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REVISTA
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Animal vaccination
Part 1: development, production and use of vaccines

Vaccination animale
Partie 1 : développement, production et utilisation des vaccins

Vacunación animal
Parte 1: desarrollo, producción y utilización de vacunas

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Preface

Animal vaccination
Part 1: development, production and use of vaccines
Part 2: scientific, economic, regulatory and socio-ethical issues

Vaccination, when available, is undoubtedly the most cost-effective means of preventing and controlling, and even eradicating, infectious diseases. In recent years vaccination has also been used for other purposes in animal health, production and welfare, e.g. immunocastration. In fact, the impact of vaccination goes far beyond the mere control of infectious diseases.

Acting through natural mechanisms, vaccination of animals serves many different purposes, such as controlling animal infections and infestations, thus improving animal health and animal welfare; controlling anthropozoonoses and food poisoning, thereby protecting public health; solving problems associated with antibiotic and anthelmintic resistance; helping to leave food-producing animals free of chemical residues; protecting the environment and biodiversity; and ensuring animal farming sustainability, thereby helping to alleviate poverty.

Vaccination will help to reach many of the objectives of the United Nations 'Millenium Development Goals Report - 2005', especially in the light of the foreseen livestock revolution.

Public perception and disapproval of some veterinary prophylactic measures, such as mass slaughtering of livestock to control epizootic diseases, serve to further promote the use of vaccination as an alternative disease control strategy, even if slaughtering of infected animals will still be necessary in many circumstances. This will be made easier, thanks to recent progress in veterinary vaccinology, such as the availability of marker (DIVA [differentiation of infected from vaccinated animals]) vaccines.

Recent progress in animal genomics and the availability of the entire genome sequences of several domestic species such as cattle and chickens, as well as recent progress in veterinary immunology will help to develop more effective and safer vaccines.

Unfortunately, there are several barriers to the development of new vaccines: economic barriers such as the lack of investment incentives, especially for vaccines against diseases that only occur in developing countries; scientific obstacles, for instance, the antigenic variability of some pathogens and the ability of parasites to circumvent immune response; regulatory hurdles due the stringent and non-harmonised regulations in place for vaccine registration; deliberate withholding by some countries of strains of pathogenic agents; and, finally, public perception of the consumption of food products derived from vaccinated animals and of technologies such as genetic engineering.

Vaccination and vaccines have always been a major topic for the World Organisation for Animal Health (OIE) since elimination or control of animal diseases, particularly zoonoses, is a global public good. This is why profitability should not be a priority when vaccination policies are established. The OIE *Terrestrial Animal Health Code (Terrestrial Code)* and the *Manual of Diagnostic Tests and Vaccines for Terrestrial Animals (Terrestrial Manual)* respectively provide recommendations on how to administer and how to manufacture veterinary vaccines. Veterinary Services should be encouraged to regularly consult these publications in order to improve animal health throughout the world.

Recently, the OIE was involved in the production of a textbook published by Elsevier *(Veterinary Vaccinology)*, and organised an international conference on the 'Control of Infectious Animal Diseases by Vaccination' in Buenos Aires in April 2004, the proceedings of which were published by the International Association of Biological Standardisation (IABS). It seemed timely, therefore, to review the different aspects of vaccination and vaccines in animal health to provide OIE Delegates with updated information to scientifically support decision making. To this end, these two issues of the OIE *Scientific and Technical Review* are designed to provide useful generic information rather than give detailed technical descriptions of specific diseases or vaccines.

I am certain that this *Review* will help all those involved in animal health, animal welfare and public health.

I would like to express my sincere thanks to all the authors who contributed to these two issues of the *Review* which is on a subject of great importance for the OIE and all its Member Countries.

I would especially like to thank Professor Paul-Pierre Pastoret, Dr Michel Lombard and Dr Alejandro Schudel for accepting our invitation to coordinate these issues of the *Review*. I am very grateful for the way in which they undertook this task and for their contribution to the development of this publication.

Bernard Vallat
Director General

Préface

Vaccination animale
Partie 1 : développement, production et utilisation des vaccins
Partie 2 : aspects scientifiques, économiques, réglementaires et socio-éthiques

Lorsqu'elle est envisageable, la vaccination est sans conteste le moyen le plus économique de prévenir et de contrôler les maladies infectieuses, voire de les éradiquer. Ces dernières années, la vaccination a trouvé d'autres applications dans les domaines de la santé animale, de la production animale et du bien-être des animaux, par exemple l'immunocastration. En réalité, l'impact de la vaccination va bien au-delà du simple contrôle des maladies infectieuses.

La vaccination fait intervenir des mécanismes naturels et peut viser diverses finalités : prophylaxie des maladies infectieuses et parasitaires affectant les populations animales pour améliorer la santé et le bien-être des animaux ; contrôle des anthropozoonoses et

des toxi-infections alimentaires pour protéger la santé publique ; résolution des problèmes liés à la résistance aux antibiotiques et aux anthelminthiques ; lutte contre la présence de résidus de médicaments dans les animaux destinés à la consommation humaine ; protection de l'environnement et de la biodiversité ; promotion de l'élevage durable afin de lutter contre la pauvreté, etc.

La vaccination est vouée à jouer un rôle crucial dans la réalisation d'un certain nombre des objectifs cités dans le rapport des Nations unies « Objectifs du Millénaire pour le développement » de 2005, en particulier dans la perspective attendue de l'augmentation de la demande mondiale de viande.

La perception généralement négative qui prévaut dans l'opinion publique à l'égard de certaines mesures de prophylaxie vétérinaire telles que l'abattage sanitaire des animaux en cas d'épizootie encourage à recourir à la vaccination même si, dans bien des cas, l'abattage des animaux infectés ne pourra être évité. Le recours à la vaccination est facilité par les progrès récents accomplis dans le domaine de la vaccinologie vétérinaire, notamment la mise au point de vaccins marqueurs de type « DIVA » (c'est-à-dire permettant de différencier les animaux infectés des animaux vaccinés).

Les avancées réalisées dans nos connaissances sur le génome des animaux et, en particulier, le séquençage intégral du génome de plusieurs espèces d'animaux domestiques dont les bovins, ainsi que les progrès récents de l'immunologie vétérinaire laissent présager la mise au point de vaccins plus efficaces et plus sûrs.

Malheureusement, cette évolution se heurte à un certain nombre d'obstacles : barrières économiques, avec l'absence d'incitations à investir, surtout s'agissant de vaccins destinés à des maladies ne sévissant que dans les pays en voie de développement ; contraintes scientifiques, relatives notamment à la variabilité antigénique de certains agents pathogènes ou à la capacité des parasites à contourner la réponse immunitaire ; obstacles réglementaires, dus aux législations en place, parfois contradictoires, applicables à l'enregistrement des vaccins ; sans parler de la rétention délibérée de souches d'agents pathogènes par certains pays et des appréhensions du public à l'égard des produits alimentaires dérivés d'animaux vaccinés ou des technologies liées à l'ingénierie génétique.

La vaccination et les vaccins ont toujours été un thème important pour l'Organisation mondiale de la santé animale (OIE), d'autant plus que l'élimination ou la maîtrise des maladies animales et particulièrement des zoonoses sont considérées un bien public international. C'est pourquoi la rentabilité ne doit pas être une priorité lorsqu'il s'agit de mettre en place des politiques de vaccination. Le *Code sanitaire pour les animaux terrestres (Code terrestre)* et le *Manuel des tests de diagnostic et des vaccins pour les animaux terrestres (Manuel terrestre)* de l'OIE fournissent des recommandations concernant respectivement l'administration et la fabrication des vaccins à usage vétérinaire. Il convient de convaincre tous les Services vétérinaires à s'y référer en permanence afin d'améliorer la santé animale dans le monde.

L'OIE a contribué, dans un passé récent, à l'élaboration d'un ouvrage de référence intitulé *Veterinary Vaccinology*, publié en 1999 par Elsevier ; l'OIE a également organisé la Conférence internationale sur la prophylaxie des maladies infectieuses par la vaccination, qui s'est tenue à Buenos Aires (Argentine) en avril 2004 et dont les actes ont été publiés par l'Association internationale de standardisation des produits biologiques. Il devenait nécessaire de refaire un bilan sur les différents aspects de la vaccination et des vaccins utilisés en santé animale afin de fournir aux Délégués des Pays Membres de l'OIE des informations réactualisées leur permettant de fonder leurs décisions sur des bases scientifiques. Les deux numéros de la *Revue scientifique et technique* consacrés à ce sujet visent à dresser un tableau général complet à cette fin, plutôt qu'à entrer dans

le détail technique de maladies ou de vaccins particuliers. Je suis certain que cette publication sera utile à tous ceux qui s'investissent dans les domaines de la santé animale, du bien-être des animaux et de la santé publique.

Je remercie les nombreux contributeurs qui ont participé à l'élaboration de ces deux numéros de la *Revue*, dont le sujet revêt une grande importance pour l'OIE et pour tous ses Pays Membres.

Ma gratitude va également au Professeur Paul-Pierre Pastoret et aux Docteurs Michel Lombard et Alejandro Schudel, qui ont aimablement accepté d'assumer la responsabilité éditoriale de ces numéros et n'ont ménagé aucun effort pour faire aboutir cette entreprise.

Bernard Vallat
Directeur général

Prólogo

Vacunación animal
Parte 1: desarrollo, producción y utilización de vacunas
Parte 2: aspectos científicos, económicos, reglamentarios y socio-éticos

Las vacunas, cuando las hay, constituyen sin duda el medio más eficaz y rentable para prevenir y controlar, o incluso erradicar, enfermedades infecciosas. Además, en los últimos años también han sido utilizadas con otros fines en los terrenos de la sanidad, la producción y el bienestar animales, por ejemplo para la inmunocastración. De hecho, las vacunas tienen aplicaciones que van mucho más allá del mero control de enfermedades infecciosas.

La vacunación de los animales, que se basa en mecanismos naturales, puede emplearse con muchos fines distintos, por ejemplo: controlar las infecciones e infestaciones y, gracias a ello, mejorar la salud y el bienestar de los animales; controlar las antropozoonosis y las toxiinfecciones alimentarias, protegiendo así la salud pública; resolver problemas ligados a la resistencia a antibióticos y antihelmínticos; contribuir a

que los animales destinados al consumo humano estén exentos de residuos químicos; proteger el medio ambiente y la biodiversidad; y hacer posible una producción ganadera sostenible, ayudando así a reducir la pobreza.

La vacunación será determinante para que puedan cumplirse muchas de las finalidades del informe 'Objectivos de desarrollo del Milenio' de 2005, teniendo en cuenta especialmente el incremento de la demanda mundial de carne que se perfila en el horizonte.

La opinión y mala acogida que en el gran público suscitan ciertas medidas profilácticas veterinarias, como el sacrificio masivo de animales para luchar contra enfermedades epizoóticas, favorecen aún más el uso de vacunas como estrategia alternativa de control zoosanitario, aun cuando en muchas circunstancias siga siendo necesario el sacrificio de los ejemplares infectados. Esta vía alternativa será cada vez más fácil gracias a los progresos que ha conocido recientemente la vacunología veterinaria, de los que es buen ejemplo la obtención de vacunas con marcador serológico (que permiten distinguir entre los animales infectados y los vacunados).

Los recientes adelantos de la genómica animal y la posibilidad de disponer de la secuencia genómica entera de varias especies domésticas como la vaca, así como los avances logrados en los últimos tiempos en el terreno de la inmunología animal, ayudarán a obtener vacunas más eficaces y seguras.

Lamentablemente, en el camino hacia la creación de nuevas vacunas quedan aún varios obstáculos por superar: obstáculos de tipo económico, como la falta de incentivos a la inversión, sobre todo para vacunas que vayan a utilizarse contra enfermedades que solo existen en los países en desarrollo; obstáculos científicos, por ejemplo la variabilidad antigénica de algunos patógenos y la capacidad de los parásitos de eludir la respuesta inmunitaria; obstáculos reglamentarios, derivados de la falta de armonización de las normas relativas al registro de vacunas; la retención deliberada de cepas de microorganismos patógenos en algunos países; y, por último, la percepción que el gran público tiene del consumo de alimentos obtenidos a partir de animales vacunados o de técnicas como la ingeniería genética.

Desde siempre, la cuestión de las vacunaciones y las vacunas ha sido uno de las principales líneas de trabajo de la Organización Mundial de Sanidad Animal (OIE), no en vano la eliminación o el control de las enfermedades animales, en particular las zoonosis, es un objetivo de interés público a escala mundial. Por este motivo la rentabilidad económica no ha de considerarse una prioridad a la hora de instituir políticas de vacunación. El *Código sanitario para los animales terrestres (Código terrestre)* y el *Manual de pruebas de diagnóstico y vacunas para los animales terrestres (Manual terrestre)* de la OIE ofrecen recomendaciones sobre, respectivamente, la administración y la fabricación de vacunas. Convendría que los Servicios Veterinarios se remitieran siempre a ellas para mejorar la sanidad animal en el mundo.

En fechas recientes, la OIE participó en la elaboración de un libro de texto titulado *Veterinary Vaccinology*, publicado por la editorial Elsevier, y organizó una conferencia internacional sobre el control de enfermedades animales infecciosas por vacunación (Buenos Aires, abril de 2004), cuyas actas publicó la International Association of Biological Standardisation. Parecía llegado el momento de pasar revista a distintos aspectos de la vacunación y las vacunas en el terreno de la sanidad animal para proporcionar información actualizada a los delegados ante la OIE y respaldar así con datos científicos el proceso de adopción de decisiones. Estos dos números de la *Revista científica y técnica* de la OIE están concebidos con ánimo de brindar información útil de carácter general, y no tanto de ofrecer prolijas descripciones técnicas de enfermedades o vacunas concretas.

Espero sinceramente que esta publicación resulte de ayuda a cuantos trabajan en salud pública o en sanidad y bienestar animales.

Quisiera expresar mi más sincera gratitud a todos los autores que han contribuido a estos números de la *Revista*, dedicado a un tema de suma importancia para la OIE y para sus Países Miembros.

Asimismo, quisiera agradecer especialmente al Profesor Paul-Pierre Pastoret, y a los Doctores Michel Lombard y Alejandro Schudel que aceptaran nuestra invitación a coordinar estos números de la *Revista*, y sobre todo la forma en que desempeñaron esa tarea y contribuyeron así al crecimiento de nuestra publicación.

Bernard Vallat
Director General

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Introduction

Animal vaccination
Part 1: development, production and use of vaccines
Part 2: scientific, economic, regulatory and socio-ethical issues

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Vaccination is without doubt the single most useful measure available to prevent infectious diseases. The advantages of vaccination are numerous. It is the only available method to prevent, or sometimes cure, viral animal infections in the absence of broad spectrum antivirals.

Vaccines are environmentally friendly and increase animal welfare by preventing suffering caused by disease or by the consequent curative treatment. Curative treatment may also result in antibiotic resistance and pharmaceutical residues in food. For the management of livestock health, vaccines are the best tool to achieve sustainability.

Veterinary vaccines can be used to protect animal health, but by preventing zoonotic infections animal vaccination also protects human health, as exemplified by wildlife vaccination against rabies.

In animal health the focus is now on animal infections rather than on animal diseases. Vaccines should be designed to prevent infection rather than to prevent clinical signs of disease and should, wherever possible, produce sterile immunity. Last but not least, available technologies allow us to design DIVA vaccines, together with their companion diagnostic tests, which make it possible to distinguish between vaccinated and infected animals even if the latter were previously vaccinated.

Smallpox, a human disease, was the first viral infection to be eradicated; eradication means the complete elimination of the disease and its infectious agent worldwide. This remarkable success was due to several factors, including the availability of an efficacious vaccine, namely vaccinia, and the absence of a wildlife reservoir. According to the World Health Organization (WHO), eradication of human poliomyelitis and measles may also soon be achievable for the same reasons.

The only animal virus disease for which the same circumstances exist is rinderpest: there are several efficacious vaccines already available and the infection seems to reach a dead-end if transmitted to susceptible wild species. The same cannot be said for other

animal viral infections, none of which are likely to be eliminated in the near future, either due to the lack of an efficacious vaccine (e.g. African swine fever) or to the existence of wildlife reservoirs such as the wild boar (*Sus scrofa*) for classical swine fever or the African buffalo (*Syncerus caffer*) for foot and mouth disease. These diseases are more prone to regional elimination than to complete eradication worldwide. The choice of method to eliminate an animal infectious disease must take into account the biological and epidemiological characteristics of the infection, the available control techniques and the emergence of new vaccination technologies such as DIVA vaccines.

A plea for veterinary vaccines

The reasons to develop veterinary vaccines are now manifold:

– to protect animal health

– to eliminate/eradicate an infection

– to improve animal welfare

– to protect public health

– to protect consumers from certain risks which may be linked to products derived from food-producing animals

– to protect the environment and biodiversity

– to avoid the emergence of pathogens resistant to available drugs

– to promote sustainable agriculture and food-producing animal production.

Unfortunately, even though the reasons for developing veterinary vaccines are many, there are still many obstacles to their development:

– scientific obstacles, such as those that prevent the development of vaccine for African swine fever, theileriosis, and many parasitic diseases

– difficulty in accessing the target species (wildlife)

– poor investment return for companies involved in vaccine development and production

– animal health regulations that prohibit the use of vaccination

– regulatory requirements for vaccine registration

– the so-called 'minor' species status of some targets

– conditions of minor importance in so-called 'major' species

– conditions of minor importance in so-called 'minor' species (the worst-case scenario).

Animal health, animal welfare, and environmental protection

Public concern for animal welfare is increasing, leading to the implementation of 'the three Rs' (*replace, reduce* and *refine* the use of laboratory animals).

The value of animal models for veterinary vaccines is not to be ignored, particularly since researchers have access to target animal models which are often more relevant, especially for challenge/protection studies. Immune protection involves complex

immunological phenomena and processes. Animal models are particularly important whenever cellular immunity plays a crucial role because it is still easier to measure antibody than cellular responses *in vitro*. Nevertheless, the trend is to replace animal models by *in vitro* systems wherever possible.

The use of veterinary vaccines has obvious benefits for animal welfare. Vaccines, unlike therapeutic treatments, are the best way of avoiding animal suffering since they prevent disease or avoid the need for slaughtering as part of the implementation of stamping out. Furthermore, due to the short lifespan of many food-producing animals, vaccine need only be administered once, while treatments generally necessitate repeated interventions. Nevertheless, there is still room for improvement by developing less reactogenic adjuvanted vaccines. Another area of animal welfare improvement is the use of vaccines for immunocastration of male pigs to avoid boar taint, instead of surgical castration.

The use of vaccines in animal production systems is also often more environmentally friendly since it reduces the use of chemicals. Of special interest is the anti-tick vaccine developed in Australia, which is based on a cryptic intestinal antigen of the parasite. One should also mention the trials carried out in Australia to reduce methane (a greenhouse gas) emission by ruminants by vaccinating them against *Archeobacteria* of the rumen, although unfortunately this has had little success as yet.

Minor species and diseases specific to developing countries

Several attempts have been made to define a 'minor' species and many definitions proposed. Simply put, minor species are animal species other than cattle, sheep (meat and wool producing), horses, pigs, chickens, dogs, cats and salmonidae. In Europe, for instance, this means milking sheep, goats, rabbits, and other fish and avian species.

It is difficult for pharmaceutical companies to develop vaccines for such minor species due to the small market size and the poor return on investment. The same obstacles apply to the development of vaccines against diseases only found in developing countries.

These problems can only be solved by public funding and sound public-private partnerships.

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Introduction

Vaccination animale
Partie 1 : développement, production et utilisation des vaccins
Partie 2 : aspects scientifiques, économiques, réglementaires et socio-éthiques

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La vaccination est sans aucun doute le moyen le plus efficace de se prémunir contre les maladies infectieuses. Les avantages de la vaccination sont nombreux. Elle est le seul moyen de prévenir, voire de traiter certaines infections virales chez les animaux, alors qu'il n'existe pas d'antiviraux à large spectre.

Les vaccins ne sont pas nuisibles pour l'environnement et ils améliorent le bien-être des animaux en leur épargnant la souffrance liée à la maladie ou aux traitements curatifs en cas d'infection. Ces traitements curatifs peuvent induire une résistance aux antibiotiques ou faire subsister des résidus de médicaments dans les denrées alimentaires. Les vaccins sont le meilleur outil pour une gestion durable de la santé du bétail.

Les vaccins vétérinaires servent, bien sûr, à protéger la santé animale, mais en vaccinant les animaux contre les agents de zoonose cette protection s'étend à la santé publique, comme c'est le cas avec la vaccination de la faune sauvage contre la rage.

Actuellement, la médecine vétérinaire met davantage l'accent sur l'infection que sur la maladie. Les vaccins devraient avoir pour objet de prévenir l'infection plutôt que de prévenir les signes cliniques de la maladie et, dans la mesure du possible, ils devraient conférer une immunité stérile. Enfin, mais non moins important, les technologies disponibles ont permis de développer des vaccins (et des épreuves diagnostiques parallèles) capables de distinguer les animaux infectés des animaux vaccinés (DIVA), même dans les cas où les animaux aujourd'hui infectés ont été vaccinés par le passé.

La première infection virale à avoir été éradiquée est la variole, une maladie humaine ; l'éradication signifie l'élimination totale de la maladie et de son agent causal de la surface de la terre. Plusieurs facteurs ont rendu possible cette réussite spectaculaire, notamment la disponibilité d'un vaccin efficace, le virus de la vaccine, et l'absence de réservoir dans la faune sauvage. D'après l'Organisation mondiale de la santé (OMS), la poliomyélite et la rougeole devraient également être éradiquées d'ici peu, grâce aux mêmes atouts.

La seule épizootie virale susceptible d'être éradiquée dans un proche avenir est la peste bovine : plusieurs vaccins efficaces sont disponibles et le virus semble s'enfermer dans un cul-de-sac épidémiologique dès qu'il atteint des espèces sauvages sensibles. On ne peut en dire autant des autres épizooties virales, dont aucune ne paraît pouvoir être éliminée dans un futur proche, soit parce qu'il n'existe aucun vaccin efficace (cas de la peste porcine africaine), soit parce que le virus a des réservoirs dans la faune sauvage, par exemple le sanglier (*Sus scrofa*) pour la peste porcine classique ou le buffle africain (*Syncerus caffer*) pour la fièvre aphteuse. Pour ces épizooties, il est plus réaliste d'envisager l'élimination région par région qu'une éradication mondiale. Le choix de la méthode à utiliser pour éliminer une maladie infectieuse doit tenir compte des caractéristiques biologiques et épidémiologiques de l'infection, des procédés de contrôle disponibles et du développement des nouvelles technologies vaccinales telles que les vaccins DIVA.

Un plaidoyer en faveur des vaccins vétérinaires

Aujourd'hui, les motifs incitant à développer des vaccins vétérinaires sont multiples :

– protéger la santé animale,

– éliminer/éradiquer une infection,

– améliorer la santé animale,

– préserver la santé publique,

– protéger les consommateurs contre certains risques associés aux produits alimentaires d'origine animale,

– préserver l'environnement et la biodiversité,

– empêcher l'émergence d'agents pathogènes résistants aux médicaments,

– promouvoir l'agriculture durable et la production d'animaux destinés à la consommation.

Malheureusement, en dépit du nombre d'arguments en faveur des vaccins vétérinaires, il subsiste encore beaucoup d'obstacles à leur développement :

– obstacles scientifiques, par exemple ceux qui freinent la mise au point de vaccins contre la peste porcine africaine, la theilériose et bien d'autres parasitoses animales,

– accès difficile à l'espèce cible (chez les animaux sauvages),

– rendement trop faible pour les laboratoires qui développent et produisent les vaccins,

– réglementations zoosanitaires interdisant le recours à la vaccination,

– exigences réglementaires encadrant l'enregistrement des vaccins,

– le statut dit « mineur » de certaines espèces cibles,

– les maladies considérées comme mineures bien qu'affectant des espèces « majeures »,

– les maladies considérées comme mineures et affectant des espèces « mineures » (le pire des scénarios).

La santé animale, le bien-être des animaux et la protection de l'environnement

Le bien-être animal étant une préoccupation de plus en plus présente, l'expérimentation animale fait désormais l'objet d'une stratégie dite des « trois R » (solutions de réduction, de raffinement et de remplacement de l'expérimentation animale).

L'utilité des animaux de laboratoire pour la mise au point des vaccins vétérinaires ne doit pas être ignorée, en particulier depuis que les chercheurs ont directement accès aux espèces animales ciblées qui offrent souvent un plus grand intérêt, notamment pour les inoculations d'épreuves pour l'évaluation de la protection. La protection immune fait intervenir des phénomènes et des processus immunologiques complexes. Les animaux de laboratoire sont particulièrement utiles lorsque l'immunité à médiation cellulaire joue un rôle déterminant, car l'apparition d'anticorps est plus facile à évaluer que les réponses cellulaires *in vitro*. La tendance, néanmoins, est de remplacer, autant que possible, les animaux de laboratoire par des systèmes *in vitro*.

Le recours aux vaccins vétérinaires présente des avantages évidents en termes de bien-être animal. Comparativement aux traitements thérapeutiques, les vaccins offrent de meilleures garanties de protection du bien-être animal, car ils empêchent l'apparition de la maladie ou permettent d'éviter d'abattre des animaux dans le cadre de mesures de police sanitaire. En outre, la plupart des animaux destinés à la consommation humaine ont une durée de vie relativement courte, de sorte qu'une seule administration de vaccin suffit, alors que les traitements nécessitent généralement plusieurs interventions. Toutefois, des améliorations peuvent encore être apportées, notamment en développant des vaccins avec adjuvant qui soient moins réactogènes. Une autre perspective prometteuse pour le bien-être animal consiste à remplacer la castration chirurgicale des verrats, visant à supprimer l'odeur de verrat, par une vaccination (immunocastration).

Dans les systèmes de production animale, le fait de recourir à la vaccination, et donc de réduire la quantité de produits chimiques utilisés, est bénéfique pour l'environnement. Citons l'exemple particulièrement intéressant du vaccin anti-tique mis au point en Australie, qui utilise un antigène cryptique situé dans l'intestin du parasite. Mentionnons également, même s'ils ne sont pas encore couronnés de succès, les efforts accomplis en Australie pour réduire la production de méthane (un gaz à effet de serre) par les ruminants en administrant à ces animaux un vaccin contre les archéobactéries méthanogènes du rumen.

Espèces mineures et maladies spécifiques des pays en développement

Plusieurs tentatives de définir les espèces « mineures » ont été faites et un grand nombre de définitions ont été proposées. Nous dirons simplement que sont considérées comme mineures les espèces animales autres que les bovins, les ovins (à laine et à viande), les chevaux, les porcs, les poulets, les chiens et les chats, et les espèces de poisson autres que les salmonidés. Par exemple, en Europe les espèces mineures sont les moutons laitiers, les chèvres, les lapins, les poissons (autres que les salmonidés) et les oiseaux (autres que les poulets).

Il est difficile pour les laboratoires pharmaceutiques de développer des vaccins destinés aux espèces mineures, car ils représentent de faibles parts de marché et leur rentabilité est médiocre. Ce même argument s'applique au développement de vaccins dirigés contre des maladies ne sévissant que dans les pays en développement.

Ces problèmes ne pourront être résolus qu'en mettant en place des financements publics et des partenariats public-privé adéquats.

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Introducción

Vacunación animal
Parte 1: desarrollo, producción y utilización de vacunas
Parte 2: aspectos científicos, económicos, reglamentarios y socio-éticos

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De todas las medidas existentes para prevenir enfermedades infecciosas, la vacunación es sin duda la más útil. Entre las numerosas ventajas que presenta destaca la de constituir, a falta de antivirales de amplio espectro, el único método disponible para prevenir, e incluso a veces curar, afecciones animales de origen vírico.

Las vacunas son poco agresivas para el medio ambiente y aportan un mayor bienestar a los animales porque previenen el sufrimiento derivado de una enfermedad o del consiguiente tratamiento curativo, tratamiento que además puede generar resistencia a los antibióticos e introducir residuos farmacéuticos en la cadena alimentaria. Las vacunas son el mejor instrumento para instaurar una gestión sostenible de la salud del ganado.

Dado que previenen infecciones zoonóticas, las vacunas veterinarias pueden proteger no sólo la salud de los animales sino también la del hombre, como demuestra el caso de la vacunación de animales salvajes contra la rabia.

Ahora mismo, en el terreno zoosanitario, las infecciones de los animales están mereciendo más atención que sus enfermedades, o dicho de otro modo: conviene elaborar vacunas pensando más en prevenir infecciones que en impedir que se manifiesten los síntomas clínicos de la enfermedad. De ser posible, además, las vacunas deben inducir inmunidad esterilizante. Por último, pero no menos importante, las técnicas existentes han abierto las puertas a la concepción de vacunas con marcador serológico (DIVA). Éstas, acompañadas de las correspondientes pruebas de diagnóstico, permiten distinguir entre animales vacunados e infectados, aun cuando éstos últimos hayan sido vacunados previamente.

La viruela, que es una enfermedad humana, fue la primera infección vírica en quedar erradicada. "Erradicar" significa eliminar completamente de la faz de la Tierra la enfermedad y su agente infeccioso. Tan destacado éxito fue posible gracias a varios factores, entre ellos la existencia de una vacuna eficaz, elaborada con el virus *vaccinia*, y la ausencia de un reservorio de la enfermedad en la fauna salvaje. Según la

Organización Mundial de la Salud (OMS), por las mismas razones cabe pensar que quizá pronto puedan eliminarse la poliomielitis y el sarampión en el hombre.

La única enfermedad vírica animal en la que concurren tales circunstancias es la peste bovina: ya existen varias vacunas eficaces, y la infección parece llegar a un callejón sin salida al transmitirse a una especie salvaje susceptible. No cabe decir otro tanto de las demás dolencias víricas que afectan a los animales, ninguna de las cuales, presumiblemente, quedará erradicada en un futuro próximo, ya sea por la falta de una vacuna eficaz (como es el caso de la peste porcina africana) o por la existencia de reservorios salvajes como el jabalí (*Sus scrofa*) para la peste porcina clásica o el búfalo africano (*Syncerus caffer*) para la fiebre aftosa. Las circunstancias son más propicias a la eliminación a escala regional de esas enfermedades que a su completa erradicación en todo el mundo. A la hora de elegir un método para eliminar una enfermedad animal infecciosa, conviene tener en cuenta las características biológicas y epidemiológicas de la infección, las técnicas de lucha existentes y la aparición de nuevas tecnologías en materia de vacunación, como es el caso de las vacunas con marcador serológico.

Alegato en favor de las vacunas veterinarias

Hoy en día sobran motivos para apostar resueltamente por el desarrollo de las vacunas veterinarias:

– proteger la salud animal,

– eliminar o erradicar una infección,

– mejorar el bienestar de los animales,

– proteger la salud pública,

– proteger a los consumidores de ciertos riesgos que pueden guardar relación con productos procedentes de animales destinados al consumo humano,

– proteger el medio ambiente y la diversidad biológica,

– evitar la aparición de patógenos resistentes a los fármacos disponibles,

– favorecer la sostenibilidad de las actividades agrícolas y de producción animal para el consumo humano.

Por desgracia, aun cuando no falten razones para obtener vacunas veterinarias, subsisten igualmente un gran número de obstáculos:

– problemas científicos, como los que impiden obtener una vacuna contra la peste porcina africana, la teileriosis o muchas enfermedades parasitarias,

– dificultades para llegar a las especies destinatarias (en el caso de la fauna salvaje),

– escasa rentabilidad para las empresas que se dedican a la creación y fabricación de vacunas,

– reglamentos zoosanitarios que prohíben el uso de vacunas,

– requisitos normativos para registrar una vacuna,

– la condición de especie (así llamada) 'menor' de algunas de las especies destinatarias,

– afecciones de pequeña importancia en las especies (así llamadas) 'mayores',

– afecciones de importancia secundaria en las especies (así llamadas) 'menores' (la peor de las combinaciones).

Salud y bienestar de los animales y protección del medio ambiente

La opinión pública muestra cada vez más preocupación por el tema del bienestar de los animales, hecho que ha llevado a instaurar los principios cardinales de la sustitución, la reducción y el perfeccionamiento (o "tres erres" por sus iniciales en inglés: '*replacement, reduction, refinement*') en el uso de los animales de laboratorio.

No cabe obviar la utilidad de los modelos animales en el terreno de las vacunas veterinarias, especialmente porque los investigadores pueden utilizar modelos basados en los animales destinatarios que en general son más adecuados, sobre todo para inoculaciones de prueba con miras a evaluar el grado de protección. La protección inmunitaria trae consigo complejos fenómenos y procesos inmunológicos. Los modelos animales revisten especial importancia en los casos en que la inmunidad celular desempeña un papel decisivo, porque sigue siendo más fácil medir el nivel de anticuerpos que la respuesta celular *in vitro*. No obstante, en la actualidad se tiende a sustituir, siempre que sea posible, los modelos animales por sistemas *in vitro*.

El uso de vacunas veterinarias trae aparejados evidentes beneficios en cuanto al bienestar de los animales. Las vacunas, a diferencia de los tratamientos terapéuticos, son la mejor forma de ahorrar sufrimientos al animal, pues previenen la enfermedad o evitan que haya que proceder a sacrificios sanitarios dentro de las medidas de policía sanitaria. Por otra parte, dado el poco tiempo que viven muchas especies destinadas al consumo humano, sólo hay que administrar las vacunas una vez, mientras que los tratamientos suelen requerir varias intervenciones. Sin embargo, todavía queda margen para mejorar elaborando vacunas con adyuvante menos reactogénicas. Otro ámbito del bienestar animal en el que se puede avanzar es el uso de vacunas para la inmunocastración de cerdos en sustitución de la castración quirúrgica, procedimiento utilizado hasta ahora para evitar el olor a verraco.

La utilización de vacunas en los sistemas de producción animal también entraña menos agresiones al medio ambiente porque reduce el uso de productos químicos. Especial interés reviste la vacuna contra garrapatas elaborada en Australia, que se basa en un antígeno intestinal críptico del parásito. También cabe destacar los ensayos realizados en Australia para reducir las emisiones de metano (uno de los gases que provocan el efecto invernadero) por los rumiantes vacunando a éstos contra las arqueobacterias del rumen, aunque lamentablemente no hayan dado hasta ahora buenos resultados.

Especies menores y enfermedades específicas de los países en desarrollo

Ha habido varias tentativas de determinar lo que es una especie 'menor', y se han propuesto varias definiciones. Expresado con sencillez, ese término se aplica a todos los

animales que no sean ganado vacuno ni ovino (productor de carne y lana), caballos, cerdos, gallinas, perros, gatos y salmónidos. En Europa, por ejemplo, entrarían en la categoría de especie 'menor' la oveja lechera y la cabra, el conejo y las demás especies de peces y de aves.

Para las empresas farmacéuticas es difícil elaborar vacunas destinadas a esas especies menores a causa de la exigüidad del mercado y de la escasa rentabilidad que ofrecen. Otro tanto cabe decir de la fabricación de vacunas contra enfermedades que sólo se dan en países en desarrollo.

La única solución para superar estos problemas estriba en la financiación pública y la creación de las adecuadas alianzas público-privadas.

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A brief history of vaccines and vaccination

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Summary

Human vaccinology, with its primary focus on the individual, seems far removed from veterinary medicine, with its concern for the health of the herd. Yet several episodes in the past (smallpox, fowl cholera, anthrax, swine erysipelas, rabies, tuberculosis, etc.) serve to illustrate the proximity between research on veterinary and human vaccines. In some cases the human vaccine was developed first, while in other cases it was the animal vaccine. The history of vaccinology clearly demonstrates the importance of these 'two medicines' working together. Foot and mouth disease (FMD) vaccines were among the first vaccines to be developed, beginning at the end of the 19th Century. Thanks to the discoveries of several researchers, including European researchers such as Vallée (French), Waldmann (German), Frenkel (Dutch) and Capstick (British), FMD vaccines began to be produced on an industrial scale from 1950 onwards, making possible vaccination of millions of animals in Europe and beyond. Vaccination strategies against FMD have always been dependent on the properties of the vaccines being used. At the beginning of the 21st Century FMD vaccines are designed in such a way that serological tests can differentiate infected from vaccinated animals, which has affected OIE regulations on international trade in animals and animal products. The history of vaccination against rinderpest, bovine contagious pleuropneumonia, and Marek's disease will also be dealt with.

Keywords

Bovine contagious pleuropneumonia – Foot and mouth disease – History of vaccinology – Human/veterinary medicine relationship – Marek's disease – Rinderpest – Vaccination – Vaccine – Veterinary vaccine.

The origins of veterinary vaccination: the human medicine viewpoint

In terms of its practices and concerns, human vaccinology, with its primary focus on the individual, seems far removed from veterinary medicine, with its concern for the health of the herd. Yet the history of vaccination provides evidence of close ties between what Dr Charles Mérieux affectionately called the 'two medicines'. It illustrates first of all their time-honoured collaboration, and it should be noted that the stock phrase in English, 'herd immunity', is directly derived from the veterinary concept of protecting the herd. After a century of totalitarianism in the name of general interest, people today are less inclined to accept measures that place the interests of society too far above those of the individual. In terms of vaccination, in recent years, the ideal of an individual vaccination 'à la carte' seems more in keeping with the demands of modern or even post-modern times. Yet, historically, vaccination has with some exceptions been predominantly a public health tool, aimed at populations rather than individuals.

What is a veterinary vaccine? A vaccine that a veterinarian applies to animals, be they companion animals, wild animals or herds of livestock. Yet the usefulness of veterinary vaccines extends beyond these limits since many of them also protect humans from

anthropozoonoses, diseases common to humans and animals.

Veterinary vaccination differs *a priori* from human vaccination in terms of the ethical issues surrounding experimentation, and the importance, and even the priority, of economic considerations when it comes to animal health. There is also a major difference in the use of alternative solutions to the vaccination or treatment of sick livestock, such as mass culling, a strategy often employed in veterinary public health, despite the high cost and the shocking image it creates. No farmer can remain indifferent to having to have his livestock culled, especially if they are healthy. And even the wholesale destruction of mere battery chickens is not an operation to be treated lightly. Anyone who has tried to save a bird caught in an oil slick will find it hard to accept that in the 21st Century the only way of avoiding an epizootic is to destroy entire populations of poultry, cows or sheep, or even stray dogs.

