosa, and tongue.[14]

Using oxygen in the field is currently standard practice, and, when given intranasally, it rapidly improves blood oxygen levels in both species. In white rhinoceros, it is most effective when given together with butorphanol and/or diprenorphine. It is of particular value in animals that have undergone marked exertion and also in females that are heavily pregnant and in animals that are compromised or in poor condition. It is usually given at approximately 10–15 L/min, although higher flow rates may be initially indicated.

White Rhinoceros

If breathing is unsatisfactory, partially reverse with 40 mg butorphanol and/or 1.2 mg diprenorphine IV; this may be repeated, but the animal may attempt to stand.

Black Rhinoceros

If ventilation is depressed, administer 5 mg butorphanol IV immediately; this may be combined with 100–200 mg of IV doxapram. Some veterinarians give 2.5 mg butorphanol IV when they get to the rhinoceros and another 2.5 mg IM. Butorphanol should be administered with caution because black rhinoceros may stand suddenly if given too much butorphanol.

For both species, if breathing stops, immediately inject IV diprenorphine or naltrexone at calculated reversal dose, put the rhinoceros in lateral recumbency, and apply pressure to the abdomen, using your knee, to compress abdominal

contents and shift the diaphragm forward. Reversal drugs may be repeated until breathing resumes and the animal recovers from anesthesia.

Muscle Tremors

Muscle tremors are rarely a problem in black rhinoceros but are frequently very marked in white rhinoceros,[2] particularly if they are in the lateral position. Apart from being caused indirectly by hypoxemia and acidosis,[11] tremors also increase oxygen consumption and heat generation,[7] which requires immediate intervention by giving butorphanol and/or low-dose diprenorphine and oxygen.

Monitoring Heart Rate and Blood Pressure

Hypoxemia is associated with a sympathetic response that increases the heart rate. Etorphine also causes hypertension and tachycardia in rhinoceros. Azaperone results in lower blood pressure when combined with etorphine.[7] Butorphanol and/or low-dose diprenorphine administration lowers heart rate, especially if combined with supplemental oxygen to counteract hypoxemia.[3,7]

Hyperthermia

To prevent hyperthermia, try to immobilize rhinoceros when ambient temperatures are less than 25°C. Aim for a quick induction and minimize exertion. Once the rhinoceros is recumbent, provide shade with a large beach umbrella and douse with copious cool water, using a handheld sprayer and leaf blower for evaporative cooling of the animal. A thermoimaging camera is valuable to indicate the hotter parts of the animal to focus cooling efforts. Never put a wet rhinoceros in a closed crate in hot, humid conditions without air flow, because heat stroke and shock may occur quickly.

The normal resting values for white rhinoceroses are given in Table 98.3.[16]

"Walking" a Rhinoceros

Rhinoceros must be securely blindfolded and have their ears plugged and a rope attached to the head and also to one of the hind legs to be used as a brake (Fig. 98.1). Eight people are typically needed to "walk" a rhinoceros, with two (or three in the case of a white rhinoceros) supporting the animal on either side and two on each of the ropes.[14]

White Rhinoceros

For animals that only need to be loaded for translocation, it is ideal to keep them standing after placing the blindfold, so they may be guided directly into a crate. If you need to walk a white rhinoceros, do not use more than 20–40 mg azaperone in the immobilizing mixture and avoid α_2 agonists. If a rhinoceros has received butorphanol at 20 mg per mg of etorphine, it may usually be stimulated to stand and walk by prodding; if unsuccessful, incremental doses of diprenorphine (1 mg IV) may be administered. Alternatively, if immobilized with etorphine and azaperone, give 10% of the standard diprenorphine reversal dose (1.2 mg for adults) IV immediately after the animal becomes recumbent, to facilitate walking the rhinoceros (D. Cooper, personal communication, 2016). This approach results in a slightly more alert animal, making walking easier, especially uphill. If α_2 agonists have been used for immobilization, give incremental doses of atipamezole IV, starting at 1 mg for every 1 mg medetomidine; keeping in mind that at 5:1, the effects of medetomidine will be fully reversed.

• **Figure 98.1** "Walking" a white rhinoceros *(Ceratotherium simum).*

Black Rhinoceros

First roll the animal on its side for a minute or two and pump the legs; then put the rhinoceros into sternal recumbency, and administer 5 mg butorphanol IV. Wait 2–5 minutes for the drug to have an effect and then stimulate the rhinoceros by rocking it or using a prod. If the rhinoceros does not stand up, give an additional 5 mg butorphanol IV and wait 2–5 minutes before stimulating again. Ten milligrams butorphanol is usually sufficient, but as much as 20 mg may be needed. Black rhinoceros that have been immobilized with a 50:50 mix of etorphine and thiafentanil may also be walked with similar doses of IV butorphanol. As with white rhinoceros, keep the dose of azaperone low if you plan to walk a black rhinoceros or alternatively use midazolam.

Nalorphine is still used to walk black rhinoceros at 10–20 mg IV instead of butorphanol, and some people are comfortable walking black rhinoceros with a very low dose of diprenorphine 0.25–0.4 mg IV.

Reversal of Immobilization

White Rhinoceros

For full reversal in the field, use IV naltrexone at 20–30 times the etorphine dose in milligrams; if medetomidine has

been used, use IV atipamezole at 5 times the medetomidine dose. Diprenorphine will not result in complete reversal in white rhinoceros and should not be used.

Black Rhinoceros

Use either IM or IV naltrexone at 20–30 times or diprenorphine IV at 2.4 times the etorphine dose.

Transporting Rhinoceros

Airlifting Rhinoceros

It is now accepted practice to airlift immobilized black and white rhinoceros by their feet out of inaccessible terrain (Fig. 98.2). They may hang safely for up to 30 minutes. Once the rhinoceros is stable, it is blindfolded and earplugs are inserted. Endless round soft polyester slings (strength of 2000 kg) are attached to each foot, and the other ends are brought together in a D-shackle, which is then hooked to a 20-m slinging chain attached to a helicopter's cargo hook. The helicopter slowly lifts the rhinoceros, and a person on the ground checks that the slings are correctly positioned before the helicopter flies off. Great care is needed when the rhinoceros is placed back on the ground, and a firm mattress may be positioned under the rhinoceros' spine as it descends.[2] In the case of white rhinoceros the equipment is the same, except a strap is used to support the head. The head strap is attached to hold the head horizontally or angle it slightly downward to allow any fluid to drain out (D. Cooper, personal communication, 2017).

• **Figure 98.2** A black rhinoceros *(Diceros bicornis)* being airlifted by helicopter. (Photo credit: H.O. Reuter.)

Transporting by Vehicle

White Rhinoceros

After the rhinoceros is in the transport crate, etorphine is partially reversed using diprenorphine IV at 2–3 times the etorphine dose in milligrams. This keeps the animal partially sedated. If used in the immobilizing combination, α_2 agonist drugs may be partially reversed, if required, by giving a lower ratio atipamezole to effect, starting with 1 mg atipamezole IV to 5 mg medetomidine.

For short distances, 80 mg zuclopenthixol IM provides adequate tranquilization. Thereafter, azaperone at 80–120 mg IM for adults may be used every 3–4 hours as needed. For long trips, or if post-offloading sedation is important, 100–150 mg zuclopenthixol IM may be used for adults. At higher dosages, rhinoceros will be heavily sedated and lie down frequently.

Rhinoceros should be transported facing toward the rear. Do not allow the rhinoceros to lie down during transport for more than 30 minutes at a time, particularly very large animals. The circulation to leg muscles may be restricted, resulting in irreversible muscle damage.

When using recommended drug combinations, head pressing is seldom a problem in white rhinoceros. A metal plate rising at an angle from the floor of the crate in front of the feet of the rhinoceros to the level of the shoulder may be used to prevent rhinoceros with long horns from leaning against the horn, which often results in horn loss. If head pressing is a problem, 3–5 mg naltrexone IM may be helpful if sufficient sedation has been given. It may be repeated after a few hours if required.

Black Rhinoceros

It is very challenging to transport black rhinoceros. If not adequately sedated, they traumatize themselves in the crate, and, if too heavily sedated, they injure themselves by straining against the front of the crate or by collapsing in an unnatural position. An experienced veterinarian should always travel with black rhinoceros, observe them frequently, and maintain the appropriate level of sedation.

Except when rhinoceros are moved very long distances between countries, almost all translocated black rhinoceros are currently immobilized in the field, transported to the new area, and released back into the field within 24 hours. The results are generally good, provided the immobilization is relatively stress free, animals are kept adequately sedated during the trip (stopping as little as possible), and they are released quietly into an area where there is adequate browse and sufficient water.

The challenge is to keep them adequately sedated for the length of transport, and the best option at this stage is to only partially antagonize etorphine with diprenorphine and/or butorphanol. The result is improved by also administering a short-acting tranquilizer or sedative (azaperone, diazepam, or midazolam) and the long-acting phenothiazine tranquilizer, zuclopenthixol acetate. In addition, a

blindfold and earplugs may be very effectively used to keep a rhinoceros calm, and a blindfold makes it easier to quietly inject a rhinoceros in a crate. Adult black rhinoceros bulls are particularly challenging. Two techniques are currently used:

1. Give 100–250 mg of the long-acting tranquilizer zuclopenthixol IM and then walk into the crate with IV butorphanol given at 20 mg per mg of etorphine or thiafentanil used in the immobilizing dose. 1,2 mg diprenorphine is usually added to the butorphanol to prevent pushing and head-pressing; this is sometimes necessary to repeat (IM or IV) after about 10 minutes if the animal continues to push. Additional tranquilization on the road is usually 20 mg midazolam IM or 60–100 mg azaperone IM, and later a low dose of etorphine (0.1–0.2 mg IM) might be necessary. Long transports have been accomplished using this technique with the rhinoceros sleeping much of the way. Some people prefer to give as much as 60–80 mg butorphanol for an adult black rhinoceros, but head pressing may be a problem and additional diprenorphine (0.3–0.6 mg IV or IM) might be necessary to alleviate this. Additional tranquilization on the road is usually 20 mg midazolam IM or 20 mg butorphanol IM/IV or 60–100 mg azaperone IM.
2. The alternative technique is to apply a cloth blindfold tightly to the head of the rhinoceros[2] and plug the ears with cotton wool, and then walk the rhinoceros into the crate, with 1.2–1.8 mg diprenorphine IV. The animal will be quite awake with this dose of diprenorphine, but because it cannot see, it will stand calmly in the crate. Midazolam or diazepam (10–15 mg IV) injected 10 minutes before waking up may also improve the quality and duration of the sedation. If the rhinoceros struggles to stand in the crate, it may be stimulated by removing the earplugs or by prodding it on the forehead after waiting 60 seconds after drug injection. An additional dose of diprenorphine (0.2–0.5 mg IV) may be used if needed to reverse sedation. After approximately 4 hours, the effects of the immobilizing dose of etorphine will start to wear off and 60 mg azaperone and a very low dose of etorphine (0.05–0.1 mg) may be administered IM every 2 hours as needed to maintain sedation. The rhinoceros should be closely monitored, and if there is a lot of ear movement, indicating that it is lightly sedated, then more azaperone and etorphine is administered. A good response is generally seen within 10 minutes.

Transporting Orphan Calves

Calves are immobilized with a low dose of IM etorphine and azaperone depending on size, age, and condition, and then partially reversed with 1–2 mg butorphanol IV until stable. Place a blindfold (a bra works well) and plug the ears with cotton wool and tape them closed. Monitor small calves during transport using a pulse oximeter on the tongue or lip, because depth and rate of respiration are hard to see in a moving vehicle. If dehydrated, administer polyionic fluids and dextrose and then use 1–2 mg of midazolam IM or IV to transport, repeating the dose if necessary. Ideally, plan to have the midazolam wearing off (approximately 1 hour from last injection) on arrival at the orphanage. Attempt to bottle-feed the calf immediately on arrival, while in the vehicle or crate, before reversing with IM or IV naltrexone and removing blindfold and earplugs. This technique may save hours of struggling and works well for both black and white rhinoceros calves as young as 2–3 weeks (M. Toft, personal communication, 2017).

Off-loading

White Rhinoceros

When off-loading or waking up a white rhinoceros in the field, use IV naltrexone at 20–30 times the etorphine dose in milligrams. Before reversal, remove all vehicles and personnel. In the case of a cow and calf, put the calf right next to the mother, facing the female. Reverse the calf first and then the mother. Retreat quietly and monitor to ensure they do not get separated.

Black Rhinoceros

They are much calmer at night; therefore arrange off-loading when it is dark, if feasible. Lights are unnecessary and usually confuse the animal when used. A red headlamp works very well. If dark and the rhinoceros is heavily sedated, put the crate on the ground; then remove vehicles and administer diprenorphine or naltrexone IM and immediately open the door.

During the day when a black rhinoceros is released from a crate, it will inevitably attack the crate and any vehicles in the vicinity and risk seriously injuring itself. To prevent this, one may either:

1. Inject the rhinoceros in the crate with an immobilizing dose of etorphine and azaperone. When it is about to collapse, open the front door of the crate and hold the rhinoceros with ropes (either attached to the hind foot and/or put across its chest) until it falls. Alternatively, let the rhinoceros become recumbent in the crate and then pull it out. If this option is used, a rubber mat on the floor of the crate makes it much easier to pull out the rhinoceros (J. Joubert, personal communication, 2017). The crate, vehicles, and people must all be removed from the area before the rhinoceros is reversed with IM/IV diprenorphine or naltrexone. Some people rub the animal's muzzle with its own dung and leave some of the dung close to the head before waking up (M. Toft, personal communication, 2017). This is also the technique used for releasing a cow and calf combination both in the day and night. Place the immobilized calf close behind the cow and wake them up with naltrexone or diprenorphine IM.

2. Alternatively, inject a low dose of etorphine (0.3–0.5 mg for an adult) IM and wait until the rhinoceros is heavily affected; then inject a full dose of diprenorphine or naltrexone IM and immediately open the crate door. The rhinoceros will wander off before the antidote antagonizes the etorphine.

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Update on Rhinoceros Nutrition

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Human greed and ignorance have led to devastation in dwindling wild populations; basic knowledge of preventative health care is integral when rhinoceros are maintained in managed populations. Although nutrition under human care never replicates the variety and exact composition of wild rhinoceros diets, informed feeding practices will serve to complement behavioral and reproductive health. Several references exist on basic rhinoceros nutrition, including previous iterations of the current text.[1–3] Much nutrition research in the past 5–10 years has focused on the challenges of feeding rhinoceros under human care, characterizing disease issues, and illustrating novel approaches. Researchers strive to make practical connections between nutrient absorption, subsequent physiologic and metabolic interplay, and repercussions on reproduction and disease. Nutrient research in rhinoceros continues to be necessary as the complex interactions of feedstuffs, genetics, and digestive physiologic needs of each species continue to be discovered.

Generalized Recommendations for Feeding Rhinoceros Under Human Care

Rhinoceros consist of five extant species, the black *(Diceros bicornis)*, white *(Ceratotherium simum)*, Sumatran *(Dicerorhinus sumatrensis)*, Indian or greater one-horned rhinoceros *(Rhinoceros unicornis),* and the Javan or lesser one-horned rhinoceros *(Coelodonta antiqitatis).* Rhinoceros are large herbivorous mammals that currently have all species on the International Union for Conservation of Nature Red List of Threatened Species, with three species classified as critically endangered.[4] They are primarily in danger from human poaching for their keratinous horn and habitat loss. Herbivorous feeding strategies vary across species, with Sumatran, Javan, and black rhinoceros as browsers, white rhinoceros as grazers, and Indian rhinoceros classified as intermediate feeders. Current worldwide population estimates (2017) are 60–63 Javan, 100 Sumatran, 3500 Indian, 5042–5455 black, and 19,682–21,077 white rhinoceros.[4] Javan and Sumatran rhinoceros are currently primarily maintained in national parks in Indonesia, and this review will not focus on nutrition of these species under human care.

Although base nutrient recommendations in all extant rhinoceros species have not been significantly altered in recent history, research in the past 10–15 years has added specific knowledge based on species' physiologic idiosyncrasies. Nutrient requirements have not been specifically detailed for each rhinoceros species; however, the horse is considered the closest digestive model used for intake recommendations. Horse digestion and absorption have been compared with rhinoceros species, with most applicability nutritionally for white and greater one-horned rhinoceros, as compared with black or Sumatran.[5] Rhinoceros have been recommended to be fed 1%–3% of their body weight (BW) on an as-fed basis (1%–2% on a dry matter [DM] basis). It must be emphasized that a maximum of one-third of total calories comes from a pelleted concentrate.[1,3,6] Pelleted concentrate may vary in energy (calorie) content, especially compared with hay species, which possibly should be avoided while estimating that pellets themselves should be one-third of the diet. Monitoring of energy intake is critical to maintaining healthy BW in all species.