Yet however unique it may be in many of its theoretical or practical aspects, veterinary or animal vaccination has a scientific history that is closely linked to that of human vaccination, for which it has served as a model, a tutor and a complement. This proximity and even interconnection illustrate how in many ways veterinary medicine offers a wealth of observations unmatched by human medicine, confined as it is to the anatomy and physiology of *Homo sapiens*. Not to mention that there are many human diseases where the reservoir is found in animals (rabies, for example), that the species barrier to infections is often crossed, and that many epizootic diseases prove to be potentially dangerous for humans, as in the case of avian influenza, all of which indicates the need for close collaboration in research (and decisions!).

Both human and veterinary medicine have certainly found a source of inspiration in the long tradition of empirical procedures where farmers have used fluids from sick animals to protect their herds. Several attempts at immunisation by inoculation were made for sheep pox, which is close to smallpox in humans, and bovine contagious pleuropneumonia. For the latter disease, the Belgian physician Willems brought this age-old practice into the scientific era when, from 1853, he inoculated animals at the base of the tail with a small amount of infective material. The tissues, and no longer just the 'humours', then came to be studied under the microscope and underwent all kinds of procedures to try to achieve a permanent, stable attenuation or neutralisation. The telling observations of animal farmers and veterinary practices thus provided the historical crucible for contemporary vaccinology.

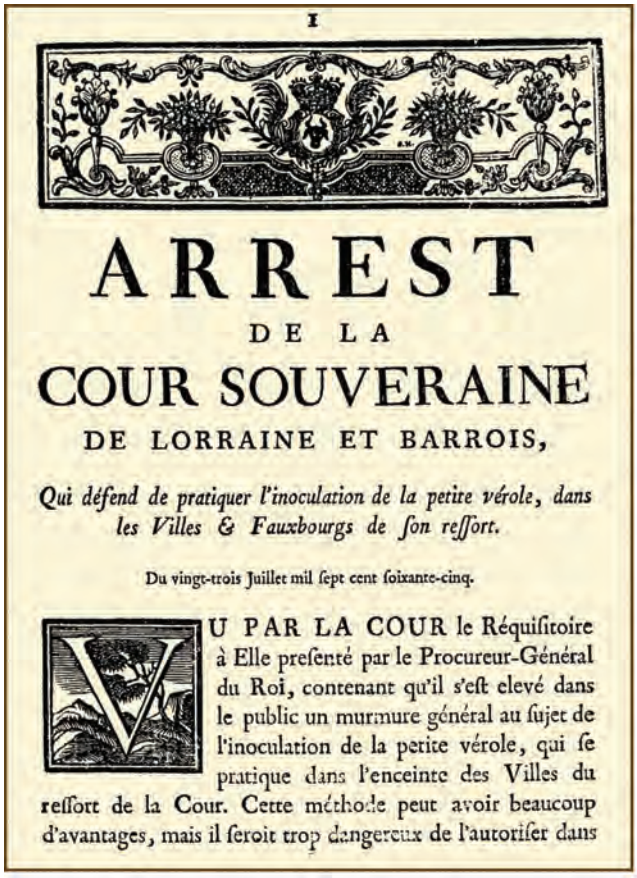
The best example of the close relationship between human and animal vaccination, and certainly the best documented, is the history of successive vaccines against smallpox. Given the importance of the eradication of smallpox (proclaimed in 1979) as a success story, and the 'long and arduous hunting down of the disease', to paraphrase French historian Pierre Darmon, it seems appropriate to recall briefly this curious story that is in many ways indicative of the links between human and animal vaccination.

Vaccination against smallpox: an example of the historic links between human vaccine and animal vaccine

The inoculation of serous fluid under the skin is a procedure that has long been known as a way of protecting flocks against sheep pox. (The French language has a term which is used to refer specifically to inoculation with sheep pox, *clavélisation*, from the French word for the disease, *clavelée*.) In particular, there is documentary evidence of its use by nomadic herders in Africa, for example among the Tulani. There can be no doubt that this practice must have drawn attention to the possibility of acquiring protection from a serious disease by contracting a form of the disease that was attenuated to a greater or lesser extent. In earlier times people were closer to their animals, and animal farmers often had the reputation among neighbouring townsfolk of being healers. Yet it is difficult to know whether it was inoculation with sheep pox that led to the idea of human variolation or vice versa. It may seem more logical to favour the first hypothesis; however, even if inoculation against sheep pox was mentioned by explorers in Africa as long ago as the 16th Century, it is highly likely that human variolation was attempted in China or India even before then.

The history of the vaccine against smallpox, a human disease with no known animal reservoir, can be summed up as the replacement of inoculation with human smallpox (variolation) (Fig. 1) with inoculation with cowpox, a procedure invented by an English doctor, Edward Jenner (1749-1823). The use of cowpox is generally seen as a remarkable advance compared to variolation. The latter technique used only human material, serous matter from pustules and scabs taken from a subject with a mild form of the disease. It generally conferred solid immunity. However, the outcome was unpredictable and post-inoculation mortality was not inconsiderable.

In contrast, inoculation with cowpox, proposed by Jenner in 1798, seemed to be less dangerous and just as effective. Through a form of cross-immunity it provided humans with satisfactory protection, though probably less solid than that produced by the inoculation of smallpox. Indeed, during the 19th Century it proved necessary to revaccinate in order to reactivate the immunity since this tended to decline over the years. The need to revaccinate



I

ARREST

DE LA

COUR SOUVERAINE

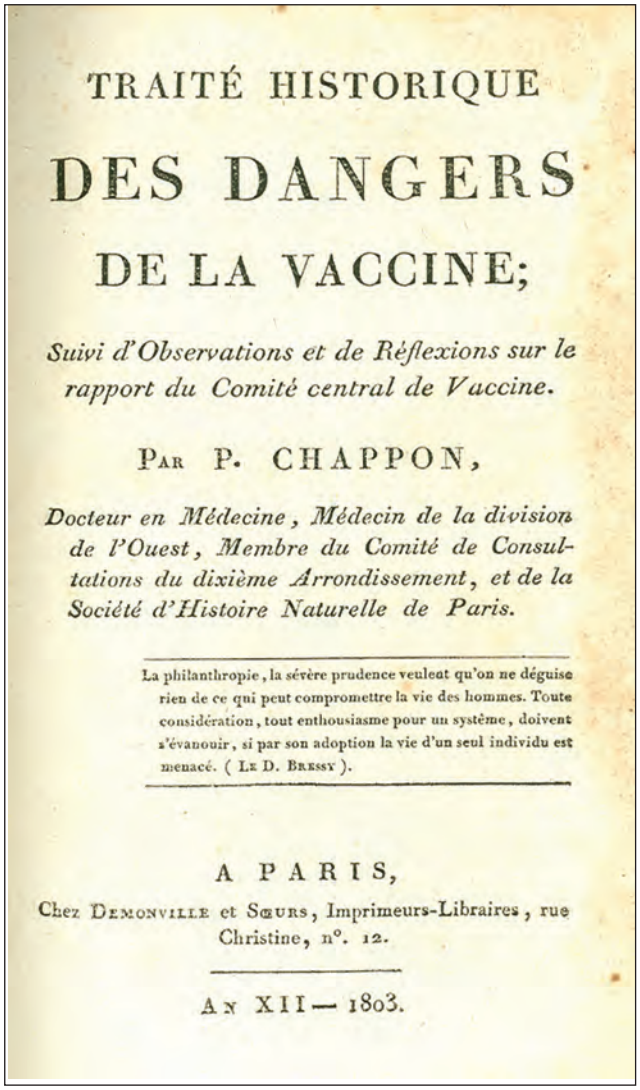
DE LORRAINE ET BARROIS,

Qui défend de pratiquer l'inoculation de la petite vérole, dans les Villes & Fauxbourgs de ſon reſſort.

Du vingt-trois Juillet mil ſept cent ſoixante-cinq.

VU PAR LA COUR le Réquiſitoire à Elle preſenté par le Procureur-Général du Roi, contenant qu'il s'eſt elevé dans le public un murmure général au ſujet de l'inoculation de la petite vérole, qui ſe pratique dans l'enceinte des Villes du reſſort de la Cour. Cette méthode peut avoir beaucoup d'avantages, mais il ſeroit trop dangereux de l'autoriſer dans

Fig. 1
Decision of the sovereign court of Lorraine and Barrois prohibiting smallpox inoculation (1765)
Source: Reproduced from *Mémoires des vaches et des bœufs* published by Equinoxe



TRAITÉ HISTORIQUE

DES DANGERS

DE LA VACCINE;

Suivi d'Observations et de Réflexions sur le rapport du Comité central de Vaccine.

PAR P. CHAPPON,

Docteur en Médecine, Médecin de la division de l'Ouest, Membre du Comité de Consultations du dixième Arrondissement, et de la Société d'Histoire Naturelle de Paris.

La philanthropie, la sévère prudence veulent qu'on ne déguise rien de ce qui peut compromettre la vie des hommes. Toute considération, tout enthousiasme pour un système, doivent s'évanouir, si par son adoption la vie d'un seul individu est menacé. (LE D. BRESSY).

A PARIS,

Chez DEMONVILLE et SŒURS, Imprimeurs-Libraires, rue Christine, n°. 12.

AN XII — 1803.

Fig. 2
Historical treatise on the dangers of vaccinia by P. Chappon, 1803
Source: P.-P. Pastoret, personal collection

complicated the task of the health services and met with incomprehension on the part of the public, obliged to repeat a procedure that they had been led to believe was permanent.

At the beginning of the 19th Century, Jenner's vaccination procedure rapidly spread around the world (Fig. 2), supported by governments favourable to a measure that could reduce the devastating effects of epidemics on their populations. The President of the United States of America (USA); the Tsar of Russia; the King of Sweden; the Emperor of France, Napoleon I; and the Pasha of Egypt, Ali Mohammed, to mention but a few, were greatly enthusiastic about the vaccine and actively promulgated it, in some cases, as with Napoleon I in 1812, going as far as to make it compulsory in the army, and even in society as a whole. When it came to putting these plans into action, however, it was of course quite a different story.

Yet it was not long before vaccination with animal vaccine underwent changes. In fact the use of lymph of animal origin that was subsequently 'humanised' soon became established: the original vaccine, derived from a cow, was first propagated from arm to arm, usually in children, who were used as vaccinifers. The method raised numerous problems. The lymph eventually lost its potency and produced hardly any pustules. Parents were also reluctant to have their offspring used as a reservoir for producing vaccine. Lastly, because repeated samples were taken from the same pustules they were soon emptied of smallpox virus, either because the pustules dried up or because they became superinfected and they then produced a fluid of dubious content.

During the latter half of the 19th Century, it seemed more natural and more practical to go back to the original source, namely cows or indeed calves, which were the only means of obtaining an authentic cowpox vaccine and ensuring an abundant and readily available supply of

lymph. This had to be organised in a completely different way. Breeding centres had to be established for animal vaccinifers, and animals had to be transported from village to village by road or rail or else the vaccinal lymph itself had to be transported, kept in an appropriate medium to conserve it and protect it from superinfection. Various excipients such as liquid paraffin, lanolin and glycerine were tested, with glycerine eventually being preferred.

As well as causing specific organisational problems, the transition from a vaccine of human origin to a vaccine of animal origin met with socio-cultural problems. In India, for example, where smallpox was a terrible scourge for the densely populated continent, variolation was an ancient tradition dating back to at least the 17th Century. Throughout the 19th Century, the British colonial administration went to great lengths to develop the vaccine but had to contend with the reluctance of Hindus. They found the use of sacred animals for this purpose abhorrent, and the presence of a fatty excipient led them to suspect the use of animal fat prohibited by culture. Furthermore, the highest castes commonly practised smallpox inoculation which, as in England in the 18th Century, was accompanied by a set of dietary measures and isolation of inoculated subjects that were considered quite satisfactory. Compared to the results of these inoculations, the variations in efficacy of inoculation with vaccine lymph were sometimes far from convincing. For a long time, the British administration steered a delicate course by using vaccination only for mass campaigns among the lower social classes. Vaccination, as a mark of solidarity against contagion, was thus confronted by the imperviousness of the caste barriers within Indian society (10).

Yet, in the case of smallpox vaccine, can one legitimately call it an animal vaccine? Right from the start, when the Jennerian procedure was first disseminated, it became difficult to determine the exact origin of the vaccine being used. In England, the practice of vaccination in hospitals formerly used for smallpox inoculation (promoted by physicians such as Pearson and Woodville) took place without the subjects being isolated, and was accompanied by a hybridisation of strains.

Moreover, in countries that were in favour of vaccination it seemed preferable to identify and use local cases of cowpox rather than having to rely on a supply from abroad. Yet in many countries in Africa (e.g. Egypt in the 1830s) or Asia (e.g. Indochina after the founding of the Pasteur Institute in Saigon in 1891), spontaneous cowpox could not be found. An alternative solution that was tried in India was to inject cows with human smallpox in the hope of obtaining an unlimited supply of attenuated material. Small institutes, in Bombay, for instance, bred calves and produced stocks of lymph for distribution to villages. The lymph was injected into subjects and then transferred from arm to arm. Children were targeted first because of their lower susceptibility, and served as 'guinea pigs' to standardise the vaccine fluid (according to the number and appearance of the pustules produced), before its use in adults. Parallel controls to test for innocuousness were sometimes performed using donkeys or rabbits.

In the history of smallpox vaccination, it is therefore very difficult to distinguish between the paths of these two fluids, one human and one animal. We can do no more than speculate about the origins of the strains that we have today. It seems likely that Jenner's original strain has been irremediably lost. Three types of virus are commonly distinguished, according to the type of cell lesions in the culture media (embryonated egg or allantoic membrane): 'historic' cowpox virus (thought to be closely related to the strain used by Jenner), vaccinia virus, and 'classic' smallpox virus. However, it seems likely that what we have today are in fact intermediate strains. Virologists are currently discussing a possible link between the smallpox vaccine and an equine virus that no longer exists in the wild (4).

We have therefore eradicated smallpox before fully elucidating the origin and behaviour of poxviruses and their vaccines throughout history. The development of a new vaccine, free from the dangers of its predecessor, to protect against any future use in bioterrorism, will probably not help us to learn more about the past (5).

The vaccine against smallpox, despite its many particularities, served as the inspiration for the development of vaccination against other diseases and as a springboard for the Pasteurian programme sometimes summed up as '*une maladie, un vaccin*' ('for each disease, a vaccine').

Veterinary vaccines and human vaccines during the Pasteurian era

In tracing the origin of modern vaccines one is inevitably confronted by the legend of Louis Pasteur (1822-1895) (Fig. 3), which presents a picture of a man of genius who knew no precursor other than himself. Yet the variety and eclecticism of Louis Pasteur's research, which ranges from the scientific basis of vinification to diseases of silkworms and human diseases, suggest that he relied more heavily on the results of his contemporaries than is generally realised. In fact, by tracing the path of his scientific research, it is easy to identify those who made his work possible and whose names were obliterated by his glory. At each stage in his career, Louis Pasteur kept himself very well informed of the scientific output of his time, even if he sometimes omitted to cite his sources (22). He obtained information from veterinary practitioners and specialists, agronomists, surgeons, farmers and herdsmen. Of these, the veterinarians and livestock farmers played a predominant role.

Fig. 3
Portrait of Louis Pasteur (1822-1895) in 1865
Source: Reproduced with kind permission of Mérial (provided by Philippe Dubourget)

In 1881, on the basis of his preliminary research, Louis Pasteur called for an extensive programme of prophylaxis against all diseases potentially of infectious origin. In an emotional speech to the French Academy of Science in the same year, he introduced the term '*virus-vaccin*' (synonymous with attenuated microbe), which he subsequently shortened to '*vaccin*':

'Nous possédons maintenant des virus vaccins. Ces vaccins peuvent protéger contre la mort sans être eux-mêmes mortels' (33).

(I.e. 'We now have virus vaccines. These vaccines can protect against death, without being lethal themselves.')

This was in spite of the fact that at the time he still only had two available candidate vaccines, both of which were veterinary vaccines, one against fowl cholera and the other against anthrax.

Veterinarians were traditionally very involved in trying to find ways of preventing the diseases that were decimating herds of livestock. The discovery of microbes under the microscope, the demonstration of their pathogenicity and, particularly, their culture in the laboratory paved the way for the development of new preventive techniques, while at the same time providing the animal models needed for experiments in human medicine.

Not all French veterinarians were immediately won over to the microbian theory of diseases, no doubt because, with their experience of working in the field, they were aware of the multitude of factors that could be involved in triggering diseases and were suspicious of the notion of a single cause. While the veterinary school in Lyons led by Jean-Baptiste Chauveau (1825-1917) subscribed to the new ideas, Henri Bouley (1814-1885), then director of the prestigious veterinary school in Maisons-Alfort, near Paris, long remained attached to the doctrine of spontaneous generation, which he defended in his publication *Recueil de médecine vétérinaire*. In 1877, however, probably under the influence of a group of young teachers working with Edmond Nocard (1850-1903), Henri Bouley did a complete about-turn and from then on conducted a regular correspondence with Louis Pasteur on all aspects of 'vaccination', both human and animal (31). The use of the word 'vaccine' as the generic term to designate all existing and future vaccines, and not just the Jennerian vaccine, came into use in the international scientific community around 1880 before being included in the French dictionary.

What were the explanations for virulent microbes becoming attenuated while retaining their protective effect and maintaining the stability of attenuation? Nowadays, we attribute these changes in virulence to genetic mutations that occur spontaneously and are then selected by changes in the synthetic media used in the laboratory. The approach adopted by the contemporaries of Louis Pasteur was above all empirical, even if they were only too eager to theorise on the basis of their initial successes.

Fowl cholera

In 1876, the French veterinarian Henri Toussaint (1847-1890) cultured a causal bacterium of fowl cholera in neutralised urine, described two years later by Perroncito (and subsequently known as *Pasteurella avicida* or *gallicida*, and now as *P. multocida*). Were the cultures of the organism that causes fowl cholera accidentally left on a laboratory bench by one of Pasteur's assistants during the holidays, as the legend goes? Was it a chance discovery that the cultures which had become acidic due to aging had acquired attenuated virulence? Whatever the case may be, the hen survived inoculation with the 'forgotten' cultures and even became resistant to a subsequent, virulent inoculation. It was in fact an empirical trial to attenuate the culture by re-seeding the medium at longer intervals devised by Emile Roux with the help of a system of continuous oxygenation to accelerate the aging process.

Anthrax

Whereas fowl cholera was not known to occur in humans and was rather more an academic exercise in exploring artificial immunisation at Pasteur's laboratory, anthrax was a constant source of concern for farmers faced with the seriousness of the outbreaks that affected herds grazing in the so-called *champs maudits* ('cursed fields') and with the risk of inadvertently inoculating themselves with the fatal black pustule while handling carcasses.

The team working with Louis Pasteur endeavoured to attenuate the bacteria in the laboratory by comparing or cumulating different methods borrowed from one another. In England, in 1878, John Burdet-Sanderson and William Greenfield, by re-seeding the culture at 35°C succeeded in attenuating the virulence of the strain without affecting its immunising potential. In 1880, Henry Toussaint proposed that if animals were vaccinated with blood heated at 55°C they could then survive an otherwise lethal inoculation. He successfully immunised five ewes using this technique.

Applying the laboratory method in the field was to prove decisive. In 1881, Louis Pasteur undertook his still famous trial at the farm in Pouilly-le-Fort, near Paris. In the presence of an extensive public consisting of farmers and veterinarians, he compared the behaviour of vaccinated and unvaccinated sheep. Initially, his vaccine had consisted of a culture attenuated simply by heating. However, Pasteur's disciples persuaded him to take the precaution of using an attenuated culture also containing an antiseptic known to inhibit the formation of spores (this was 'the secret of Pouilly-le-Fort'), and in so doing saved the day. The carefully staged-managed experimental trial ended in triumph, with the death of the unvaccinated animals. This success was the prelude to the Pasteurian vaccine being distributed to livestock-producing areas in the world affected by anthrax. Even though the years that followed were not without controversy, since the results of vaccinations sometimes proved difficult to interpret regarding the 'natural' immunity of some herds, this date marked a decisive turning point in the history of the fight against animal diseases.

Swine erysipelas

Although Louis Pasteur relied heavily on professors of French veterinary schools, he also mobilised the network of livestock farmers and veterinarians in the provinces. In 1881, at the invitation of a modest veterinarian from Bollène (a village in the south of France), Louis Pasteur conducted research into an attenuated vaccine against swine erysipelas, a disease caused by a bacillus that had recently been discovered by Louis Thuillier. This attenuated vaccine was lapinised, in other words attenuated by serial passages through rabbits.

The observation of an increase in virulence when a disease is passed from one individual to another during an epidemic is common to both physicians and veterinarians. In contrast, the notion of *in vivo* attenuation of virulence when germs affecting one species are passed through another species is an empirical observation of long date made by veterinarians. It proved to be a fruitful source of research for the Pasteurian school.

Rabies

In 1879, Louis Pasteur, left fowl cholera, anthrax and swine erysipelas to one side to concentrate on this rare, but invariably fatal disease: *'Si la rage pouvait être attribuée à l'action d'un organisme microscopique, il ne serait peut-être pas au-dessus des ressources naturelles de la science de trouver le moyen d'atténuer l'action du virus de la terrifiante maladie, pour la faire servir ensuite et en préserver d'abord les chiens et ensuite l'homme'* (34). (I.e. 'If rabies could be attributed to the action of a microscopic organism, it would perhaps no longer be beyond the natural resources of science to find a means of attenuating the action of the virus of this fearful disease, and thereafter put it to use, first to protect dogs and then to protect humans.')

It was with the vaccine against rabies, the cornerstone of Pasteurian science, that collaboration with veterinarians was to prove most crucial. It involved a human vaccine against an animal disease. Humans only become infected as an unfortunate accident and do not play a role in maintaining the natural cycle of rabies, because once the disease has developed in a human patient it is virtually never transmitted to others. Today, contrary to the hopes of Louis Pasteur, rabies has still not been eradicated, and is unlikely to be so in the near future since its animal reservoir is not restricted to domestic carnivores but now includes wild animals, such as foxes, among which Lyssaviruses, a group of viruses that includes rabies, are known to circulate. The reservoir also includes other wildlife species that are currently being identified, such as the many species of bats.

At the time of Louis Pasteur, veterinarians alone had the necessary expertise to study rabies. It was the veterinarians who monitored the disease in towns and the countryside, looking for evidence of rabies lesions during the post-mortem examination of dogs suspected of biting humans. They also provided dogs from the animal pound for use in experiments. It was the veterinarian Pierre-Victor Galtier (1846-1908), a pupil of Chauveau at the Lyons veterinary school (France), who showed rabies to be an affection of the nervous system, with a variable incubation period. In 1879, he suggested that laboratory dogs could be replaced by rabbits, which develop a paralytic form of the disease with a faster course than in dogs, thus making them more manageable. Moreover, after studying rabies immunity in

sheep injected with blood from a rabid dog, he put forward the idea of a 'preventive treatment, undertaken before the onset of lesions of the nerve centres', which amounted to a treatment for the disease!

In 1881 and 1882, Louis Pasteur and his pupils Charles Chamberland, Emile Roux and Louis Thuillier entered the fray and modified Galtier's technique by inoculating nervous tissue from a rabid animal directly into the brain after trephination. By successive passages in dogs, they obtained a virus of maximum virulence coupled with a fixed incubation period of around 10 days. They then needed to attenuate the virulence of the causal microbe and measure the degree of attenuation indirectly by passages through rabbits. The chosen attenuation procedure was invented by Emile Roux. It consisted of suspending the spinal cord of a rabid rabbit in a flask, in a warm dry atmosphere, to achieve slow desiccation. Using animals as a live propagating medium, Pasteur and his team succeeded in producing 'attenuated viruses of different strengths', in short a standardised range of viruses, the weakest of which could be used to prepare a vaccine. Inoculating dogs with a sequence of spinal cords of increasing virulence rendered them resistant to inoculation with medulla of absolute virulence (42). The dog could then without danger be exposed to a street virus. This was the protocol that Louis Pasteur successfully applied to the young Joseph Meister on 6 July 1885 even though the experiments on dogs were still in progress (the dogs had not yet been subjected to the final test with an infective bite) and two trials had not been wholly conclusive.

Throughout the experiments with rabies, there was constant collaboration between veterinarians and physicians, even if Louis Pasteur is the name that tends to remain in the collective memory. The clinical know-how of veterinarians proved very important during all stages of rabies research, which they followed closely before returning to their main concern, namely animal rabies, the cornerstone of all programmes aimed at eliminating rabies in humans. The same vaccine was for a long time used to protect humans and animals, until genetically engineered oral vaccines were developed which could be distributed in baits by plane or helicopter as a means of immunising foxes.

The success of antirabies vaccination led to the founding of the Pasteur Institute, which to this day still conducts internationally recognised research. Edmond Nocard, who became Director of the veterinary school in Maisons-Alfort (France), worked tirelessly with Pasteur and his group, first at the laboratory in the rue d'Ulm in Paris and then at the Pasteur Institute. His presence was considered indispensable on the French mission to Egypt in 1883 during the cholera epidemic in Alexandria, which was to cost Louis Thuillier his life. On the well-known group photo at the Institute library, it is certainly no accident that Edmont Nocard is seated to the right of Pasteur, with Emile Roux on the Master's left (Fig. 4).

Fig. 4
Louis Pasteur with his team in 1894
Back row from left to right: Eugène Viala, Paul Reboud, Marcel Mérieux, Auguste Chaillou, Amédée Borrel, Louis Marmier, Auguste Marie, Andrien Veillon, Ernest Fernbach, Auguste Fernbach. Front row from left to right: Albert Calmette, Louis Martin, Emile Roux, Louis Pasteur, Edmond Nocard, Henri Pottevin, Félix Mesnil
Source: Reproduced with kind permission of Mérial (provided by Philippe Dubourget)

Sera was used for preventive or curative purposes in both human and veterinary medicine from the late 19th Century onwards. Serum therapy for children suffering from diphtheria was introduced in Germany and France in 1894, by Emil von Behring and Emile Roux, respectively. Serum therapy for anthrax was used by Sclavo and Marchoux in 1895. Seroprotection of cattle against foot and mouth disease (FMD) was attempted by Friedrich Löffler (1852-1915) in 1897 and applied on a large scale in Denmark. The sera proved to be of variable efficacy and many were abandoned. Some were later used in association with vaccines in the belief that they rendered the vaccines more effective.

At the beginning of the 20th Century, a new vaccine developed by the Pasteur school bore witness to the constant intermingling of the history of human and veterinary vaccines, but this time it was for a disease that was very different from the earlier ones, namely, tuberculosis.

Bovine and human tuberculosis, the same fight?

Vaccination against tuberculosis is still based on the historic vaccine of Calmette and Guérin whose initials it bears (BCG vaccine [bilious bacillus vaccine of Calmette and Guerin], the fruit of collaboration between a physician and a veterinarian.

In 1882, Robert Koch (1843-1910) described the tubercle bacillus responsible for tuberculosis in humans. Tubercular infection was also well known in cattle. However, Theobald Smith in the USA drew attention to differences between the bovine and human bacilli: their chemical characteristics and differences in virulence in experimental animals. This marked the beginning of the controversy over the role of bovine tuberculosis in human tuberculosis, notably through the ingestion of milk, and vice versa. Animal tuberculosis like human tuberculosis often went undetected, due to the frequently insidious nature of the disease and its chronic course, complicating the task of epidemiologists at that time. However, the analogies between the two diseases resulted in virtually parallel lines of research.

Initially, vaccinating cattle with human bacilli, considered to be less adapted to animals and less virulent, appeared to be simpler, and perhaps more urgent. Koch suggested inoculating a calf with human tubercle bacilli treated with phenol. Working on behalf of the firm Hoechst, Emil von Behring prepared a bovo-vaccine based on desiccated human bacilli reduced to a powder. At around the same time, a physician in Berlin named Friedmann suggested using a tuberculosis bacillus in humans that was not thought pathogenic since it came from an animal of a distant species, a turtle. This vaccine, which was reputedly both preventive and curative, was extremely popular for a number of years.

In France, the veterinarians Vallée and Rossignol (the son of the veterinarian who had organised Pasteur's anti-anthrax vaccine trials at Pouilly-le-Fort) carried out trials in cattle in 1904. The results were equivocal, the protection afforded being relatively short-lived and not consistent. A quarter of the animals were not protected and contracted active tuberculosis. Gaston Calmette, at the Pasteur Institute in Lille, was particularly interested in these observations, which suggested that in some cases an abortive infection resulted in immunisation against further contamination, acting like an attenuated vaccine, and which he believed constituted a protective infection.

In 1897, Albert Calmette and Camille Guérin, a veterinary pupil of Nocard, began working together. A bovine bacillus, isolated by Nocard in a sample taken from the udder of a tuberculous cow, was cultured by passages through glycerinated bile potato medium, eventually resulting in an attenuated form. The tubercular bacillus has a fatty capsule which makes it difficult to blend. The idea of using bovine bile in the culture medium most likely came from the veterinarian Vallée, who had used delipidated bacilli in his vaccination trials: at that time, ideas were readily passed from team to team. The bacillus, from 1908 to 1921, was subsequently transformed by serial passages (230 passages) without regaining virulence in susceptible animals. The vaccine was called 'BCG' (which stands for '*vaccin bilié de Calmette et Guérin*'). In 1921, amid concerns at the upsurge in tuberculosis after the First World War, two experiments took place that, today, with the benefit of hindsight, are striking in their parallelism. Together with his co-workers, Henri Vallée, newly appointed director of the veterinary school in Maisons-Alfort near Paris, experimented with the BCG vaccine at a farm near Fécamp in Normandy. They tested the vaccine under different conditions, such as adding powdered pumice to the attenuated bacilli inserted under the skin and using intravenous injection. The trials were not judged to be entirely conclusive. The cattle did not acquire 100% protection even though every precaution had been taken during the experiment. It had taken place in a model farm with the best possible conditions of hygiene, far removed from conditions existing elsewhere in the country at that time.

Also in France in 1921, the first clinical trial of BCG took place, involving a newborn child in a family with a history of tuberculosis. The paediatrician, Weill-Hallé, administered several doses of BCG with a spoon. Faced with the prospect of almost inevitable contamination, the

well-off parents had preferred to try an unknown vaccine rather than have to send the child away from home (9).

During the years that followed, scientific research was marked by a constant cross-over between human and bovine tuberculosis. For his part, Calmette demonstrated the reduced mortality from tuberculosis in children vaccinated with his vaccine after a follow up of several years, and the expansion of human BCG provided an argument in favour of bovine vaccination. Conversely, while BCG gave results that were far from satisfactory in herds of cattle, it showed no tendency to regain virulence and reassured the medical profession regarding the genetic stability of the strain for use in human medicine.

In 1928, an international veterinary commission, comprising Italy, the Netherlands, Austria, Poland, France and Germany, recommended extending the use of BCG in cattle. In 1929 over a hundred vaccinated children died in the town of Lübeck, Germany, which led to intense discussions over the safety of BCG. The official verdict attributed the deaths to the accidental contamination of the vaccine with a virulent strain, but, due to a lack of genetic knowledge on the subject, questions remained as to a possible reversion to virulence. The court case in Lübeck probably resulted in the world being divided into two camps over the use of BCG in clinical medicine, both for humans and for animals. To this day, some countries such as the USA have never used BCG, even though it is included in the UNICEF Extended Vaccination Programme for children throughout the world. The use of the BCG vaccine was not included in French legislation for the protection of bovines against tuberculosis, voted in 1933, and remained at the discretion of farmers.

Veterinary use of BCG continued after the Second World War but gradually declined, having to compete with the systematic slaughter of tuberculous cattle (known as Bang's method, after the name of a Danish veterinarian), and despite the cost of such a measure for governments and especially for farmers. The argument in favour of the latter method was not only that vaccination gave uneven results but that tuberculin tests could not differentiate between an allergic reaction indicating previous sensitisation to the bacillus and actual infection with tuberculosis. France, in the face of increasing isolation and problems with exporting meat from vaccinated animals, eventually stopped using the vaccine in 1954.

Up to the present day, BCG vaccination in humans has continued to plough a lone furrow. It has not yet been superseded by a genetically engineered vaccine, though several teams are actively engaged in research, notably at the Pasteur Institute in Paris. Due to its innocuousness, as clearly demonstrated during a century of use in humans, BCG has also been thought of as a possible vector, through the use of genetic engineering, of vaccine antigens to prevent diseases other than tuberculosis.

Adjuvants

Another famous example of the fruitful exchange between human and animal medicine, concerns the discovery of adjuvants of immunity by Gaston Ramon (1886-1963) (Fig. 5), a veterinarian at the Pasteur Institute who became one of the first Directors General of the World Organisation for Animal Health (OIE) (then known as the Office International des Epizooties), following its creation in Paris in 1924.

Gaston Ramon developed an anti-tetanus vaccine in 1924 (38), consisting of the tetanus toxin treated with formaldehyde and heat, which he called 'anatoxin' (i.e. toxoid). This discovery was to prove a model for many subsequent applications. He also proposed that the efficacy of this 'anatoxin' could be enhanced by using, in addition

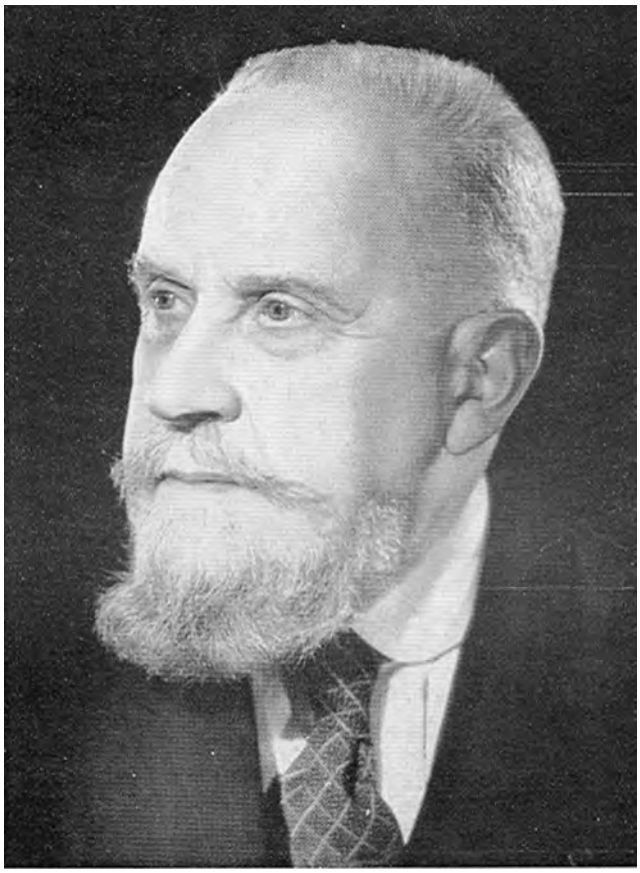


Fig. 5
Gaston Ramon (1886-1963)
Ramon was a veterinarian at the Pasteur Institute and also held the position of Director General of the World Organisation for Animal Health (OIE) from 1949 to 1959
Source: OIE

to the specific antigens, substances known as adjuvants of immunity, such as aluminium hydroxide, thereby creating the first adjuvanted vaccine. Gaston Ramon had reached this conclusion after observing differences in the effectiveness of the various immunisation protocols he had been using in horses in order to produce anti-diphtheria and anti-tetanus immune sera, an activity he was in charge of at the Pasteur Institute annex in Marnes-la-Coquette.

The use of these toxoids in association with aluminium hydroxide in a suitably adapted vaccination programme, helped to prevent the dreaded occurrence of the form of infantile diphtheria still known today as '*croup*', which had long been a scourge across rural areas of Europe, and tetanus, a disease that in those days often proved fatal when even the most superficial of wounds became infected with the bacillus. During the Second World War, the disease took a heavy toll among soldiers wounded during battles fought over tetanigenic terrain. With hindsight, it would appear unjust that this fundamental advance in the prevention of toxin infections has not brought its discoverer more universal recognition.

The independent history of veterinary vaccinology

Rinderpest

Rinderpest is one of the great historic plagues that have ravaged human livestock for centuries. In Europe, rinderpest was the major plague of cattle up to the end of the 19th Century, when it was eliminated. At about the same time rinderpest was introduced with devastating effects in Africa where it decimated the cattle and buffalo populations *(Syncerus caffer)*, along with those of other susceptible domestic ruminants and many wildlife species (3, 35).

It is remarkable that rinderpest was eliminated from Europe by the end of the 19th Century by the simple application of sanitary measures, before the nature of the infectious agent was known. In fact, the ability to control rinderpest effectively was often considered to be a measure of the quality of a country's veterinary services. When rinderpest was reintroduced in Belgium in 1920, it was again eliminated purely by sanitary measures within seven months and without spread to neighbouring countries. The history of medical prophylaxis (vaccination) against rinderpest illustrates the evolution of medical thinking (24). The Italian Lancisi (1654-1720) wrote very lucidly: 'if such a dreadful disease were to threaten our cattle, I would be in favour of destroying all sick or suspect animals, rather than allowing the contagion to increase, simply to gain time in the hope of achieving the honour of discovering a specific remedy, which is often a fruitless quest…'. His contemporaries did not necessarily share this view and Ramazzini was convinced that rinderpest, being a disease similar to smallpox, could be controlled by homologous inoculation. This lead to a whole series of unsuccessful inoculation trials. Some clearly stated that inoculation should only be recommended for areas already contaminated, otherwise there was a risk of spreading the disease further. Among all the inoculation trials against rinderpest carried out during that period, it is worth mentioning the work of Geert Reinders (1737-1815) in the Netherlands. He was a farmer in the Province of Groningen and a self-taught man. During his experiments Reinders noticed that calves from recovered cows were resistant to infection. This was most probably the first recognition of the phenomenon of maternally-derived immunity, since, according to him, this resistance was not of hereditary origin, depending solely on the immunity of the dam. He also noticed that the transferred protection gradually disappeared, leaving the calves just as susceptible as those from dams who had not had the disease. He also took advantage of this temporary resistance to inoculate calves with minimal risk and realised that he increased his chances of successful inoculation by repeating the procedure at different ages, because in some of the calves the first inoculation would not 'take' (i.e. have the desired effect).

Nevertheless, in the end it became obvious that inoculation was not a valid solution for rinderpest control. Not only were the losses after inoculation too high but, more importantly, the procedure perpetuated the circulation of the causative agent in the cattle population. At least all these experiments proved that smallpox was not unique in being preventable by inoculation and that the procedure, when successful, provided lifelong protection.