Good-quality forages (hay and browse) should provide primary nutrients for rhinoceros under human care, with concentrate feeds used to balance energy, protein, mineral, or vitamin needs.[2,6] For browsing or intermediate species, maximizing the amount of browse offered will be ideal; however, replicating natural browse variety consumed in the wild is likely not practical or possible. All rhinoceros are adapted to use energy from fibrous plant material through microbial fermentation. Animals should have ad libitum access to hay, clean water, and salt.[7] Feedings should be at least twice daily due to relatively fast transit time.[1] Trace mineralized salt would not be recommended, especially for iron-sensitive species such as black and Sumatran rhinoceros. Diets should be balanced through prioritizing adequate roughage, limiting pelleted compound feeds, and designing with mineral needs in mind for each species. It should also be emphasized that not all pelleted feeds are the same or interchangeable in terms of nutrients, and use of cereal grain in pellets for all these species should be minimized. Abrasive pellets have been postulated to be connected to dental damage and tooth wear in rhinoceros under managed care, unlike wild counterparts.[8] However,

TABLE 99.1 Mean Serum Mineral and Vitamin Values (±SD; N) for Black Rhinoceros Held Under Human Care or Free Ranging

Parameter	Units	Under Human Care (1999–2016)*	Under Human Care†	Free-Ranging†	Under Human Care[13]
Vitamin E	µg/mL	1.0 (0.9; 198)	0.84 (1.0; 85)	0.6 (0.2; 86)	
Vitamin A	ng/mL	43.2 (28.3; 172)	80 (80; 85)	40 (10.0; 86)	
Vitamin D	nmol/L	174.1 (43.3; 54)	0.24 (0; 2)		
Na	mEq/L	130.3 (14.9; 354)	131.0 (7; 12–34)	145.2 (10.8; 27)	133 (3; 81)
K	mEq/L	4.6 (0.4; 350)	5 (1.0; 12–34)	4.9 (0.6; 27)	4.7 (0.6; 82)
Cl	mEq/L	96.0 (2.7; 125)			96 (0.3; 82)
Ca	mg/dL	12.2 (0.7; 354)	12.7 (2.8; 12–34)	13.9 (2.8; 27)	12.7 (1.0; 90)
P	mg/dL	4.3 (1.2; 352)	4.1 (1.1; 12–34)	3.4 (1.2; 27)	4.8 (1.1; 90)
Mg	mg/dL	2.5 (0.3; 353)	2.1 (0.6; 12–34)	3.0 (0.9; 27)	3.3 (3.5; 27)
Co	ng/mL	1.0 (0.8; 115)			
Cu	µg/mL	1.4 (0.3; 344)	2.1 (0.7; 12–34)	1.5 (0.4; 27)	
Fe	µg/dL	208.1 (54.1; 374)	270.0 (11.1; 12–34)	215 (61.0; 27)	227 (66)
Mn	µg/mL	30.4 (82.6; 115)	0.001 (0.001; 12–34)	0.007 (0.004; 27)	
Mo	ng/mL	16.6 (13.5; 115)	22.1 (13.8; 12–34)	5.5 (4.1; 27)	
Zn	µg/mL	0.9 (0.1; 344)	1.6 (1.3; 12–34)	1.6 (0.4; 27)	
Se	µg/mL	114.7 (12.1; 114)	195.4 (56.1; 12–34)	114.8 (37.4; 27)	

*Unpublished data from black rhinoceros under the care of Disney's Animal Kingdom (1999–2016).
†All vitamin values (see reference 12); all mineral values (see reference 11).

excessive starch and sugar content in some pellets and produce may also be connected to this common issue of severe dental plaque in rhinoceros under human care. Black rhinoceros appear particularly prone to proliferative gingivitis, not always associated with degree of calculus accumulation, also indicating dietary challenges.[3] Therefore forage and browse, dependent on species, are integral to long-term health maintenance. It should be noted that browse silage has been successfully fed to white and black rhinoceros species but may not be a practical production product due to labor and cost.[9,10]

Although supplementation needs are limited in rhinoceros under human care, attention should be paid to mineral and vitamin balance in the diet for these species. For example, vitamin E in circulation appears lower across rhinoceros species compared with other mammals.[1] It should be ensured that total dietary vitamin E levels reach the 150–200 IU/kg diet mark recommended by Dierenfeld for all rhinoceros species.[1] Regular monitoring of vitamin and mineral serum status in rhinoceros may serve to refine recommendations for feeding; however, bioavailability across feed items for these nutrients is often variable. Reference values for animals both under human care and free ranging for minerals and vitamins may serve as guidelines, but regular testing within institutions is critical for individual evaluations (Tables 99.1 and 99.2). Best practices are recommended in the species-specific sections later.

For all species, regular monitoring of BW and condition is also recommended because obesity under human care is well recognized as a risk factor and health detractor across species. Use of body condition scoring (BCS) with a team of animal caretakers with varied exposure to the animal (keeper, veterinarian, nutritionist, manager, etc.) will better maintain health goals during growth, pregnancy, or maintenance. Communication across institutions to standardize BCS would also be beneficial due to its practical application across disciplines.[14]

Rhinoceros milk, as described by Blakeslee and Zuba (2002), describes a low level of total solids, high sugar, low fat, and moderate protein that may be formulated for hand-raising rhinoceros successfully using cows' milk or commercial milk replacer (Zoologic Milk Matrix 20/14, PetAg, Inc. Hampshire, IL).[15] Analysis of milk across three lactation period of a white rhinoceros found similar overall nutrient values for fat and protein but altered fatty acid composition compared with Milk Matrix 20/14, with higher levels of capric, lauric, and myristic acid.[16] Guidelines for colostrum feeding of neonates and subsequent feeding rates are clearly reviewed in the previous edition of this volume and used with success at institutions across white, black, and Indian rhinoceros species.[3]

TABLE 99.2 Mean Serum Mineral and Vitamin Values (±SD; N) for White Rhinoceros Held Under Human Care or Free Ranging

Parameter	Units	Under Human Care (1999–2016)*	Under Human Care†	Free-Ranging†	Under Human Care[13]
Vitamin E	µg/mL	0.9 (0.6; 118)	0.6 (0.48; 57)	0.8 (0.3; 5)	
Vitamin A	ng/mL	60.5 (59.7; 100)	70 (40.0; 57)	60 (20.0; 5)	
Vitamin D	nmol/L	155.6 (50.1; 49)			
Na	mEq/L	131.4 (13.1; 120)	146.7 (28.9; 2–3)	123.8 (4.0; 4)	134 (5; 74)
K	mEq/L	4.3 (0.5; 120)	4.8 (0.5; 2–3)	4.2 (0.3; 4)	4.7 (0.8; 74)
Cl	mEq/L	93.8 (2.6; 79)			95 (4; 74)
Ca	mg/dL	11.5 (1.3; 120)	13.9 (2.2; 2–3)	10.6 (1.6; 4)	11.8 (0.9; 78)
P	mg/dL	4.3 (1.2; 118)	4.0 (1.2; 2–3)	3.6 (0.9; 4)	4.0 (0.9; 76)
Mg	mg/dL	2.7 (0.4; 119)	2.4 (0.4; 2–3)	2.1 (0.4; 4)	
Co	ng/mL	0.5 (0.3; 66)			
Cu	µg/mL	1.4 (0.3; 108)	2.5 (1.3; 2–3)	1.2 (0.2; 4)	
Fe	µg/dL	144.2 (35.8; 113)	166 (23.0; 2–3)	177 (66.0; 4)	
Mn	µg/mL	6.4 (10.2; 60)	0.002 (0.002; 2–3)	0.003 (0.002; 4)	
Mo	ng/mL	19 (7.0; 68)	19 (0; 1)	28.3 (15.4; 4)	
Zn	µg/mL	0.8 (1.1; 108)	1.5 (0.3; 2–3)	1.4 (0.2; 4)	
Se	µg/mL	97.4 (29.5; 68)	232 (144.4; 2–3)	200.8 (46.1; 4)	

*Unpublished data from black rhinoceros under the care of Disney's Animal Kingdom (1999–2016).
†All vitamin values (see reference 12); all mineral values (see reference 11).

White Rhinoceros Nutrition

White rhinoceros should be offered high-quality grass hay (e.g., timothy, Coastal Bermuda grass) as their primary energy source, rather than legume hays (e.g., alfalfa/Lucerne). Horse requirements for nutrients offered apply to this species.[17] White rhinoceros in the wild have been documented consuming a variety of grasses dependent on season, with high levels of crude fiber (~36%) and low to moderate crude protein (4.5%–14.9% DM).[18,19] Monocot grass species available under human care are more limited than the wild. However, fresh grass and dry forage are still necessary as the predominant diet ingredient, rather than a high starch or protein pelleted feed. Produce is commonly used for training and enrichment; however, use of sugary items such as ripe bananas, corn, and melons should be discouraged, especially in excess.[20] All produce and diet items used for training should be tracked and accounted for as part of the animals' diet to avoid unintentional weight gains. White rhinoceros under human care have been documented with reproductive challenges.[21] High levels of digestible sugars and energy in captive diets are speculated to negatively impact energy and glucose response and promote obesity.[20] Lower glycemic index feed items produced similar glucose responses in white rhinoceros, whereas more work is needed to look at the impact of high-sugar training items on metabolic disturbances in this species.[20] White rhinoceros also appear to store vitamin E in adipose tissue rather than liver (as seen in black rhinoceros), potentially indicating antioxidant need in that tissue.[1] The interrelationship of diet impact on health and reproductive status continues to warrant investigation.

A recent study evaluated the impact of phytoestrogenicity of dietary components across multiple zoological institutions on breeding and reproductive success in white rhinoceros.[21] Dietary estrogenicity and fertility of captive-born female southern white rhinoceros had a low to moderate negative correlation across multiple institutions. Bermuda grass and timothy hay appeared to have lower estrogenicity than alfalfa or Sudan hay, or pellet extracts.[21] Limiting estrogenic compounds, although the impact on fertility is unclear, agrees with recommended feeding practices for white rhinoceros in terms of maximizing grass forage, or even fresh forage, without a need for high protein levels common in alfalfa hay or some pelleted diets. However, supplementation with a complete balanced pelleted feed is often needed under human care, especially considering seasonal needs and hay quality. It has been suggested to supplement with forage-based pellets rather than grain-based ones to achieve this balance.[6] An example of a forage-based pelleted diet used in a population with high reproductive success is described in Table 99.3.