After Jenner's discovery of vaccination against smallpox in 1796, and due to the suspected analogy between the two diseases, there were trials to vaccinate cattle against rinderpest using the smallpox vaccine. This practice was passionately supported in England during the epizootics of 1865 to 1867 (18); finally, one of the main advocates of this practice, a Dr Murchison (1830-1879), wrote to *The Times* (30 January 1866) saying that: 'the analogies between smallpox and rinderpest were so obvious that it was logical to try to vaccinate cattle against rinderpest; but it is becoming also obvious that, despite all the trials, there are nowadays sufficient evidence that vaccination does not confer a continuous protection against rinderpest'. In fact, Henri Bouley demonstrated the total lack of cross-protection between rinderpest, smallpox and vaccinia in 1865. For this purpose he sent eight cows to England, where the rinderpest epizootics were raging. These cows, which had already been used in France to produce the anti-smallpox vaccine, all contracted rinderpest.

Later on, Robert Koch, working in South Africa, suggested that cattle could be protected by subcutaneous injection of blood and bile from an infected animal. This highly dangerous method was soon replaced by the use of immune serum and later by a mixture of immune serum and virulent virus. Subsequently, the technique was improved by serial passages of the bovine virus through goats, which enabled Edwards to produce a caprinised vaccine in India in the 1920s. Trials with inactivated vaccines also took place. Finally, the successful isolation of the virus in cell culture (37) led to the *in vitro* development of an attenuated strain and from this the production of a safe and highly effective vaccine.

The Plowright tissue culture vaccine has been used with great success over the past forty years to vaccinate against rinderpest and has been the major reason behind the success of the global campaigns to eradicate the disease. We may justifiably hope that rinderpest will follow smallpox into oblivion, as only the second great plague to be eliminated on earth.

Bovine contagious pleuropneumonia

Another important disease of cattle, bovine contagious pleuropneumonia (CBPP), also played a key role in the history of veterinary vaccinology.

The disease was a real scourge in Europe during the 19th Century, reaching Belgium in 1828, the Netherlands in 1833, and the United Kingdom (UK) in 1841 (28). Louis Willems, a Belgian physician, tried to use inoculation to prevent the disease (45). Willems was the son of a distiller in Hasselt; his father had a huge fattening cowshed where he held cows coming from many different herds, which provided the ideal conditions for the transmission of a contagious disease such as bovine pleuropneumonia. Willems chose to inoculate cattle at the tail, provoking large abscesses (45); the animals presented general clinical signs but not the typical signs of the disease (pleuropneumonia) and became protected when exposed again.

It must be noted that a procedure similar to that proposed by Willems was empirically developed in Western Africa, where cattle were vaccinated with virulent pleuropneumonia tissues; vaccination sometimes provoked exostosis leading to a horny protrusion on the nasal bone. The skulls of such vaccinated animals even led to false identification of a new species named *Bos triceros* (11, 12, 21).

Bovine contagious pleuropneumonia also led to the discovery of *Mycoplasma* by Nocard and his colleagues in 1898 (32); for a long time *Mycoplasma* were called pleuropneumonia-like organisms (PPLO), a term which was applied to the group of microorganisms similar to *Mycoplasma mycoides*, the cause of pleuropneumonia in cattle.

Foot and mouth disease

The protection of herds against the consequences of FMD has been a concern for cattle breeders for centuries, probably since antiquity. Vaccination is a recent development (between the two World Wars) in the history of farm animal breeding, and was preceded by various alternative measures, all of them oriented to protect the herd from losses induced by the threatened disease.

The oldest known strategy used by cattle breeders in the distant past to confer active protection on their herd was to practise 'aphtisation' as soon as the first case of FMD was observed in the herd or in the neighbourhood. The simultaneous inoculation of all animals in the herd by rubbing muzzle or lips with virulent saliva taken from the lesions of FMD-ill animals conferred a very early, strong and long lasting post-infectious immunity. The clinical disease triggered was comparable to the spontaneous disease, but nevertheless, there were some positive aspects like the brevity of the clinical signs, the synchronisation of infection in the full herd, the absence of aggravation of virulence by passages and finally the aim of the operation, the immunity (monovalent) conferred for several years. This kind of general method for 'prevention' is well documented as having been carried out in both animal species and humans (small pox) for centuries in Asia, Africa and Europe (12, 25, 30).

The next step just before vaccine use, was the injection of therapeutic immune serums for preventing or curing FMD symptoms in cattle. Friedrich Löffler, the co-discoverer of the filterable nature of the FMD agent (1897), pioneered this new preventive means to protect herds, which was then further developed by many other researchers (12). After the First World War, the production of cattle immune serum was organised at industrial level in many European countries, for example, records show that nearly 13,000 cattle were treated in one year in France and that 112,000 litres of immune serum were used in nine years in Denmark in the 1920s (25). It is interesting to note that several authors promoted the use of immune serum associated with aphtisation to minimise the consequences of the inoculated disease (25).

The history of vaccination as a whole is very interlinked with the history of FMD vaccines, the progress in industrial vaccine technology offering new opportunities to modify the opinion on vaccination or on the way to use it. For this reason, the history of the development of FMD vaccines will be presented here in some detail.

History of foot and mouth disease vaccine technology

The pioneers

The first published attempt at using a protective FMD vaccine was that of French researchers Vallée, Carré and Rinjard in 1926 (43). Since 1922 they had been testing the action of formaldehyde on different agents of infectious diseases. In 1925 they published an article on the first vaccine, which was made from ground mucosal FMD lesions in saline buffer that had been filtered and inactivated at 20°C for 4 to 7 days with formaldehyde at 0.5%. The protection given was irregular but when present was reported as good for the standards of that time.

In 1932 in Denmark, Schmidt completed the laboratory process by the simultaneous use of aluminium hydroxide gel, a compound that had been used with formaldehyde in the domain of tetanus and diphtheria toxins by Ramon since 1924 at the Pasteur Institute in Paris.

Semi-industrial production of FMD vaccine began after the technique was further improved by the team of Professor D. Waldmann (44), working at the German Institute of Riems Island in the Baltic Sea. In 1937, they published a paper in which they highlighted the beneficial role of certain key factors, i.e. ensuring a pH>9 during the inactivation process, using a lower concentration of formaldehyde (0.05%), and maintaining the material at a higher temperature (25°C) for 48 hours. Thus, the first modern technology for turning FMD viruses into antigens for vaccines was born, and it was used with almost no modification for 50 years up until the 1970s, when attempts were made in industrial production to use other inactivants like glycidaldehyde, or aziridines.

Live attenuated vaccines against FMD have not succeed, even if there has been some semi-industrial production of such vaccines at certain times (reported by Kemron in Israel in eggs, Gribanov and Onufriev in the USSR in baby rabbits and Villegas in Venezuela in eggs).

Industrial development

Once the difficult process of turning the virulent FMD viruses into safe antigens was mastered, the second difficulty to solve was to obtain enough virus material for vaccine production.

Once again, it was the Riems Island research team which found a solution to this problem by developing an original technology for harvesting larger quantities of virulent material, which has been known ever since as the Waldmann's method. This method was used in Europe until the 1950s and it was still being used in South America in the 1970s. To encourage the standardisation of this method worldwide, the OIE organised an International Meeting in Bern in 1947 (25). According to the method, the virulent material is obtained from infected cattle which are kept in a restricted stable, inoculated at the same time at several points in the tongue, and slaughtered when the tongue lesions are at their worst. All tongues are isolated and scraped to collect lymph and epithelial lesions. The carcasses are kept in the fridge for lactic maturation for 48 hours before rejoining the commercial circuits for fresh meat. The virulent tongue lesions are ground in saline buffer, centrifuged, then diluted before the inactivation step. At the earliest stage in the development of the method, one cattle dose of monovalent vaccine was a volume of 60 ml and each cattle tongue allowed for the preparation of 40 to 50 commercial cattle doses. One disadvantage of the Waldmann's method was the necessity to use FMD-free cattle to develop large lesions after inoculation. So, as the use of vaccination progressed across the country, fewer susceptible animals were available for the production of vaccine.

The second breakthrough in FMD vaccine production was made by Professor Frenkel, a Dutch scientist from the Amsterdam Veterinary Institute. Taking advantage of the work of Maitland on tissues maintained in a special medium, Prof. Frenkel had the brilliant idea of collecting epithelia fragments taken from the tongues of healthy cattle immediately after slaughter in normal abattoirs. Maintained for 48 hours or more in an appropriate medium at 37°C under oxygen bubbling, the small pieces of epithelia (the surface areas of which were equivalent to that of a hand) were infected with a virulent seed virus. The virus multiplied in the epithelial cells and at the end of the culture time virus was present both in the epithelia and in the maintenance medium. The process was presented as experimental at the OIE meeting in Bern in 1947, but industrial development started in 1950. The concept was revolutionary for this time because the source of raw materials (tongues) was without limit in normal abattoirs, the vaccination status of the animal had no effect on virus multiplication, and the yield of FMD virus harvested per animal was 100 times more than in the Waldmann's method (400 commercial doses). In Chile, in 1951, Espinet discovered that saponins could be used as an effective adjuvant in the aluminium hydroxide gel (19), which led to the first modern vaccine available for vaccination campaigns. To meet the demand, 500 l culture tanks were used for vaccine production which induced economy of scale and made each vaccine batch bigger and each vaccine dose cheaper. And the cherry on the cake was that the vaccine prepared with bovine homologous material did not induce allergic reactions in repeated vaccination campaigns, an issue which subsequently became a huge problem with the vaccines obtained using heterologous hamster cells for virus growth.

The third major technical step in the progression of FMD vaccine production was the use of cells, first in monolayer, then in suspension, to satisfy the huge demand for millions

of litres of vaccine for vaccination campaigns in development in Europe or in South America. Cells in monolayer were used on an industrial scale mainly in Italy. At first, the cells used were primary or secondary kidney cells (from calves, piglets, lambs) taken from abattoirs. Subsequently, the advantages of a clean cell line like the baby hamster kidney cell line (BHK 21) isolated by Macpherson and Stocker became apparent. But soon, the capacity of plants to produce vaccine using cell monolayer culture in roller bottles was exceeded by demand; additionally, the harvest of thousands of bottles was not without risk of bacterial contamination. Consequently, the culture of cells in suspension became the method of choice for manufacturing huge volumes of vaccines.

The credit for the development of the BHK 21 cell line in suspension must go to Capstick and Telling, who carried out their work at the Pirbright Laboratory in the UK in 1962. The main advantage of this new technology was that everything could now be done in a completly closed circuit: cell growth, the infection of cells with sterile seed virus, the clarification in line of the virus harvest, its inactivation, concentration and formulation with adjuvant, and, finally, the filling of vaccine vials. At a time in the 1970s when FMD outbreaks were rare after successful mass vaccinations, virus escapes from manufacturing plants were seen as scandalous; consequently, the new process which was safely contained in a closed circuit, itself located in an appropriate containment unit, was the beginning of real biosecurity. The unique but huge disadvantage of this process was the presence of allergens from cell culture in the vaccine and the allergic reactions this provoked during regular vaccination campaigns. It took a decade to fine-tune purification steps so that a potent, non-allergenic vaccine could be produced in huge volumes without impairing the virus yield (1).

After the search for new adjuvants for potent FMD vaccines for pigs by McKercher and his group at Plum Island in the USA after 1965, it became obvious in the early 1970s, that oil-adjuvanted vaccines for cattle could have a promising future in regions such as South America where cattle breeding was extensive. In that region the oil-adjuvanted vaccines were well accepted both from an immunological and a political point of view, because they offered a new approach to rectify the errors of the past in disease control. Oil-adjuvanted vaccines administered by intra-muscular route protected cattle under a great variety of breeding conditions and appeared to provide longer-lasting immunity than the previous aqueous vaccines (41). Injected by vaccinators in planned programmes, oil-adjuvanted vaccines proved to be more efficient than classical vaccines bought by cattle breeders to comply with legislation but rarely injected. In fact, the great successes observed in FMD control in infected areas of South America are essentially due to the intensive use of oil-adjuvanted vaccines of good quality.

Scientific discoveries

It had been well known since the paper by Moosbrugger in 1948 (29) that after inactivation with formaldehyde FMD vaccines could remain virulent for a few days after their date of manufacture. The kinetic studies of inactivation in the 1950's confirmed that formaldehyde was not an inactivant of the first order. In 1959, Brown and Crick (15) explored the properties of a new family of inactivants: the aziridines, which were first used in the vaccine industry in 1971 by Pay *et al.* (36). But the breakthrough came in 1973 from Bahnemann (2), working for PANAFTOSA (Pan American Foot-and-Mouth Disease Center) in Rio de Janeiro, who demonstrated that in a very simple chemical reaction, an aziridine, the cyclised ethylene-imine, can be synthesised by vaccine manufacturer using a halo-ethylamine, most often the 2-bromo-ethylamine, just before the inactivation process starts. The method was immediately adopted worldwide and often repeated for biosecurity reasons in a double inactivation step. Of the hundreds of billions of vaccine doses that have been tested worldwide for safety since the introduction of this method, not one has been reported to be virulent.

Later, in the middle of the 1990s, in laboratories involved in FMD research, new studies shed light on the role of FMD virus non-structural proteins (NSPs) in the immune response and on their potential use for diagnosis (20). These findings were a revolution, as a vaccine that did not contain NSPs could be used in vaccination programmes without hampering the serological diagnosis of virus infected/carrier animals. That was the wish of all the vaccine manufacturers, who were being blamed because their products were hiding potential infection behind the protection conferred by vaccination. The DIVA system (Differentiating Infected from Vaccinated Animals) was finally applicable to FMD vaccination. That was a real change, with many consequences for the image and use of FMD vaccination.

The purification of antigens became a double necessity for manufacturers using BHK cells, firstly to remove the heterologous proteins of cell culture origin because of their allergenic role and secondly to remove the FMD virus NSPs of virus culture origin for their interference with the new serological method of diagnosis. Technical discoveries like chromatography or the use of poly-ethylene-glycols and high polymers of oxide of ethylene helped to solve this industrial challenge, without affecting the potency of FMD vaccines.

A beneficial consequence of the high purification process of the FMD antigens was the high degree of concentration of antigens, from 250 to 1,000 times (1); moreover, these concentrated antigens could be frozen and stored in vaccine banks as strategic reserves for emergency vaccinations. The possibility of obtaining on request, in just a few days, several million doses for emergency

vaccination brought a big change in vaccination strategies. Vaccine producers were also able to create their own banks to enable them to respond, within a very short time, to any request for vaccine for the formulation of multivalent vaccines anywhere in the world (27).

Regulation

After the publication of reports by Beck and Strohmaier (6) of repeated outbreaks of FMD in Germany, the sources of which were vaccines with residual virulence and virus escapes from vaccine plants, the European and the international communities reacted. They promulgated various regulations concerning biosecurity, good manufacturing practice, and marketing authorisations. More recently, to prevent transmissible spongiform encephalopathies, an EU directive has been introduced on the control of the origin of biological raw materials. Export controls on FMD products and equipment are also in force to prevent dual-use. Verification of compliance is carried out by national or international inspectors at vaccine plants.

Nowadays, in Europe and South America, FMD vaccines are the most inspected and controlled vaccines of all veterinary vaccines. When free of FMDV NSPs they are very useful tools for controlling the disease in endemic areas. Vaccines that do not contain NSPs mean that serological surveys can be used to differentiate vaccinated from infected animals, so this type of vaccine is also useful during outbreaks in previously FMD-free countries when stamping out is not sufficient to stop the disease progression.

History of foot and mouth disease vaccination

This brief summary of the various ways in which vaccination has been used over the years provides an overview of the different phases of the history FMD control in many regions of the world. It is clear that the history of FMD vaccination strategies is closely linked with the evolution of vaccine technology in general. Joubert (25) describes four distinct phases in the history of FMD vaccination and it will be useful to include them here:

a) The initial phase is characterised by the absence of national or regional level health plans and by limited funds for controlling the disease. Very often this is accompanied by ignorance amongst rural populations, vaccines of questionable quality, and an unreliable cold chain. This situation is observed in countries or regions after serious political conflicts, as seen in Europe in the past or in other continents presently. The result is the use of FMD vaccines on an individual basis by some farmers, vaccine being obtained by purchase or donation. The vaccination strategy is absent and vaccination takes place in scattered areas of the country. Sanitary measures are not applied. This method of conducting FMD vaccination is inefficient and surprisingly costly because there is no return on investment.

b) The second phase is characterised by a better awareness of the benefit gained in controlling the disease and vaccination is always the first option. The strategy is generally limited by the lack of funds; consequently the vaccine is used where considered useful, i.e. around outbreaks, following a ring or a zone vaccination strategy. Vaccine batch control is often inadequate due to absence of structure, expertise and funds. The required sanitary measures are known, but are rarely in force due to a lack of funds or of qualified personnel. In such countries, FMD vaccination is always initiated after the appearance of viruses and always takes time to stop virus progression. The consequence of this is a failure to protect national livestock from the disease. Additionally, new FMDV isolates demonstrate the constant genomic evolution of current virus(es) after selection through the 'filter' of partially immunised or convalescent animals. There is no return on the investment made in controlling FMD. Many developing countries are still in this phase of their history of FMD vaccination.

c) The third phase is characterised by a true national willingness to control and eradicate the disease. Often a National Commission for FMD Control is created for centralising information and directives. With more money allocated or/and collected from farmers or from commercial meat circuits, the control of FMD proves to be rapidly effective if farmers are educated and convinced. The 'winning trilogy' for full FMD control using vaccination is the following:

– the national programme should be enshrined in law and 100% of vaccine batches should be controlled, with failing batches destroyed and not only refused;

– throughout the country (or zones to be controlled), vaccination should be compulsory at the same period(s) of the year, with more than 90% coverage of the designated species for each campaign, and vaccination should be carried out by registered personnel, not farmers. Vaccinated large ruminants should be individually identified and recorded;

– farmer's associations should be encouraged and regularly informed and educated. During outbreaks, fair compensation for elimination of animals should be given to encourage breeders to declare suspicion of disease and respect the sanitary legal measures in force.

When the French National Vaccination Programme (promulgated on 23 August 1961) was extended to include almost 100% of the (individually identified) French cattle population in 1962, and small ruminant populations along borders in 1972, the number of outbreaks fell dramatically in a short period of time (Fig. 6). The same causes induced

the same effects when Germany started its National Programme in 1966 (Fig. 7).

Such extensive national vaccination strategies were successfully used in all European countries between 1960 and 1992, up until the EU decided to ban FMD vaccination, immediately followed by other European countries. A similar vaccination strategy is currently being employed at regional level in South America to enable countries in this region to obtain the status of a country that is 'FMD free with vaccination', a status created by the OIE in the 1990s and described in the OIE *Terrestrial Animal Health Code* (47). In such recognised countries, the use of FMD vaccines that do not contain NSPs is of prime importance: it allows for conclusive serological surveys on the absence of FMD virus circulation, facilitates the export of cattle products, and can enable countries to achieve 'Free of FMD without vaccination' status more quickly.

d) The fourth phase is the end of the long process of vaccination or is adopted right away by countries geographically protected from FMD threat. This phase is characterised by the absence of vaccination in livestock, the existence of national/regional antigen reserves for emergency vaccination and the implementation of very strict sanitary prophylaxis, i.e. sanitary controls at borders and inside national territory. This phase could be recognised by the OIE as 'Free of FMD without vaccination' (47). Of course, any strategic reserves for emergency vaccination (27) should be made from purified antigens without detectable levels of NSPs; using this type of vaccine will take advantage of the new OIE rules concerning the use of serological testing (46) for re-gaining 'FMD-free without vaccination' status in six months.

This fourth phase, including storage of strategic reserves, was reached some years ago by European continental countries after 25 to 27 years of medical prophylaxis as described in phase 3, but it was adopted straight away by the UK, Norway, the USA, Canada, Australia and New Zealand due to their favourable geographical situation.

Other veterinary contributions

A major step in the development of vaccines took place in the USA, when Salmon and Smith (1886) (40) demonstrated that pigeons could be protected against infection with a hog *Salmonella* (at the time called 'hog cholera virus') by inoculation with a heat-killed preparation of a culture of the organism. This method of vaccination proved to be widely applicable for bacterial infections, and by the end of the 19th Century killed vaccines had been developed for typhoid, plague and cholera in humans and for several bacterial diseases of animals.

The discovery by Roux and Yersin (1888) (39) of a soluble product in a culture of diphtheria bacilli (diphtheria toxin) that could produce all the symptoms of diphtheria in experimental animals, and the subsequent demonstration by von Behring and Kitasato (1890) (8) of antitoxic potency in sera of animals which had recovered after inoculation with such toxins, were of major significance in the development of both immunology and vaccinology. Initially, toxins were used for vaccination by preparing balanced toxin-antitoxin mixtures (7) but, while success could be achieved in experimental animals, it was not a practicable procedure for use in the field. However, the production of toxoids by formalin inactivation of toxins by Glenny and Hopkins in 1923 (23) and independently by Ramon in 1924 (Fig. 8) (38) added another tool to vaccinology, one which could combat bacteria whose virulence was mediated through toxins, e.g. those causing

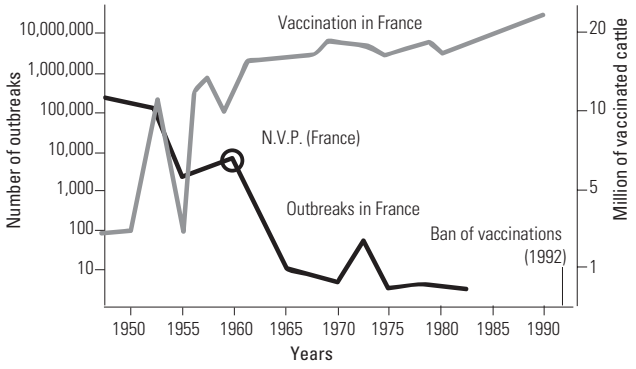


Fig. 6
The effect of the National Vaccination Programme (NVP) (which covered 100% of the cattle population) on reported outbreaks in France between 1962 and the ban of vaccination in 1992

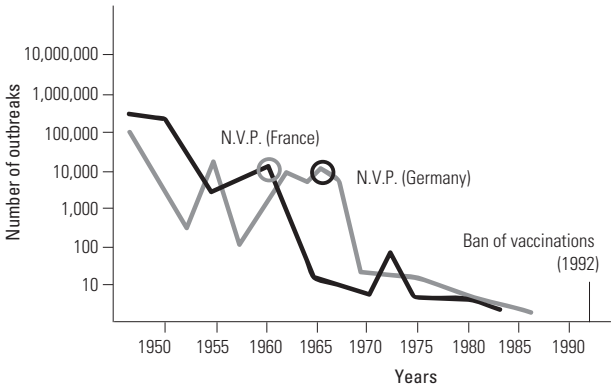


Fig. 7
The comparative effects of National Vaccination Programmes (NVP) in France and Germany

Fig. 8
Gaston Ramon at the World Organisation for Animal Health (OIE) in 1959
Source: OIE

diphtheria and tetanus in humans, and tetanus and a range of other clostridial diseases in animals.

Vaccination against Marek's disease in poultry is considered to be the first example of widespread use of a vaccine to effectively control a naturally occurring cancer agent. Although Marek's disease vaccine was primarily developed for protecting chickens, its importance extends beyond the field of animal health and it has contributed to our understanding of related human diseases and fundamental biology (17). Within the last decade there has been a dramatic change in the method of delivery of Marek's disease vaccines in commercial broiler chickens. Previously, they were administered at hatching by the subcutaneous route. Today, most major commercial hatcheries use the *in ovo* delivery system. With this system, live vaccine viruses are administered to embryonated eggs before hatching. Injection in ovo is given at the time eggs are transferred from the incubator to the hatchery, usually around embryonation day 18. Automated, multiple head injectors deliver a precise quantity of vaccine simultaneously to an entire tray of eggs (16). This simultaneous inoculation of large numbers of eggs saves on the labour costs associated with injecting individual chicks after hatching. There is no apparent adverse effect from *in ovo* injection on either the hatchability of the eggs or the long-term performance of the chickens.

A recent development in veterinary medicine is the extension of vaccination to wildlife. Through the systematic vaccination of the European wildlife reservoir, the red fox (*Vulpes vulpes*), terrestrial rabies was eliminated from most of Western European countries (14). Wild boars (*Sus scrofa*) are presently vaccinated to eliminate classical swine fever *Pestivirus* from this wildlife reservoir (26); even vaccination of wildfowl against highly pathogenic avian influenza virus, or vaccination of African buffaloes (*Syncerus caffer*) against foot and mouth disease are envisaged.

Conclusion

These few episodes in the past illustrate the close relationship between veterinary and human vaccines that still holds true today, and a whole book could be written on the subject. Nowadays, as in the past, when there are both human and animal forms of a disease, sometimes it is the human vaccine that arrives first and sometimes the animal vaccine. Whichever comes first serves as a guide for the other. An area where advances in veterinary vaccines are particularly well developed is in parasitic diseases. For instance, although a human vaccine against human schistosomiasis is still not available, there is a satisfactory vaccine against bovine schistosomiasis, even though the parasite involved is very similar to *Schistosoma mansoni*. There is also a vaccine against bovine lungworm, based on irradiated larvae. We are still awaiting one or more of the promised vaccines against malaria, whereas a vaccine against canine babesiosis is already on the market.

Where there is a risk of epizootic diseases passing to humans as a result of a reassortment involving different strains, as in the case of avian influenza, physicians see the animal vaccine as the first line of defence in avoiding a possible pandemic. The very latest human vaccine against rotaviruses, the result of a cross between an avian strain and an attenuated bovine strain, is a reminder of what the history of vaccination has revealed: the movement of pathogens between species can pose a very real threat but can also be exploited for prophylactic purposes.

Another line of convergence between human and veterinary vaccines has arisen in recent years. In the legislation to ensure greater reliability and safety of vaccines, we see the extent to which veterinary vaccines are now controlled at all stages of trials before being licensed, in a way that does not fundamentally differ from the situation with human medicine. This tendency to converge merely confirms the historic vocation of these 'two medicines' to work together. Nowhere is this more apparent than in the history of vaccinology, to which so many veterinarians have contributed.

Foot and mouth disease, which was among the first diseases recorded centuries ago in literature, has always mobilised shepherds, farmers, veterinarians and government authorities to find a means to minimise the consequences of the disease for livestock. Vaccination against the disease appeared possible between the two World Wars, but not until the 1950s, when vaccines began to be produced on an industrial scale, did it become

feasible to make vaccine available in large quantities for mass vaccination strategies. Vaccination has been a successful alternative to the more risky practices of aphtisation and serotherapy.

From the First World War up to the middle of the 1950s, FMD was observed throughout most of the European continent in the form of widespread epizootics occurring at intervals of approximately six years (13). This situation is over in Europe thanks to vaccination, but is nowadays still prevailing in some parts of the Asian and African continents. The history of FMD eradication in Europe, culminating in the ban on vaccination in 1992, should be an encouragement and a model for countries were FMD remains a scourge that impairs the development of the dairy and meat industry at a time when the Livestock Revolution has already started.

■

Une brève histoire des vaccins et de la vaccination

M. Lombard, P.-P. Pastoret & A.-M. Moulin

Résumé

La vaccinologie humaine, qui s'intéresse d'abord à l'individu, semble très éloignée de la médecine vétérinaire dont l'objet principal est la santé du troupeau. Pourtant, nombre d'épisodes du passé (variole, choléra aviaire, fièvre charbonneuse, rouget du porc, rage, tuberculose, etc.) illustrent la proximité de la recherche sur les vaccins à usage vétérinaire et humain. Dans certains cas, le vaccin humain fut le premier à être développé, dans d'autres ce fut le vaccin à usage vétérinaire. L'histoire de la vaccinologie révèle l'importance de la collaboration entre ces « deux médecines ». Les vaccins contre la fièvre aphteuse ont été parmi les premiers à être mis au point, dès la fin du XIXe siècle. Grâce aux découvertes de plusieurs chercheurs, notamment européens, tels que Vallée (un Français), Waldmann (un Allemand), Frenkel (un Néerlandais) et Capstick (un Britannique), la production à échelle industrielle des vaccins contre la fièvre aphteuse a démarré dans les années 1950, permettant de vacciner des millions d'animaux, en Europe et ailleurs. Les stratégies de vaccination contre la fièvre aphteuse ont toujours été tributaires des propriétés des vaccins utilisés. En ce début de XXIe siècle, les vaccins contre la fièvre aphteuse sont conçus de telle sorte que les tests sérologiques sont désormais capables de différencier les animaux infectés des animaux vaccinés, ce qui a modifié les prescriptions de l'OIE en matière d'échanges internationaux d'animaux et de produits d'origine animale. Les auteurs abordent également l'histoire de la vaccination contre la peste bovine, la péripneumonie contagieuse bovine et la maladie de Marek.

Mots-clés

Fièvre aphteuse – Histoire de la vaccinologie – Maladie de Marek – Péripneumonie contagieuse bovine – Peste bovine – Relation entre les médecines humaine et vétérinaire – Vaccin – Vaccin vétérinaire – Vaccination.

■

Una breve historia de las vacunas y la vacunación

M. Lombard, P.-P. Pastoret & A.-M. Moulin

Resumen
La vacunología humana, centrada sobre todo en el individuo, parece muy alejada de la medicina veterinaria, que se ocupa esencialmente de la salud de los rebaños. Sin embargo, en el pasado ha habido numerosos episodios (de viruela, cólera aviar, carbunco bacteridiano, erisipela porcina, rabia o tuberculosis, por ejemplo) que han puesto de relieve los estrechos vínculos que existen entre la investigación sobre las vacunas humanas y la dedicada a las vacunas veterinarias. En algunos casos la vacuna humana ha precedido a la animal, mientras que en otros ha ocurrido lo contrario. La historia de la vacunología demuestra a las claras la importancia de que estas 'dos medicinas' trabajen conjuntamente. Las vacunas contra la fiebre aftosa se cuentan entre las primeras que empezaron a fabricarse, desde finales del siglo XIX. Gracias a los descubrimientos de una serie de investigadores, entre ellos varios europeos como Vallée (francés), Waldmann (alemán), Frenkel (neerlandés) y Capstick (británico), a partir de 1950 se empezaron a fabricar a escala industrial, cosa que sirvió para vacunar a millones de animales tanto en Europa como en otras regiones. Las estrategias de vacunación contra la fiebre aftosa han dependido siempre de las propiedades de la vacuna empleada. Hoy en día, en los albores del siglo XXI, las vacunas están concebidas de tal manera que una prueba serológica permite distinguir entre un animal infectado y uno vacunado, hecho que ha influido en los reglamentos de la OIE sobre el comercio internacional de animales y productos de origen animal. Los autores también abordan la historia de la vacunación contra la peste bovina, la perineumonía contagiosa bovina y la enfermedad de Marek.

Palabras clave
Enfermedad de Marek – Fiebre aftosa – Historia de la vacunología – Perineumonía contagiosa bovina – Peste bovina – Relación entre la medicina humana y la veterinaria – Vacuna – Vacuna veterinaria – Vacunación.

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Genomics and vaccine development

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Summary

The current explosion in new high-throughput technologies arising from microbial and animal genomics studies is enabling the analysis of the genome, transcriptome, and proteome and offers the opportunity to gain a better understanding of the molecular pathways underlying pathogen biology, the host immune system, and host–pathogen interactions. These new tools can be applied to veterinary pathogens to overcome some of the current hurdles in the discovery of highly effective vaccines for farmed livestock and poultry.

Keywords

Animal genomics – Immunogenomics – Microarray – Microbial genomics – Vaccines – Vaccinogenomics.

Introduction to microbial genomics

The field of microbial genomics provides exciting new opportunities in the control and prevention of a wide range of veterinary diseases. Genomics, and the functional analysis of genomic data, are leading to novel approaches for vaccine discovery, and improved methods for diagnosis and epidemiology. Genomes of several viral and bacterial pathogens that impact veterinary medicine have been sequenced. Each of these studies has provided new information and unique views into viral and bacterial pathogenesis. In this introduction, the authors provide a brief overview of how bacterial genomic sequences are deduced and how genes are identified from these data. In the following section, they provide a brief overview of some bacteria of importance to veterinary medicine for which genomic sequences have been determined and they describe how genomic data can be exploited to understand the ecology and epidemiology of pathogenic bacteria, a critical element for disease control and prevention.

Currently, two basic approaches are used for determining the sequence of bacterial genomes. Both methods use a 'shotgun' approach, whereby random segments of the genome are sequenced. In the traditional method, plasmid libraries of cloned DNA fragments are constructed, and portions of the cloned DNA adjacent to plasmid vector sequences are determined by primer extension reactions (28). In an alternative pyrosequencing method, short DNA fragments are attached to microbeads, the fragments amplified, and a series of extension reactions are done that record the sequence of each fragment (60). Both techniques have strengths and weaknesses. The traditional method is labour intensive, but yields individual sequencing reads of 800 to 1000 bp in length that are paired with the complementary sequences obtained from the opposite end of the cloned fragment being physically linked. This facilitates construction of a scaffold on which

the entire sequence assembly can be constructed. In contrast, the pyrosequencing method is rapid, generating about 2×10^7 bp of data in a single 5.5 h run, but each sequence is limited to about 100 bp and is not physically linked to other fragments (60). The output from these two methods is processed by various computer programs that identify regions that have identical and overlapping sequence, and these are assembled into a series of contiguous blocks of genomic sequence. These contiguous segments of the genome are referred to as contigs, and the next step in the assembly process is to join adjacent contigs together until the chromosome or plasmid sequence (commonly referred to as a replicon because it is an autonomous replicating unit) is completely connected. Polymerase chain reactions (PCR) and primer walking of selected templates are used to improve sequence quality, and should ultimately yield a single contig per replicon.

Genomic data are processed by a variety of software programs that help identify individual genes, and translate them into the predicted protein products. Different proteins with a common function often share segments with a similar sequence of amino acids. Protein segments having shared sequences, and presumably similar functions are referred to as motifs. For example, the presence of arginine-glycine-aspartic acid (RGD) motifs in proteins facilitates binding with integrins, a feature that can be exploited by pathogens such as *Mycobacteria*, which uses the RGD-containing protein encoded by the gene *iipA* for macrophage invasion (32). The presence of common sequence motifs among lipoproteins and other proteins secreted from the cytoplasm is useful for identifying potential membrane and secreted proteins. The accumulation of this information establishes a framework for subsequent biochemical and pathogenesis studies that can lead to characterisation of previously unidentified virulence factors and antigens.

Using genomics to understand the ecology and epidemiology of infectious diseases

The sequencing and analysis of microbial genomes is fundamentally changing our understanding of the ecology and epidemiology of important pathogenic microbes and providing new insights into predictive biology and the discovery of effective countermeasures for disease control and eradication.

The genomes of some veterinary bacterial pathogens have now been sequenced, including those of several important zoonotic agents. Comparative analysis of *Mycobacterium* spp. revealed evidence of genome reduction in *M. bovis*, the cause of tuberculosis in cattle, with a general loss of functional redundancy (33). The more recent analysis of *M. avium* subspecies *paratuberculosis*, the cause of Johne's disease in ruminant species, helped identify potential targets for diagnostic tests and yielded new data regarding metabolism (54). Leptospirosis is caused by diverse pathogenic members of the genus *Leptospira*, and four strains of *Leptospira* have been sequenced, including two *L. interrogans* strains and two strains of *L. borgpetersenii* (11, 69, 87). Comparative analysis revealed substantial genetic differences that probably affect how these two *Leptospira* species are transmitted between animals (11). Comparative analysis of three *Brucella* species, *B. abortus* (40), *B. melitensis* (23), and *B. suis* (75), suggests that changes in the function of transcriptional regulatory proteins and expression of outer membrane proteins have led to differences in host specificity (14). *Bordetella avium*, a pathogen of poultry, is distantly related to the mammalian pathogens *Bo. bronchiseptica*, *Bo. pertussis*, and *Bo. parapertussis* (73) and comparison of the genomic sequences have shown distinct evolutionary patterns of adaptation to avian vs. mammalian hosts (14). Among the *Bordetella* species that infect mammals, host restriction appears to be a result of gene loss (92); these studies provided a platform that was useful in understanding the evolutionary changes that have occurred in *Leptospira* (11).

Development of an annotated genomic sequence establishes a framework through which targets for epidemiological analysis can be identified. Genomic sites that contain tandem repeated sequences often vary in the number of copies of the core repeating sequence among different strains due to errors that occur during DNA replication. Because changes in copy numbers within different variable nucleotide tandem repeats (VNTR) accumulate at independent rates, simultaneous analysis of multiple VNTRs provides a powerful method for differentiating similar strains of bacteria. Development of VNTR-based tools using PCR for epidemiological studies of several bacterial pathogens has been made possible through access to genomic sequences, and is especially useful in characterising organisms that are otherwise difficult to differentiate, including *Brucella* (7, 53, 105), *Mycobacteria* (54), and *Leptospira* (59, 90, 96, 97) species.

Another application of genomic sequencing data is the development of microarrays that provide hybridisation targets representing the entire genome, all placed on a microscope slide. Microarrays allow investigators to assess genetic variation between isolates and characterise global patterns of gene expression. For microarray analysis, RNA or DNA samples are differentially tagged with chemical labels and used to hybridise with DNA targets on the array. Unhybridised material is removed by washing and the retained, tagged samples are modified with a chemical that fluoresces when excited by lasers in a specialised instrument. The intensity of each spot, representing a

hybridisation target, usually a specific gene, is measured and compared to control samples to determine either genetic diversity (DNA input) or differential gene expression (RNA input). For example, genetic differences among *M. avium* subspecies *paratuberculosis* were identified by microarray studies (61, 76), resulting in valuable information on bacterial adaptation to different mammalian hosts. Microarray analysis of *Pasteurella multocida* gene expression during growth in chickens revealed a subset of genes induced by infection that are also expressed in response to iron limitation (6). Similar studies using *in vitro* models have also helped characterise changes in gene expression (e.g. temperature response in *L. interrogans* [57] and invasion of bovine epithelial cells by *M. avium* subsp. *paratuberculosis* [76]) and are helping to expand our understanding of how bacteria respond and adapt to growth in the natural host. A key point in using microarrays to study gene expression is that many putative genes identified by genomic analysis encode proteins of unknown function. By identifying genes that respond to environmental stimuli rather than selecting genes based on a bias formed by presumed function, it may be possible to identify bacterial proteins essential for survival in the host. This information is critical for rational selection of proteins for development as subunit vaccines.