TABLE 99.3 Example of White Rhinoceros Diet Design With Energy Contributions at Disney's Animal Kingdom

Diet Item	% Total Diet		% Gross Energy of Total Diet		Purpose
	Female	Male	Female	Male	
White Rhinoceros (Year-Round)					
Grass hay (Coastal Bermuda)	77.4	86.4	78.4	87.1	Maintenance
Grass hay–based Pellet*	11.4	9.1	11.0	8.7	Maintenance
Wheat bran/psyllium fiber	0.7	0.1	0.6	0.1	Supplement
Vitamin E—Emcelle Tocopherol†	0.1	0.1	-	—	Supplement
Alfalfa hay	7.2	2.6	7.0	2.5	Training
Timothy/alfalfa cube	1.4	0.9	1.4	1.0	Training
High starch/sugar pellet‡	1.7	0.7	1.6	0.6	Enrichment
Produce (apple/sweet potato)	0.1	0.1	—	—	Enrichment
Body condition score (1–5)	3.5	3.0			
Total weight diet (as fed kg)	27.1	38.2			
Total fed as % BW	1.4	1.7			

*Mazuri Zulife Browser Rhinoceros Cube (5Z1P) Mazuri, Land O'Lakes, St. Louis, MO.
†Stewart Products Inc. Bedford, TX.
‡Omolene; Purina, Land O'Lakes, St. Louis, MO/DAK Mazuri Petting Zoo (5MJZ) Mazuri, Land O'Lakes, St. Louis, MO.
BW, Body weight.

Greater One-horned/Indian Rhinoceros Nutrition

Indian rhinoceros have been found to benefit from more limited intake of no more than 1.1% of BW on a DM basis, due to a propensity for obesity and associated disease states of chronic foot problems and uterine leiomyomas under human care.[22] Even some roughage-only diets without added concentrates exceeded energy needs in a multiinstitutional digestibility study. This would suggest that ad libitum hay access may not be ideal for Indian rhinoceros.[22] Although this study confirmed that Indian rhinoceros diets that meet horse maintenance requirements for minerals are adequate, roughage-only diets will likely need a specific blend of mineral supplementation for balance, without added energy and protein.[23] This may be critical, considering possible connections between unknown excess zinc or biotin needs as they relate to foot issues in this species. Zinc and biotin supplementation has been shown to be integral to a complete mineral balance for hoof health in horses and potentially elephants, although not a cure all.[24,25]

Recent work comparing Indian rhinoceros offered a diet including fresh browse and forage with dried forage found health benefits in circulating nutrients with the diet including fresh herbage.[26] Access to fresh browse and forage is recommended to increase 25-hydroxy vitamin D and alpha tocopherol, although it also increased circulating nonesterified fatty acids, cholesterol, and triglycerides. Also demonstrated was variability in serum mineral levels probably related to bioavailability of minerals in different fresh or dried forage.[26] Work tracking plant species consumption in wildlife reserves in Manas National Park, Assam, India confirms previously reported adaptability of Indian rhinoceros as more of an intermediate feeder than their grazing white rhinoceros counterparts.[27] A total of 139 species of plant from 39 families were documented over 6 years (2008–2013) of observations, with only 24% of diet observed as grasses, and trees, shrubs, herbs aquatic plants, and agricultural products also represented.[27] This wide variety of diet emphasizes that, although Indian rhinoceros under human care are often considered primarily grazers, varying the forage and browse options may better optimize health, while keeping in mind the need to limit total dietary energy offered to this species. More work determining optimal balance of minerals and vitamins and impact on foot issues, as well as successful controlled diet plans to combat obesity, is recommended.

Black Rhinoceros Nutrition

Although diets offered to black rhinoceros under human care vary among institutions, recommendations about nutrient and diet specifics have been made specifically for black rhinoceros.[28] The captive diet appears to be a poor replica of the nutrient and antinutrient composition found in wild browse.[6] Compared with an all fresh browse diet in the wild, with more than 240 species of plants documented as consumed, most managed care institutions cannot offer

TABLE 99.4 Example of Black Rhinoceros Diet Design With Energy Contributions at Disney's Animal Kingdom

Diet Item	% Total Diet		% Gross Energy of Total Diet		Purpose
	Winter	Summer	Winter	Summer	
Male—Body Condition Score 3					
Browse*	26.8	30.6	14.3	11.6	Maintenance
Timothy hay	30.9	30.7	40.7	42.0	Maintenance
Coastal Bermuda grass hay	13.7	13.6	18.6	19.2	Maintenance
Grass hay–based pellet†	19.6	19.4	25.3	26.1	Maintenance
Wheat bran	1.0	1.0	—	—	Supplement
Vitamin E—Emcelle Tocopherol‡	0.1	0.1	—	—	Supplement
Sodium phosphate monobasic	0.1	0.1	—	—	Supplement
Greens§	4.1	4.1	0.2	0.2	Enrichment
Produce¶	3.0	2.9	0.5	0.5	Training
Other training items**	0.8	0.8	0.4	0.4	Training
Total weight diet (as fed kg)	31.3	31.5			
Total fed as % BW	2.5	2.5			

*Browse consists of three species in the winter (*Elaeagnus* and *Acacia* spp.) and three in summer (*Willow, Banana,* and *Pennisetum* spp.).
†Mazuri ZuLife Browser Rhinoceros Cube (5Z1P); Mazuri, Land O'Lakes, St. Louis, MO.
‡Stuart Products Inc. Bedford, TX.
§Greens include bok choy, romaine, kale, endive, and green leaf Lettuce.
¶Produce includes squash, celery, green beans, sweet potato, carrots, cauliflower, cucumber, zucchini, and bean paste.
**Other training items include pinecones, low-starch primate biscuit, and herbivore gel.
BW, Body weight.

substantial quantities of browse.[29] Black rhinoceros diets under human care may consist of pelleted feed, alfalfa and grass-legume mixes, browse, and ideally a minority of enrichment such as produce.[28] Alfalfa hay should be limited, due to high protein, calcium, and iron in many harvests, as well as creating diarrhea and colic.[2,3,28] Black rhinoceros develop iron overload disorder (IOD) under human care, where there is an excess of iron in circulation, measured repeatedly by iron biomarkers, and excessively stored, causing damage to tissues as demonstrated by necropsy.[28,30,31] There also may be a recently found mechanism of iron absorption in play in black rhinoceros, which uses plant ferritin, a protein holding up to 4500 atoms of iron, which exists only in legumes such as alfalfa and absorbs it whole through clathrin-mediated endocytosis.[32] The possibility that legume iron stores may be absorbed outside of typically understood regulated absorption in the gut may be a key factor in contributing to IOD in this species with a need for further study. As previously reviewed, excess generalized mineral supplementation should be avoided.[5] Although a well-formulated pellet should provide adequate phosphorus and not overly high calcium, extra supplementation as monosodium phosphate may be necessary, based on serum evaluations and for preventative reasons.

Essential in formulating complete diets under human care is limitation of high sugar and high vitamin C produce, both for concerns with obesity and metabolic syndrome, as well as increasing bioavailability of iron. Caution and tracking should be exercised to limit training items, as well as high sugar and high iron items like molasses. Pellet formulations for black rhinoceros are recommended to be low in starch and soluble sugars, with NDF values from 40%–60%.[28] An example of a successful diet for black rhinoceros under human care that improved measures of iron stores, primarily ferritin, is shown in Table 99.4.[33] Based on the practical limitations and availability of feed items such as low-iron pelleted feed and browse, iron concentration in the diet was recommended to not exceed 300 mg/kg DM or approximately 6 g of iron per day for a 1300 kg black rhinoceros fed 1.5% BW in DM.[2,28] The commercial process of milling grains produces high-iron pelleted commercial feeds, even in grain that is moderate in iron concentration.[17,34] There have been positive correlations found between amount of grain-based products in the diets of European-held black rhinoceros and omega-6 polyunsaturated fatty acids.[35] Excess levels of omega-6 fatty acids are established precursors to proinflammatory pathways, which could exacerbate inflammation in an already oxidant-sensitive species.

Much work has focused on diseases of unknown etiology in the black rhinoceros, many of which may be tied to inability to replicate wild nutrition for this species under

human care. This appears to include the interplay of stress (both oxidant and environmental), improper fatty acid balance, lack of antioxidant, lack of polyphenolic antinutrients, excess of dietary iron, excess of starch and other glucose supplies, and inadequate fiber in a diet under human care.[11,28,35] Antioxidant and phosphate supplementation appear to be preventative diet supplements for black rhinoceros.[1] Physiologic findings indicate rhinoceros have a reduced capacity to neutralize oxidants, especially black rhinoceros.[36] Genetic testing of the black rhinoceros genome has identified specific mutations that may begin to explain the fragility of black rhinoceros red blood cells due to a mutation in their adenosine coding.[37]

Theories exist on interconnection of obesity, insulin/glucose metabolism, metabolic dysregulation, and connection to iron loading and inflammation in black rhinoceros under human care.[38] Baseline assays on banked samples from zoo-managed (n = 86) and free-ranging (n = 120) black rhinoceros were assessed for biomarkers of inflammation; with TNFα, serum amyloid A, insulin, insulin to glucose ratio, and ferritin found statistically higher in zoo-managed versus free-ranging populations.[38] This supports the need for investigation via longitudinal serial sampling in zoo-managed black rhinoceros into factors impacting inflammation, including nutrient and diet impact, as well as body condition and activity levels of these animals. Fluctuations of vitamin D with seasonal exposure to sunlight (highest in summer) have been seen, despite vitamin D supplementation in a pelleted feed year-round.[39] Vitamin D, a known genetic regulator across species, also may fit into the complicated picture of metabolic regulation in this species.

Comparisons between human iron overload and black rhinoceros IOD indicate that black rhinoceros IOD appears multifactorial, with both dietary iron intoxication and intrinsic metabolic dysregulation as possible contributors.[40,41] Although lowering dietary iron and increasing natural dietary chelators to combat IOD in black rhinoceros may be a valid preventative approach to one aspect of this disorder, classical human treatments of phlebotomy and iron-targeted synthetic chelation are equally valid options with varied success.[33,42] Measurements of iron biomarkers in free-ranging black rhinoceros (n = 194) found circulating ferritin values, the only known marker of iron storage in the liver, to be 290 ng/mL ± 18 ng/mL (mean ± SEM), markedly lower than common under human care.[43] A black rhinoceros ferritin species-specific test is currently under development based on a fully sequenced ferritin gene; however, assessment of iron load should be made using ferritin and transferrin saturation (serum iron/total iron-binding capacity).[44]

Chelators as a dietary therapy for IOD have been investigated. Condensed tannins (proanthocyanidins) are one of a larger array of phenolic compound classes, which may work to decrease iron absorption in the digestive tract.[45] It has been demonstrated that inclusion at 0.5%–1.5% of diet DM as quebracho (a proanthocyanidin), but not tannic acid (a hydrolysable tannin), increased total antioxidant capacity but did not affect the apparent absorption of iron.[46,47] Using natural sources of tannins, such as grape pomace or tea leaves, there is doubt on the ability to supplement enough phenolic compounds to mimic levels observed in wild diets.[6] Grape seed extract was added as a form of natural chelator to both horse and black rhinoceros fecal continuous culture, analyzing impact on the fecal microbial population.[48] Grape seed extract did not change nutrient digestibility or fermentation, and similar microbial populations were found between species; the increased inclusion of the extract did increase condensed tannins and iron-binding capacity and stimulated microbial growth.[48] Although there were no negative effects on the hindgut in vitro, more research is warranted to understand in vivo effects, including possibly binding and limiting absorption of microminerals. Recent work examined the use of an iron-specific drug chelator, known as HBED (N,N′-Bis(2-hydroxybenzyl) ethylenediamine-N,N′-diacetic acid), for oral use to increase iron excretion in black rhinoceros under human care.[42] The chelator was effective in increasing iron excretion through urinary output; however, due to one study, animal's post study health crisis, administration of HBED is not recommended for black rhinoceros with documented health problems.[42] Iron-specific synthetic chelators may still provide a future for preventative solutions, perhaps at lower or tapered doses.[42] Although IOD is not the only chronic disease state affecting black rhinoceros long term under human care, understanding the interplay between inflammatory pathways and disease progressions may lead to more concrete diet design for black rhinoceros. Future research must integrate practical husbandry concerns with epidemiologic perspective. Veterinarians, nutritionists, and husbandry professionals may then approach IOD preventative measures with a similar mindset that emphasizes sharing and centralizing information.

Nutrition Moving Forward

Taking a preventative approach and trying to think long term in nutritional care serve to minimize nutritionally related disease states. Working across disciplines and thinking of how nutrition, reproduction, behavior, and genetics are integrated in rhinoceros metabolism are necessary to move to sustainable populations. Practical diet guidelines serve as a starting off point for balancing rhinoceros diets to maximize animal health.

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Health of the Forest Rhinoceros of Southeast Asia: Sumatran and Javan Rhinoceros

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Introduction

The rhinoceros living in the rainforests of Southeast Asia now survive almost entirely within the boundaries of Indonesia and represent the most threatened species of the Rhinocerotidae family. Following Bergmann's rule, the Sumatran rhinoceros *(Dicerorhinus sumatrensis)* and Javan rhinoceros *(Rhinoceros sondaicus)* are smaller than their African and Asian relatives living outside of tropical environments.[1] For both species for which conservation has historically included (Javan) or currently includes (Sumatran) attempts at a managed breeding program, our understanding of their biology and associated threats from disease draws us to a singular conclusion: their health is integrally linked to the native forests. Invariably, captive browsing rhinoceros, Sumatrans included, are susceptible to captive-induced disease in the form of gastrointestinal ailments, infectious disease, enhanced susceptibility to ocular disorders, and most concerning of all—due to its insidious onset and irreversible progression—iron storage disease (ISD). The objective of this chapter is to summarize in one place the most current state of health knowledge in captive and wild settings for these unique forest rhinoceros representing two diverse genera (Table 100.1).

Sumatran Rhinoceros (*D. sumatrensis*, Fischer 1814)

Taxonomy

Known by its colloquial name of the "hairy rhinoceros," three subspecies of Sumatran rhinoceros are recognized. *D. sumatrensis sumatrensis* is found in just three protected areas on the island of Sumatra (Way Kambas, Bukit Barisan Selatan, and Gunung Leuser National Parks), whereas *D. sumatrensis harrissoni* is found in exceedingly small numbers in Sabah (Malaysian Borneo) and Kalimantan (Indonesian Borneo) on the island of Borneo.[2] Although there are unsubstantiated claims that a small population persists in Mynamar, *D. sumatrensis lasiotis* is presumed extinct.

Infectious and Emerging Disease

Bacteria, Viruses, and Preventative Medicine

Sumatran rhinoceros mortality from necrotizing enteritis and septicemia following gastrointestinal infection with *Escherichia coli*, *Klebsiella*, and *Salmonella* spp. was prevalent in captive animals before advances in zoo husbandry (see Table 100.1).[3,4] No viral diseases have been described in the Sumatran rhinoceros, although West Nile virus and equine herpesvirus type 1 (EHV-1) infections have caused disease in captive greater Asian one-horned rhinoceros.[5,6] Fecal samples should be collected for salmonella culture and serotyping in cases of acute diarrhea. Skin tuberculin testing is recommended during quarantine and in high-risk environments. Vaccination against tetanus, rabies, West Nile virus, and leptospirosis are based on perceived risk and local veterinary practice.

Captive management of Sumatran rhinoceros requires routine health monitoring and basic animal husbandry practices, including physical exam with measurement of body weight, condition scores, screening for endoparasites and ectoparasites, and serial hematology and biochemistry (Table 100.2).[7] Blood may be collected readily in small volumes from the auricular vein located on the outside of the rhinoceros's pinna, from the tail or coccygeal vein, or from the radial vein.[8] It is relatively easy to collect blood from standing nonsedated rhinoceros in a chute while hand-feeding fruit (jackfruit, durian, melon, and bananas are favorite treats).

TABLE 100.1 Records of Disease Outbreaks With Associated Parasites and Pathogens in Captive and Wild Sumatran *(Dicerorhinus sumatrensis)* and Javan Rhinoceros *(Rhinoceros sondaicus)*