Genomic analysis is also helping to develop a more comprehensive understanding of signals used to direct proteins to extracytoplasmic locations, including the outer membrane. Outer membrane proteins (OMPs) are often considered ideal vaccine candidates, and improved methods for identifying protein motifs that direct proteins to the outer membrane are essential to assign presumptive locations of proteins with unknown function. This problem is of particular importance in identifying putative OMPs in spirochetes, a distinct group of bacteria with an unusual cell wall/cell membrane structure. Models for predicting OMPs based on other bacteria were potentially misleading when applied to spirochetes. Availability of the genomic sequences for several pathogenic spirochetes enabled Setubal *et al.* (94) to develop an algorithm with improved predictive power to identify potential OMPs in this group of bacteria. This information is being used to help select and analyse potential vaccine candidates for a wide variety of spirochete diseases, including leptospirosis (31).

Viruses, due in part to their small size, are more easily compared using genomic approaches than bacteria, and new studies are providing useful information on strain variation. For example, comparison of the genomes from 45 strains of variola (smallpox) virus provided improved epidemiological analysis which would be invaluable in the case of virus release, and helped to identify proteins that may affect virulence (27). A similar comparison of 103 foot and mouth disease virus (FMDV) isolates revealed highly conserved, and presumably essential regions of the genome (13). Analysis of these FMDV isolates also found evidence for recombination, leading to increased diversity (13), potentially confounding epidemiological analysis and resulting in the discovery of vaccines that may be effective under experimental conditions but ineffective in the field.

These analyses illustrate how genomic sequencing is increasing our understanding of the interaction of important pathogenic microbes with their environment and facilitating the identification of relevant targets for designing vaccines that are effective under field conditions. In the next section, the authors describe approaches for analysing genomic sequences to enable the rational design of new vaccines and identify sensitive diagnostic targets.

Whole genome analysis of pathogens in vaccine discovery

Although there are comparatively far fewer completely sequenced genomes for bacterial pathogens of farmed livestock than there are for human pathogens, several completed sequences of these pathogens have recently become available for studies in vaccine development. Cattle pathogens such as *Bacillus anthracis* (83), *Mycobacterium avium* subspecies *paratuberculosis* (54), *Brucella abortus* (40) and *Leptospira* (11, 69, 87), as well as swine and poultry pathogens including *Pasteurella multocida* (62) and *Bordetella avium* (92) are among the veterinary pathogens with published sequences. Thus far, very few vaccine developments flowing from this genomic information have been published owing to the recent completion of the sequencing projects. However, two veterinary vaccines, identified through genomic approaches, have shown promise and are worthy of mention.

Eimeria is a protozoan parasite that causes coccidiosis in livestock and is especially costly to the poultry industry, with estimated annual worldwide losses at US$ 800 million (106). Efforts to develop a vaccine against this parasite have been difficult not only because of the several thousand genes and hence potential antigens encoded by this parasite, but also because the majority of candidate molecules are immunogenic, but few of these same molecules are immunoprotective (stimulate a protective immune response) (3). By using linkage analysis of DNA markers combined with an understanding of the parasite infection cycle, investigators have identified four key regions within the *Eimeria* genome capable of stimulating protective immunity (2, 3). Some progress has also been made with *Brucella abortus*, a facultative intracellular pathogen that causes abortions in livestock. Researchers analysed sequence data of *Brucella* and found the *exsA* gene (88). Further bioinformatics analysis showed

ABC transporter motifs present within the gene product, which suggest polysaccharide transport functions are critical to *Brucella* virulence. A deletion mutant of *exsA* was constructed and this attenuated strain showed protection in mice (88).

Examples of genome-wide applications with human pathogens are more common and provide a pathway that studies involving veterinary pathogens will no doubt follow. For example, Group A *Streptococcus* (GAS) has long been known to cause a variety of human diseases that range from pharyngitis to skin invasion/infection (19, 86), but the molecular basis for the phenotypic differences underlying these widely varying disease presentations were unknown until recently. By comparing complete transcriptomes of a GAS pharyngeal isolate and GAS skin isolate, investigators found 89 genes differentially expressed, 24 of which were virulence genes (103). These investigators followed up on this observation by completely re-sequencing the pharyngeal isolate and found a 7-bp frameshift mutation in a two-component regulator encoded by *covRS*. This mutation resulted in a truncation of the CovS protein, a histidine sensor (103). This mutation was exclusively responsible for the diverse disease phenotypes of each GAS isolate and has resulted in solid vaccine leads and intervention strategies.

Perhaps one of the best-known examples of genomic application to vaccine development is with *Neisseria meningitides* Group B. Investigators sequenced the complete genome of this human pathogen and recombinantly expressed 350 genes predicted to encode surface-exposed or secreted proteins (77). Immunological assays quickly trimmed the list of vaccine candidates to seven, of which some were later shown to be protective in animal studies (104). Thus, the term 'reverse vaccinology' was coined to reflect how genomic approaches allow for the design of vaccines starting from the prediction of all antigens *in silico* (performed by computer simulation), independently of their abundance and without the need to grow the micro-organism *in vitro* (82).

Accordingly, the genomic approach that can most readily be applied to vaccine discovery is the creation of subunit vaccines. In most cases, the production of killed or whole-cell protein preparations and attenuated live vaccine strains does not need genomic technology, but rather a protein chemist or microbiologist. However, the identification of suitable antigens is crucial to successful vaccine development based on subunit approaches. Using a combination of proteomics, genomics and bioinformatics, investigators can quickly narrow the list from thousands of genes down to a few dozen vaccine candidates (Figure 1). The genomics/proteomics methods define the coding capacity, and then the bioinformatic analyses trim off pseudogenes (nonfunctional or noncoding) and sequences similar to human proteins and make predictions about secreted and surface-located proteins. Then it is back to the laboratory to recombinantly produce these proteins and determine their immunogenicity (Fig. 1), although bioinformatics can make limited predictions along these lines as well. From these exercises emerge the short list of solid vaccine candidates to test in an animal model for protection.

Genomics also has the capability to make DNA vaccination studies much more efficient. Before the genome sequence was available for *Mycobacterium avium* subspecies *paratuberculosis*, DNA vaccination was attempted for this cattle pathogen using the expression library immunisation procedure (47). This study revealed two pools of DNA that were shown to be protective in mice and limited efforts were made to identify the relevant DNA in those pools (44). Random expression library immunisation was used because the genome sequence was not available at the project's inception. This random cloning method meant that the majority of clones would be in the opposite orientation relative to the coding strand or would be out of frame with the coding sequence. Therefore, many additional clones were needed to make the library truly representative of every coding sequence in the genome. An approximate total of 16,500 clones were used to immunise mice in that study (44). With the genome sequence now complete (54), a directed expression library immunisation project, in which each clone faithfully represented a single coding sequence, could be initiated. This method has the advantage that fewer clones are needed, making resulting clone pools less complex and there is no 'garbage' or nonfunctional clones such as those in the opposite orientation or out of frame. For such a study, only 4,350 clones would be needed because that is the total number of genes present in the *M. avium* subspecies *paratuberculosis* genome (54). An added benefit is that fewer mice would be needed to test the clone pools.

Genomic approaches can also identify the best targets for knockout mutations that enable engineering of attenuated vaccine strains. However, as yet, there are no published studies for bacterial pathogens that demonstrate a genome-wide approach that can identify a target, knockout this target, and show both attenuation and protection in an animal host. Rather, the literature reports studies in which genomics has been used to genetically define a known vaccine strain. The *Salmonella typhimurium* vaccine strain is protective in mice and lacks the transcriptional regulator RfaH. Use of whole genome microarrays identified the RfaH-dependent genes giving investigators insight into the mechanism of attenuation for this vaccine strain (68).

The most famous example of the use of genomics to define an attenuated bacterial vaccine strain is *M. bovis* BCG (Bacillus Calmette-Guérin – named after the the French scientists Calmette and Guérin), which is the most widely used global vaccine to prevent human tuberculosis (TB).

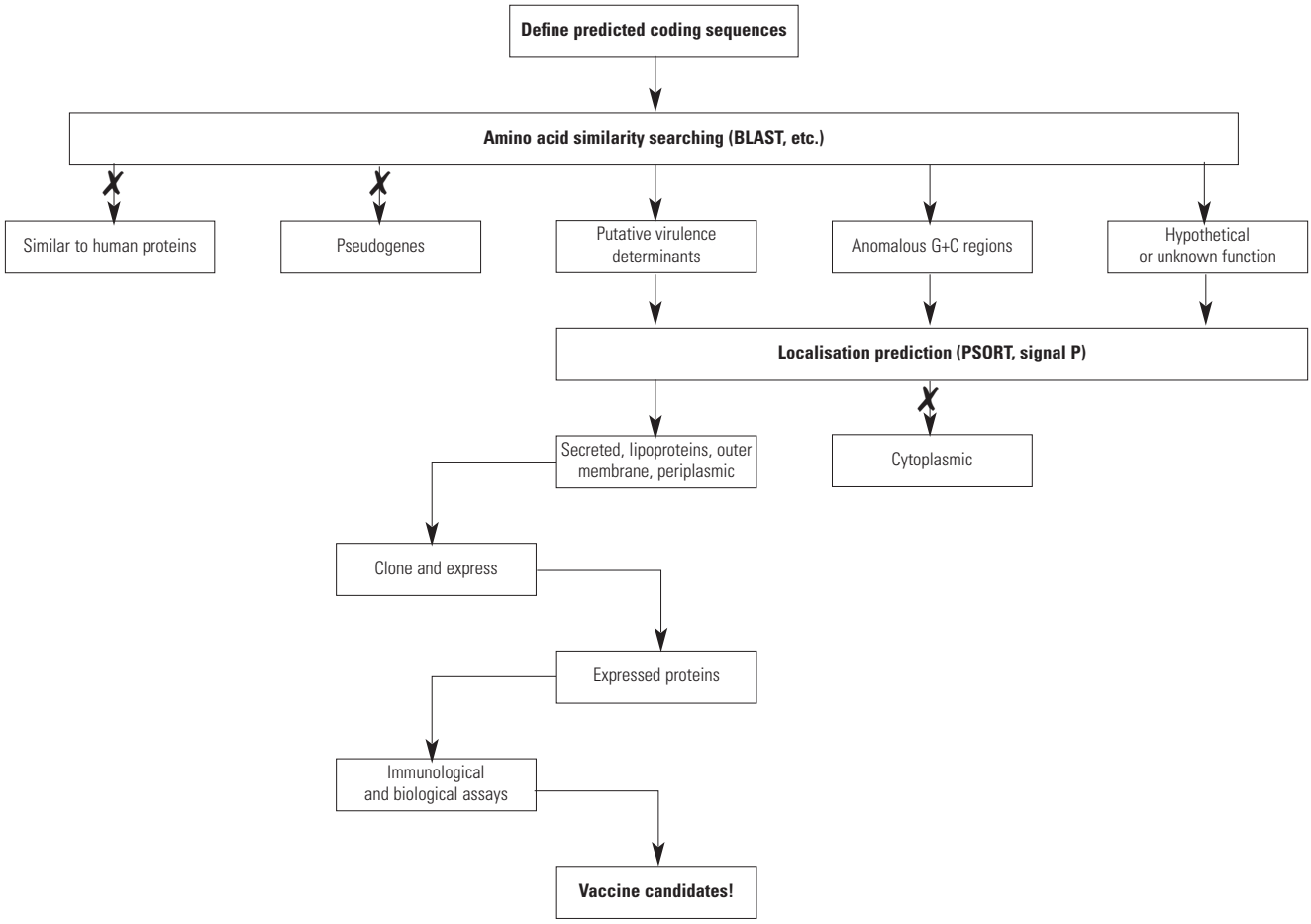

Fig. 1
Schematic flow diagram showing the genomic approach to obtaining vaccine candidates for use in subunit vaccine approaches
The initial step involves annotating the genome to define its coding capacity and hence all potential antigens. Bioinformatics similarity searches should then be performed to discard pseudogenes and anything resembling human proteins. The remaining list of genes is then cloned and expressed and analysed immunologically for vaccine candidates

Over 3 billion individuals have been vaccinated with BCG without major side effects (4). The BCG vaccine strain was derived long ago from a fully virulent isolate of *M. bovis* by prolonged serial passage of the bacterium resulting in its attenuation (66). However, the molecular basis for this attenuation was never understood until the complete genome sequences of *M. tuberculosis*, the causative agent of TB, and *M. bovis* BCG became available (16, 29, 33). Genomic comparison of these two species revealed one region of deletion in BCG, termed RD1 (8, 79). This region contains the well-known antigen ESAT-6, a secreted antigenic target that strongly induces Th1 immune responses (100).

More than any other benefit, whole genome analysis of pathogens enables the targeted selection of protective immunogens encoded by the disease-causing pathogen. This allows investigators to move away from empirical approaches in vaccine development towards a more focused, logical development and discovery of protective DNA segments and proteins. In the next section, the authors describe the applications of bioinformatics in the design of the ideal vaccine.

Bioinformatics and computational vaccinology

Designing an ideal vaccine depends greatly on several factors associated with targeted pathogens and host responses, including knowledge at the molecular level of the immune response, pathogenesis, host–pathogen interaction, and genetic and physiological variation among animals and pathogens. Recently discovered genome sequences of food animals and pathogens together with rapid advances in biotechnology will allow us to collect an unprecedented amount of information on hosts and pathogens that may have significant implications for

vaccine discovery. However, transforming this information into practical understanding requires intensive data-mining using sophisticated computational and bioinformatic tools. Highly intensive computation using high-speed central processing unit, multi-thread, and 64-bit technologies have greatly facilitated this process. Using computational approaches in vaccine design has become known as 'computational vaccinology' (30).

Applying bioinformatics algorithms to facilitate vaccine design is a very powerful approach that is changing many paradigms of vaccine discovery (81, 91, 93). As discussed in the previous section, a new approach is reverse vaccinology, the process of using *in silico* analysis of genetic information instead of pathogens themselves as the starting point (81). This approach has resulted in several successful vaccines that conventional methods would have taken much longer or failed to produce (81, 93). Thus, we can now use a genome-based approach in reverse vaccinology where the genome sequence of a pathogen is screened with bioinformatic tools to identify open reading frames that may encode candidate proteins. Proteins predicted to be surface-exposed or secreted are considered as vaccine candidates for further laboratory testing. Some proteins having structures similar to known toxins can also be included in the candidate list. If the genome sequences of different strains (virulent and avirulent) or serotypes are available, a pan-genome approach can also be used to identify candidate vaccines by comparative genomics. The applications of these approaches in vaccine development have been reported (77, 86).

If candidate antigens are identified, peptide vaccines can be developed based on the epitopes of the antigens. 'Immunoinformatics' – the new science of epitope prediction – applies bioinformatics to the design of peptide vaccines (50). Antigen processing and presentation in the adaptive immune response are well-known at the molecular level. B-cell epitopes can be either linear or discontinuous amino acid residues dependent on the conformation of protein antigens (surface accessibility), whereas T-cell epitopes are short linear peptides that are processed by proteases and presented by class I and II major histocompatibility complex (MHC) molecules. These epitopes can be mapped using laboratory procedures, which are costly and labour intensive. The epitopes can also be predicted using various bioinformatic algorithms. Currently, T-cell epitopes are more predictable than B-cell epitopes due to the linear nature of the former. The prediction of T-cell epitopes can be based on anchor motifs in the binding pockets of MHC molecules (71), or on training sets of laboratory tested data, using statistical methods such as a hidden Markov model or machine learning methods, e.g. artificial neural networks and support vector machines (10). A protein called 'transporter associated with antigen presentation' (TAP) selectively transports endogenous antigenic peptides into the endoplasmic reticulum (ER) for class I MHC antigen presentation. This selectivity can be taken into consideration in the prediction of class I MHC epitopes (58, 108).

In contrast to T-cell epitopes, B-cell epitopes remain much less predictable (5, 30). Recently, using recurrent neural network (89), machine learning classifiers (98, 99), and structural-energetic analysis (12) improved the prediction of continuous B-cell epitopes, whereas the combination of protein 3D structures and statistics has been used to predict discontinuous B-cell epitopes (42). Although the technical difficulties of predicting B-cell epitopes remain to be overcome, combining laboratory and bioinformatic analysis, such as phage display and mimotope analyses, can increase the accuracy of predicting continuous and linear epitopes (65, 78). Mimotopes were first described as peptides that mimic native epitopes of foot and mouth disease virus and can bind to the same antibody as native antigens (34, 35). Candidate vaccines can be identified based on mimotopes that can induce antibody capable of binding to native antigens of pathogens (74). This approach may be useful for developing multi-epitope vaccines to fight against pathogens with several serotypes, such as foot and mouth disease virus.

One of the challenges of epitope-based vaccines is population coverage due to MHC polymorphism. Different MHC molecules display distinct peptide-binding specificity (84, 85). However, it has been shown that certain MHC alleles share overlapping peptide-binding specificity and the alleles can be grouped into supertypes based on their common binding specificity (95). Predicting peptides that bind to MHC supertypes for vaccine development can avoid the complication of MHC polymorphism. MHC alleles can also be grouped into supertypes based on the bioinformatics analysis of MHC protein structures and sequences (24), and supertypic MHC ligands can be predicted for multi-epitope vaccine development to increase population coverage (84). It has been estimated that targeting only 3 to 6 class I HLA alleles should cover ~90% of the human population because of linkage disequilibrium in the MHC loci (39). MHC genes are also tightly linked in food animals (51, 52).

Another application of bioinformatics in vaccine development is the interpretation of data collected with functional genomics approaches to gain detailed understanding of the immune response, pathogenesis, and host–pathogen interaction. The knowledge obtained can be implemented in vaccine design. DNA microarray and proteomic analyses are two common approaches used in the studies of functional genomics, measuring transcript and protein expression levels, respectively. Because gene expression levels are collected in a genome-scale, the data

must be stored in databases in order to be managed and analysed effectively. The data also contain a large portion of technical variation introduced by laboratory procedures. The variation must be removed or minimised by data normalisation before statistical analysis (80). Because multiple statistical tests are used in the data analysis, significant thresholds must be adjusted to balance between false positives and false negatives in detecting differentially expressed genes (25). Differential gene expression can be further analysed to infer biological conclusions based on known molecular pathways and gene functions (20). Bioinformatic analysis will play a very important role in animal health by generating the detailed knowledge needed for rational vaccine development.

In summary, bioinformatics has become an additional powerful approach in vaccine design. The impact of the application of bioinformatics on rational vaccine design will be very significant in the future as research in this field progresses. Short synthetic peptides have been considered to be the next generation vaccines (93); however, there are several technical difficulties in using peptides as vaccines (41). Many of the obstacles could be overcome by bioinformatic approaches. Currently, there are many challenges confronting animal health in the areas of disease prevention and eradication. Bioinformatics may allow us to take all relevant information into consideration, including the genetic diversity of hosts and pathogens, to formulate vaccines that have broader effects regardless of these variations. Combining genomics and biotechnologies, bioinformatics can provide us with the detailed knowledge needed for vaccine development. However, the tools and infrastructure to facilitate these applications in animal health have yet to be fully developed. The next section provides an update on the animal genome initiatives.

Animal genomics

In the past two decades, molecular biology has changed the face of agricultural animal research, primarily in the arena of genomics and the relatively new offshoot areas of functional genomics, proteomics, transcriptomics, metabolomics and metagenomics. Development of genetic and physical genome maps in the past 15 years has given rise to the possibility of being able to understand the molecular nature of the genetic component of phenotypic variation. While quantitative geneticists have been successful in improving production traits, genomic technology has potential to lead to more accurate and rapid animal improvement, especially for phenotypic traits that are difficult to measure.

In the mid-1980s, a new window of opportunity opened in livestock production science. In 1986, the term 'genomics' was coined to name a new journal in which science generated from the new technologies that had been developed and applied to the study of mammalian DNA (principally for the Human Genome Initiative) could be published. Technologies that were being used at that time included such things as the application of bacterial restriction endonucleases for rudimentary visualisation of differences in the sequence of DNA, in particular chromosomal locations through 'restriction mapping'. This was followed quickly by the development of the polymerase chain reaction (PCR) in 1987, which opened up an entirely new world for the study of differences in the DNA sequence of animals. Coupled with the discovery of short tandem repeat DNA markers, PCR became a powerful tool that quickly allowed the development of genetic maps of the livestock genomes, primarily based on linkage of microsatellite DNA markers. These maps were developed and published in the early 1990s.

By the time genetic linkage maps were in place, a number of research groups around the world had developed resource family populations that were being employed to identify regions of the genome appearing to harbour genes giving rise to phenotypic variation in complex economically important traits (so-called Quantitative Trait Loci [QTL]). Once DNA markers anchoring these QTL regions were identified, it was postulated that 'marker-assisted selection' could be used to make directed genetic change in the desired traits using this technology.

By the end of the 20th Century, it was recognised that more genomic tools and resources were necessary for the fulfilment of the promise of livestock and poultry genomics. Although a large number of putative QTL had been identified for a wide spectrum of traits, only a handful of causative mutations had been elucidated through this approach. In all of these successful cases, the fine mapping of the identified genes had relied on comparative mapping approaches to make use of the denser information available in the human, mouse, and rat maps. Despite having some genomic resources, such as bacterial and yeast artificial chromosome libraries, it became clear that without the availability of the whole genome sequence as a scaffold from which to work, the time and expense of fine QTL mapping was much greater than initially envisioned. Fortunately, new high-throughput technologies were being developed that made the sequencing of whole genomes more practical, efficient, and cost effective. The human genomics research community quickly recognised this opportunity and the government and privately funded human genome sequencing projects were launched.

As the 21st Century began and the human genome moved toward an initial draft sequence, other new technologies also became available that allowed livestock and poultry researchers to move into large-scale gene expression studies for the first time. By coupling expressed sequence

tags (ESTs) with new microarray technologies, researchers were able to visualise changes in levels of expression of hundreds or thousands of genes in specific tissues under a wide variety of conditions. This began to broaden genomics research into the functional realm and initiated open discussions on how genomics might be used to bridge various disciplines into a 'systems biology' framework.

Recently, the agricultural research community has been able to capitalise on the infrastructure built by the human genome project (17, 46) by sequencing two of the major livestock genomes (*Gallus domesticus* [45, 107] and *Bos taurus* [36]). The 2006 calendar year marked a major milestone in the history of agricultural animal research since annotated draft genome sequences were completed for chickens and cattle and sequencing was initiated for the porcine and equine genomes. We now have in place a powerful toolbox for understanding the genetic variation underlying economically important and complex phenotypes.

Developed concomitantly with these genome projects has been a suite of associated tools, including:

– EST libraries

– bacterial artificial chromosome maps

– integrated physical and linkage maps

– full-length complementary DNA (cDNA) libraries

– microarrays or gene chips

– identification and validation of a large number of single nucleotide polymorphism markers.

Currently, major efforts are underway to develop haplotype maps of these genomes in order to fine map QTL and enable whole genome selection for quantitative traits (48).

While the maturing field of livestock genomics has been largely centred on improvement of production traits up to the present time, it is widely recognised that the highest potential of these technologies resides in difficult to measure and expensive traits such as efficiency of nutrient utilisation and resistance to disease. In particular, genomics holds great promise for unravelling the interactions between various hosts and pathogens (38). Understanding host–pathogen interactions at the molecular level will increase our understanding of viral and bacterial pathogenesis and the mechanisms pathogens use to evade host immune responses, both of which are paramount to the discovery of the ideal vaccine for control and eradication of animal diseases. The next section describes the role of functional genomics and the application of microarray technologies to understand host–pathogen interactions at the genomics level.

Host–pathogen interactions at the genomics level

Recent progress in sequencing the genomes of microbial pathogens and their hosts is providing sophisticated strategies for unravelling the biological complexity of host–pathogen interactions (18). Elucidating these interactions at the molecular level, however, remains largely unrealised because understanding of gene function lags behind gene expression analyses obtained through high-throughput, large-scale functional genomics approaches. Nonetheless, functional genomics is rapidly revolutionising the analysis of whole genome responses of pathogens and hosts. This will lead to a better understanding of disease processes, the mechanisms through which pathogens evade host immunity and the genetic basis of host–pathogen interactions, which will ultimately result in the discovery of novel vaccines. Collectively, the integration of these approaches in vaccine research (vaccinogenomics) is likely to fundamentally change the way scientists approach the challenges of discovering safe and effective vaccines.

DNA microarray technologies allow high-throughput measurement of global gene transcription patterns on a whole-genome or tissue-specific basis, thereby enabling the investigation of the transcriptional status of complex biological systems underlying host–pathogen interactions. Specifically, genomic technologies combined with immunology (immunogenomics) permit in-depth analysis of complex immunological processes based on large-scale whole genome approaches. Unlike conventional methods of differential gene expression (e.g. SAGE [serial analysis of gene expression] and differential display) that enable functional annotation of sequenced genomes, DNA microarray hybridisation analysis stands out for its simplicity, comprehensiveness, data consistency, speed, and high-throughput methodologies.

Global profiling of host and pathogen gene expression is an attractive approach to identifying the novel genes involved in disease processes since, in general, genes are transcribed only when and where their function is required. Thus, determining the conditions under which a given gene is expressed allows inferences to be made about its function. For example, this approach has led to the annotation of the function of multiple microbial genomes, probing a microbe's physiological state, identifying candidate virulence factors, pharmacogenomics (drug-specific signature gene expression), and molecular genotyping (molecular diagnostics for genotyping polymorphisms in related pathogens). Similarly, host genomic analyses have led to a better understanding of the response to pathogenesis, the development of diagnostic gene expression profiles, the dissecting of the genetic basis of disease susceptibility, and the characterisation of genetic polymorphisms associated with diseases.

Gene expression profiling

A variety of human DNA and oligonucleotide microarrays are commercially available. The most commonly used host microarrays are largely composed of ESTs. DNA arrays have become popular because they are generally considered to be easier to use than other gene expression profiling methods, and they allow the simultaneous quantification of thousands of genes from multiple samples. DNA array technologies rely on nucleic acid hybridisation between labelled free targets derived from a biologic sample, and an array of DNA fragments (the probes, representing genes of interest) tethered to a solid surface (9, 101, 102). The targets, often produced by reverse transcription of messenger RNA (mRNA) and simultaneous labelling of the corresponding cDNAs, form a complex mixture of fragments that hybridise with their cognate probes during the assay. The signal generated on each probe reflects the mRNA expression level of the corresponding gene in the sample. After detection, quantification, and integration of signals with specialised software, intensities are normalised for technical deviations, providing a gene expression profile for each sample that may be compared with the profiles of other samples. Standard, robust statistical methods are required for assigning significance values to gene expression measurements and to infer meaningful information. Although most global gene expression analyses have used some form of clustering algorithm to find genes coregulated across the dataset, under a primary assumption that shared gene expression often implies shared function, more sophisticated data mining techniques and specialised analysis tools may be needed to extract meaningful biological insights.

When applied to host–pathogen interaction studies, gene expression profiling has been commonly used to analyse altered expression patterns during disease states, thereby elucidating the mechanisms of disease and pinpointing the functional pathways involved in the host response to infection. Furthermore, comparison of temporal gene expression patterns during microbial infections has facilitated novel gene discovery for use in candidate vaccines and biotherapeutics. One underlying assumption of DNA microarrays is that genes are preferentially expressed when their functions are required. Given this assumption is correct, application of expression profiling in host–pathogen studies allows one to examine the functions of the genes of both hosts and pathogens: by using pathogen arrays, one can monitor the expression of microbial genes, characterise the functions of unknown genes, identify virulence-associated genes, measure physiological adaptations under various environmental conditions, and evaluate the effects of drugs and vaccines. Similarly, by using host gene microarrays, one can explore host responses at the level of gene expression and provide a molecular description of the events that follow infection. Host profiling may also identify gene expression signatures unique for each pathogen and in genetically disparate hosts, thus providing novel tools for diagnosis, prognosis, and clinical management of infectious diseases. Together, this information will guide the future design of a new generation of molecular vaccines.

Microarray applications in host–pathogen interaction studies

Strategies to investigate host–pathogen interactions using high-throughput gene expression analysis have been described utilising various *in vitro* and *in vivo* models with whole genomic or tissue-specific microarrays. The main objective of these studies is to identify groups of genes that are involved in the activation or repression of key regulatory pathways of interest. Additionally, high-throughput gene expression arrays allow one to investigate the temporal sequences of induction or repression of transcription, a prerequisite for determining the order of events following host–pathogen interaction. In most cases involving complex disease processes, it is difficult to investigate all of the interacting factors *in vivo*. Thus, in order to reduce the complexity of whole animals, and to facilitate the interpretation of genomic data, *in vitro* systems have been exploited (e.g. homogeneous cell lines that are relevant to the type of study), the results of which are compared to the results obtained with *in vivo* studies. In either case, the interpretation of gene expression changes will be challenging, and it is important that the results of microarrays are validated using other methods, such as reverse transcription PCR or Northern blotting.

In vitro studies

The first reported application of whole genome expression arrays to analyse host–pathogen interactions used primary human fibroblast cells infected with human cytomegalovirus (CMV) (109). RNA samples collected at 40 min, 8 h, and 24 h after CMV infection were used to interrogate gene chips containing oligonucleotides corresponding to >6,600 human mRNAs (GeneChip microarray, Affymetrix, Santa Clara, California, USA). At 40 min post-infection, 27 mRNAs showed significant alterations in expression, and at 8 h and 24 h, the number of altered genes increased to 93 and 364, respectively. These high numbers of genes were in contrast to previous results obtained by differential display that identified 15 interferon-inducible genes activated by CMV.

Although CMV replicates in many different cell types and the response may be different from those seen in primary human fibroblasts, it can be speculated that many of the genes identified using the GeneChip array are involved in early response of host cells to this virus. Therefore, it is not

surprising that data analysis using GeneChip software showed that substantial transcription changes began very early after infection involving the activation of many early transcription factors and proinflammatory signalling molecules, including cytokines, chemokines, stress-inducible proteins, and interferon-inducible proteins. These results were consistent with the expected host cellular response to CMV infection. In particular, CMV modulated the expression of genes involved in the production of prostaglandin E2 from arachidonic acid, indicating that prostaglandin E2-mediated inflammation is part of the host response to CMV infection. The data also revealed altered expression of immune-related genes. For example, upregulation of HLA-E mRNA by a factor of 6 was observed. Interestingly, genes encoding HLA-A, HLA-D, and HLA-G were not changed. HLA-E is a nonclassical class 1 major histocompatibility molecule whose expression has been associated with pathogen evasion of host NK cell recognition. Thus, identification of key host genes whose functions provide tantalising hints of potential mechanistic roles in disease processes underscores the utility of gene array technologies in the study of disease pathogenesis.

In a second example of the use of whole genome expression arrays, gene expression analysis was used to investigate proinflammatory responses of human intestinal epithelial cells infected with *Salmonella* (26) and human promyelocytic cells infected with *Listeria monocytogenes* (15). In both cases, genes involved in the early proinflammatory response to intracellular pathogens were significantly induced, including IL-1β, intracellular adhesion molecule-1 (ICAM-1), and macrophage inflammatory protein-1β (MIP-1β). Moreover, an interesting contrast was noted between the ability of the two microbes to induce host genes. Thus, *Salmonella* induced apoptosis-promoting genes whereas anti-apoptosis genes were modulated by *L. monocytogenes*. These studies typify the power of expression profiling in detecting different virulence strategies that microbes employ in host–pathogen interactions at the molecular and cellular levels.

Macrophages are important cells of the host immune system and play an important role in dictating the quantity and quality of immunity to microbial pathogens. Thus, gene expression profiling of macrophages has been used to characterise host immune responses. For example, RNA samples from an established macrophage cell line (HD11) infected with the intracellular pathogens *Salmonella* or *Eimeria* were used to investigate the underlying mechanisms of host innate immunity against these micro-organisms using a microarray containing approximately 5,000 macrophage ESTs (55). Analysis of the transcriptional profiles of HD11 cells infected with *S. enteritidis* at 2 h, 5 h, and 24 h identified 338 genes that exhibited at least 2-fold increased or decreased expression. Among these genes, the chemokine ah294 consistently showed highest expression at all time points examined; ah294 is a CC chemokine that activates innate immune responses and prevents the apoptosis of virus-infected macrophages. Other immune-related genes with enhanced gene expression following *Salmonella* infection included immune-responsive protein 1, interleukin-6 (IL-6), inducible T-cell costimulator, anti-apoptotic NR13, matrix metalloproteinase-9 (MMP-9), and glutamate-cysteine ligase (GCLM). By contrast, genes associated with cell adhesion and cell proliferation were downregulated following *S. enteritidis* infection. In the case of *Eimeria*-infected HD11 cells, upregulated expression of several important immune effector genes was reported, including the proinflammatory cytokine IL-1β, the chemokines ah221 and MIP-1β, and osteopontin. Interleukin-1β is secreted by macrophages and other cells upon activation by a variety of different stimuli and, in turn, induces the expression of other chemokines, thereby amplifying the immune response. Among these, MIP-1β and K203 belong to the CC chemokine family and are involved in the recruitment of macrophages to sites of infection.

In a related study, gene expression analysis with the macrophage microarray was used to characterise the innate immunity of three antigenically distinct species of *Eimeria*, namely, *E. acervulina*, *E. maxima*, and *E. tenella* (22). All of these species of *Eimeria* elicited similar gene expression response profiles, characterised by pronounced induction of many common genes involved in innate immunity. In particular, a set of 25 core response genes was identified. In addition, 60-67 genes that were unique to the individual *Eimeria* species were induced or repressed. In summary, while a shared similarity in transcript quality existed among the three *Eimeria* micro-organisms, differences were evident in the magnitude, direction, and timing of the immune responses to each individual species.

Another example is that of Marek's disease virus (MDV), a herpesvirus which causes T-cell lymphoma by infecting $CD4^+$ T cells and inducing immunosuppression. Herpesvirus of turkeys (HVT) has been successfully used as a vaccine to prevent Marek's disease in chickens. To investigate the mechanism of this protective response, expression gene analysis of chicken embryonic fibroblasts infected with the HVT vaccine was performed (49). Transcript levels upregulated by HVT included those encoded by genes known to be induced by interferon, as well as others modulating protein kinases and scaffolding proteins of signal transduction cascades. Many of these genes are known to function at critical steps in the host protective response to viral infection. In summary, all of the studies mentioned above are illustrative of the power of using new high-throughput molecular/genetic tools to investigate complex interactions between hosts and pathogens.

In vivo studies

Influenza A/Texas/36/91 virus causes a human-like influenza syndrome in pigtailed macaques and this animal model has been successfully used to study influenza virus infection at the genetic level (1). Transcriptional analysis of lung and tracheobronchial lymph nodes of pigtailed macaques infected with a genetically reconstructed strain of human influenza H1N2 A/Texas/36/91 virus was carried out to study host–virus interactions and to compare the antiviral response of macaques and humans. A commercially available human cDNA array (Agilent Technologies, Palo Alto, California, USA) containing duplicate spots of 13,026 unique clones was used in this study. Significant transcriptional activation of inflammatory cells with the activation of interferon, B cell, and apoptotic pathways accompanied by overt clinical signs was observed in the lungs of H1N2-infected macaques, which coincided with gross and histopathological signs of inflammation and tissue damage. The results of this cDNA microarray study provided insights into the molecular and cellular mechanisms associated with local innate immunity to influenza virus which were consistent with clinical signs of disease. Furthermore, gene expression profiling of influenza-infected lungs revealed new views of the role of cytotoxic T cells and natural killer cells in clearing influenza virus from the lung.

Another example of the use of functional genomics studied at the *in vivo* levels concerns avian coccidiosis due to infection of the gut with *Eimeria* parasites. The immune response to *Eimeria* is complex and involves many different types of locally situated intestinal intraepithelial lymphocytes (IELs) (21, 55). Different species of *Eimeria* show preferential invasion of distinct sites in the intestine and induce a species-specific host immune response. Two major species of *Eimeria*, *E. maxima* and *E. acervulina*, preferentially invade and develop in the jejunum and duodenum, respectively. To investigate local host immune responses induced by *Eimeria* infection, global transcriptional changes in IELs induced by oral inoculation of chickens with *E. acervulina* or *E. maxima* were monitored using a cDNA microarray containing 400 unique immune-related genes (63, 64). RNA samples from the jejunum and duodenum were obtained at 4 different time points following primary and secondary infections in order to characterise response kinetics. The results demonstrated that multiple immune-related gene transcripts were significantly upregulated or downregulated following primary or secondary infection with *E. acervulina* or *E. maxima*. In general, infection by either parasite resulted in the altered expression of more genes in naïve hosts than in immune hosts, and *E. acervulina* induced more changes compared with *E. maxima*. On the other hand, similar changes in the levels of several cytokine mRNAs were observed in both *Eimeria* species following primary infection. Also identified was a set of transcripts whose expression was commonly enhanced or repressed in the intestinal IELs of chickens infected with either parasite.

A third example of the use of gene microarrays to study host–pathogen interactions *in vivo* involves MDV. Liu *et al.* (56) used expression profiling to investigate the underlying genetic basis for disease resistance to MDV using two genetically disparate avian hosts. Transcriptional differences seen between two inbred chicken lines (lines 6 and 7), which were MDV resistant and susceptible respectively, provided insights for these investigators into the mechanisms of disease resistance. Furthermore, the nature of host proteins that interacted with specific MDV proteins was identified using a supplementary approach based on a yeast two-hybrid assay. Specifically, the growth hormone gene (GH1) was identified as a candidate gene associated with MDV resistance and further studies indicated that GH1 variation correlated with a number of Marek's disease-associated traits.

The long-term goals of using functional genomics and microarray technologies in infectious disease studies include obtaining a detailed molecular understanding of host–pathogen interactions and identifying critical target molecules and pathways for better diagnosis and design of preventive measures. Importantly, applying an integrated systems biology approach using diverse techniques such as immunome, proteome, *in vitro* and *in vivo* transcriptome analyses, comparative genomics analyses and bioinformatics analyses (as described by Musser and DeLeo [67]) will yield new insights into microbial pathogenesis and the host response. This will enable the identification of potential candidate vaccine and therapeutic targets more quickly and efficiently than otherwise possible by conventional approaches. The final section of this article provides a peek into the future application of genomics for selecting good responders to vaccination.

Selection of good responders to vaccination

An important application of animal genomics will be the evaluation of genetic influences on individual animal responses to vaccination. Veterinarians involved in vaccine clinical trials have long observed disparity in the response of individual animals to infection and vaccination in well characterised animal challenge models. These empirical observations have highlighted the need for sound biometric analyses as well as robust regulatory standards such as good laboratory practices (GLP) and good clinical practices (GCP) to eliminate experimental and

environmental biases in clinical trials. With the elimination of these biases remains the effect of host genetics on the actual safety and efficacy profile of vaccines in various livestock and poultry animal populations.