Species	Disease	Dates	No. Affected	Evidence	References
Sumatran Rhinoceros	*Trypanosoma evansi*	2003, 2006	6 (C)	• 5 died, *T. evansi* in blood smears, histopathology confirmed; poor hygiene proposed by critics—*E. coli* overgrowth without pathology • Trypanosomes found in rhinoceros blood (disease survey)	6, 14
	Klebsiella pneumoniae	1986	1 (C)	• Died after showing gastrointestinal signs • *Klebsiella* cultured from GI tract, necropsy indicated septicemia	2
	Ectoparasites	2006, 2007	- (C)	• *Haemaphysalis hystricis, Amblyomma testudinarium, Aponomma* spp.	4, 9
	Necrotizing Enteritis	1989	1 (C)	• Death after GI signs, necropsy suggests *Salmonella* enteritis	3
	Hemoparasites	2006, 2007	- (C)	• Microscopy: *Anaplasma marginale, Anaplasma centrale*, *Theileria* spp., and *Babesia* spp; Molecular techniques: *Theileria bicornis*	6
	Helminths	1986, 2006, 2007	- (C)	• *Fasciolopsis buski* infestation identified • *Fasciolidae*, *Oxyuris, Paramphistomidae*	5*, 6, 8
	Protozoa	2006, 2007	- (C)	• *Cryptosporidium, Entamoeba, Balantidium, Ophryoscolecidae, Spirodinium* spp.; families Buetschliidae, Cycloposthidae	6
	Iron Overload Disorder (Hemochromatosis, Iron Storage Disease)	1992–2017	9	• Biochemical & necropsy histopathology (6), biochemical (transferrin saturation & ferritin) (3)	Don Paglia, personal communication, 2018
	Ocular Disease	1990, 2004–2005	6 (C)	• Corneal opacity and ulceration, loss of vision in eye(s) • *Staphylococcus* and *Flavobacterium* spp. cultured	4, 16
	Lacerations	1990	- (W, C)	• Lacerations from snares, wood floors	4
	Hyperkeratosis	1990	- (C)	• Hyperkeratosis, *E. coli* pyoderma a common sequela	4
	Abscesses	1990	- (C)	• Injection sites, puncture wounds, horn abscesses	4
	Hoof Cracks	1990	- (C)	• Husbandry related	4
	Myiasis	1990	2 (C)	• No details given	4
	Phimosis	1990	1 (C)	• Attachment of penis to prepuce, approximately 5 cm of penis free • Improved markedly over 3–4 months	4
	Uterine Pathology	1986, 2001	- (C)	• Histology—Uterine leiomyoma, cystic endometrial hyperplasia • Ultrasound—tumors, uterine cysts, multiple corpora lutea	2, 25
Javan Rhinoceros	**Helminths**	1980, 2006	- (W)	• *Strongyloides, Bunostomum, Trichostrongylus, Fasciola, Schistosoma, Anaplocephalidae, Oesophagostomoma, Plagiotaenia*	31, 29
	Protozoa	2006	- (W)	• *Balantidium, Entamoeba, Cryptosporidium, Eimeria, Cycloposthium, Lavierella* spp.	31
	Ectoparasites	1980	10 (W)	• *Amblyomma* spp.	30
	T. evansi	2011	1 (W)	• Circumstantial—tabanids had positive *T. evansi* on PCR • Soil also positive for *C. difficile* (normal soil contaminant)	32
	Hemorrhagic Septicemia (HS) or Anthrax	1982	5 (W) 33* (W)	• Circumstantial-350 goats & 50 • buffalo died of HS in adjacent villages • History of anthrax 3 decades before	

(C) and (W) indicate captive and wild rhinoceros, respectively. References marked with an asterisk (*) are not peer reviewed.
Table prepared by Virgina Mule, DVM.
PCR, Polymerase chain reaction.

TABLE 100.2 Hematology and Biochemistry Values for Adult Captive Sumatran Rhinoceros *(Dicerorhinus sumatrensis)[*]**

Analyte	Mean ± SD	Median	IQR	Min	Max	CV_g (%)	C_{Vti} (%)	Index of Individuality[†]
Hemoglobin (g/dL)	13.2 ± 1.1	—	—	10.5	16.1	3	8	2.53
White blood cell count ($\times10^3/\mu L$)	7.1 ± 1.6	—	—	3.2	12.2	17	16	0.89
Red blood cell count ($\times10^6/\mu L$)	5.1 ± 0.5	—	—	4.1	6.5	4	8	2.33
Platelets ($\times10^3/\mu L$)	133 ± 59	—	—	18	280	24	40	1.68
Hematocrits (%)	39 ± 3	—	—	32	48	2	8	3.53
Mean corpuscular hemoglobin (pg)	26 ± 1	—	—	23	28	4	3	0.67
Mean corpuscular hemoglobin concentration (g/dL)	34 ± 1	—	—	32	37	2	2	0.98
Mean corpuscular volume (fl)	76.5	—	—	62.7	94.1	—	—	—
Protein (g/dL)	8.1 ± 1.1	—	—	5.2	10.8	3	13	4.66
Albumin[‡] (g/dL)	–	3.9	3.6–4.5	1.9	7.3	1	17	24.70
Globulin (g/dL)	3.9 ± 1.3	—	—	0.5	6.6	3	33	12.39
Aspartate aminotransferase (U/L)	72 ± 23	—	—	40	140	27	22	0.79
ALT[‡] (U/L)	–	21	16–31	7	69	5	14	3.05
LDH[‡,§] (U/L)	–	884	684–1217	212	3583	<1	8	16.64
Bilirubin_Total (mg/dL)	0.6 ± 0.2	–	–	0.3	1.2	14	31	2.25
Bilirubin_Direct[§] (mg/dL)	0.3 ± 0.1	–	–	0.01	0.6	2	52	23.68
Bilirubin_Indirect (mg/dL)	0.4 ± 0.2	–	–	0.1	0.9	17	48	2.85
Serum urea (mg/dL)	21 ± 7	–	–	6	42	20	31	1.55
Creatinine[‡] (mg/dL)	–	1.1	1.0–1.5	0.6	2.9	146	130	0.89

*Hematology and biochemistry values with estimates of central tendency (mean or median), variability (standard deviation [SD] or interquartile range [IQR]), minimum and maximum reference interval values, between-animal coefficient of variation (CV_g), within-animal coefficient of variation (CV_{ti}), and index of individuality for adult captive Sumatran rhinoceros *(Dicerorhinus sumatrensis)* at the Sumatran Rhino Sanctuary, Lampung, Indonesia.
[†]Estimated with the use of the equation CV_{ti}/CV_g.
[‡]The median and IQR are reported rather than the mean and SD because of the skewed distribution of these analytes; however, log-transformed variables were used in mixed ANOVA models to calculate CVs and the index of individuality.
[§]Ratu is excluded because of low sample numbers in order to estimate the between-individual animal variance.
Modified from Andriansyah, Candra D, Riyanto MA, et al. Hematology and serum biochemistry of Sumatran rhinoceros *(Dicerorhinus sumatrensis)* in a rainforest sanctuary in Way Kambas National Park, Indonesia. *J Zoo Wildl Med* 44(2):280–284, 2013.

Endoparasites

Internal parasites of the Sumatran rhinoceros include roundworms, flatworms, and protozoa of the genera typical of large ungulates with *Fasciolidae*, *Paramphistomidae*, *Strongyloidae*, *Oxyuridae*, *Cryptosporidium*, *Entamoeba*, *Balantidium*, *Ophryoscolecidae*, and *Spirodinium* spp. identified in captive animals (see Table 100.1).[9,10] Routine screening of animals at the Sumatran Rhino Sanctuary in Lampung Province, Indonesia was conducted using direct smears, magnesium sulfate, and acid techniques, which proved ideal for identifying fluke eggs because sugar flotations collected too much debris. Acid sedimentation techniques were superior to use of the McMaster chamber for quantification of egg counts.[11]

Ova of *Fasciola* sp. (liver fluke) was detected in feces of four of five sanctuary rhinoceros, whereas adults and ova of a previously identified *Paramphistome* sp. (stomach fluke) were found in feces of one of five rhinoceros with ova measuring 125 × 60–65 µm and 150 × 60–65 µm, respectively. Even though no apparent disease was attributable to these fluke infections, treatment was initiated with praziquantel at 3 mg/kg orally, with a slight decrease in egg

counts observed following a single trial. Because reinfection was likely, control of flukes in a wet rainforest environment must also target the snail intermediate host through environmental interventions, such as clearing of vegetation around day stalls where rhinoceros feed. A *Lymnea* sp. snail was identified in a wallow frequented by the rhinoceros at the sanctuary.[10–12]

Ticks and Tick-borne Disease

External parasites, including ticks, flies, and leeches, are common in the warm humid environments where Sumatran rhinoceros live. In addition to taking a blood meal (which may offer a natural mechanism for iron reduction), these parasites are vectors for important diseases. Of note are the hemoparasite infections carried by ticks in the family Ixodidae. In a survey of four captive Sumatran rhinoceros living in native rainforest habitat in Way Kambas National Park, two species of ticks were identified, *Haemaphysalis hystricis* (81%) and *Amblyomma testudinarium* (19%), with predilection for the neck and shoulder skinfolds of the animals. At the time of the 2008 tick survey, simultaneous microscopic hematoparasite examination of Giemsa-stained blood smears collected from the captive rhinoceros revealed tick-borne diseases, including *Anaplasma marginale* (27%), *A. centrale* (10%), *Babesia* sp., and *Theileria* sp. (15%).[10,13] Further molecular analysis using reverse line blot hybridization (RLB) and nested polymerase chain reaction (PCR) revealed *Theileria bicornis* in a single Sumatran rhinoceros. *T. bicornis* was first described as a novel blood parasite in free-ranging black rhinoceros *(Diceros bicornis)* in South Africa and, although fatal babesiosis from infection with *Babesia bicornis* was described in three black rhinoceros, there was no evidence that *T. bicornis* was associated with disease in African rhinoceros.[14]

In an effort to boost immunity against tick-borne pathogens, one captive-born Sumatran rhinoceros destined for repatriation back to Indonesia from an American zoo received three doses of a babesia-anaplasma vaccine (lyophilized protein of *Babesia bigemina*, *B. bovis,* and *A. marginale* of bovine origin) prior to the translocation.[15] Although no postvaccine titers were measured, the rhinoceros made a smooth transition into the tick-endemic environment of Way Kambas, with mild subclinical hemoparasite infections documented in serial blood smears.