Most immunogenetics studies in livestock and poultry species have focused on disease resistance (a good review of these efforts can be found in a previous issue of the OIE *Scientific and Technical Review* [43]). Scientific studies providing evidence that an individual animal's genotype may predetermine immunological responses to vaccination are more limited. One landmark study by Newman *et al.* demonstrated in large half-sibling families differences in antibody responses induced in cattle by vaccination with *B. abortus* Strain 19, a live attenuated bacterial vaccine (70). The data were analysed using a parametric statistical model that incorporated the effects of sire, bovine major histocompatibility complex (BoLA) types, and parameters related to experimental design. Variation between individual animals was not only significant but the study also identified individual animals and families with high or low antibody production phenotypes. In several cases, these traits were significantly correlated with individual bulls, suggesting the existence of sire effects, or individual BoLA types.

Elizabeth Glass at the Roslin Institute in the United Kingdom reported that BoLA haplotypes are associated with FMDV-specific T-cell and antibody responses (37). In a fully pedigreed cattle population genotyped with 186 microsatellite markers derived from a cross between two extremes of cattle, Holtstein dairy and Charolais beef cattle, a first cohort of females immunised with a 40-mer FMDV peptide in Freund's incomplete adjuvant demonstrated a wide variation of immune responses ranging from complete non-responders to very high responders. Of all the immune responses measured, significant sire effects were seen for INF-γ, IgG_2, and IgG_1:IgG_2 ratio, suggesting that genetic influences other than the MHC genes may be regulating host responses to the FMDV peptide.

In another study (72), a commercial bovine respiratory syncytial virus (BRSV) vaccine was tested in the same Holstein-Charolais crossbred study population described above. BRSV-specific IgG antibody responses associated with protection were measured by ELISA. The analysis included the separation of heritable factors (e.g. breed-cross and sire effects) from non-heritable factors (e.g. year of birth, age and sex effects) to quantify their respective contributions to the variation in antibody response. Although this study could not determine any breed differences between Holstein and Charolais calves, the results established a significant calf–sire heritable influence on BRSV-specific IgG antibody levels.

These studies suggest that the heritability of complex traits such as vaccine responsiveness is polygenic and unlikely to be under the control of a single gene. When considering the complexities of host–pathogen interactions, it is expected that the many genes that control vaccine responses will be highly variable and individual genes will potentially display polymorphisms that collectively will determine the level of vaccine responsiveness in individual animals.

Conclusion

Genomic-based approaches are driving fundamental changes in our understanding of microbiology. Comparative analysis of microbial strains is providing new insights into pathogen evolution, virulence mechanisms, and host range specificity. Most importantly, gene discovery and genetic variations can now be used in genotyping analyses and the rational design of vaccines.

New research strategies employing high-throughput gene expression analysis are providing novel platforms for more comprehensive understanding of host–pathogen interactions. In particular, functional genomics is rapidly revolutionising the analysis of whole genome responses of host and pathogens, which will ultimately lead to a better understanding of disease processes and the mechanisms through which pathogens evade host immunity; identification of the genetic basis of host–pathogen interactions; and discovery of novel vaccines, drugs, and biotherapeutics.

Ultimately, we will be able to monitor the two way conversation between hosts and pathogens with the rapidly developing public database of the completely annotated genomic sequence datasets of many hosts and pathogens, the use of sequence-based high-throughput expression profiling technologies, and integrated bioinformatic tools to analyse and interpret genomic data. Through these multiple and combined approaches, we will obtain a complete picture of infectious diseases, microbial pathogenesis and protective host immune mechanisms using an integrated systems biology that will be crucial in developing a new generation of intervention strategies against pathogens infecting humans and animals. Microarray-based technologies for studying genome-wide transcriptional profiling hold exceptional promise for infectious diseases studies, since transcriptional control plays a key role in host–pathogen interactions. Rapidly advancing microarray technology platforms (expression profiling) will allow greater flexibility by providing this technology with increasing array element densities, better detection sensitivities, and more highly cost-efficient protocols. Future challenges for microarray researchers

will include developing databases and algorithms to manage and analyse the vast genomic-scale datasets and extracting meaningful biological information from them.

Vaccinogenomics, the integration of pathogen and host genomics in vaccine research, is likely to revolutionise the way scientists approach the challenges of discovering safe and effective vaccines. The availability of the genomics tools described in this review provides unprecedented opportunities for the rational design of highly effective veterinary vaccines. Identifying genes and genetic variances that control mechanisms of immune evasion, disease resistance, and vaccine responsiveness will in the future fundamentally change vaccine discovery research and enable vaccinologists to design vaccines to control and eradicate pathogens in targeted animal populations. For example, the use of chicken lines with defined genetic backgrounds in modern production systems provides unique opportunities for applying vaccinogenomic approaches to enable the development of vaccines that perform consistently under field conditions. Paradoxically, the heterogeneity found in outbred livestock populations may also present opportunities for vaccinogenomics by enabling marker-assisted selection of good responders to vaccination. Ultimately, genetic markers of protective immunity may one day lead to practical applications in selective breeding programmes to significantly increase disease resistance in farmed livestock and poultry, thereby improving animal welfare and the safety of our food supply.

La génomique et la mise au point de vaccins

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Résumé
Grâce au développement spectaculaire des nouvelles technologies à haut-débit dérivées de l'étude des génomes microbiens et animaux, il est devenu possible d'analyser le génome, le transcriptome et le protéome, ce qui ouvre de nouvelles perspectives pour mieux comprendre les processus moléculaires à l'œuvre dans la biologie des agents pathogènes, dans le système immunitaire de l'hôte et dans les interactions hôte-agent pathogène. L'application de ces nouveaux outils au domaine vétérinaire devrait permettre de surmonter certains obstacles parmi ceux qui freinent encore la mise au point de vaccins performants destinés au bétail et aux volailles.

Mots-clés
Génomique animale – Génomique microbienne – Immunogénomique – Microdamier – Vaccin – Vaccinogénomique.

Genómica y desarrollo de vacunas

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Resumen
Gracias al asombroso desarrollo de las nuevas tecnologías de alto potencial derivadas del estudio de la genómica microbiana y animal, actualmente se está analizando el genoma, el transcriptoma y el proteoma. Esos estudios posibilitarán una mejor comprensión de las vías moleculares de la biología de los agentes patógenos, el sistema inmunitario de los huéspedes y las interacciones entre huéspedes y patógenos. La aplicación de esas nuevas herramientas a los agentes patógenos de los animales debería permitir superar algunos de los obstáculos actuales al descubrimiento de vacunas eficaces para el ganado y las aves de corral de criadero.

Palabras clave
Genómica animal – Genómica aplicada a las vacunas – Genómica de la inmunidad – Genómica microbiana – Micromatrices – Vacunas.

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Vaccines and viral antigenic diversity

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Summary

Antigenic diversity among ribonucleic acid (RNA) viruses occurs as a result of rapid mutation during replication and recombination/reassortment between genetic material of related strains during co-infections. Variants which have a selective advantage in terms of ability to spread or to avoid host immunity become established within populations. Examples of antigenically diverse viruses include influenza, foot and mouth disease (FMD) and bluetongue (BT). Effective vaccination against such viruses requires surveillance programmes to monitor circulating serotypes and their evolution to ensure that vaccine strains match field viruses. A formal vaccine strain selection scheme for equine influenza has been established under the auspices of the World Organisation for Animal Health (OIE) based on an international surveillance programme. A regulatory framework has been put in place to allow rapid updating of vaccine strains without the need to provide full registration data for licensing the updated vaccine. While there is extensive surveillance of FMD worldwide and antigenic and genetic characterisation of isolates, there is no formal vaccine strain selection system. A coordinated international effort has been initiated to agree harmonised approaches to virus characterisation which is aimed at providing the basis for an internationally agreed vaccine matching system for FMD supported by the OIE. The emergence and spread of BT in Europe have resulted in an intensification of vaccine evaluation in terms of safety and efficacy, particularly cross-protection within and between serotypes. The most important requirement for producing vaccines against viruses displaying antigenic diversity is a method of measuring antigenic distances between strains and developing an understanding of how these distances relate to cross-protection. Antigenic cartography, a new computational method of quantifying antigenic distances between strains has been applied to human and equine influenza to examine the significance of viral evolution in relation to vaccine strains. This method is highly applicable to other important pathogens displaying antigenic diversity, such as FMD.

Keywords

Antigenic cartography – Antigenic diversity – Bluetongue – Cross-protection – Foot and mouth disease – Influenza – Serotype – Surveillance – Topotype – Vaccine strain selection.

Introduction

Understanding the genetic diversity of viral pathogens and how it is modulated by host immunity, transmission bottlenecks, epidemic dynamics and population structures is essential for the development of effective control measures (26). Ribonucleic acid (RNA) viruses with their short replication times, particular propensity to mutate during replication, and other strategies for diversification, are a particular challenge (27). The best-known example of antigenic diversity of a virus and its importance for vaccines is that of human influenza for which there is in-depth knowledge of virus serotypes, their evolution and their significance for vaccine efficacy. The global

surveillance and monitoring of human influenza and emergence of new viruses from animal reservoirs are embedded in the Global Influenza Programme of the World Health Organization (WHO) (67) and the basic requirements for effective surveillance, outbreak response and updating of vaccine strains are well established. The development of the programme has required the coordination of a network of reference laboratories, an annual strain review mechanism, acceptance of recommendations on vaccine strains by national authorities, internationally accepted standards for vaccines and an updating mechanism that can respond rapidly to changing epidemiological conditions. This article focuses on diseases of veterinary species which have similar requirements and reviews progress in understanding pathogen diversity and in establishing systems to identify appropriate vaccine strains in response to changing epidemiological situations.

There are a number of viral diseases affecting animals which are antigenically diverse and require similar approaches to control by vaccination. Probably the most studied in relation to vaccine strain selection are (i) influenza, and in particular equine influenza (43), and (ii) foot and mouth disease (FMD) (69). Both diseases are caused by viruses demonstrating a high degree of antigenic diversity and evolution. Additionally, there are other veterinary viruses which, although they do not show the same degree of antigenic evolution, do display multiple serotypes. Such viruses include, for example, the orbiviruses, bluetongue (BT) and African horse sickness (AHS), where serotype identification is important for appropriate vaccine strategies.

Mechanisms producing viral diversity

During replication of viruses, mistakes occur in the process of producing copies of viral nucleic acid which are known as mutations. Viruses containing ribonucleic acid (RNA) generate a higher rate of mutation than viruses containing deoxyribonucleic acid (DNA) because there is no effective proof-reading mechanism in the replication strategies employed by RNA viruses (20). As a result, 'clouds' of mutants or quasi species are generated during infection, however, many fail to transmit, a phenomenon known as transmission bottle-necks.

If random mutations have some selective advantage in terms of viral fitness (ability to replicate within the host and transmit and spread in a population) or avoidance of the immune response (ability to avoid neutralisation by antibody generated by earlier related strains) then these mutations may become fixed in the population of progeny viruses (7). These processes are well recognised in a number of RNA viruses such as influenza, FMD and BT.

Genetic and antigenic drift

The progressive accumulation of random genetic mutations is known as *genetic drift* which may or may not result in changes in amino acid sequence of viral proteins. If the genetic code for amino acid changes then this results in altered antigenic characteristics and is known as *antigenic drift*. There are a number of factors which drive the selection of antigenic variants and in some populations antigenic variants co-exist while in others emerging variants replace earlier viruses. These processes are known as viral evolution and understanding its basis and predicting likely trends are an important aspect of controlling virus diseases (7).

Antigenic diversity arising from recombination and reassortment

Genetic and associated antigenic changes can also occur as a result of deletions and genetic rearrangements caused by nucleic acid splicing and recombination events, as has been reported for foot and mouth disease virus (FMDV). In influenza virus infections for example, a key event arising from the segmented genome is the reassortment of genes during mixed infections of the same cells with two different viruses. This is an important mechanism for the emergence of new influenza viruses and has been reported for human, avian and swine influenzas (11, 37). The process of generating a new influenza virus with a unique combination of surface glycoproteins by reassortment is called antigenic shift. This process has also been reported in BT virus (BTV). Although virus virulence is not being considered in detail here, it is noteworthy that reassortment of surface glycoproteins from one virus on a novel background of internal genes from another virus can significantly alter the pathogenicity of influenza viruses.

Selection and survival of variants

Key factors affecting the selection of variants relate to the virus, the host immune response and the population size and structure. For example, viruses with a high infectivity have a selective advantage as they are more successful in transmission. Viruses with altered antigenic sites, particularly those involved in virus–cell attachment, may be capable of avoiding neutralising antibody present in a population as a result of previous infection. This phenomenon of immune selection of variants is particularly important for infections where immunity is not lifelong, where there is a high rate of mixing within host populations and where animals are exposed to repeated infections by closely related viruses.

Selection and survival of variant viruses is also affected by host population structure and size, which is well illustrated by considering influenza of different species. Viral

evolution in human influenza has been extensively studied and within the subtypes, one strain largely replaces another on a global scale, as viruses replicate in a partially immune population creating the need to escape the immune response.

A similar pattern is seen in equine influenza, although there is not such a strong effect due to lower population densities and lower rates of infection. Additionally, there may be little mixing among different populations, which encourages evolution of discreet co-existing lineages under potentially different selection pressures.

By comparison, influenza in pigs and domestic poultry held in isolated environments is, in general, reliant on the rapid introduction and constant availability of young immunologically naïve hosts within the breeding and farming structures. There are few opportunities for re-infection as stock is slaughtered at a young age. Maintenance of infection in such naïve populations and absence of partially immune older animals does not provide the same driving forces for immune selection and lack of mixing between farms encourages development of multiple lineages. For example, swine influenza, while showing antigenic diversity with multiple strains co-existing, shows less immune-driven evolution.

Thus, antigenic variability is driven not only by the ability of the viruses to mutate and their ability to transmit between hosts, but also by the opportunities for survival that present themselves as a result of the immune environment and population size and structure.

Equine influenza: addressing issues of antigenic diversity in relation to vaccines

Background to vaccination with equine influenza

In 1956 the H7N7 subtype of equine influenza was first isolated in Prague and the prototype was designated as A/equine1/Prague/56. Within a 7 year period a second equine influenza virus of the H3N8 subtype was isolated from horses in Florida and was designated A/equine2/Miami/63. Both subtypes caused major epidemics and in the mid 1960s vaccination against equine influenza was introduced. The early vaccines contained the prototype strains of the H7N7 and H3N8 subtypes grown in eggs, inactivated and combined with an oil adjuvant. The early products were not widely accepted as they were highly reactogenic, but as acceptable adjuvants were found vaccination became the accepted means of control, particularly in performance animals such as race horses.

In well-vaccinated populations vaccine breakdown attributable to H7N7 viruses was rare or non-existent, however, repeated infections with the H3N8 subtype have been reported over a long period. Much research has been undertaken to establish the contribution of vaccine potency and antigenic variation to this observed vaccine failure (43, 44).

Virus structure and variability

Influenza viruses are single-stranded RNA viruses with segmented genomes comprised of 8 segments (genes) coding for structural components of the virus particle and non-structural components important for replication within host cells. The two most important structural proteins demonstrating genetic and antigenic variation which are relevant to protection and vaccination are the envelope glycoproteins, the haemagglutinin (HA) and the neuraminidase (NA). Of these, the HA is particularly important as it mediates virus attachment to the host cell and antibody induced against the HA neutralises virus infectivity. The ability of the virus to evolve in terms of the antigenic character of the HA (antigenic drift) is crucial for avoidance of population immunity and immunity derived from inactivated vaccines, which is largely reliant on antibody to HA. The NA is involved in elution of virus from cells and the spread of infection between cells, but although the NA is known to vary, there is little information on the impact of its antigenic drift on vaccine efficacy.

Antigenic and genetic variation of equine influenza viruses

As with other influenza A viruses, both subtypes of equine influenza exhibit genetic and antigenic variation. The evolution of the HA gene has been well studied because of its importance in relation to virus neutralisation and protection. Attention has been focused on the A/equine/2 (H3N8) virus as this has been the predominant strain circulating since the 1960s and more importantly because there have been repeated reports of vaccine breakdown in the field. The majority of studies on the antigenic character of the HA and its relationship to viral neutralisation have been conducted using haemagglutination inhibition (HI) tests, exploiting the fact that influenza viruses naturally agglutinate erythrocytes and that antibody inhibiting agglutination is a measure of virus neutralisation (VN). Much antigenic analysis of influenza viruses has relied on the use of ferret sera as this species is susceptible to infection with influenza and provides strain specific antisera which can discriminate between strains in HI tests.

In 1983, Hinshaw reported that there had been major antigenic drift in viruses isolated between 1979 and 1981 as compared with the prototype virus Miami/63 (31). However, they also recognised, based on antigenic analysis with ferret sera in HI tests, that some viruses similar to the prototype Miami/63 virus were co-circulating with the more recent variants. On the basis of this data they recommended that additional strains (Fontainebleau/79 or Kentucky/81) should be included in vaccines, which at the time contained only the prototype H3N8 virus, Miami/63. At that time surveillance and virus collection was sporadic and there was no certainty that the strains selected as vaccine strains were representative of the predominant strains circulating.

Subsequent genetic analysis (32) based on sequencing the HA gene of a larger panel of viruses from around the world, revealed that the A/equine/2 HA gene was evolving essentially as a single lineage, however, antigenic analysis revealed that the resultant changes to the amino acid sequence gave rise to viruses which were both similar to and distinct from the prototype H3N8 virus, Miami/63. It was noted that the pattern of evolution was similar to that seen in human influenza and it was proposed that it was driven by immunological pressure, i.e. the existing immunity to historical viruses present in the older population. This study demonstrated that not only was the degree of antigenic drift important, i.e. the number of mutations which had arisen and become fixed in the HA molecule, but their identity and location was also important because genetically distant viruses could nevertheless react in a similar way in HI tests.

In both these studies antigenic differences between prototype and recent strains were measured using HI tests and post infection ferret or rabbit sera or monoclonal antibodies. Where fourfold differences in reactivity of sera with different virus strains could be detected it was concluded that the viruses were significantly different in terms of antigenicity, which may have implications for vaccines. At that time no attempt was made to assess the significance of such antigenic differences for vaccine efficacy in the target species. The significance of fourfold differences in HI tests was assumed to have immunological relevance based on experience with human influenza viruses.

The conclusion that the antigenic drift might compromise vaccine efficacy was not accepted by others. Burrows *et al* (13) concluded that the antigenic differences detected between the prototype strain Miami/63 and the new variants Fontainebleau/79 and Kentucky/81 (demonstrated using ferret sera and monoclonal antibodies) were unlikely to be important because post vaccination sera from horses vaccinated with Miami/63 was highly cross-reactive with the recent 1979 isolates (13). This lead to a debate about the relevance of antigenic differences detected using post infection and post vaccination sera from laboratory animals as compared to sera from target species (43) and hindered progress in the understanding of significance of antigenic variation in equine influenza viruses in relation to vaccine efficacy.

Genetic and antigenic drift has been periodically reported from a number of different countries (34, 52). However, a particularly important observation was made in a joint study by OIE Reference Laboratories in the United Kingdom (UK) and in the United States of America (USA). These laboratories examined viruses from 1963 to 1994 and revealed that genetic and antigenic variants were co-circulating as a result of a divergence in the single lineage of the H3N8 viruses (originally described by Kawaoka *et al*. [32]) into two sublineages representing isolates originating from the Americas on the one hand and viruses from Europe and Asia on the other (15). However, these lineages did not remain geographically separate and in the early 1990s American-like viruses were identified in Europe probably reflecting the significant traffic of horses from the USA to Europe for racing (Fig. 1).

At that time vaccines manufactured in America contained American isolates and most vaccines manufactured in Europe contained European viruses. Thus, horses vaccinated with European viruses were reliant on cross-protection when exposed to viruses from the American lineage and vice versa.

The two sublineages of the H3N8 viruses have continued to evolve and sequencing has revealed the appearance of a number of clades (subgroups) within the lineages, some of which have geographic origins, e.g. the South American branch of the American sublineage (34).

Evidence of antigenic drift affecting vaccine efficacy in the field

Vaccine breakdown has been reported during a number of outbreaks of influenza A/equine/2 over many years, but this had been largely attributed to poor vaccine efficacy, or vaccination schedules which did not accommodate the short duration of immunity provided by the early inactivated vaccines. In 1976 (68) and 1979 (12) vaccinated horses became infected, but those horses which succumbed to infection had low or undetectable antibody at the time of exposure. Thus, at this stage there was no firm evidence for antigenic drift being the explicit cause of vaccine failure.

In contrast, in 1989 a major epidemic of equine influenza A/equine/2 occurred in the UK and elsewhere and first cases were identified in regularly vaccinated army horses with high levels of antibody prior to infection (36). Although the infection was generally mild in well

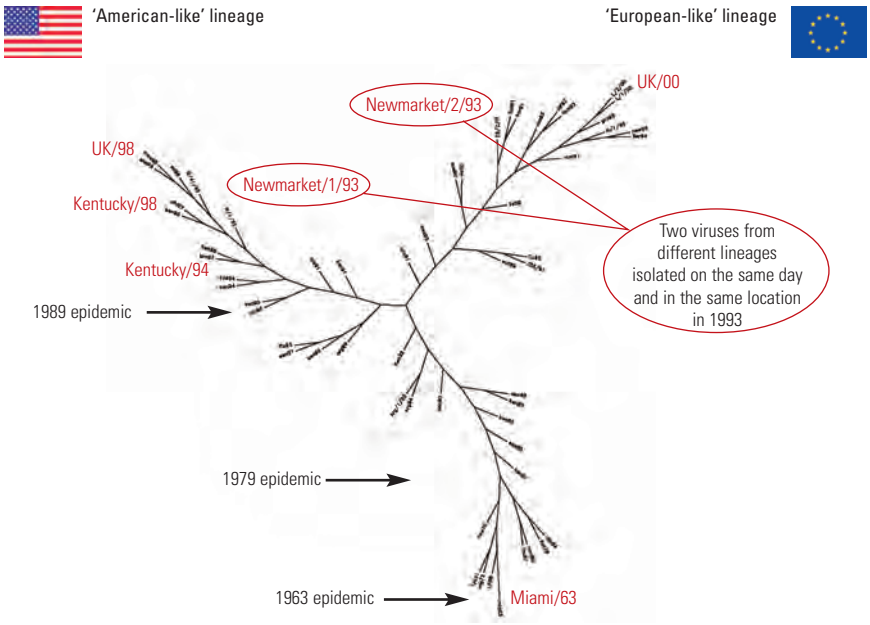

Fig. 1
Phylogenetic tree of H3N8 equine influenza viruses showing divergence from a single lineage into the American and Eurasian sublineages

vaccinated horses it spread rapidly through populations indicating that levels of virus shedding were significant even in the absence of severe clinical signs. At the time of the outbreak, available vaccines contained the prototype strain Miami/63 and a strain from the 1979-1981 epidemic such as Fontainebleau/79, Kentucky/81, Brentwood/79 or Borlange/79.

In the intervening ten years between 1979 and 1989 there had been a major improvement in vaccine potency as a result of the introduction of challenge models in the target species to assess vaccine efficacy and establishment of acceptability thresholds for vaccines in terms of antigen content (measured as µg HA) and levels of antibody (measured by Single Radial Haemolysis [SRH]) that are consistent with protection. As a result many of the European vaccines available at that time had demonstrable efficacy against homologous strains as judged by HA content, serological responses generated and protection against challenge infection (46, 47). These observations further supported the conclusion that significant antigenic drift had occurred in 1989.

Significance of antigenic variation measured by haemagglutination inhibition tests in relation to vaccine efficacy in the target species

As knowledge of the genetic and antigenic diversity and evolution of equine influenza grew and field observations suggested antigenic drift may have played a role in vaccine breakdown, it became essential to establish the significance of antigenic variability as measured by HI tests with ferret sera for vaccine efficacy in the target species.

A series of four viruses spanning a period of 26 years (Miami/63, Fontainebleau/79, Kentucky/81 and Suffolk/89) were examined in a cross-protection study in ponies in which groups of ten ponies were vaccinated with two doses of inactivated vaccines prepared from each strain containing equivalent HA content and challenged with a recent isolate Sussex/89 (43). Protection was measured in terms of serological responses, virus excretion and clinical signs following challenge. The key findings from this study were that vaccines derived from both recent and historic viruses provided equally effective clinical protection in terms of reduction in pyrexia and coughing in vaccinates as compared to unvaccinated controls. In contrast, the ability of vaccines to protect against infection and suppress virus excretion following challenge was directly related to the antigenic relatedness of the challenge and vaccine viruses, with the Miami/63 vaccine allowing significantly more virus excretion than the Suffolk/89 virus most closely related to the challenge virus Sussex/89 (Table I). This difference in protection could not be attributed to differences in potency because similar levels of HI antibody to the challenge virus (Sussex/89) were stimulated by the Miami/63 and the Suffolk/89 vaccines (Table II). Furthermore, SRH antibody levels to Sussex/89 were higher in the Miami/63 vaccine group than in the Suffolk/89 vaccine group (Table III).

Table I
Virus excretion following aerosol challenge with A/equine/2 (H3N8) virus Sussex/89 from ponies vaccinated with monovalent vaccines

Vaccine group	Number of ponies excreting virus	Mean duration (days)
Miami/63	9/10	3.6 *
Fontainebleau/79	9/10	3.3 *
Kentucky/81	8/10	2.5 **
Suffolk/89	5/9	1.6 ***
Controls	10/10	5.1

* p<0.05, **p<0.01, ***p>0.001 (compared to controls)

Table II
Cross-reactivity of haemagglutination inhibition (HI) antibody stimulated by two doses of monovalent vaccine

Vaccine	Mean HI titres to virus strains			
	M/63	**F/79**	**K/81**	**S/89**
Miami/63	**1.58***	0.95	1.32	0.7
Fontainebleau/79	0.9	**1.15**	1.40	0.95
Kentucky/81	1.08	1.11	**1.54**	0.9
Suffolk/89	0.7	0.85	1.0	**1.0**

* HI titre $\log_{10}$

Table III
Cross-reactivity of single radial haemolysis (SRH) antibody stimulated by two doses of monovalent vaccine

Vaccine	Mean SRH antibody to virus strain			
	M/63	**F/79**	**K/81**	**S/89**
Miami/63	**125.7***	148.2	103.7	143.7
Fontainebleau/79	41.6	**54.0**	46.2	55.6
Kentucky/81	74.0	75.2	**82.7**	69.6
Suffolk/89	18.0	46.1	48.9	**75.2**

* mean area of zone of haemolysis to specified strain (mm^2)

This study was the first to demonstrate that antigenic differences between equine influenza strains detected by HI tests with ferret antisera were significant for vaccine efficacy in the target species, particularly with respect to protection against infection and virus excretion. However, it also demonstrated that vaccines containing viruses ill-matched to epidemiologically relevant strains provided a degree of clinical protection which could mask infection while allowing copious amounts of virus to be excreted. These data supported the conclusion that for the control of influenza at the herd level it is important that vaccines contain virus strains which match currently circulating strains in order to minimise virus shedding.

These observations also raised the question of geographic variation and its importance for vaccine strain selection. While the majority of vaccines available in the USA, Europe and centres of thoroughbred racing around the world are produced by large multinational companies, other vaccines are made locally for specific populations, for example in South America, Japan, Eastern Europe and India. It became important to explore whether antigenic differences between viruses of different locations are likely to affect vaccine efficacy.

Competition animals travel extensively and internationally and it is likely that such horses are exposed to viruses from different locations. While originally it was held that equine influenza evolved as a single lineage, the observations made in the early 1990s revealed that the A/equine/2 lineage diverged into American and Eurasian sublineages. Subsequent to that observation a further sublineage of the American-like viruses has been recognised as originating from South America (34) (Fig. 2). It is central to international control of equine influenza to understand the significance of the antigenic differences between these subpopulations (or clades) for vaccine efficacy.

With this objective in mind a series of vaccination and challenge studies in the target species have been performed to examine cross-protection between strains arising from the American and Eurasian lineages. The prototype viruses Newmarket/2/93 (Eurasian) and Newmarket/1/93 (American) were selected and used in cross-protection studies in horses (16, 82). As with the study to examine the significance of temporal antigenic drift, it was found that the vaccines containing viruses from the two lineages provided a significant degree of cross-protection against each other in terms of suppression of clinical signs such as coughing and pyrexia. Interestingly it was also found that the American lineage virus protected equally well against the European virus as against the homologous American virus in terms of infection and reduction in virus excretion (82). In contrast, the European lineage virus vaccine was not as effective in protecting against infection and virus excretion when challenged with the American lineage virus as compared with the protection afforded against a homologous challenge (16).

While the differences between the protection observed using the different vaccines were subtle under experimental conditions in limited groups of ponies, it has been demonstrated, using mathematical models, that the likely impact of such variations in suppression of virus excretion on immunity in a population is significant (53) (Fig. 3). Furthermore, field observations have supported this conclusion. In a limited outbreak of the European lineage virus, it was found that horses vaccinated with a product containing a European virus and with SRH antibody levels above the protective threshold were protected against infection (50). In contrast, in a similar

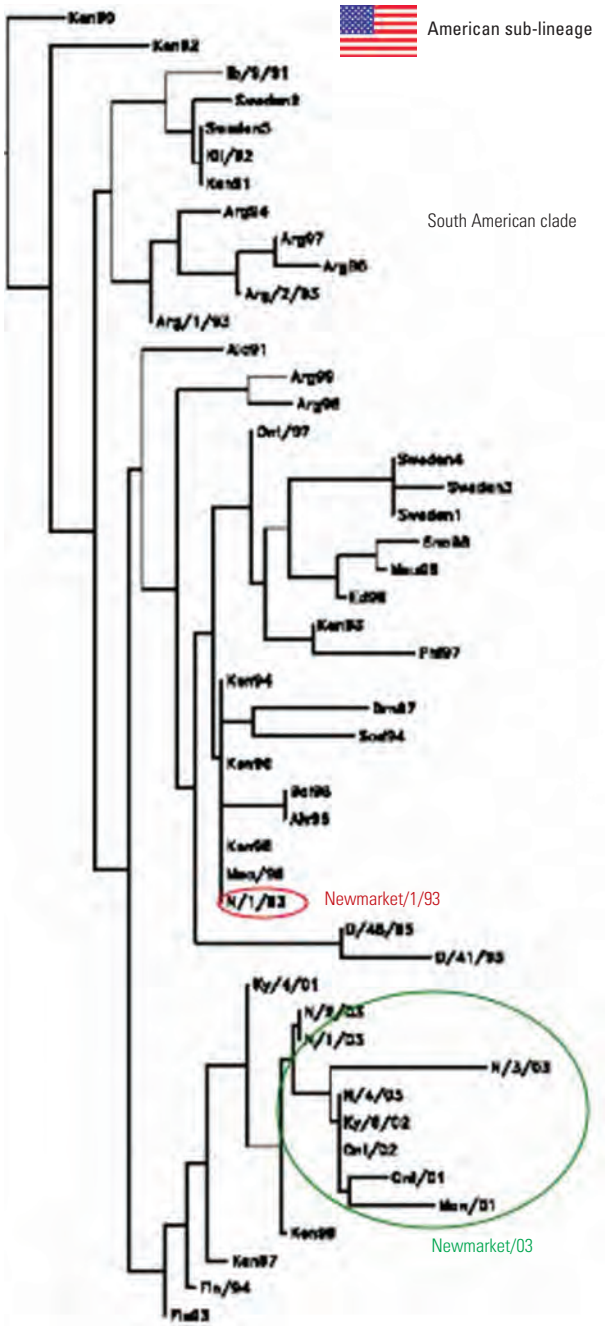

Fig. 2
Phylogenetic tree of the American lineage of H3N8 equine influenza viruses showing South American clade

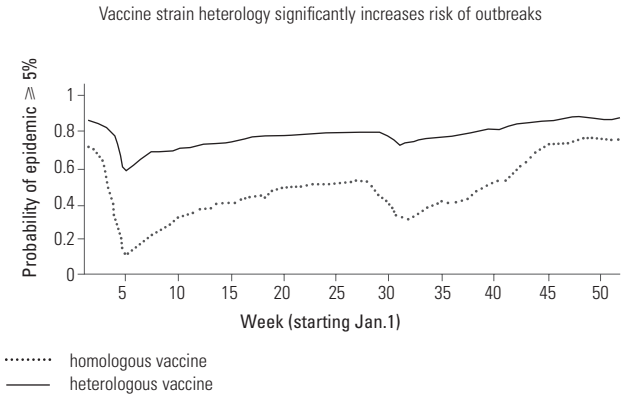

Fig. 3
A model of the probability of outbreaks occurring throughout a year in which horses are vaccinated on a 6 monthly basis with either a strain that matches the outbreak strain or a heterologous strain

outbreak caused by an American lineage virus, horses vaccinated with a European virus vaccine were not protected even when antibody levels were above the protective threshold (49) (Fig. 4). Thus, predicting the likely efficacy of a vaccine is based not only on potency but also suitability of the vaccine strains in the field.

Annual review of vaccine strains and criteria for changing strains

While cross-protection studies in the target species are the ultimate test of the significance of antigenic drift, it is not practical to base vaccine strain selection on such studies because of the difficulty of accessing influenza-free ponies, and the cost and time required to undertake large animal experimentation. This holds true for many virus vaccines. Therefore, in order to identify a reliable predictor of significant antigenic drift, there has been considerable effort to examine the relationship between protection in the target species, protection in hamsters as a small animal model and antigenic differences discriminated by HI tests using ferret, horse and hamster sera (16).

As already mentioned, ferrets produce highly strain-specific sera following infection with influenza strains, whereas horse sera are more cross-reactive. However, analysing the reactivity of post infection ferret sera in HI tests remains a useful way to compare the antigenic differences between strains and it provides an indication of cross-protection (Fig. 5).

Surveillance and equine influenza expert surveillance panel

As there is considerable international traffic of *Equidae*, it is important to conduct surveillance on a global scale and there are continuing efforts to collect viruses from around the world for sequencing and antigenic analysis. While the numbers of viruses screened are low by comparison with human influenza, surveillance has provided a picture of the evolution of equine H3N8 strains and the importance of inadequately vaccinated animals in the transmission of viruses globally. Based on the WHO model for surveillance,

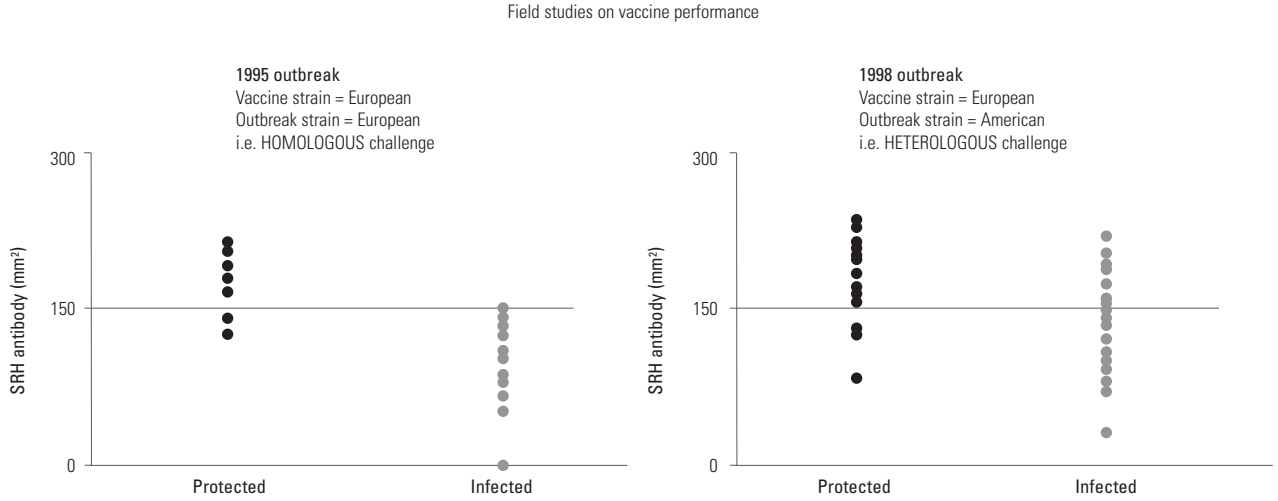

Fig. 4
Prechallenge single radial haemolysis (SRH) antibody in protected and susceptible horses in an outbreak where the field and vaccine strains were homologous or heterologous

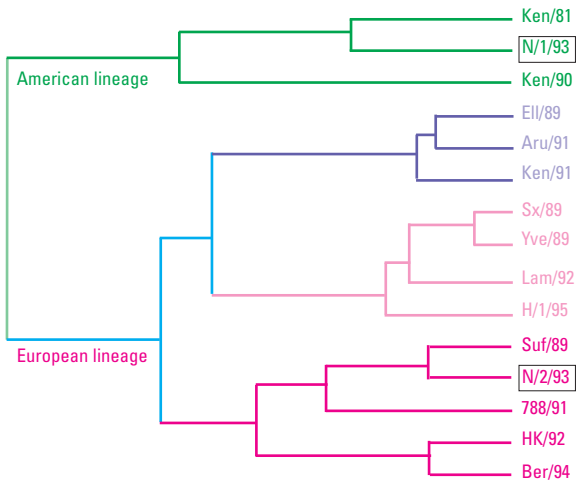

Fig. 5
Antigenic distances between equine H3N8 viruses measured with haemagglutination inhibition tests using post infection ferret sera

analysis of viruses and vaccine strain selection, an Equine Influenza Expert Surveillance Panel has been set up under the auspices of the OIE to review on an annual basis outbreaks of equine influenza, vaccine performance, antigenic and genetic character of new virus isolates and to take decisions on the need to update vaccine strains. The panel includes experts from the WHO collaborating laboratories at the National Institute of Medical Research and the National Institute for Biological Standardisation and Control in London, the three OIE Equine Influenza Reference Laboratories in Germany, the UK and the USA and other experts involved in equine influenza surveillance. Their conclusions are reported annually by the OIE.

Developing criteria

Originally, the criteria that were applied to decisions about the need to change vaccine strains were based on those used for human influenza and included vaccine breakdown in the field, fourfold differences detected in HI tests with ferret sera between vaccine strains and predominant field isolates, discrimination between vaccine and field viruses by post-vaccinal equine sera and genetic sequence of the HA1 molecule. Additionally, these criteria have been judged against cross-protection studies in horses and hamsters in order to validate their relevance to the criteria applied to equine influenza viruses. It has become clear that post-vaccinal horse sera are generally unable to discriminate between viruses unless there are major antigenic differences, therefore this test has become less important in the decision-making processes. Decisions to change vaccine strains are normally conservative and are only recommended when there are measurable antigenic differences as a result of significant genetic mutations between vaccine and predominant field strains and evidence of vaccine breakdown. For example, European lineage viruses which can be discriminated from vaccine strains based on fourfold differences with ferret sera but which have not established and spread in the equine population have not warranted a recommendation to change vaccine strains.

To date, strain differences identified with ferret sera appear to correlate well with limited cross-protection studies conducted in horses, however, patterns of cross reactivity between panels of ferret sera and viruses are complex and difficult to interpret by eye. Recent advances in computational methods are revolutionising the way such data can be analysed and a method known as antigenic cartography has been applied to historical data from human influenza and equine influenza (65). This

technique provides a visual image of the antigenic distances between viruses and how they cluster and is beginning to provide a method to assess whether variants analysed are evolving along a main lineage or whether they are unusual variants distant from the predominant antigenic types. Such information is very useful in deciding which strains are most suitable for selection as vaccine viruses, i.e. which have the widest cross-reactive repertoire with viruses in the field. The method has been adopted for the annual selection of human influenza vaccine strains and is being developed for equine influenza.

Regulatory framework for updating vaccine strains

The OIE *Manual of Diagnostic Tests and Vaccines for Terrestrial Animals (Terrestrial Manual)* (81) provides detailed recommendations for vaccine strains and vaccine potency testing. The standards it contains are generally in line with the European Pharmacopoeia Monograph on inactivated equine influenza vaccines and the standard under development by the United States Department of Agriculture (USDA).

The majority of equine influenza vaccines are inactivated whole virus (46) or viral subunits (47) combined with an adjuvant. The immune response of the horse to vaccination is relatively short-lived and multiple doses are required to maintain complete protection against infection, although a degree of clinical protection is provided with fewer doses.

The basis of vaccine potency for inactivated vaccines is well understood and relates to the amount of immunologically active HA contained in the vaccine and the efficacy of the adjuvant in enhancing circulating antibody to HA (80). Many studies in immunologically naïve horses have demonstrated a direct relationship between µg HA in vaccines (79) and antibody responses in horses measured using an SRH test (44). Furthermore, the level of SRH antibody stimulated is indicative of the level of protection acquired against challenge infections in vaccinated horses, with 150 mm^2 being identified as the threshold for protection, provided that the vaccine contains a virus antigenically similar to that being used to test the vaccine by challenge infection (44). Furthermore, this threshold for protection against experimental infection is valid for a field situation (50). Therefore, the efficacy of a vaccine in a field situation can be predicted based on accurate measurement of immunologically active HA in the vaccine, SRH antibody stimulated by the HA in combination with adjuvant and protection against challenge infection; however, the predictions will only be accurate if the virus used as a standard for the single radial diffusion (SRD), or as antigen in the single radial haemolysis (SRH), or as challenge virus for experimental infection, is antigenically indistinguishable from the vaccine strain.

The requirements of the European Pharmacopoeia for licensing equine influenza vaccines are described in Monograph No. 249 and utilise these relationships. Testing requires measurement of vaccine antigen, antibody responses in horses, and challenge infection studies with at least one virus included in the vaccine (23).

Vaccine strains are recommended by the Equine Influenza Expert Surveillance Panel and are published by the OIE. Currently, it is recommended that vaccines should contain representatives of the Eurasian and American sublineages of the H3N8 virus. Inclusion of H7N7 virus is no longer recommended on the basis that such a virus has not been isolated for more than 20 years. Viruses originating between 1989 and 1993 are still accepted for the European lineage, however, recent antigenic drift and field outbreaks caused by American lineage viruses have lead to a recommendation that vaccine viruses should be updated to representatives from 2003 such as South Africa/2003. The selection of virus strain is not prescriptive but selected strains must be shown to be antigenically similar to those recommended.

Fast track licensing system

Once new recommendations are made it is highly desirable that vaccines are updated as quickly as possible and to this end a fast track licensing system has been developed for updating vaccine viruses in Europe. These Guidelines, which have been developed by the Immunological Working Group of the European Medicines Evaluation Agency (22), recognise the well-established relationship between µg immunologically active HA in the vaccine, levels of SRH antibody generated in the target species and protection against challenge infection. They operate on the principle that if a vaccine has been licensed according to the European Pharmacopoeia standards, which also use these relationships in their requirements for potency and efficacy testing, and that in the process of updating a vaccine strain no other parameter of the vaccine is changed, then manufacturers are only required to demonstrate safety and the ability of the final product to generate protective levels of antibody in the target species against the new strain. This obviates the need for challenge studies and generation of duration data, significantly reducing the testing required to license the updated vaccine.

International considerations for vaccine strain selection and standardisation of licensing procedures

The majority of vaccines are made in the USA or Europe, and efforts are ongoing to harmonise licensing procedures between the European Pharmacopoeia and the USDA.

A series of WHO/OIE consultations have been held to work towards international harmonisation of vaccine standards (45). In recent years, challenge tests have been accepted by the USDA as useful for efficacy and a document is now under review to provide a fast track licensing system for updating strains for vaccines produced in the USA (70).

Influenza of other species

The same basic principles apply for influenza of other species, but control processes other than vaccination may be more suitable. The relevance of antigenic diversity has been examined for swine influenza vaccines (72) and good cross-protection has been demonstrated between divergent strains. This has been attributed to the use of very potent adjuvants in swine vaccines which may compensate for antigenic differences. Thus, to date, vaccine strain selection has not become an important issue for swine influenza.

With the recent outbreaks of H5 and H7 avian influenza there is an increasing interest in vaccination as a method of control to avoid massive slaughter of infected flocks. The main aspect of genetic variation studied in avian influenza has been the switch to highly pathogenic virus from viruses with low pathogenicity of the same serotype. However, there have been some recent reports of antigenic drift occurring under the immune pressure of vaccination (35). Therefore, it is likely that if vaccination becomes widely used to protect poultry against avian influenza more attention to strain evolution and vaccine strain selection will be required.

Foot and mouth disease virus

Introduction

The potential impact of antigenic diversity on the control of FMD is well recognised, however, the task of setting up adequate response systems is enormous and needs to take account of the number of serotypes and subtypes, host range, political and socio-economic constraints. Since the 2001 outbreak of FMD in Europe caused by serotype O, there have been renewed efforts to improve the procedures in place for surveillance of FMD on an international scale. These include collection and submission of viruses to reference laboratories, and development of the scientific and technical approaches to examining antigenic diversity among FMDV strains and assessment of its relevance for vaccine strain selection. These issues have been examined in a number of reviews on FMDV vaccines (17, 18) and vaccine strain selection (54) and are addressed in the most recent foot and mouth disease chapter in the OIE *Terrestrial Manual* (Chapter 2.1.1.). The importance of having an early warning system for emergence of variant strains is well recognised. The ability to rapidly analyse new viruses and measure their antigenic relatedness to existing vaccine strains is crucial to providing effective vaccines used in rapid response control programmes and for laying down new viruses in vaccine banks.

Genetic and antigenic variability

The molecular basis of antigenic variation in FMDV has been extensively studied and it is well known that FMDV exhibits a high degree of genetic and antigenic variation (21). As with other RNA viruses such as influenza, this high level of variation is attributable to the error-prone replication of viral RNA and the lack of a proof-reading mechanism associated with the viral replicase (20, 66). Thus, mutations are constantly being produced in progeny viruses and subsequently selected for or against as the virus is transmitted within a population, depending on whether the mutations are beneficial for virus survival (29, 38). There are 7 serotypes of FMDV known as O, A and C (historically regarded as European types), Asia-1, and SAT 1, 2 and 3 (from the South African Territories) (6, 75). Within each serotype there are varying degrees of diversity with subtypes recognised in some serotypes. There is a particularly high diversity among SAT 1 and 2 viruses which has been ascribed to generation of variants in persistently infected buffalo (75). Antibody generated by infection or vaccination against one serotype fails to cross-protect against all other types. Furthermore, antigenic differences within a serotype may be so great that there is little or no cross-protection between strains of the same serotype (3).

During infection some mutations are selected under the influence of immune pressure (10), while others become fixed even in the absence of immune pressure (19, 64). This viral evolution can occur in distinct populations of susceptible animals in separate geographic locations (Fig. 6) (77), resulting in the maintenance and evolution of distinct lineages within an FMDV virus serotype (40, 75). These so-called topotypes are an important feature of FMDV as they may have significantly different antigenic characteristics which could impact on vaccine efficacy (Fig. 7) (60).

Virus structure and antigenic sites

Foot and mouth disease virus is a small non-enveloped positive-stranded RNA virus belonging to the *Picornaviridae* family. The single-stranded RNA is comprised of a large open reading frame (ORF) encoding a single polypeptide which undergoes proteolytic cleavage to form non-structural proteins involved in virus replication and four structural proteins (VP1, VP2, VP3, VP4) which

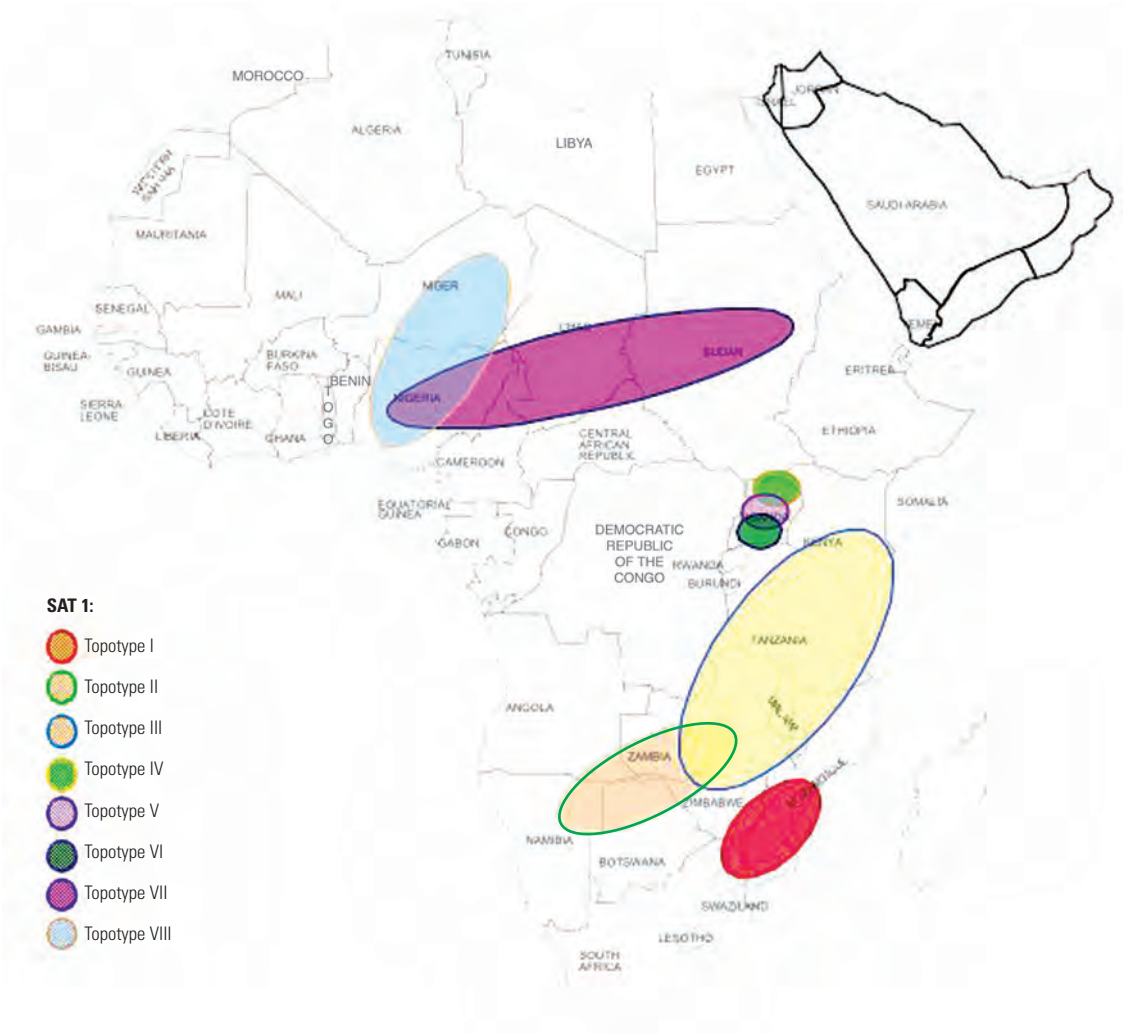

Fig. 6
Location of different topotypes of SAT 1 strains of foot and mouth disease in Africa
Source: W. Vosloo

are incorporated into the virus capsid. VP1, VP2 and VP3 are exposed on the viral surface and carry major antigenic sites. An important cell attachment site with a conserved structure is located between variable regions on the highly immunogenic loop of VP1 which protrudes from the capsid surface. This region is capable of eliciting neutralising antibody and its variable nature leads to both intra- and inter-typic antigenic variation (73). Some other epitopes (or antigenic sites) are dependent on the tertiary structure of the virus particle (41) and are only present in the intact virus known as the 146S particle (named on the basis of its sedimentation coefficient). Additionally, different FMDV types are able to attach to different cell types using a range of cellular receptors (25) and different host species may preferentially recognise different antigenic sites (1). Thus, the virus epitopes involved in attachment to cells and virus neutralisation are complex.

Foot and mouth disease virus vaccines and vaccine banks

Foot and mouth disease virus vaccines are generally purified inactivated whole-virus particles combined with adjuvants (their production and use is reviewed by Doel [17, 18] and Ahl *et al* [2]). During the manufacturing process the antigenic content of the vaccine is measured as the amount of 146S particles. Following inactivation and combination with adjuvant, potency is measured in terms of ability to generate virus neutralising antibody, with the ultimate test of efficacy being challenge infection of vaccinated cattle with a challenge virus homologous with the vaccine virus. While there is some data on the relationship of antigenic content, antibody responses and protection against infection, it has not been possible to describe these relationships for all the serotypes and

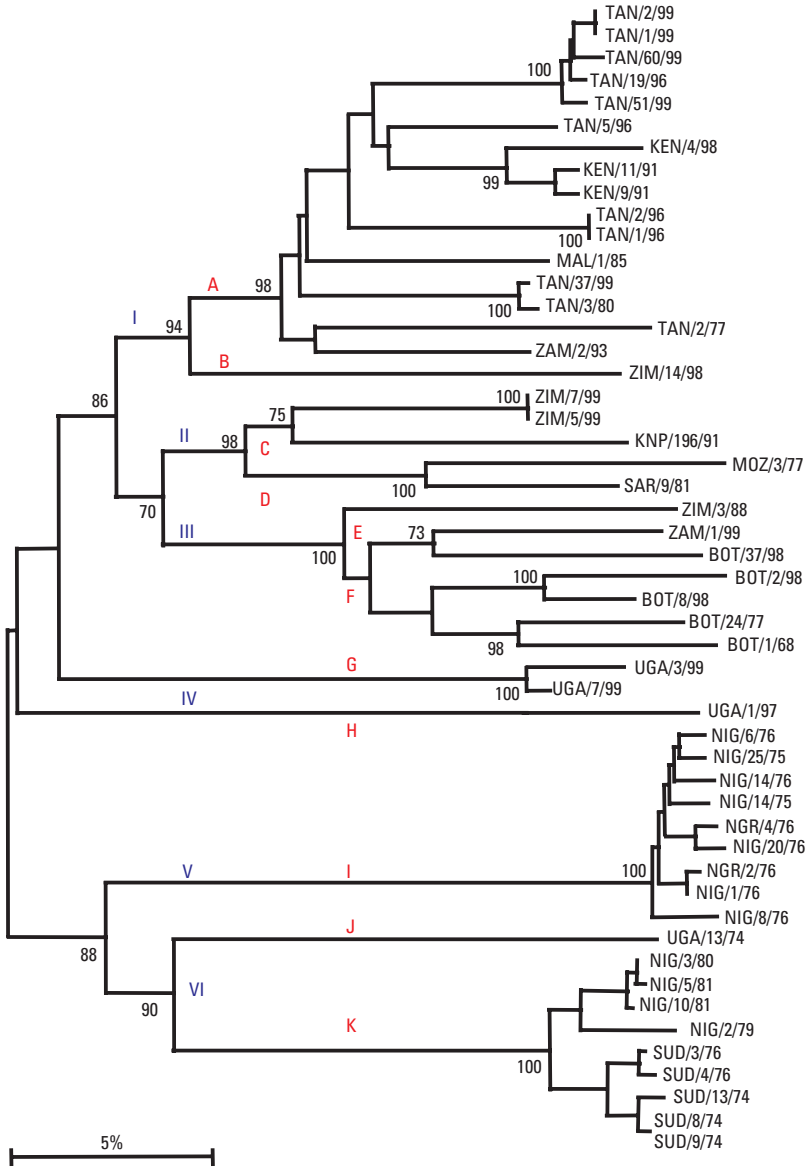

Fig. 7
Phylogenetic tree of SAT 1 viruses isolated in East Africa between 1971 and 2000
Source: W Vosloo

subtypes within them. There are international standards for potency recommended by the OIE and these relate to normal routinely used vaccines. Vaccines are manufactured and supplied by local laboratories around the world as well as by multinational companies, and depending on the source of vaccine there is more or less adherence to recommended standards. Efficacy under field conditions is highly variable depending on quality and potency of vaccines, strain matching tests and species infected. However, modern vaccines properly standardised are reported to be efficacious (5).

As well as vaccines designed for routine use there is a requirement for stockpiles of emergency vaccines in vaccine banks maintained in disease-free countries such as those in Western Europe, North America and Australasia (4, 24). The vaccines are stored as a safeguard against incursions of disease against which the population will have no immunity. Since it is not possible to predict which serotypes may cause an outbreak, it is desirable for vaccine banks to store a full spectrum of serotypes and subtypes to respond to any potential eventuality. These vaccines are stored as virus concentrate over liquid nitrogen and in an

emergency are diluted to concentrations higher than normal vaccines as the aim is to arrest spread of infection with a single dose. Understanding the impact of strain diversity between vaccine strains and field strains is very important for predicting the likely contribution of emergency vaccination strategies to the eradication of the infection. There is also an important interplay between vaccine potency and strain diversity, as highly potent vaccines containing a heterologous strain may be as effective in control as a well matched vaccine strain in a low potency vaccine and at present there is little data to inform governments of the best vaccines to select in a crisis.

Vaccine strains

Vaccine strains are selected on the basis of a number of characteristics, but good growth characteristics and the ability to elicit an antibody response which is broadly cross-reactive within a subtype are the most important (17). This is a major challenge for vaccine manufacturers globally, but particularly for the providers of vaccine banks which hold a range of vaccines or vaccine concentrates to enable disease-free countries to respond to incursions of FMDV with vaccination programmes.

Epidemiology of foot and mouth disease and the use of vaccines

Inactivated vaccines are in routine use in some regions where FMDV is endemic and the virus types included in the vaccines reflect those which are prevalent in the region. In general the SAT1, SAT2 and SAT3 types have been restricted to sub-Saharan Africa, with only occasional incursions into North Africa and the Middle East. Serotypes O, A and C have also been reported from the African continent (Fig. 8). In South America there have been intensive efforts to eradicate FMD through a vaccination policy, but type O and A viruses continue to be isolated (3), and there has been a need in recent years to modify vaccine strains in response to a variant virus of the A serotype. In Asia there are large unmonitored reservoirs and types O, A and Asia 1 are endemic in some regions (62). In South Eastern Europe types O and A, and occasionally Asia 1, have also been reported in recent years (61). Of particular note is the dramatic spread of type O (pan-Asian lineage), which was first reported from Northern India, but spread east to Taipei China and west to the Middle East and the Balkans. Eventually it was shipped to South Africa in 2000 and reached Europe in 2001.

The ability for this virus to spread rapidly through populations and to be transported in the form of contaminated products is a clear indication of the importance of horizon scanning as part of a preparedness policy (59). It is essential to maintain an awareness of current virus types and the strains within those types which are circulating and this is a major challenge given the diversity of strains even within one continent (Fig. 8) (76). A cornerstone of effective vaccination programmes to control and eradicate the disease in endemic areas and to prevent incursions into normally disease-free areas is the use of vaccines containing strains that are well matched to the outbreak strains. The huge logistical problems to achieving this on a global scale are reviewed by Paton *et al.* (54). In some regions such as South America there are well coordinated surveillance programmes and vaccine strain selection systems, whereas in other regions there is little attempt to monitor circulating strains or submit viruses to national or reference laboratories for characterisation.

Initial characterisation and selection for vaccine matching tests

Isolates collected from around the world are submitted to OIE reference laboratories and the Food and Agriculture Organization (FAO) World Reference Laboratory for foot and mouth disease (Institute of Animal Health [IAH], Pirbright, UK) for identification, genetic analysis and serological typing. Identification is normally achieved by enzyme-linked immunosorbent assay (ELISA) with a panel of type specific antisera. Sequencing of part of the VP1 gene allows comparison with other viruses already typed and submitted to the database (33). This comparison very often allows the origin of the outbreak strain to be located as virus topotypes can be identified in this way. As an example there are at least 8 topotypes of serotype O. This data can give an indication of whether the virus strain submitted has been isolated before or whether it is unusual and warrants vaccine matching tests, given the high mutation rate and consequent variable nature of FMDV, where possible several isolates from the same outbreak are characterised and submitted for vaccine matching.

Foot and mouth disease virus international surveillance and virus typing

In parallel with genetic studies cross-neutralisation tests with reference sera that have been prepared to previously characterised viruses are conducted to examine the cross-reactivity between outbreak strains and the available vaccine strains. ELISA tests are also used to examine antigenic relationships. The purpose of this exercise is to identify the virus type and ascertain whether the isolates are closely related to currently held vaccine strains of the relevant type or are antigenically distinct. As already mentioned, it is important, particularly for procurers of vaccines, to appreciate that within a single type there may

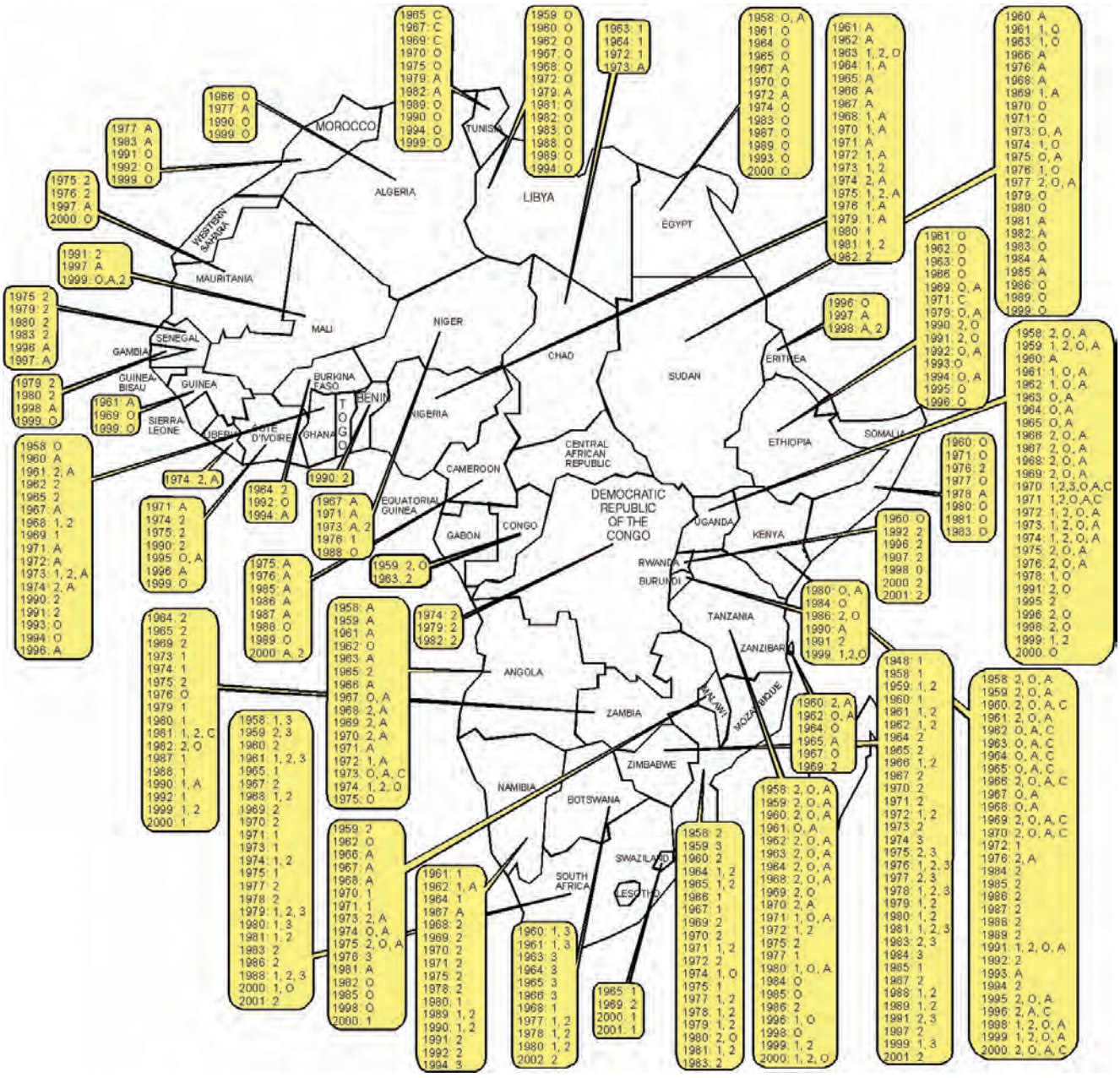

Fig. 8
Location of foot and mouth disease outbreaks and their serotypes recorded in the African Continent

be a wide spectrum of strains, some of which barely cross-react and therefore would not cross-protect.

Laboratory tests used internationally to characterise viruses and match them to vaccine strains

As a result of the independent regional efforts to address the problem of vaccine strain selection and disparate approaches used by local and multinational vaccine manufacturers, a number of tests have been developed for comparing field isolates with vaccine strains. These include the calculation of R values (relationship values) from serological cross-reaction studies using VN, complement fixation and ELISA tests to compare the reactivity of outbreak and vaccine strains with antisera to vaccine virus (54). Additionally, in South America this approach of comparing vaccine and field viruses serologically has been refined by using sera from vaccinated cattle which were subsequently challenged, thus allowing a prediction of protection to be made based on the serological cross-reactivity (54). The major drawback with all these tests is that there has been little standardisation or harmonisation

of techniques and reagents or provision of international reagents for standardisation. Furthermore, there is only very limited data from cross-protection studies using emerging viruses as challenge viruses against heterologous vaccine strains. Thus, interpretation of such data in terms of vaccine efficacy in a field situation remains uncertain.

Future initiatives

It is clear that with increasing international trade and travel, FMDV has the means by which to spread rapidly around the world. It is essential, particularly for the disease-free regions, to maintain effective horizon scanning so that they are prepared for the emergence of new strains.

To date, there has been no internationally coordinated programme of collection and review of FMDV isolates as is conducted for equine influenza. However, the laboratory at the IAH, Pirbright, which is the FAO World Reference Laboratory for foot and mouth disease, has characterised many viruses from around the world. Other laboratories have played similar roles at a regional level. It has been recognised that to respond to the challenges of the strain diversity of FMDV these resources need to be pooled (54).

Following the 2001 outbreak in Europe a 'coordinated action' has been funded by the European Union to enable OIE reference laboratories round the world to create a network of information and reagents in order to harmonise approaches to virus characterisation and comparison with vaccine strains. This will bring together expertise from the UK, South America, Russia and sub-Saharan Africa and provide an opportunity for international harmonisation. The aims are to develop standardised methods of best practice; collect, characterise and archive viruses which represent FMDVs global diversity; exchange reagents and information to facilitate efficient vaccine matching and to report annually to the OIE and FAO.

Orbiviruses: bluetongue and African horse sickness

Structure and variability

Bluetongue and AHS viruses are members of the *Orbivirus* genus in the family *Reoviridae*. They are arthropod-borne (*Culicoides* sp.) viral diseases of ruminants and equidae respectively (14, 74). Orbiviruses have double-stranded RNA segmented genomes and as such have the potential for displaying broad antigenic diversity, as evidenced by the 24 serotypes of BT and 9 serotypes of AHS. As expected, the replication of the RNA genome of orbiviruses is also prone to errors due to lack of a proof-reading polymerase. Diversity is also generated by gene segment swapping during mixed infections (27). However, the rate of evolution in arthropod-borne viruses is lower than in single-host pathogens such as equine influenza and it is hypothesised that it is limited by the alternating host replication cycles (*Culicoides* sp. and ruminants) which demand a compromise in fitness levels to enable the virus to replicate in both vertebrate and invertebrate cells (78).

The 10 genome segments code for seven structural proteins (VP1-7) and three non-structural proteins (NS1-3). VP2 is the major component of the outer capsid and the main antigen responsible for cell attachment and virus neutralisation, although VP5, another component of the capsid, also plays a minor role. There is some cross-protection between serotypes within each virus and this is attributed both to a degree of cross-neutralisation between serotypes with similar VP2 antigenic structures and also to cell-mediated immunity driven by the less variable internal antigens.

The gene segments evolve independently of one another by genetic drift in a host-specific fashion generating quasispecies populations in both ruminants and insects. It has also been shown that random mutations occurring in vertebrate cells may become fixed when ingested by *Culicoides* sp. (9). Thus, there are many complex opportunities for genetic and antigenic diversity.

The genetic diversity of BT has been exploited for epidemiological studies. Analysis of genes coding for the conserved VP3 or the NS3 proteins can be used for geographic typing and tracing (9, 27) whereas the VP2 gene segregates strains according to serotype (8). Nevertheless, in a recent investigation of BT in the Mediterranean Basin complete sequence analysis of the VP2 gene has proved very useful in identifying topotypes within a serotype and in tracing sources of infection (56).

Vaccines and antigenic diversity

Currently, most available vaccines for BT and AHS are classical attenuated vaccines developed by passaging viruses in embryonated eggs (BT) or mice (AHS) and are produced in tissue culture (74). These attenuated vaccine strains are not without risk and their main use has been to control the diseases in sub-Saharan Africa, therefore, knowledge of the impact of viral diversity on vaccine efficacy is limited. The low levels of cross-reactivity between serotypes have been exploited for vaccination against both BT and AHS. Thus, it is not necessary to include all serotypes in live vaccines in order to provide relatively broad protection against a range of serotypes (14, 74). In general, the success of this strategy has been assessed from field rather than experimental studies. The current inactivated vaccine contains serotypes 2 and 4 and

cross-protection against other serotypes has not been reported.

The recent outbreaks of BT, serotypes 1, 2, 4, 8, 9 and 16, in the Mediterranean Basin (28) have focused attention on genetic and antigenic diversity of BT (56) and how it may relate to vaccine efficacy in the field. The use of the live attenuated BT vaccines in Europe and subsequent spread of the vaccine virus has also revealed the potential safety issues relating to live vaccines. The recent spread of BT serotype 8 in northern Europe (42) further focuses attention on appropriate vaccine strategies to respond to changing the epidemiological situation in Europe. Historically there has been much research to develop subunit vaccines as alternative vaccine candidates to both BT and AHS (58, 57) and to explore common antigens between serotypes. However, if inactivated vaccine strategies are pursued, antigenic diversity within and between serotypes will have much greater importance.

While it is recognised that VP2 is highly variable across and within serotypes, it is also recognised that the VP2 genes retain common regions across serotypes which may explain the degree of cross-reactivity observed between some serotypes. Similar observations have been made for AHS (55). The challenge is to assess how important the observed diversity is in terms of neutralisation and protection in the target species. To date, there have been few studies to examine this question. However, it was observed that there was a high homology at the molecular level between Italian isolates and the vaccine strain for BTV-2 (51), which was consistent with observed protection in the field (28, 63). In contrast, there was low genetic homology between the BTV-9 isolated in Italy and the vaccine strain, although cross-protection was demonstrated in a challenge study (G. Savini, unpublished findings). Interestingly, when amino acid sequences, as opposed to nucleotide sequences, were compared there was a higher degree of homology between the two BTV-9 strains. Thus, it appears that important epitopes relating to cell attachment may have been preserved in spite of the propensity for the virus to diversify genetically (56). Also, the observed protection may be in part due to the fact that live attenuated vaccines generate neutralising antibody to a number of surface epitopes on other viral proteins as well as elicit cell-mediated immunity.

Clearly, with the increasing importance of BT (and potentially AHS) in the changing global climatic conditions, there is a need to increase our understanding of vaccine efficacy against intra- and inter-typic variants of these viruses. This will require more cross-protection studies in the target species and analysis of protection in relation to antigenic characteristics.

Summary and conclusions

This article refers to the antigenic diversity of three different types of RNA viruses and briefly reviews its potential significance for different vaccination strategies. Although the genetic basis of virulence has not been addressed in this chapter it is crucial to the understanding of vaccine efficacy given that the immunity provided by vaccines can be overcome if infections are rapid within host or create high virus doses and spread rapidly through populations.

There are obviously many more viruses displaying similar characteristics which are generating intensive research efforts to examine antigenic diversity in relation to control. The appearance of bat lyssaviruses in Europe has initiated efforts to understand the antigenic significance of different lineages with respect to vaccination (48). Similarly, the explosion of infectious bursal disease infections in poultry has created huge interest in this avian birnavirus, where it is essential to understand the relative contribution of changes in virulence and antigenicity to the epidemiology of the disease (30, 71).

Ribonucleic acid viruses will remain an enormous challenge in disease control as new variant viruses emerge. However, prospects of responding more effectively are increasing. Collaborations between virologists, computational experts and mathematicians are opening up exciting new opportunities for monitoring viral diversity and predicting likely changes. As genome sequencing becomes a routine and rapid technique it becomes easier to track large numbers of viruses and assess genetic distances between isolates, and, consequently, compiling large databases becomes possible. As genetic data accumulates in parallel with antigenic data it is becoming possible to identify amino acid changes which are silent and those which have significant antigenic impact. Such studies are already ongoing for influenza and where profound changes in antigenicity of the HA have been associated with single amino acid substitutions, the causal nature of the observations are being examined using reverse genetics.

The development of microarray-based identification of antigenic variants of FMD virus provides prospects for speeding up the analysis of antigenic variation among large numbers of strains and, eventually, of vaccine strain selection (39).

To date, antigenic analysis of FMD viruses has relied on examination of R values based on VN tests or ELISA, and analysis of influenza has been based on the examination of cross HI data. The development of a sophisticated computational method called antigenic cartography (65) for measuring antigenic distances between strains has provided a step change in the way epidemiological data for

human influenza is reviewed annually and vaccine strains selected. This approach can provide a multidimensional image of the antigenic distances between viruses, how they cluster and the direction of their evolution. It has great potential for other viruses requiring this process of review and selection. It can be applied to historical data of serological reactions between viruses and sera used to compare strains. When linked with challenge data demonstrating protection by vaccines, as is possible for equine influenza, antigenic cartography is providing real insight into the important antigenic changes affecting cross-protection.

Clearly, success in this field will depend on multidisciplinary teams including clinical virologists, epidemiologists, molecular biologists and mathematicians to exploit the new opportunities available.

Les vaccins et la variabilité antigénique des virus

J.A. Mumford

Résumé

La variabilité antigénique des virus à acide ribonucléique (ARN) est le résultat de la mutation rapide qui intervient lors de la réplication et de la recombinaison/réassortiment de matériel génétique de souches apparentées, pendant une co-infection. Les souches variantes bénéficiant d'un avantage sélectif en termes de capacité de se propager ou de contourner l'immunité de l'hôte s'établissent au sein des populations. Le virus de l'influenza, le virus de la fièvre aphteuse et le virus de la fièvre catarrhale du mouton sont des exemples de virus présentant une variation antigénique. Pour être efficaces contre ces virus, les stratégies de vaccination doivent s'accompagner de programmes de surveillance visant à détecter les sérotypes en circulation et à retracer leur évolution afin d'assurer un parfait appariement entre les souches vaccinales et les souches sauvages. Sous les auspices de l'Organisation mondiale de la santé animale (OIE), un dispositif de sélection de souches vaccinales du virus de la grippe équine a été mis en place, fondé sur un programme international de surveillance. Un cadre réglementaire autorise désormais la réactualisation rapide des souches vaccinales sans qu'il soit nécessaire de fournir toutes les données d'enregistrement de ces vaccins réactualisés. La fièvre aphteuse fait l'objet d'une surveillance rigoureuse partout dans le monde, recourant à la caractérisation antigénique et génétique des isolats, mais il n'existe aucun système formel de sélection des souches vaccinales. Une initiative a été entreprise à l'échelle internationale pour harmoniser les méthodes de caractérisation des virus, dans le but d'établir la base d'un futur système d'appariement des vaccins vis-à-vis de la fièvre aphteuse, accepté sur le plan international et soutenu par l'OIE. En raison de l'émergence et de la propagation de la fièvre catarrhale du mouton en Europe, l'évaluation de l'innocuité et de l'efficacité de vaccins contre cette maladie a été intensifiée, notamment en ce qui concerne la protection croisée vis-à-vis de chaque sérotype et entre sérotypes. Le principal critère pour produire des vaccins dirigés contre des virus présentant une variabilité antigénique est de disposer d'une méthode permettant de mesurer la distance antigénique entre les souches et de mieux appréhender les relations entre ces distances et les mécanismes de protection croisée. Une nouvelle méthode de modélisation informatique permettant de chiffrer la distance entre souches, appelée cartographie antigénique, a été appliquée aux virus de la grippe humaine et équine dans le but d'élucider l'évolution de ces

virus par rapport aux souches vaccinales. Cette méthode est parfaitement applicable à d'autres agents pathogènes présentant une variabilité antigénique, tels que le virus de la fièvre aphteuse.

Mots-clés
Cartographie antigénique – Fièvre aphteuse – Fièvre catarrhale du mouton – Grippe – Protection croisée – Sélection de souche vaccinale – Sérotype – Surveillance – Topotype – Variabilité antigénique.