Tabanids and Trypanosomes

The emergence of animal trypanosomiasis (surra) in Sumatran rhinoceros highlights the growing threat of pathogens transferred to novel hosts that have not adapted (or poorly adapted) to the agent.[16] Trypanosomes evolved on the African continent, and African rhinoceros have evolved a relatively stable host-parasite relationship, with disease observed primarily during periods of stress or following translocation of naïve animals into tsetse fly zones.[17] However, Asian rhinoceros are particularly susceptible and suffer high mortality.

A 2003 outbreak in a captive population of Sumatran rhinoceros housed in peninsular Malaysia at the Sungai Dusun Conservation Center was attributed to infection with *Trypanosoma evansi*.[18] The epidemic was characterized by a biphasic die-off of animals with clinical signs that varied from anorexia and depression to incoordination, rear limb paralysis, and recumbency. Pathology at the time of the outbreak showed overgrowth of *E. coli* and *Klebsiella* bacteria from multiple organ systems, generating significant debate about the level of hygiene and husbandry at the sanctuary.[19] However, subsequent histopathology revealed that the bacteria were not associated with disease but rather consistent with overgrowth. Furthermore, trypanosomes invaded tissues and were found in various organs (including the brain), together with unique lesions in the spleen consisting of enlarged periarteriolar sheaths with lymphoid depletion, pathologic lesions characteristic of surra in other mammals.[16,18]

Noninfectious Disease

Eye Disorders

The Sumatran rhinoceros has a propensity for ocular disorders that is greater than that observed in other captive rhinoceros species. Excessive exposure to ultraviolet light (UV) is the primary factor implicated in the ocular syndrome, although a multifactorial etiology is suspected given the broad presentation of clinical signs in a variety of environments, including confinement within range countries. Eye conditions progress from mild corneal edema to severe opacity, uveitis, and blindness, with secondary bacterial and fungal infections common sequelae. A case summary of a breeding pair of Sumatran rhinoceros in Sabah Malaysia compared development of clinical eye disease with indoor and outdoor locations in an attempt to elucidate causation from light intensity or other environmental factors. A seasonal pattern was noted, with all eye disorders appearing in the months of July and August, although no correlation could be found to excessive UV exposure or dry conditions.[20]

The difference in light intensity in captive environments compared with natural tropical forest architectures is likely significant, given that chronic recurrent eye syndromes are also prevalent in the captive Malayan tapir *(Tapirus indicus)* coming from the same region. The rainforest environments where these rhinoceros live consist of a complex forest structure in four layers, namely the emergent, canopy, understory, and forest floor. The extensive canopy and emergent layers filter direct sunlight before it reaches the forest floor. In a study of forest structure in Costa Rica, canopy architecture and light transmittance in both secondary and old growth rainforests were compared—diffuse transmittance of light at 1–2 m above the forest floor (the understory level where Sumatran rhinoceros live) was less than 3%.[21] Although shade structures in captive environments appear to help reduce eye disease in this species, it is not always sufficient. One captive Sumatran rhinoceros housed in an environment with extensive shade structures and high humidity

developed eye disease during the winter months when cloudy conditions predominated.

The integument of the black rhinoceros has been implicated as the primary organ in which allergic or disease conditions manifest under a variety of circumstances, suggesting that their epidermis is highly sensitive to disruption of metabolic homeostasis.[22] The corneal epithelium of the Sumatran rhinoceros eye may respond to disruptions in a similar manner with nutritional deficiency, UV exposure, and dry conditions leading to increased disease states and reduced healing—all of which are compounded with life in captive environments. The first sign of eye disease presents as corneal edema; then, if not treated with aggressive topical medication, peripheral vessel ingrowth occurs and pigmentation follows. Some animals progress to recurrent anterior uveitis similar to moon blindness in horses (S. Citino, personal communication, March 8, 2017). A vicious inflammatory cycle of reinjury drives pathogenesis of ocular disease, with the initial insult causing inflammation that augments further injury to the ocular surface—invasion of leukocytes and release of immune mediators from damaged cells leads to cyclic damage and reinjury. Therefore the best response to treatment may be achieved using topical cyclosporine ointment, an immunomodulator that inhibits T-lymphocyte proliferation (S. Citino, personal communication, March 8, 2017).

Iron Overload Disorder (Hemachomratosis, Iron Storage Disease)

The induction of toxic overburdens of elemental iron in captive black rhinoceros was first noted by Smith et al. (1995).[23] Subsequent evidence extended these findings to include Sumatran rhinoceros, the only other browser rhinoceros currently managed in captivity.[24,27] Sumatran rhinoceros develop progressive iron overloads even more rapidly than do African black rhinoceros, reaching tenfold elevations in body burdens within as little as 3 years of captive birth or transfer into captive conditions and increasing in direct relation to time in captivity.[24,27]

Measurements of serum *ferritin* concentrations and *transferrin saturation* (the ratio of serum iron to total iron-binding capacity [TIBC]) provide the least invasive means to assess iron status. It is widely acknowledged that serum ferritin concentrations reflect total body iron stores with an accuracy exceeded only by direct quantitative analyses of tissue samples.[25] Most rhinoceros studies have relied on the assay developed by Smith et al. (1984),[26] which is available through the Kansas State University Veterinary Diagnostic Laboratory. In separate studies, serum ferritin values measured by this assay in African black and white rhinoceros *(Ceratotherium simum)* free-ranging in their natural habitats were less than 100–200 ng/mL.[27] By contrast, specimens from 14 captive Sumatran rhinoceros averaged greater than 850 ng/mL, with individual values ranging as high as 2000–4000 ng/mL.

Despite its widespread use and verified correlation with quantitative tissue assays and necropsy histopathology, the Smith et al. ferritin assay has been criticized by some because it requires species-specific reagents with variable cross-reactivity among diverse species and because results sometimes seem highly variable in individual animals. The latter is likely due to serial dilutions of plasma that are required for exceptionally high ferritin concentrations in species with captivity-induced ISD. In addition, results may be confounding because ferritin is an acute-phase reactant that elevates secondarily in a number of inflammatory, neoplastic, or other conditions.

Transferrin saturation, the amount of iron bound to the plasma transport-protein transferrin, provides a simple, qualitatively reliable, supplement or alternative if ferritin assays are equivocal or unavailable. Transferrin saturation correlates well with ferritin concentrations, with quantitative tissue analyses, and with histopathology using ferric-specific stains such as Prussian blue.[24,27] Transferrin saturation in most vertebrates is approximately 35%. US captive Sumatran rhinoceros measure 90%–100%, clearly indicating iron in sufficient excess to overwhelm carrying capacity of protective proteins.[24,27] Alternative systems for measuring ferritin and assessing ISD status have been proposed, but these have not yet been validated by studies directly comparing both assay systems or their relation to demonstrable histopathology.[28,29]

Nutrition is fundamental to the health of the browsing rhinoceros, whether they are managed in captive or semicaptive environments. A comparison of browse diversity in Sumatran rhinoceros housed in three diverse settings (North American zoo, Malaysian center, Indonesian sanctuary) demonstrated marked differences in nutritional management and predicted that these disparities relate directly to differences in iron loading.[30] Browse diversity was measured across five areas: number of locally available plant species, number of plant species fed daily, access to a free-range browse environment (i.e., native rainforest), transit time from plant cutting to feeding, and percentage of nonbrowse items in diet (i.e., hay or pelleted ration). When comparing traditional zoo rhinoceros with sanctuary animals, zoo rhinoceros were fed fewer species of browse (8 vs. 100 species); fed fewer species on a daily basis (2–3 vs. 8–10 species); spent fewer hours browsing (0 vs. 6 hours); ate browse that had been in transit longer (>72 vs. <12 hours); and fed significantly more nonbrowse items as percentage of diet (20%–38% vs. 0%). These same groups differed in iron stores, with zoo rhinoceros having higher mean ferritin than sanctuary rhinoceros managed in range countries (2835 ± 295 ng/mL vs. 680 ± 168 ng/mL, respectively) (see also Chapter 99).

The inevitable morbidity and mortality of chronic progressive iron toxicity can be prevented by induction of negative iron balance through periodic phlebotomies, as validated by extensive experience with an equivalent human disorder, hereditary hemochromatosis. The clinical effectiveness of this procedure has been validated in African black rhinoceroses at multiple institutions, but has not yet been applied appropriately to Sumatran rhinoceroses (D. Paglia, personal communication, 2018).