Vacunas y variabilidad antigénica de los virus

J.A. Mumford

Resumen
Las rápidas mutaciones originadas por la replicación y recombinación/reordenamiento de material genético de cepas afines en infecciones simultáneas provocan la variabilidad antigénica de los virus ARN. Aquellas variantes cuya ventaja selectiva les permite propagarse, o evitar la inmunidad del huésped, se establecen en las poblaciones. Entre los virus que presentan variabilidad antigénica pueden mencionarse los responsables de la influenza, la fiebre aftosa y la lengua azul. Para que la vacunación contra esos virus sea eficaz es preciso recurrir también a programas de vigilancia de los serotipos circulantes y su evolución a fin de asegurarse de que las cepas vacunales neutralizan a los virus de campo. Se ha establecido un sistema oficial de selección de cepas vacunales contra la influenza equina, bajo los auspicios de la Organización Mundial de Sanidad Animal (OIE), basado en un programa de vigilancia internacional. Ese marco reglamentario permite actualizar rápidamente las cepas vacunales sin necesidad de presentar todos los datos para obtener la autorización de comercialización de la vacuna actualizada. Si bien la fiebre aftosa es objeto de una estrecha vigilancia en todo el mundo, caracterizándose los antígenos y genes de las muestras, aún no se dispone de un sistema oficial de selección de cepas vacunales. Con el apoyo de la OIE, se ha dado inicio a una iniciativa internacional conjunta para armonizar los métodos de caracterización de virus y echar los cimientos de un sistema de comparación de cepas vacunales contra la fiebre aftosa aceptado internacionalmente. La aparición y propagación de la lengua azul en Europa condujeron a intensificar la evaluación de la inocuidad y eficacia de las vacunas, en particular, la protección cruzada contra cada serotipo, y entre ellos. La condición más importante para producir vacunas contra virus que muestran variabilidad antigénica consiste en recurrir a un método de medida de las distancias antigénicas entre cepas y comprender la relación entre esas distancias y la protección cruzada. La cartografía antigénica, un nuevo método informático para medir las distancias antigénicas entre cepas, se ha aplicado a los virus de la influenza humana y equina con objeto de estudiar la importancia de su evolución en relación con las cepas vacunales. Este método puede aplicarse muy fácilmente a otros importantes agentes patógenos que presentan variabilidad antigénica, como el virus de la fiebre aftosa.

Palabras clave
Cartografía antigénica – Fiebre aftosa – Influenza – Lengua azul – Protección cruzada – Selección de cepas vacunales – Serotipo – Topotipo – Variabilidad antigénica – Vigilancia.

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Les vaccins face à la diversité antigénique des bactéries

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Résumé

Les agents pathogènes bactériens ont élaboré tout un éventail de stratégies anti-immunes pour surmonter à la fois l'immunité innée et l'immunité acquise de leurs hôtes. Ces stratégies jouent un rôle crucial dans la capacité des agents pathogènes à provoquer la maladie et rendent compte des difficultés rencontrées lors du développement de vaccins et de la lutte contre ces micro-organismes. L'un des principaux problèmes réside dans le fait que les bactéries possèdent un niveau élevé de diversité antigénique. Pour faire face à cette variabilité, que l'on commence à bien connaître grâce au séquençage des génomes bactériens, les stratégies vaccinales consistent à utiliser soit plusieurs variants d'une (ou de plusieurs) protéine(s) apte(s) à induire des anticorps protecteurs, soit des protéines (ou des fragments protéiques) ou des épitopes relativement bien conservés, notamment du fait de leur implication dans le métabolisme de l'agent pathogène. L'approche la plus élaborée fait appel à la vaccinologie inverse « pan-génomique », qui analyse le profil protéique comparé d'un grand nombre d'isolats de diverses souches d'une même espèce, afin de mettre en évidence les protéines exprimées en surface présentes dans tous les isolats. Parmi ces protéines, celles qui sont exprimées lors de la transmission à l'hôte sont ensuite évaluées afin de déterminer leur capacité d'induire une protection immunitaire. À ce jour, cette approche a été utilisée avec succès contre des bactéries en médecine humaine et la voie est ouverte pour son application en médecine vétérinaire, grâce aux progrès accomplis dans le séquençage génomique des agents pathogènes d'importance vétérinaire.

Mots-clés

Bactéries pathogènes – Vaccinologie vétérinaire – Variabilité antigénique.

Introduction

Les surfaces des bactéries sont des structures complexes qui, du point de vue de l'hôte, présentent de multiples cibles antigéniques. L'une des difficultés majeures pour les bactéries consiste à cacher à la surveillance immunitaire cette surface complexe, où interviennent des protéines et des hydrates de carbone, tout en exposant des molécules clés comme les adhésines ou les invasines. Les agents pathogènes qui y parviennent ont élaboré tout un éventail de stratégies anti-immunes pour surmonter à la fois l'immunité innée et l'immunité acquise, qui mettent en œuvre la reconnaissance par des récepteurs immunologiques de surface, la sécrétion de molécules anti-microbiennes effectrices, l'internalisation puis la dégradation par les phagocytes et l'activation tant du système immunitaire humoral que cellulaire (20). Ces stratégies jouent donc un rôle capital dans la capacité des agents pathogènes à provoquer la maladie, et rendent compte des difficultés propres au développement des vaccins et au contrôle de ces bactéries.

L'un des principaux problèmes liés aux infections bactériennes est que les bactéries présentent un niveau élevé de diversité antigénique. De fait, la plupart de ces

micro-organismes possèdent différents sérotypes qui, dans bien des cas, ne confèrent pas de protection croisée. Pour augmenter encore le degré de complexité, des variants sont souvent retrouvés parmi les souches du même sérotype.

Bien qu'une variation des molécules antigéniques soit habituelle d'une souche à l'autre, le terme spécifique de « variation antigénique » se réfère aux changements qui ont lieu au niveau de quelques antigènes appartenant à une même souche, que ce soit pour maintenir une infection en cours ou pour réinfecter des hôtes ayant éliminé une première infection (74). Ce phénomène est toutefois plus communément observé pour les infections virales et parasitaires que pour les infections bactériennes.

L'objectif de la présente revue est d'évaluer succinctement le problème de la diversité antigénique en tant que facteur d'échec de la vaccination. Seront examinées successivement la variation antigénique à l'intérieur d'une espèce bactérienne (les sérotypes), la variation de souche à l'intérieur d'un même sérotype et la variation antigénique proprement dite.

Existence de divers sérotypes au sein d'une même espèce bactérienne

Les antigènes bactériens les plus importants sont ceux qui sont exposés à la surface des bactéries. L'un de ces antigènes majeurs est représenté par la production d'une capsule. Ce mécanisme est utilisé par la plupart des agents bactériens extracellulaires, à coloration de Gram négative et positive, qui circulent de façon systémique dans le corps. Des agents pathogènes affectant l'homme et/ou les animaux, tels que *Streptococcus pneumoniae, Haemophilus influenzae, Escherichia coli* systémique, *Streptococcus agalactiae, Streptococcus suis, Actinobacillus pleuropneumoniae* et d'autres, comptent sur leur capsule pour prévenir le dépôt des anticorps et du complément sur leur surface, échappant ainsi à l'opsonisation et à la phagocytose (19, 66). Pour ces espèces bactériennes, la composition de la capsule polysaccharidique détermine ou participe à la spécificité du sérotype et, la plupart du temps, des anticorps spécifiques de ces polysaccharides capsulaires sont nécessaires pour obtenir une protection.

Les bactéries qui expriment une capsule à leur surface possèdent aussi des adhésines filamenteuses (*fimbriae* et *pili*) qui traversent la surface capsulaire, permettant aux adhésines de se lier aux récepteurs de l'hôte sans dévoiler la surface bactérienne. Chez certaines espèces de bactéries (*E. coli* par exemple), ces structures participent également à la diversité antigénique au niveau d'une surface bactérienne beaucoup plus complexe (17, 41).

Les lipopolysaccharides (LPS) sont une composante majeure des bactéries à Gram négatif et jouent un rôle clé tant du point de vue de l'agent pathogène que de l'hôte. Certains éléments de ces molécules, par exemple le lipide A, sont présents dans la plupart des organismes à Gram négatif et de ce fait jouent un rôle central dans l'activation des récepteurs de l'hôte. Néanmoins, la partie externe du LPS est constituée d'hydrates de carbone très variables, conférant à chaque souche un sérotype particulier (antigène O). Ainsi, diverses souches de la même espèce peuvent réinfecter le même hôte du seul fait de différences dans les antigènes de surface. L'importance de la diversité de ces antigènes est illustrée ci-après avec l'exemple de deux bactéries, l'une à Gram positif, *Streptococcus suis*, et l'autre à Gram négatif, *Actinobacillus pleuropneumoniae*.

Infections à *Streptococcus suis*

Streptococcus suis est l'un des agents pathogènes les plus importants du porc, à l'origine de pertes économiques considérables partout dans le monde. À ce jour, 35 sérotypes ont été décrits en se fondant sur la composition des antigènes capsulaires, avec une prédominance du sérotype 2, qui est le plus virulent (31). *Streptococcus suis* est responsable d'une grande variété de maladies porcines dont la septicémie, la méningite, le syndrome du choc toxique, l'arthrite, l'endocardite et la pneumonie (31). *Streptococcus suis*, particulièrement le sérotype 2, a été décrit comme un agent important de zoonoses touchant les personnes qui sont en contact direct avec des porcs infectés ou des produits dérivés du porc (33). De fait, les cas humains d'infection recensés récemment en Chine, avec un taux élevé de mortalité, étaient directement liés à une épizootie d'infection à *S. suis* chez des porcs (81).

On retrouve *S. suis* partout où l'industrie porcine est importante ; depuis plus de quinze ans, des infections associées à ce micro-organisme sont observées aussi bien dans les exploitations de type traditionnel que dans les exploitations intensives modernes. La présence d'un grand nombre de sérotypes complique le contrôle de l'infection à *S. suis*. Le sérotype 2 est considéré comme le plus important car il prédomine dans nombre de pays (31). Toutefois, la situation peut varier suivant la localisation géographique. Par exemple, le taux de prévalence de ce sérotype retrouvé sur des animaux malades au Canada reste relativement faible (en-dessous de 25 %) (30). Cette situation est très différente de celle observée dans certains pays européens, où le sérotype 2 prédomine en France, en Italie et en Espagne (8, 78). Au Japon, on retrouve

également une prévalence relativement élevée de ce sérotype (28 %) (38).

D'autres auteurs suggèrent que le contrôle des infections à *S. suis* ne devrait pas se limiter au sérotype 2, car la majorité des souches isolées de porcs atteints appartiennent à un plus grand nombre de sérotypes, allant le plus souvent du sérotype 1 au sérotype 8 (22, 30, 32, 38, 60). De plus, certaines souches appartenant à des sérotypes moins communs ont été associées à des cas d'infection sévère. Le sérotype 9 est décrit comme le plus fréquemment isolé en Belgique, aux Pays-Bas et en Allemagne (78), où il est associé au déclenchement de septicémies, de méningites et de pneumonies chez les porcs sevrés (56, 24). Au Royaume-Uni, le sérotype 14 est fréquemment isolé de porcs dont certaines manifestations cliniques et pathologiques ressemblent à celles associées au sérotype 2 (29). De plus, ce sérotype a également été isolé chez l'homme (77). Il convient de noter que plusieurs sérotypes peuvent être présents chez le même animal. Au cours d'une étude sur des porcs (51), il s'est avéré que 31 % d'entre eux présentaient un seul sérotype au niveau des cavités nasales, 38 % en avaient deux ou trois et 6 % en avaient plus de quatre. L'isolement de plusieurs sérotypes chez les animaux atteints a également été décrit.

Bien que nos connaissances sur les facteurs de virulence soient restreintes, le candidat antigénique majeur chez *S. suis* est la capsule, car elle joue un rôle de facteur anti-phagocytaire important (25). En fait, des anticorps dirigés spécifiquement contre la capsule se sont révélés protecteurs parce qu'ils augmentent la mort bactérienne (2, 11). Les anticorps dirigés contre la capsule semblent par conséquent nécessaires pour une bonne protection contre l'infection. Les vaccins disponibles sur le marché sont en fait des bactérines, c'est-à-dire des suspensions de bactéries totales inactivées. Dans certains pays, on utilise aussi des vaccins autologues (ou auto-vaccins), préparés sur le même principe. L'un des problèmes rencontrés avec cette bactérie est la diversité antigénique des divers sérotypes, car la vaccination contre un sérotype ne sera pas protectrice vis-à-vis d'un autre sérotype. Il est donc rare que les vaccins soient réellement efficaces sur le terrain. Pour couvrir cette diversité, certains vaccins autologues sont composés de six sérotypes différents (observations non publiées). Certaines protéines (de surface, extracellulaires, voire de toxines) ont également été utilisées comme immunogènes (31). Bien qu'une certaine protection ait été constatée lors d'infections expérimentales, seule une faible proportion de souches de *S. suis* (et pour très peu de sérotypes), dans des régions géographiques bien déterminées, produisent ces protéines, ce qui rend ces candidats vaccinaux peu prometteurs (31). La difficulté de trouver un antigène protecteur commun à plusieurs sérotypes et plusieurs souches de *S. suis* n'a donc pas encore été résolue.

Infections à *Actinobacillus pleuropneumoniae*

Actinobacillus pleuropneumoniae est l'agent étiologique de la pleuropneumonie porcine, une affection pulmonaire très contagieuse chez les porcs, qui occasionne des pertes économiques considérables pour les éleveurs partout dans le monde. Les manifestations cliniques sont une grave insuffisance respiratoire aboutissant, dans certains cas, à une mort brutale en 24 à 48 heures ou à une infection chronique persistante (27). On reconnaît deux biotypes : le biotype I requiert du nicotinamide adénine di-nucléotide pour sa croissance, tandis que le biotype II, beaucoup moins courant, n'en nécessite pas (27). *Actinobacillus pleuropneumoniae* du biotype I a été divisé en 13 sérotypes et le biotype II en 2 sérotypes, soit au total 15 sérotypes. Des épizooties ont été décrites dans pratiquement toutes les régions d'Europe et en divers endroits des États-Unis d'Amérique et du Canada, en Amérique du Sud, au Japon, en Corée, à Taïwan et en Australie (27). Bien que certains sérotypes soient plus répandus dans certains pays (par exemple le sérotype 2 en Suisse, au Danemark, en France et en Suède et les sérotypes 1 et 5 aux États-Unis, au Canada et au Mexique), il arrive souvent que plusieurs sérotypes soient retrouvés dans une même région. Certains sérotypes, par exemple le sérotype 3, considérés comme peu virulents et sans importance épidémiologique dans certaines régions, pourraient être facteur d'épizootie dans d'autres (10, 15). De nombreuses publications ont donné des informations sur la répartition des sérotypes au niveau d'un pays déterminé (16). Même à l'intérieur d'un pays, la distribution peut être particulière à certaines régions, comme par exemple, la Catalogne en Espagne, où principalement les sérotypes 1, 2, 4, 7, 9 et 11 ont été identifiés, et le Québec au Canada, où les sérotypes 1, 5 et 7 prédominent (16). Il arrive également que divers sérotypes soient trouvés dans une même ferme. En fait, la plupart des troupeaux commerciaux sont infectés avec plus d'un sérotype d'*A. pleuropneumoniae* (26). La répartition des différents sérotypes sur le plan international est particulièrement intéressante comme indicateur de la transmission survenue lors des échanges internationaux d'animaux.

La spécificité de sérotype d'*A. pleuropneumoniae* est déterminée par la capsule, faite d'unités répétées d'oligosaccharides. La capsule est aussi l'élément principal de protection de la bactérie vis-à-vis des défenses de l'hôte. Elle est responsable de l'aspect iridescent caractéristique des colonies sur milieu clair. La composition chimique et la structure de la capsule ont été mises en évidence (57). Elles sont généralement constituées d'unités répétées d'oligosaccharides (sérotypes 5a, 5b et 10), de polymères d'acide téichoïque réunis par des ponts phospho-diesters (sérotypes 2, 3, 6, 7, 8, 9, 11), ou de polymères d'oligosaccharides réunis par des ponts phosphates (sérotypes 1, 4, 12) (57). Les capsules sont chargées négativement du fait des résidus phosphates et acides

carboxyliques, certains étant partiellement O-glycosylés. Il ressort des études de détermination de structures effectuées sur les souches de référence des 12 premiers sérotypes que les capsules diffèrent assez pour que les anticorps dirigés contre cet élément constituent des antisérums pour le typage spécifique (58). Comme pour *S. suis*, la capsule d'*A. pleuropneumoniae* a des propriétés anti-phagocytaires qui protègent la bactérie contre les défenses cellulaires de l'hôte (35, 64). Des mutants dépourvus de capsule du sérotype 5, mais non du sérotype 1, sont facilement détruits par des antisérums porcins normaux, tandis que les souches capsulées résistent à une mort inhérente à l'action du complément (61, 76). La capsule assure la résistance en limitant la quantité d'anticorps et de C9 déposés à la surface bactérienne dans le sérum normal (76).

Les LPS sont des composants structuraux de toutes les bactéries à Gram négatif et constituent un déterminant de virulence. Du point de vue structural, la majorité des LPS comprennent trois régions distinctes : le lipide A, le cœur oligo-saccharidique ou le cétodésoxyoctonate, un sucre spécial à huit carbones, et le polysaccharide O qui est constitué d'unités répétées d'oligosaccharides. Perry et coll. (1, 43, 57) ont réalisé des études structurales sur les chaînes O latérales des souches de référence de chaque sérotype d'*A. pleuropneumoniae*, pour les treize premiers sérotypes. Ces études ont montré que la composition et la structure des chaînes latérales O sont spécifiques pour presque chaque sérotype. Néanmoins, certains sérotypes ont des épitopes communs : c'est le cas des sérotypes 1, 9 et 11, des sérotypes 3, 6 et 8 et des sérotypes 4 et 7 (16). Bien que la capsule soit présente à la surface de ce micro-organisme, d'autres études ont révélé que le LPS peut traverser l'épais matériel capsulaire et atteindre la région la plus externe de la cellule (7). Cette observation est de première importance si l'on considère que le développement d'un vaccin devrait être basé sur des molécules facilement accessibles aux cellules impliquées dans la réponse immunitaire de l'hôte et aux anticorps, durant le processus infectieux. De plus, les LPS jouent un rôle primordial dans les premières étapes de la colonisation bactérienne (36).

De façon générale, les vaccins qui contiennent des cellules bactériennes inactivées (ou bactérines) d'*A. pleuropneumoniae* sont d'usage courant pour contrôler la maladie. Ces vaccins produisent des anticorps dirigés principalement contre la capsule et le LPS. Ils sont capables de réduire la morbidité lors d'une infection par un sérotype homologue mais ils ne peuvent prévenir la maladie ou le développement de l'état de porteur, et ne confèrent pas de protection lors d'une exposition à l'infection avec des souches hétérologues (4, 28). Lorsqu'on vaccine des animaux avec des sérotypes dont les polysaccharides d'antigène O ont en commun certains épitopes, un certain degré de protection croisée a lieu (55). D'autres études ont par ailleurs montré que des porcs pouvaient aisément être réinfectés avec *A. pleuropneumoniae* appartenant à des sérotypes antigéniquement non reliés (54, 62). Dans la recherche d'antigènes protecteurs, un vaccin sous-unitaire renfermant des toxines et des protéines communes à tous les sérotypes a été développé et commercialisé (71). Ce vaccin peut être utilisé dans n'importe quelle ferme, quel que soit le sérotype présent. Cependant, des résultats récents indiquent une faible protection contre le dernier des sérotypes décrits, le sérotype 15 (71).

Diverses souches appartenant au même sérotype : épidémiologie prédictive

Tel qu'évoqué précédemment, des souches appartenant au même sérotype sont parfois différentes. Les méthodes biochimiques et sérologiques ne sont d'aucune utilité pour établir une distinction entre des clones individuels ou des souches, et les antibiogrammes (profils de sensibilité aux antibiotiques) sont, dans ce cadre, d'un intérêt limité. Le génotypage est la méthode courante pour distinguer les souches appartenant au même sérotype. Plusieurs laboratoires font appel à l'électrophorèse sur gel en champ pulsé (PFGE) comme méthode de base, seule ou en association avec d'autres méthodes qui font appel ou non à l'amplification en chaîne par la polymérase (PCR) (18, 44). Des stratégies alternatives d'empreintes génomiques ou de typage incluent des approches par hybridation ou ribotypage (69) ou par analyse avec des enzymes de restriction (13). Les stratégies s'appuyant sur la PCR comprennent l'analyse par amplification aléatoire de l'ADN polymorphe (RAPD) (49) et par amplification de séquences répétitives (rep-PCR) (5). La répartition des séquences ERIC (*enterobacterial repetitive intergenic consensus*) a été évaluée en utilisant des séquences consensus d'oligo-nucléotides dans les essais PCR (75). Les amorces ERIC permettent de générer directement des empreintes génomiques qui sont, sans ambiguïté, spécifiques d'espèces et de souches. La méthode rep-PCR est basée sur l'observation que des couples d'amorces complémentaires des extrémités des séquences répétitives dispersées, permettent l'amplification de fragments d'ADN dont la taille est représentative de la distance entre ces éléments. La séparation par électrophorèse permet d'établir des patrons génomiques spécifiques de souches bactériennes individuelles. Plusieurs de ces éléments répétitifs dispersés se retrouvent chez divers genres de bactéries, ce qui permet d'utiliser le même couple d'amorces pour plusieurs micro-organismes. Les

empreintes génomiques obtenues avec des sondes basées sur les séquences répétitives dispersées permettent de faire la distinction entre des organismes non apparentés, car les distances entre ces séquences sont caractéristiques de souches bactériennes individuelles. Depuis le développement de la rep-PCR, des séquences palindromiques répétitives extragéniques (*repetitive extragenic palindrome* : REP), des séquences ERIC et des séquences BOX (découvertes par B. Martin et coll. en 1992) (46) ont été utilisées pour obtenir les profils génomiques de bactéries à Gram négatif et de bactéries à Gram positif (40, 48, 73, 75). La rapidité des approches par empreintes génomiques bénéficie de la vitesse d'amplification des acides nucléiques et des méthodes de détection, propices à des analyses en temps réel. L'optimisation des réactions de PCR en faisant appel à des réactifs de référence, y compris pour les amorces, a mené au développement de trousses commerciales, avec une reproductibilité et une précision accrues. Ces stratégies moléculaires sophistiquées peuvent être mises à profit pour réaliser des études d'épidémiologie moléculaire et pour contribuer à identifier les bactéries pathogènes.

Ici encore l'exemple de *S. suis* peut être souligné. Il a été montré que jusqu'à six patrons génotypiques de la même souche du sérotype 5 peuvent être mis en évidence chez les truies porteuses de *S. suis* au niveau d'une seule ferme (12). Une hétérogénéité encore plus grande a été retrouvée chez un nombre plus restreint d'isolats provenant des cavités nasales, comparativement à ceux d'origine vaginale. Cependant, un seul clone a été associé aux cas cliniques, car tous les isolats provenant des animaux atteints ou morts (avant ou durant l'étude) se sont révélés appartenir au même génotype. Un an plus tard, aucune caractéristique distincte de ce patron particulier associé à l'infection n'était détectée dans la ferme, en l'absence de signes cliniques (12). D'autres études portant sur des souches de sérotype 2 ont montré que dans des troupeaux infectés et maintenus en confinement, un seul clone était responsable de la maladie (47, 50, 60, 70).

En dépit du fait que diverses souches provenant du même sérotype sont généralement présentes dans le même troupeau, il est difficile de prédire la protection croisée qui a lieu entre ces souches. Tel qu'évoqué précédemment, lorsque les anticorps dirigés contre la capsule sont importants et que le sérotype est déterminé par la structure de cette capsule, on peut présumer qu'une protection croisée intervient entre les diverses souches du même sérotype. Cependant, pour certaines espèces bactériennes, la protection dépend de divers antigènes de surface qui ne sont pas reliés au sérotype. Dans ces cas, il est difficile de prédire la protection conférée vis-à-vis de souches appartenant au même sérotype mais différentes de celles utilisées dans le vaccin.

Variabilité antigénique chez les bactéries

Bien qu'une variabilité des molécules antigéniques soit courante d'une souche à l'autre, la variabilité antigénique fait référence aux changements affectant spécifiquement certains antigènes au sein d'une même souche ; ce processus permet que l'infection se maintienne ou que l'hôte soit réinfecté, même après l'éradication réussie de la première infection (20). Trois critères doivent être remplis pour que la variabilité soit considérée comme variabilité antigénique (19) :

– les changements antigéniques interviennent dans l'évitement du système immunitaire ou dans un créneau de sélection ;

– il s'agit d'un changement comportant plusieurs phases ;

– le mécanisme relève de la conversion génique.

Les mécanismes moléculaires utilisés par les bactéries pour générer la variabilité antigénique sont multiples (19).

Il est important de noter que la majorité des études ont été menées en utilisant des agents pathogènes pour l'homme. Les mécanismes impliqués relèvent d'un des trois processus suivants :

– la présence de plusieurs copies différentes de la même molécule, chacune d'elle pouvant être activée de façon autonome ;

– la présence d'un locus d'expression associé à plusieurs copies silencieuses d'un même gène, avec un perpétuel changement du choix de la copie du gène exprimé ;

– la présence d'une région très variable d'une molécule qui évolue constamment.

L'espèce *Neisseria* (agent causal de la méningite et de la gonorrhée chez l'humain) est peut-être l'un des meilleurs exemples de la variabilité antigénique chez les bactéries, illustrant les trois concepts évoqués ci-dessus et soulignant les raisons de l'insuccès des vaccins contre ce type d'organismes. Le gonocoque possède 10 ou 11 protéines Opa (pour opacité) de la membrane externe, de profils antigéniques différents. L'expression de chaque protéine Opa dépend du contrôle indépendant de chacun des gènes correspondants. Durant l'infection, plusieurs protéines Opa sont exprimées suivant diverses combinaisons. Le pilus de *Neisseria* a comme composante structurale majeure la protéine piline, qui est l'objet d'une extrême variabilité. La base moléculaire de cette variabilité est l'existence d'un système multi-génique dont les membres sont soumis à une recombinaison intragénique (c'est-à-dire à une recombinaison affectant une partie de gène). Plusieurs gènes silencieux de piline (*pilS*), présents dans le

génome, font don de minicassettes variées au gène situé dans le locus exprimé (*pilE*), et un pilus constamment différent est généré (21). Comme ces organismes sont par nature compétents, ils acquièrent d'autres séquences du gène de piline et les incorporent au niveau des loci *pilS* silencieux. *Neisseria meningitidis* modifie aussi la structure de ses lipo-oligosaccharides (LOS, semblables aux LPS) suivant un mécanisme de variation de phase. La bactérie peut exprimer jusqu'à 13 immunotypes différents par modification de la structure des divers sucres terminaux. Ceci est le résultat du changement d'expression de divers gènes de biosynthèse des hydrates de carbone. Par exemple, l'activité de la glycosyltransférase est régulée suivant un mécanisme de mauvais appariement par glissement de brin d'ADN (*slipped strand mispairing*), ce qui aboutit à une incorporation de sucres variés dans les LOS (59).

Comme cela a été déjà évoqué pour la variabilité des pili, il faut garder à l'esprit qu'un facteur de variabilité antigénique des bactéries est dû à la transmission horizontale d'informations génétiques entre agents pathogènes (80). Trois types de transfert peuvent avoir lieu :

– la transformation, qui implique l'acquisition, par la bactérie, d'un ADN qui se trouve dans son environnement. Depuis la reconnaissance de l'aptitude à la transformation des pneumocoques par Griffith en 1928, celle-ci a été mise en évidence chez d'autres espèces naturellement « transformables », telles que *Haemophilus influenzae, Neisseria gonorrhoeae, Streptococcus pneumoniae* et *Bacillus subtilis*. Le processus de transformation implique que ces bactéries acquièrent un état physiologique de « compétence » grâce à l'expression régulée de certains gènes et que l'ADN étranger présente une homologie de séquence avec un fragment du chromosome bactérien ; cette homologie de séquence permet qu'il y ait recombinaison, puis intégration de l'ADN étranger dans le chromosome bactérien ;

– la conjugaison, qui permet le transfert de gènes entre deux cellules de différenciation sexuelle appropriée. Le transfert d'ADN s'effectue grâce aux pili des bactéries à Gram négatif et des adhésines de surface pour les bactéries à Gram positif ;

– la transduction, qui est un transfert génétique au cours duquel un ou plusieurs gènes sont transmis d'une bactérie donatrice à une bactérie réceptrice par l'intermédiaire d'un bactériophage transducteur. Ces gènes sont portés par le bactériophage à la suite de sa multiplication dans la bactérie donatrice, génétiquement différente de la bactérie réceptrice.

La transmission des plasmides de résistance aux antimicrobiens, par exemple, peut s'effectuer par l'un quelconque de ces mécanismes de transfert horizontal entre pathogènes (80). De ces trois processus, *N. gonorrhoeae*, qui présente une extrême plasticité génomique, n'utilise que la transformation pour un échange horizontal continu de séquences chromosomiques. Le déséquilibre de liaison des gènes qui en résulte est particulièrement marqué pour cette bactérie ; il l'est moins par exemple pour *N. meningitidis*, qui présente un déséquilibre de liaison de gènes intermédiaire entre celui de *N. gonorrhoeae* et celui d'autres bactéries comme *E. coli* ou *Salmonella* (21). L'impact de cette plasticité génomique est par exemple crucial au niveau de la variabilité antigénique des pili. En ce qui a trait à *S. suis*, un transfert horizontal du gène codant pour la suilysine (hémolysine produite par certaines souches) a été mis en évidence lors d'une étude portant sur l'analyse du locus de la suilysine – par PCR et/ou hybridation de Southern – au niveau de 68 souches de *S. suis* (67).

La composition vaccinale a-t-elle un impact sur la prévalence des sérotypes ? Peut-elle influencer l'émergence de nouveaux sérotypes auparavant de faible prévalence ?

Ceci est une question intéressante, à laquelle il n'a pas encore été répondu, du moins en médecine vétérinaire. En médecine humaine, les effets de la vaccination visant un sérotype spécifique sur l'émergence de nouveaux sérotypes sont sujets à discussion. Néanmoins, il y a quelques années, Lipsitch (42) a utilisé un modèle mathématique pour étudier la dynamique de la transmission de deux sérotypes, et plus, de bactéries colonisant une population, en accordant une attention particulière aux effets de la vaccination contre un ou plusieurs sérotypes. Ce modèle prédit que des vaccins spécifiques composés d'un sérotype auront pour effet d'augmenter la prévalence des sérotypes exclus de la composition vaccinale. Cela comprend également l'émergence de nouveaux sérotypes qui au préalable étaient incapables d'entrer en compétition avec le sérotype ciblé. Dans un système à deux sérotypes, l'augmentation de la prévalence de l'un ou l'autre des sérotypes sera toujours moindre que le déclin de la prévalence du sérotype vaccinal ; donc, dans un tel système, le nombre total d'individus indemnes vis-à-vis de l'un quelconque des sérotypes augmentera toujours avec la vaccination. Cependant, dans un système à plus de deux sérotypes (situation plus courante en médecine vétérinaire

de populations), la vaccination spécifique dirigée contre un sérotype risque d'augmenter la proportion des hôtes porteurs d'un des sérotypes non ciblés, plus qu'elle ne réduira la proportion de porteurs du sérotype ciblé.

Quelle stratégie vaccinale ?

Au cours de l'évolution, nombre de bactéries pathogènes sont arrivées à la même stratégie de variation antigénique pour surmonter les défenses de l'hôte. Cette variation antigénique a des conséquences importantes pour le développement de vaccins contre ces pathogènes. Si l'antigène variable est la cible de l'immuno-prophylaxie, le vaccin devrait théoriquement posséder un degré de multivalence pratiquement impossible à obtenir. Pour relever ce défi, deux approches sont envisageables.

La première consiste à restreindre la multivalence en sélectionnant, parmi les variants, ceux qui sont les plus représentatifs. De fait, on constate parfois la prédominance de certaines combinaisons antigéniques, ce qui limite l'ampleur de la diversité antigénique « originelle ». Ce type d'analyse a été mené par R. Urwin et coll. (72) sur la bactérie *N. meningitidis*. Trois protéines de la membrane externe (PorA, PorB et FetA) sont des candidats vaccinaux pour lesquels se pose le problème du choix des variants à inclure dans la préparation vaccinale, compte tenu de la grande variabilité génétique et de la diversité antigénique des populations de méningocoques. Cette équipe a séquencé les gènes des trois protéines d'intérêt sur une collection rassemblant les 78 souches les plus invasives et liées aux maladies les plus endémiques et épidémiques des cinquante dernières années. Il ressort de cette étude qu'il existe une certaine structure d'association de variants antigéniques. C'est ainsi qu'il suffirait qu'un vaccin associe six variants de PorA et cinq variants de FetA pour conférer une protection contre les 78 isolats examinés.

La deuxième approche envisageable consiste à mettre l'accent sur les domaines fonctionnels de la ou des protéines variables. Dans la pathogénèse, les régions variables de la protéine ont d'autres fonctions (l'adhérence par exemple) que la seule évasion immunitaire. Ces régions, assujetties à des contraintes de structure plus strictes, c'est-à-dire codées par des séquences d'ADN mieux conservées, seraient ainsi propices à produire des anticorps à réactions croisées.

En exemple particulièrement parlant des difficultés de mise au point d'un vaccin devant faire face à la variabilité antigénique de la bactérie en cause est celui de *Streptococcus pneumoniae*. Cette bactérie responsable de méningite, de septicémie et de pneumonie chez l'homme est à l'origine d'un million de décès annuels chez les enfants de moins de cinq ans (37). La surface externe de ce pneumocoque est une paroi couverte d'une capsule polysaccharidique, avec plus de 100 sérotypes capsulaires décrits. Les polysaccharides de la capsule sont très immunogènes mais les anticorps produits protègent uniquement contre le sérotype homologue. Certains polysaccharides étant communs, il n'est toutefois pas exclu que des réactions croisées aient lieu. Outre les polysaccharides capsulaires, certaines protéines de surface qui traversent la capsule se sont révélées efficaces pour déclencher une réponse immunitaire protectrice chez des animaux de laboratoire.

Depuis 1911 jusqu'à nos jours, diverses approches vaccinales ont été tentées, qui ont fait l'objet d'une excellente revue par D. Bogaert et coll. (9). Après une première tentative de vaccin à base d'une bactérine représentative d'un sérotype, le premier vaccin constitué de polysaccharides capsulaires s'est révélé assez efficace pour enrayer une épidémie de pneumonie au Massachusetts (États-Unis d'Amérique) en 1931. Ce vaccin s'est vu retiré du marché, supplanté par l'efficacité des antibiotiques. Lorsque la résistance à la pénicilline est apparue, à partir de 1947, les recherches en vue d'un vaccin ont repris et abouti à la production (en 1977) d'un vaccin polysaccharidique 14-valent puis d'un vaccin 23-valent en 1983. Le vaccin Pneumovax 23® (Merck, West Point, États-Unis d'Amérique), renferme 23 antigènes polysaccharidiques capsulaires purifiés, assurant une protection théorique contre 85 % à 90 % des pneumocoques responsables d'infections chez les adultes et les enfants de plus de deux ans. Par contre, ce vaccin n'induit qu'une réponse partielle dépendante des cellules T, ce qui implique une quasi-absence de cellules B mémoires et limite la durée de la protection. Pour augmenter l'immunogénicité, l'étape suivante a donc été un vaccin conjugué, dans lequel les polysaccharides capsulaires sont liés à une protéine porteuse, telle que l'anatoxine tétanique. Le vaccin conjugué Prevenar® (Wyeth, Paris) contient sept variants de polysaccharides capsulaires conjugués à une protéine mutante de toxine diphtérique. Bien qu'aux États-Unis ce vaccin fasse partie du calendrier de vaccinations depuis octobre 2000, certaines études ont mis en évidence l'augmentation de l'incidence d'otites moyennes, qui seraient imputables au phénomène déjà évoqué dans la section précédente, à savoir l'émergence de nouveaux sérotypes non représentés dans la préparation vaccinale (39). Au cours des dix dernières années, les recherches s'orientent vers des protéines de surface, telles que la protéine A de surface (PspA), la pneumolysine, la protéine liant la choline (PspC), la neuraminidase, l'autolysine et l'adhésine A (PsaA). Aucune de ces protéines n'est capable d'induire une protection à large spectre, du fait de l'existence de la variabilité allélique (34). Quelles sont, dès lors, les perspectives vaccinales ? Plusieurs variants d'une même protéine, ou encore la combinaison de plusieurs protéines, ou la conjugaison d'une de ces protéines avec des

polysaccharides capsulaires sont des alternatives pour limiter l'aptitude des pneumocoques à contrecarrer les défenses de l'hôte. L'utilisation, par exemple, de deux protéines ayant des fonctions complémentaires dans la virulence pourrait conférer des rôles additifs de protection (comme PsaA contre la colonisation et PspA contre l'invasion).

Un deuxième exemple de stratégie vaccinale astucieuse pour contourner le problème de la variabilité antigénique est celui qui s'applique dans le cas d'agents pathogènes transmis par des vecteurs. En ce qui concerne la maladie de Lyme chez l'homme, causée par *Borrelia burgdorferi*, il existe une diversité considérable des séquences du gène codant la protéine C de surface externe (OspC) qui définissent les différentes souches (6). Un vaccin basé sur la protéine OspC devrait être multivalent. Ce n'est pas la stratégie qui a été retenue. Le vaccin utilise une seule protéine (OspA), qui est exprimée dans l'intestin du vecteur (la tique), mais avec laquelle l'hôte réceptif ne peut avoir de contact. Pour cette raison sans doute, et du fait que le vecteur ne possède pas de système immunitaire adaptatif, on retrouve peu de divergence entre les séquences de OspA (65). Le vaccin fonctionne apparemment par la production d'anticorps qui inhibent ou détruisent les spirochètes dans les tiques avant que ne soient exprimés des gènes plus polymorphes (tels que OspC) chez l'hôte (14).