Chronic Renal Disease

A 30-year-old male Sumatran rhinoceros named *Torgamba* housed at the Sumatran Rhino Sanctuary in Lampung, Indonesia developed renal disease characterized by progressive azotemia, hypercalcemia, and hypophosphatemia (data summarized over a 4-year period showed progressive deterioration in renal function as measured by BUN 30.3 ([10–52 µg/dL]; creatinine 3.2 [0.87–20.7 µg/dL]; Ca 15.8 [8.9–28.3 mg/dL]; Ph 3.3 [1.3–9.7 mg/dL]; and Ca to Ph ratio 5.6:1 [1.2:1–11.8:1]. Radcliffe, RW and Candra D: unpublished data, 2009). The disease was monitored with serial measurement of body weight and serum biochemistry analysis on a weekly basis. Nutritional management of the disease focused on feeding a highly palatable selection of browse that included hand-feeding during periods of inappetence together with supplementation of elemental phosphorus (Equi-phos; Uckele Health & Nutrition, Blissfield MI 49228: guaranteed analysis of Ph 19%; Na 4.5%–5.5%, and Ca 0.1%–0.2%, with each ounce supplying 5.4 g of elemental Ph), electrolyte water, and a salt lick. Phosphorus supplementation ranged from 1–4 oz per day, with dosing changes based on the most recent biochemistry panel; in general, the dose was increased by 1–2 oz per day when the Ca to Ph ratio exceeded 5:1. The condition was managed successfully for half a decade before the rhinoceros finally deteriorated and died from complications of the disease.

Reproductive Pathology and Allee Effect

The development of reproductive pathologies in female Sumatran rhinoceros impacts both captive and wild conservation programs. Uterine tumors, such as leiomyomas and cystic endometrial hyperplasia, have been visualized on ultrasound and confirmed on histopathology.[31] These diseases are more common in older animals and may be related to physiologic states related to long-term estrogen influence from cycling without pregnancy, a condition observed in other captive rhinoceros. Uterine and ovarian masses have also been observed in recently captured wild female rhinoceros, inferring that the same pathologies may be developing in wild animals. With their slow breeding rate (long gestation and intercalving interval), small populations of rhinoceros are particularly susceptible to stochastic factors and the Allee effect (i.e., solitary nature and reduced mating opportunity, reproductive pathologies from prolonged periods of nonparity, skews in sex ratios, and inbreeding depression), with the end result being fewer births than deaths.[19]

Javan Rhinoceros *(R. sondaicus*, Desmarest 1822*)*

Taxonomy

The Javan rhinoceros or one-horned lesser rhinoceros *(R. sondaicus)* is one of the most critically endangered terrestrial mammals in the world. There are three distinct subspecies, of which only one is extant; the Indonesian Javan rhinoceros *(R. sondaicus sondaicus)* solely inhabiting Ujung Kulon National Park (UKNP), with 67 individuals recorded in 2016; the Indian Javan rhinoceros *(R. sondaicus inermis)* now extinct but once common throughout Bengal, Bangladesh, and Burma; and the Vietnamese Javan rhinoceros *(R. sondaicus annamiticus)* recently declared extinct in Cat Tien National Park, Vietnam and formerly also found in Cambodia, Laos, Thailand, and Malaysia. Currently, many challenges threaten the last population of Javan rhinoceros, including infectious disease at the wildlife–domestic animal interface and noninfectious disease (toxic plants, invasive arenga palm, parasitism, and feeding competition with sympatric ungulates), all compounded by the significant demographic risk of natural disaster and inbreeding depression inherent in a single small population. If not addressed, these challenges may create an irreversible extinction vortex.

Javan Rhinoceros Mortality Events and Infectious Disease

The first population census of UKNP was conducted in 1955 by IUCN Director-General, Dr. Lee Talbot, and repeated a dozen years later by WWF researcher, Professor Schenkel; both recorded fewer than 30 Javan rhinoceros.[32,33] Since then, the population has fluctuated between 58 and 69 individuals. In 1982 the first of several mortality events was reported, with five carcasses discovered with horns intact, representing 8.9% of the population (see Table 100.1).[33] The investigation focused on infectious disease because the park lies adjacent to local agricultural communities with a significant population of water buffalo *(Bubalus bubalis)*. The sequence leading to death was deduced from traces at the site—walking and feeding, diarrhea, lying down, convulsive leg movements, and death. One comparatively fresh carcass showed prolapse of rectum and foamy mucus at the mouth and nostrils. Hemorrhagic septicemia (HS) is an infectious disease caused by the gram-negative bacteria *Pasturella multocida.* HS is a fatal disease of cattle, yak, camel, and water buffalo. In 1981 an HS outbreak was responsible for the death of 350 domestic goats and 50 buffaloes in the region around the park. Anthrax outbreaks were also recorded locally several decades previously. Despite inconclusive laboratory findings from the soil samples collected at the site, Schenkel concluded that anthrax was most likely the causative agent because the spores are long lived and clinical signs were typical of acute outbreaks.

Following the 1982 die-off, the local government sponsored an HS vaccination program to districts that were affected by the outbreak, although the implementation remains intermittent and irregular. For a 1-year period from June 2012 to July 2013, a disease surveillance study was conducted to investigate the prevalence of HS.[34] The study was conducted in 19 buffer villages surrounding the national park due to a high risk of cross-infection between the villagers' water buffalo and ungulates in the park, including the Javan rhinoceros. Blood samples for serology ($n = 770$) and nasal swabs for culture ($n = 85$) were collected

from water buffalo and compared with perceived risk factors for buffalo herd management. A low seroprevalence of 1.8% (14 of 770 animals) was observed, suggesting that carrier animals could contribute to ongoing outbreaks in the park. Husbandry practices associated with a positive serologic response in water buffalo were: lack of a permanent area to house buffalo at night; low body condition score (BCS = 2); high body temperature (fever ≥40°C); a history of clinical signs or sudden death in the previous year; and a grazing system that accessed significant forage inside the park. Serologic response was not associated with sex, age, vaccination status, or season.[34]

Historic surveillance for endoparasites in Javan rhinoceros fecal samples has demonstrated cestode, nematode, and trematode infections with *Strongyloides*, *Bunostomum*, *Trichostrongylus*, *Fasciola*, *Schistosoma*, *Anaplocephalidae*, *Oesophagostomoma*, and *Plagiotaenia*.[35–37] Protozoans isolated include *Balantidium*, *Entamoeba*, *Eimeria*, *Cryptosporidium*, *Cycloposthium*, *Lavierella*, and *Ophryoscolecidae*, some of which are known pathogens in other species.[37]

Vector-borne disease is emerging as a significant potential threat to the UKNP Javan rhinoceros population, given the disease reservoir of buffalo that are grazed inside the park. Tabanid flies of the genus *Tabanus* are common hemoparasites known to transmit animal trypanosomiasis or surra. Trypanosomiasis infects cattle, water buffalo, horses, elephants, camels, and rhinoceros, with Asian species being highly susceptible.[16,18] Surveillance for *T. evansi* in 2014 demonstrated a high prevalence (90%) of trypanosomiasis in the livestock of two villages intersecting directly with the park boundary. A comprehensive study of tabanid vector biology, including trypanosome infection rate and host blood meal analysis, is underway to better understand host-parasite-vector dynamics in the UKNP ecosystem.[38,39]

Noninfectious Disease

One of the most challenging aspects of conserving the Javan rhinoceros is the insufficient data regarding the species and the habitat that shelters it. The Javan rhinoceros population has been on the rise since 1937; however, for the past 2 decades, population growth has been stagnant. A variety of extrinsic and intrinsic factors may contribute to this population plateau.

Because vegetation analysis data in UKNP is limited, it is possible that toxic plants have contributed to rhinoceros mortalities. An invasive palm *(Arenga obtusifolia)* known locally as "langkap" may lower the carrying capacity for browsers in the park. The invasive palm crowds out sunlight and reduces secondary growth that provide the natural food plants for the Javan rhinoceros. With the disappearance of open grasslands in the park, banteng *(Bos javanicus)* may be competing with Javan rhinoceros for available browse. The UKNP banteng population has been increasing from 200 in 1983 to more than 800 individuals in 2000.[40]

Finally, the Allee effect may further limit Javan rhinoceros population growth rates. The idea that population size may impact fitness is significant for both species of Indonesian rhinoceros.[19] Cooperative rhinoceros behaviors such as feeding, breeding, territorial defense, and communication through dung middens are less effective at low population size, leading to decreased survivorship. Likewise, the per capita risk from predation and disease are heightened in small populations; a recent camera trap recording documents predation of a banteng juvenile and a Javan rhinoceros bull followed closely by a pack of Javan dhole *(Cuonalpinus javanicus)*.

Demographic Risks to a Single Population

The loss of the Vietnamese subspecies of Javan rhinoceros in 2011 leaves UKNP as the last habitat for Javan rhinoceros in the world. UKNP lies at the western most tip of Java Island in the heart of the Sunda Arc, an area of converging tectonic plates that commonly produces earthquakes and triggers tsunamis.[41] In 1883 the eruption of Krakatoa devastated Ujung Kulon and its surrounding area, making way for the Javan rhinoceros to colonize the region. Ironically, the same threat that gave the Javan rhinoceros its last refuge is looming with Strombolian eruptions of Anak Krakatau (Child of Krakatoa) actively spewing lava into the sea.[42]

The creation of a second population of Javan rhinoceros, remote from Ujung Kulon in Cikepuh Wildlife Reserve, has been proposed to the government of Indonesia.[43] Ecologic, biological, and socioeconomic viability assessments are underway to evaluate the readiness of Cikepuh to host a founder population of four select Javan rhinoceros based on distinct mean kinship. The second population strategy is planned for execution in 2023.

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