Le troisième exemple de stratégie vaccinale est l'approche mise en œuvre pour un vaccin contre *N. meningitidis* du groupe B. Ce sérogroupe est responsable d'environ la moitié des cas de maladies (humaines) dues au méningocoque à travers le monde. Il s'agit du seul sérogroupe pour lequel l'infection ne peut être prévenue par l'utilisation de vaccins capsulaires, car cette capsule est un polymère α(2-8) d'acide N-acétyl-neuraminique, qui est retrouvé sur les tissus humains. Diverses tentatives ont fait intervenir des protéines de membrane externe (Omp), mais c'est l'approche la plus récente (23, 82), qui fait appel à la « vaccinologie inverse », qui sera retenue ici. Le séquençage du génome de *N. meningitidis* a ouvert la voie à l'identification d'antigènes potentiels. À partir du génome et à l'aide de certains algorithmes développés en bioinformatique, il est possible d'identifier et de caractériser les gènes, d'analyser leur localisation et le niveau d'expression des protéines correspondantes (protéomique et transcriptomique). C'est ainsi que 600 antigènes ont été recensés, codant pour des protéines exposées à la surface. La moitié de ces antigènes ont fait l'objet d'expression dans *E. coli*, les protéines recombinantes ont été purifiées et leur immunogénicité a été évaluée. Des 91 immunogènes retenus, 29 se sont révélés être des antigènes protecteurs. Certains de ces candidats vaccinaux sont en cours d'essais cliniques. Cette approche a été suivie pour d'autres agents pathogènes tels que *S. pneumoniae* (79), *Porphyromonas gingivalis* (63), *Chlamydia pneumoniae* (52) et *Bacillus anthracis* (3). Toutefois, pour intégrer le problème de la diversité antigénique des bactéries dans la conception d'un vaccin, on ne peut pas se limiter à une seule séquence. Un concept clé est de faire appel à un « profil » séquentiel multi-génomique, pour intégrer le facteur de variabilité génétique. Ce concept est à la base de la vaccinologie inverse « pan-génomique » (par comparaison avec la vaccinologie inverse « classique » telle que décrite précédemment). Le principe de cette méthode est d'analyser la diversité génétique d'une espèce ; pour ce faire, elle recourt à l'hybridation génomique comparative par rapport à une séquence déterminée et à la comparaison des séquences de plusieurs souches. Cette méthode a été mise en œuvre pour *S. agalactiae, Streptococcus* du groupe B (45, 53, 68) et a permis de restreindre à 396 (sur 589) le nombre des protéines de surface à évaluer, 193 d'entre elles n'étant pas exprimées dans l'une quelconque des souches analysées. Cette approche de vaccinologie inverse « pan-génomique » pourra être appliquée à d'autres agents pathogènes dont le génome aura été séquencé, en incluant ceux qui jouent un rôle important en médecine vétérinaire. Actuellement, plus de 300 génomes bactériens sont séquencés en totalité et plus de 500 sont en cours de détermination. L'un des avantages de la vaccinologie inverse est que la plupart des étapes peuvent être menées en amont des études d'immunogénicité chez l'animal.

■

Vaccine development: strategies for coping with the antigenic diversity of bacteria

M. Gottschalk & S. Laurent-Lewandowski

Summary
Bacterial pathogens have evolved a whole range of anti-immune strategies to overcome both the innate and acquired immunity of their hosts. These strategies play a crucial role in the capacity of pathogens to trigger disease and also explain why it is so difficult to develop vaccines and to control these micro-organisms. One of the main problems is that bacteria are highly antigenically diverse. The vaccination strategies for coping with this variability, which we are starting to understand more fully as a result of sequencing bacterial genomes, consist of using either several variants of one or more proteins capable of inducing protective antibodies, or else proteins (or protein fragments) or epitopes that have been relatively well preserved notably because they are involved in the pathogen's metabolism. The most sophisticated approach calls upon 'pan genomic' inverse vaccinology which compares the protein profiles of a large number of isolates from various strains of a single species in order to reveal the surface-expressed proteins present in all the isolates. Of these proteins, the ones which are expressed when the host is infected are then evaluated in order to determine their capacity to induce a protective immune response. So far this approach has been successful in controlling bacteria in humans and the way is now open for its application in veterinary medicine, thanks to progress with the genomic sequencing of pathogens of veterinary importance.

Keywords
Antigenic variability – Pathogenic bacteria – Veterinary vaccinology.

Las vacunas ante la diversidad antigénica de las bacterias

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Resumen
Los agentes patógenos bacterianos han elaborado todo un arsenal de estrategias para luchar contra la inmunidad, tanto innata como adquirida, de los organismos que infectan. Tales estrategias, que son un componente básico de la aptitud de dichos patógenos para provocar una enfermedad, explican las dificultades existentes a la hora de fabricar vacunas y de luchar contra esos microorganismos. Uno de los principales problemas estriba en la gran diversidad antigénica que presentan las bacterias. Las estrategias de vacunación para combatir esta variabilidad, que empezamos a conocer bien gracias a la secuenciación de genomas bacterianos, consisten en utilizar: bien distintas variantes de una (o varias) proteína(s) susceptible(s) de inducir una respuesta de anticuerpos protectores; o bien proteínas (o fragmentos proteicos), o epitopos relativamente bien conservados, sobre todo porque intervienen en el metabolismo del patógeno. Los métodos más elaborados son los que recurren a la vacunología inversa "pangenómica", procedimiento que consiste en analizar

y comparar el perfil proteínico de un gran número de muestras de varias cepas de una misma especie a fin de determinar las proteínas de superficie que están presentes en todas ellas. A continuación, de entre todas esas proteínas, se analizan y evalúan las que se expresan cuando la bacteria infecta al huésped, con objeto de determinar su capacidad de inducir una respuesta inmunitaria protectora. Hasta la fecha, este método ha sido utilizado con éxito contra bacterias que infectan al hombre, y, gracias a los progresos realizados en la secuenciación genómica de patógenos de importancia veterinaria, la vía está expedita para aplicarlo en medicina veterinaria.

Palabras clave
Bacteria patógena – Vacunología veterinaria – Variabilidad antigénica.

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Control of parasitic disease using vaccines: an answer to drug resistance?

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Summary

Antiparasitic drugs have been used successfully to control parasitic diseases in animals for many years, as they are safe, cheap and effective against a broad spectrum of parasites. One drawback of this success appears to be the emergence of drug resistance in many target parasites. Moreover, issues of residues in the food chain and environment have arisen, which threaten their sustained use. Control methods in which vaccines would have a central role provide attractive alternatives. However, while attenuated parasite vaccines have been successful, sub-unit vaccines are still rare. The advent of new techniques in molecular biology allows the elucidation of entire parasite genomes and the identification of individual genes. It is envisaged that a further understanding of parasite genes and the role of their products in parasite biology may lead to the identification of useful antigens, which could then be produced in recombinant systems. However, for this aim to be realised, continued investment in basic research on the complex interplay between parasite and host will be necessary.

Keywords

Antiparasitic drug – Control – Drug resistance – Host – Parasite – Parasite vaccine – Residue – Vaccine.

Introduction

Until now, chemotherapeutic drugs have predominated over vaccines in the prevention and treatment of parasitic disease in livestock and companion animals (63). Traditionally, a *therapeutic cure* was sought for diseased animals and people, an approach which is still reflected in traditional medicine. The realisation that disease could be *prevented* (e.g. through such measures as hygiene) developed much later, and the principle of vaccination was systematically exploited only from the beginning of the 20th Century. When chemical industries expanded in the second half of the last century, a series of chemical compounds were developed to protect crops. A number of these compounds were also tested in screening assays for antiparasitic activity, and highly effective compounds were further developed as parasitic drugs. In contrast, the science of immunology, which provides the basic knowledge for the development of vaccines, was only defined as a discipline in the mid-1900s. Although there has been a continuous flow of vaccines to the market, the number of antiparasitic vaccines has remained low (63). This is a point of concern, in light of the alarming increase in drug resistance among different parasite species. In this review, the authors discuss the opportunities and obstacles in the development of antiparasitic vaccines. Together with drugs and other management practices, such vaccines could form part of an integrated strategy to control parasitic disease.

Resistance against antiparasitic drugs

In almost every use of antiparasitic drugs, the emergence of resistant strains has been reported. It is not known whether resistance is induced by the drug or whether the use of that drug leads to the selection of resistant strains that were present in the initial population. Whatever the case, the net result is the occurrence of drug-resistant parasite strains. Resistance has been reported among endoparasites (from unicellular protozoa to multicellular metazoa) as well as ectoparasites (Table I).

Resistance to coccidiostatic drugs among *Eimeria* parasites, which infect chickens, is widespread. To delay further development/selection for resistance, alternating rotation and shuttle programmes, using different coccidiostatic drugs, have been implemented (58). Drug resistance is now reported in *Trypanosoma* (19) and resistance to the anti-babesial drug, diminazene, has been implied in a survey of canine babesiosis in South Africa (7), while resistance to anti-malarials in humans is long established.

Anthelmintic resistance is widespread in the gastrointestinal nematodes of sheep and goats. The efficacy of the three major classes of anthelmintics against *Haemonchus contortus*, the most important gastrointestinal sheep nematode, has fallen to disastrous levels (25). Worryingly, drug resistance is now also prevalent in *Teladorsagia circumcincta* in sheep, *Cooperia* spp. and *Trichostrongylus* spp. in cattle (26). In horses, benzimidazole resistance is increasingly recognised as a problem that requires careful management of anthelmintic use (6).

Table I
Reported emergence of parasite resistance to drug treatment

Parasite	Host	Compound
Eimeria species	Poultry	Chemical drugs, ionophores
Trypanosoma brucei	Cattle	Diminazene, isometamidium
Trypanosoma congolense	Cattle	Diminazene, isometamidium
Babesia rossi	Canines	Diminazene aceturate
Plasmodium falciparum	Humans	Multi-drug resistance
Haemonchus contortus	Sheep, goats	Multi-drug resistance*
Teladorsagia circumcincta	Sheep	Multi-drug resistance*
Trichostrongylus species	Cattle	Benzimidazoles, levamisole, macrocyclic lactones
Cooperia oncophora	Cattle	Benzimidazoles, macrocyclic lactones
Small strongyles	Horses	Benzimidazoles
Boophilus microplus	Cattle	Multi-drug resistance
Lucilia cuprina	Sheep	Organophosphates
Psoroptes ovis	Cattle, sheep	Organophosphates, pyrethroids
Ctenocephalides felis	Canines, felines	Carbaryl, chlorpyrifos, malathion, pyrethroids

* Combined resistance to benzimidazoles, levamisole and macrocyclic lactones

In ectoparasites, multi-drug resistance has been reported in *Boophilus microplus* ticks. It has also been shown that these ticks have a reduced sensitivity to the older acaricides, organophosphates, synthetic pyrethroids and amidines. Resistance against the newer acaricide, ivermectin, has been reported in Brazil (33) and suspected in Colombia. In addition, resistance against organophosphates has been found in the sheep blowfly (*Lucilia cuprina*) in Australia.

The intensive use of antiparasitic drugs also increases the risk of drug residues in animal products (13). There is now considerable public concern about such residues, as demonstrated by increasing consumer demand for organic food products (17). Although it can be scientifically argued that such consumer concerns are overstated, their commercial impact is real.

Antiparasitics and their metabolites also accumulate in the environment through animal excretion. Although the environmental impact is not high (3, 57), this has been highlighted as a major source of public concern by the Organisation for Economic Co-operation and Development (OECD) (40, 42). Clearly, current parasite control strategies are not sustainable, and preventing these infections must become the objective. To comply with the requirement for prime quality animal products, farmed in a way that is minimally harmful to the environment, immunological control of these infections is the most rational way forward. The World Health Organization, the Food and Agriculture Organization and the OECD all regard vaccines as among the most cost-effective methods for promoting human and animal health (16, 41, 72, 73).

The prospects for discovering new antiparasitic drugs may be diminished by the increased difficulties of discovery in a time of mechanism-based screening (66). To date, existing drugs have been identified by random screening of existing molecules with no definition of the mode of action. There has been a perception that expanding knowledge, at the molecular level, of how the parasite survives in the host would readily lead to targeted approaches to drug design. However, this approach has proven to be time consuming. Moreover, it has led to the development of more complex drugs (18), with associated increases in production costs, which affect profitability and their adoption ('uptake') by the livestock producer.

Current status of parasitic vaccines

With the advent of recombinant deoxyribonucleic acid (DNA) technology in the early 1980s, there was general optimism that sub-unit vaccines against many of the major parasitic diseases affecting humans and animals were very near, in fact, 'just around the corner'. The reality is that this early confidence has dissipated. Table II highlights the fact that most parasitic vaccines are still live vaccines that stimulate an immune reaction in the hosts, mimicking natural infections. Table II also shows that progress in developing commercial vaccines against protozoa far outstrips progress in vaccines against metazoa. However, it is worth drawing attention to the spectacular achievements in vaccines against cestodes and ticks (see below). These studies emphatically demonstrate that it is possible to develop recombinant sub-unit vaccines against complex metazoans.

Protozoa

Vaccination by controlled low-level infection that stimulates the development of protective immunity has been used successfully, as reviewed by Cornelissen and Schetters (8). In the case of protozoal vaccines, this has been achieved by using parasite strains selected for:

– complete but shortened life cycles (e.g. precocious *Eimeria* strains) (65, 71)

– truncated life cycles (e.g. the *Toxoplasma gondii* S48 strain, which does not form tissue cysts) (4)

– virulence attenuated by repeated passage through splenectomised calves (e.g. *Babesia bovis* and *B. bigemina* strains) (14, 53) or *in vitro* culture (e.g. *Theileria annulata* and *T. hirci*) (53).

Alternatively, infections can be controlled by the simultaneous administration of chemotherapeutic drugs, as in the case of East Coast fever in cattle, caused by *T. parva* (36). Except for coccidiosis vaccines, the majority of live vaccines are not produced commercially, but manufactured and distributed by governmental organisations, mainly for reasons of market failure. There are an increasing number of antiprotozoal vaccines available that are based on killed parasites or refined parasite antigen fractions. A vaccine based on killed *Neospora caninum* tachyzoites is available, which reduces *N. caninum*-induced abortion (48). Sub-unit vaccines

Table II
Antiparasitic vaccines commercially produced and/or manufactured or distributed by governmental organisations

Parasite	Host	Type of vaccine	Comments	References
Eimeria spp.	Poultry	Non-attenuated	Low (non-pathogenic) dose infection immunity	65, 71
Eimeria spp.	Poultry	Attenuated for precocity	Infection immunity	65, 71
Eimeria maxima	Poultry	Sub-unit vaccine of gametocyte antigen	Induction of maternal immunity	67
Toxoplasma gondii	Sheep	Attenuated for truncated life cycle	Reduces abortion	4
Neospora caninum	Cattle	Killed tachyzoites	Reduces abortion	48
Babesia canis	Canines	Antigens from *in vitro* culture supernatants	Reduces disease	35, 49
Babesia bovis and *B. bigemina*	Cattle	Attenuated by repeated passage through splenectomised calves	Live infection immunity Manufactured locally	14, 53
Theileria parva	Cattle	Non-attenuated wild type	Chemotherapeutically controlled infection Manufactured locally	36
Theileria annulata	Cattle	Attenuated by *in vitro* culture	Manufactured locally	53
Giardia duodenalis	Canines	Disrupted axenically cultured whole trophozoites	Reduces disease and cyst shedding Commercially available in the USA	39
Leishmania infantum	Canines	Sub-unit vaccine (FML)	Antiparasite activity and possibly therapeutic	11
Taenia ovis	Sheep	Recombinant antigen	Registered but not marketed	30, 46
Dictyocaulus viviparus	Cattle	Irradiated L3 larvae (truncated life cycle)	Limited to Europe	44
Boophilus microplus	Cattle	Recombinant tick gut antigen (Bm86)	Limited to Australia, Cuba and some countries in Central and South America	69

FML: fucose mannose ligand

based on soluble parasite antigens from one or more *Babesia* species reduce clinical disease in dogs due to *B. canis* (35, 49). A vaccine to prevent clinical signs of giardiosis and reduce cyst shedding in dogs and cats is commercially available (39). The vaccine was obtained by disrupting axenically cultured *Giardia* whole trophozoites. At the end of 2004, a vaccine against canine leishmaniosis, caused by *Leishmania infantum*, was introduced onto the market. The vaccine is based on the fucose mannose ligand (FML) of *L. infantum* (11). Finally, a sub-unit vaccine that induces maternal immunity in broiler breeders against coccidiosis, and is based on gametocyte antigens of *E. maxima*, has been developed and marketed (67).

Helminths

A vaccine against the bovine lungworm, *Dictyocaulus viviparus*, was the first available anti-metazoan vaccine and is still used in Europe today (44). The vaccine contains irradiated L3-larvae that do not mature to adult worms. A similar approach was used to develop a vaccine against the canine intestinal nematode *Ancylostoma caninum* (34). Irradiation-attenuated larval vaccines were also developed against several gastrointestinal nematodes but they did not protect young, susceptible stock against infection and were, therefore, never commercialised (27). In general, these vaccines are difficult to produce as larvae must be harvested from the manure of infected animals.

Effective recombinant vaccines were developed against the cestodes *Taenia ovis*, *T. saginata*, *T. solium* and *Echinococcus granulosus*. These vaccines are based on antigens of the parasite stage that adheres to the gut wall. When used for vaccination, these antigens induce immune responses that interfere with successful attachment. To date, although the vaccine against the cestode *T. ovis* has been registered in Australia and New Zealand, it has not been marketed. This could reflect the marginal commercial benefit of this vaccine and/or debate about the fundamental principles of cestode control in the intermediate versus the primary host. However, such developments prove that it is possible to achieve a reliable, high level of protection against a complex metazoan parasite, using defined recombinant antigens (30, 46).

Ticks

The vaccine against the cattle tick, *B. microplus*, is a recombinant vaccine based on a protein (abbreviated as Bm86) found in the tick at the surface of the gut wall. This protein is an example, along with several derived from *H. contortus*, of a 'hidden' antigen (the term 'hidden' meaning that the protein is not recognised by the systemic antibody response during natural infection). Vaccination stimulates the production of specific circulating antibodies that are ingested by the target parasite during blood feeding (28). The vaccine effectively suppresses the population of tick larvae available for infestation, rather than protecting individual cattle (69), with a chemical control being applied if tick numbers rise above acceptable limits (70). Vaccinating cattle with the recombinant *B. microplus* vaccine induces almost total immunity to *B. annulatus*, demonstrating immunological cross-protection. This immunity is sufficiently strong to inhibit *Babesia* transmission (43).

Barriers to vaccine development

Apart from the fact that vaccines began to be developed much later than chemotherapeutic drugs, a number of additional factors have affected the progress of parasitic vaccine development. Not least was the implementation in the 1990s of legislation on the authorisation of veterinary medicinal products in Europe (50). Moreover, and in contrast to viruses and bacteria, even the simplest parasites and their life cycles are highly complex, and there is a general lack of precise understanding of the host/parasite interaction.

Scientific challenges

Owing to the complex nature of parasites, the immune system is confronted with a highly diverse and plastic antigen repertoire. A number of biological characteristics perpetuate this diversity. First, many parasites go through a phase of sexual reproduction, with the associated exchange of genetic material from the parent strains (e.g. crossing-over). This results in progeny with a different genetic and phenotypic make-up. Secondly, there is a differential expression of genes during the successive life-cycle stages, as if the host has been infected with a number of different parasites. Finally, a number of species can express antigenically distinct variants of stage-specific molecules. This ability allows them to avoid the defensive responses of the host. These factors impose considerable challenges in screening for potential vaccine antigens.

In addition, the site of infection affects the nature of the protective immune response and may constrain research on vaccine development. For instance, many gastrointestinal parasites are not invasive and dwell only in the gastrointestinal tract, the interface with the host being the epithelial lining of the gut lumen. Since little is known about the immune effector mechanisms that function in immune hosts, there are few immunological tools to aid in selecting potential vaccine antigens. Consequently, research is guided by general biological criteria (e.g. mucosal antigen delivery) and has been mainly empirical. More basic research in mucosal immunology is required.

Clearly, the ability to produce parasite antigens through genetically modified micro-organisms has improved the feasibility of some parasitic vaccines. However, producing protective recombinant parasite antigens has proven difficult. Efforts have been inhibited by the fact that recombinant proteins may be incorrectly folded and/or lack critical post-translational modifications, particularly the glycans that are attached to several of the native candidate antigens. This issue is a major challenge in vaccine production and has been discussed recently (10).

Finally, in general, vaccines can be expected to induce a narrow spectrum of protection, often restricted to a single species or strain, whereas, in many cases, the actions of chemotherapeutics transcend the species level. Broadening the spectrum of protective immunity is a major issue in vaccine development.

The marketplace

The market size for products that control these parasites is often not impressive. The commercial viability of a vaccine depends on such factors as development and production costs, and specific characteristics, such as storage/transport conditions and shelf life. Perhaps the biggest barrier is the fact that current drugs have efficacies approaching 100%. It will not be easy to persuade users that a vaccine which is less than 100% effective can usefully control the disease. In addition, as patents expire on many anti-parasiticides, there is a market trend in favour of generic drug companies, which spend little on research and development and essentially do not invest in discovering new drugs or vaccines (18). Reasons for this are many and varied, with the demand for quick, high returns on investment reducing the opportunity for long-term discovery projects. As a result, very few animal health companies are currently committed to the discovery and development of antiparasitic vaccines.

Reasons for optimism

Progress in science and technology, along with political trends and economic forces, creates new opportunities for vaccine development.

Continued vaccine development

Experimental and first generation vaccines against a number of protozoal diseases have been described (Table II), and it is likely that, of these, the sub-unit vaccines will be developed further to improve not only efficacy profiles but also production processes. *Giardia, Babesia* and *Leishmania* vaccines based on antigens from *in vitro* culture, for example, are likely to be developed into recombinant antigen vaccines (22). A recombinant sub-unit vaccine against *Theileria* spp. is probable in the near future (24).

Effective vaccine candidates have been identified and tested, in native form, from:

i) H. contortus:

– H11 (37)

– H-gal-GP (55)

ii) Ostertagia ostertagi:

– sub-fractions from parasite excretory-secretory products (20, 60)

iii) Fasciola hepatica:

– cathepsin Ls and haemoglobin (9).

The levels of protection (60% to 90% reduction in egg output and/or worm burdens) are higher than those required to provide full disease control, as predicted by epidemiological analyses and mathematical modelling (2, 62). Developing equally effective recombinant versions of these vaccines, however, is proving elusive. It is suggested that post-translational processing and, in particular, glycosylation, is crucial (29). Further research is being devoted to these issues and it is expected that improved expression systems will become available (54).

In the field of tick vaccines, most success has been recorded with slow-feeding species, which have prolonged contact with the host immune system. There are grounds to think that better tick vaccines could be developed fairly easily. The potential for increased efficacy, by using more than one recombinant antigen in a formulation, has been demonstrated experimentally, while the number of antigens available for trial is steadily increasing (68).

New scientific developments

In the meantime, the search for new useful antigens continues (22, 45, 54, 68). In principle, the available genomes provide access, *in silico*, to the full complement of potential protein antigens and/or novel targets, as well as supplying the database needed for micro-array and proteomics-based analyses of expression. The number of genomes being fully sequenced is rapidly increasing. Gene knockout and ribonucleic acid interference offer the prospect of performing *in vitro* and *in vivo* gene 'knockdown', which may identify possible targets (see Scarselli *et al.* for a review [47]). Proteomic approaches could also be used to define protein/protein interactions, including those between parasite protein and immune effector molecules (the area of 'immunomics') (12).

Another factor which appears crucial for the induction of protective immunity, along with the identification of

protective antigens, is the way in which these antigens are delivered to and/or presented at the host interface. A variety of microbial vectors are being used to target antigens to specific sites in the host; e.g. *Salmonella* spp. are being employed to target *Eimeria* antigens to the gut epithelium (64). The inclusion of genes encoding molecules with adjuvant- or immuno-modulating activity is being intensively studied to improve the effectiveness of recombinant vaccines (15, 38). In addition, more effort is being devoted to understanding how parasites evade the host immune response, with these effector molecules themselves becoming vaccine targets (32).

Economic factors

In the developed world, by far the greatest losses associated with parasitic infections are sub-clinical or economic. Antiparasitic drugs are used more often to maximise profits than to salvage clinically sick animals (61). Such practices may be threatened in the future, due to a growing awareness that the extensive use of antibiotics could lead to the rapid emergence of drug-resistant pathogens, some of which could also pose a threat to humans. Consequently, a more recent approach has been to reduce the prophylactic use of drugs as much as possible, with a concomitant reduction of drug residues in biological products. The reasonable alternative is disease prevention by improved management practices, in which vaccination could play a pivotal role.

It will be important to tailor the vaccination regime to normal farm management procedures for the target species, and to deliver vaccines at an acceptable cost. There is considerable scope for improving vaccine delivery. First, the vaccination schedule should not impose significant management constraints on the producer, over and above those associated with current control practices. As an example, the conventional method of delivering a live coccidiosis vaccine to chickens was through their drinking water, or by spraying the vaccine onto their feed. To facilitate broiler production management, these vaccines are now preferably administered by spraying the chickens at one day of age. In the future, administration *in ovo* is a clear possibility (52). Secondly, alternatives have been developed to replace the use of needles for vaccines that must be administered parentally, such as DNA vaccines (21). These alternative devices can also be used to administer conventional vaccines, and are convenient in pig farming. Oral and mucosal delivery systems are also being exploited (for example, delivering vaccines to grazing ruminants in their forage is one exciting possibility) (1, 51). Vaccines are preferably delivered as a single shot, i.e. not requiring repeated booster vaccinations, to reduce the costs of animal handling and veterinary services. Different delivery systems, such as microspheres, liposomes, pumps and implants, have been used. The results indicate that, contrary to conventional thinking in immunology, continuous antigen delivery is capable of inducing immunity and providing affinity maturation, isotype switching and immune memory (31). It is highly likely that, given the short life span of many food animal species, single dose delivery will become a reality for selected veterinary vaccines.

In conclusion, it is reasonable to assume that, in the near future, more parasitic vaccines will become available for use as a practical tool in the control of parasitic disease.

The role of vaccines and drugs in parasite control

At present, vaccines against parasitic diseases are relatively expensive when compared to the costs associated with drug treatment. The incentive to use vaccines is, in some cases, related to a lack of efficacy in the parasitic drug. This is particularly evident in controlling coccidiosis in broilers. The emergence of drug-resistant *Eimeria* parasites has been well documented.

To reduce the emergence of resistant strains, it has been suggested that coccidiostat treatment and vaccination should be alternated in successive rounds. Vaccination with live parasites could lead to the replacement of field strains with drug-sensitive vaccine strains (5). It is more likely, however, that large-scale use of the live coccidiosis vaccines will eventually replace the use of coccidiostatic drugs.

Another example comes from the retrospective analysis of the use of a vaccine against *B. microplus* in Cuba. Its introduction was accompanied by a change in approach to the disease: the objective was no longer the total eradication of ticks; treatment was conducted only when the number of adult ticks per animal exceeded a low threshold. The result was an 87% reduction in acaricide treatments and an 82% reduction in the national consumption of acaricides, accompanied by an overall reduction in the incidence of clinical babesiosis. The large number of cattle involved – more than half a million – gave confidence in the results (59). The long-term impact on drug resistance is suggested by work in Australia, where a statistical analysis of factors associated with acaricide resistance identified the frequency of treatment as a major factor. The integrated use of a vaccine, plus restricted drug treatment as needed, should postpone the emergence of resistance (23).

Combination vaccines against *Haemonchus* that contained two highly protective antigen complexes expressed in the intestine of L4 and adult worms, namely H11 and

H-gal-GP, were evaluated under field conditions in South Africa (56). Vaccination reduced the mean egg output by > 82% and, simultaneously, the degree of anaemia and number of deaths due to haemonchosis. There was a surge in egg output during a period of irrigation, but revaccination cleared the animals of the newly acquired infection, restoring protection to the levels observed beforehand. Anthelmintic intervention was required to control the infection in some control animals but not in the vaccinated animals. Thus, it seems probable that vaccination against haemonchosis would also dramatically reduce dependency on anthelmintic drugs and selection pressure towards drug-resistant worms.

Conclusions

Antiparasitic drugs will remain important for a long time yet, though the development of resistance could limit their use. The continuous threat of drug resistance, the issue of residues entering the food chain and a lack of new drugs are all major reasons to focus research (and money) on vaccine development. Indeed, efforts towards vaccine development should be pursued intensively while drug-based infection control persists; it is pointless to wait until effective control is lost. Many vaccines may find their greatest and most immediate application in integrated control strategies. The synergies offered by a combination of vaccines and parasiticides should be thoroughly explored, as this approach may lead to a substantial reduction in the use of parasiticides.

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Prophylaxie des maladies parasitaires au moyen de la vaccination : une réponse à la résistance aux médicaments ?

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Résumé

Les médicaments antiparasitaires sont utilisés avec succès depuis longtemps pour lutter contre les maladies parasitaires affectant les animaux, car ce sont des produits sans danger, peu onéreux et à large spectre. L'inconvénient de ce succès semble être l'apparition, chez plusieurs espèces de parasites, d'une résistance aux médicaments. Le problème de la persistance de résidus dans la chaîne alimentaire et dans l'environnement se pose également, suscitant des doutes quant au bien-fondé d'une utilisation durable de ces médicaments. Des méthodes prophylactiques centrées sur la vaccination semblent offrir une alternative prometteuse. Or, si les vaccins basés sur des parasites atténués ont une efficacité avérée, très peu de vaccins sous-unitaires ont été mis au point. Grâce au développement des nouvelles techniques de la biologie moléculaire, il est désormais possible de séquencer des génomes entiers de parasites et de caractériser certains gènes en particulier. L'approfondissement de nos connaissances sur les gènes des parasites et sur le rôle joué par leurs produits dans la biologie des parasites devrait nous permettre de caractériser des antigènes intéressants, lesquels pourront ensuite être produits dans des systèmes recombinants. Néanmoins, avant de réaliser cet objectif il sera nécessaire de continuer à investir dans la recherche fondamentale sur les interactions complexes entre le parasite et son hôte.

Mots-clés

Hôte – Médicament antiparasitaire – Parasite – Prophylaxie – Résidu – Résistance aux médicaments – Vaccin – Vaccin antiparasitaire.

El control de enfermedades parasitarias por las vacunas como posible solución al problema de la farmacorresistencia

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Resumen

Hace ya muchos años que se vienen empleando con buenos resultados medicamentos antiparasitarios para luchar contra las infestaciones en los animales, puesto que esos fármacos son seguros, baratos y eficaces contra un amplio espectro de parásitos. Uno de los inconvenientes del éxito obtenido parece ser la aparición de farmacorresistencias en muchos de los parásitos en cuestión. Además, han surgido problemas ligados a la presencia de residuos de esos fármacos en la cadena alimentaria y el medio físico, hecho que pone en peligro su utilización sostenida en el futuro. Los métodos de lucha basados en el uso de vacunas ofrecen interesantes alternativas. Sin embargo, aunque las vacunas basadas en parásitos atenuados se han demostrado eficaces, aún hay pocas vacunas de subunidades. Gracias al advenimiento de nuevas técnicas de biología molecular, es posible ahora caracterizar la totalidad del genoma de un parásito e identificar genes concretos. Se espera que el hecho de conocer mejor esos genes y la función de las correspondientes proteínas en la biología del parásito sirva para encontrar antígenos útiles, que después cabría sintetizar con sistemas de ADN recombinante. Tal objetivo, sin embargo, requiere una inversión duradera en investigación fundamental para estudiar las complejas relaciones entre los parásitos y sus huéspedes.

Palabras clave

Control – Farmacorresistencia – Huésped – Medicamento antiparasitario – Parásito – Residuo – Vacuna – Vacuna antiparasitaria.

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Antigen and vaccine banks: technical requirements and the role of the European antigen bank in emergency foot and mouth disease vaccination

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Summary

Antigen and vaccine banks are stocks of immunogenic materials ready to be formulated into vaccines (bulk antigens) or ready to use (vaccines) in case of need by one or more of the parties of the bank. These stocks were primarily developed by foot and mouth disease [FMD] free European countries to control unexpected severe FMD episodes after the cessation of routine vaccination in the 1990s. For various reasons, including the lack of suitable antigens or of discriminatory tests to be used following emergency vaccination, such banks have so far not been developed to control other transboundary diseases, although over the last few years stocks of vaccines have been collected by the European Community to support control measures for bluetongue or classical swine fever.

The FMD virus antigens in the banks are stored at ultra-low temperatures (usually –130°C) to guarantee a shelf life of at least five years compared to a shelf-life of one to two years for vaccines stored at +4°C. When concentrated, a 50 l volume of antigens can contain up to 15 million cattle doses as per the standard potency specifications in the OIE *Manual of Diagnostic Tests and Vaccines for Terrestrial Animals*. Selecting antigen/vaccine strains for storage in a bank and selecting the appropriate strain(s) to be used in the case of emergency vaccination is the responsibility of FMD disease experts. The paper discusses the role of serological testing for the detection of infected animals in a vaccinated population, which is necessary for the recognition of FMD status. Technical advantages and disadvantages of antigen and vaccine banks in general are also outlined in this article. Finally, the experience of the European Community in organising, renewing, and controlling a sizeable FMD antigen bank since 1993 is discussed, and the use of the European Union (EU) antigen bank for international actions outside the EU is presented.

Keywords

Antigen bank – Control strategy – DIVA method – Emergency vaccination – European Community – Foot and mouth disease – Non-structural protein – Strategic reserve – Vaccine bank – Vaccine strain selection.

Introduction

Nowadays, the terms 'antigen bank' and 'vaccine bank' are better understood than in previous years by those working in the field of infectious or contagious disease control. The history of the foot and mouth disease (FMD) episodes in 2000 in Japan and South Korea, and the devastating epidemic in 2001 in parts of Western Europe remain in the collective memory of many animal health experts (40, 41). In particular the culling of vast numbers of animals, which was the dominant control strategy in 2001, and the limited use of emergency vaccines available from antigens held in antigen banks have triggered an intensive discussion about the most effective and ethically sustainable disease control strategy.

Known worldwide as vaccine banks, antigen banks or strategic reserves, these collections of immunogenic material ready to be used or ready to be rapidly reconstituted into the final vaccine product have, to date, performed well on several occasions. However, these materials have only been utilised, thus far, for the control of FMD outbreaks in order to protect countries that have been free of the disease without vaccination for a long period of time before the outbreak.

The first mention of strategic reserves was made after the devastating outbreak of FMD in Great Britain in 1967-1968 by a high-level commission established by the British Government and chaired by the Duke of Northumberland to examine the outbreak and make recommendations for the future. One of the Commission's recommendations was to maintain a stock of FMD vaccine for use if a similar outbreak of FMD occurred again. Following the recommendation of the Commission, subsequently referred to as the Northumberland Commission, the British Government purchased annually several hundred thousand doses of completely formulated FMD vaccine types O, A and C and established the first strategic antigen bank in the world. Because the vaccine was completely formulated, it had to be discarded and replaced at the end of its shelf life. In addition to the establishment of a vaccine bank, the British Government encouraged the private sector to invest in vaccine production through providing financial support to the State Laboratory Animal Virus Research Institute (AVRI, now called the Institute for Animal Health, IAH) in Pirbright in the United Kingdom (UK). Consequently, a centre of excellence for FMD vaccine manufacturing developed within the Institute, and during the following years several scientific and technological breakthroughs by researchers at the Institute contributed to the improvement of FMD vaccines.

During the early 1970s, several European manufacturers developed different technologies to concentrate, purify, and store FMD viruses, which have the valuable characteristic of being able to resist freezing when mixed with appropriate buffers and preservatives.

In 1974, a French manufacturer published the first patented process for the concentration and purification of the FMD virus prior to inactivation using a chemical named Polyox as the active agent (1).

In 1979, Lei and McKercher (33) published the results of a two-year study in Denmark investigating the production of strategic reserves using a virulent form of the FMD virus precipitated on diatomea filters and ready for the processes of inactivation and formulation. The inactivation of virulent virus concentrates was a lengthy process that was full of difficulties due mainly to the occurrence of virus aggregates. The advantages of establishing strategic reserves using already inactivated bulk antigens, which can more quickly be turned into vaccines than virulent viruses, thus, became rapidly evident.

In early 1979, the United States Department of Agriculture (USDA) decided to establish a large strategic reserve of FMD bulk antigens as an alternate source of protection for the livestock industry. This did not imply a change in the policy recommending stamping out as the primary eradication strategy should FMD ever reach the United States of America (USA). However, the potential for a large-scale outbreak, the impacts of such an outbreak, and the related environmental and animal welfare issues were already identified in the late 1970s and dictated the use of vaccination as part of the eradication procedures. Later, Mexico and Canada joined the Bank, referred to as the North American FMD Vaccine Bank, which is presently located at the Plum Island Animal Disease Center in New York in the USA.

In 1985, another joint FMD antigen bank, designated as the International Vaccine Bank (IVB), was established as a strategic reserve at the AVRI (now the IAH). This reserve was established in response to an agreement signed by the governments of Australia, Finland, Ireland, New Zealand, Norway, Sweden, and the UK. Several years later Malta joined the agreement.

In the early 1990s, as a consequence of the cessation of routine vaccination against FMD in the European Community (followed rapidly by similar bans by other governments in Central and Eastern Europe) there was a high demand for the establishment of strategic antigen banks for use in the event of a reappearance of the dreaded disease. Several governments negotiated contracts with manufacturers to establish their own national reserves. In 1992, the European Union (EU) launched an ambitious programme to store several million doses of important representative strains of the FMD virus (12, 30).

From a regulatory perspective, the establishment of strategic reserves led the European Pharmacopoeia to adapt their procedures regarding the emergency release of vaccines prepared from previously controlled antigens (at that time, standards pertaining to the emergency release of vaccines had not yet been included in the *Manual of Diagnostic Tests and Vaccines for Terrestrial Animals* [*Terrestrial Manual*] published by the World Organisation for Animal Health [OIE] [45]).

Banks of manufactured bottled vaccines

Keeping stocks of vaccines in bottles ready for use and in appropriate locations is a common preventive measure against health threats which have the potential to become animal health disasters, particularly if sufficient amounts of vaccine would otherwise be unavailable in an emergency situation. There is no need for very specialised premises and all types of vaccines against any disease can be stored if they have been manufactured according to standard marketing authorisation procedures.

The main advantage of bottled vaccines is the availability for immediate use for the full duration of the shelf life of the vaccine. Vaccine banks are normally subjected to regular inspection by or on behalf of the owner and the vaccines can be potency tested at the end of the shelf life, if the owner so wishes, to see if the validity period can be extended. One of the administrative disadvantages of vaccine banks that are comprised of ready-to-use bottled vaccines is the need to renew the stocks at the end of the shelf life of the product (between 12 and 24 months). If renewal orders are received too late by the manufacturer, there is a gap between the expiry date of the current bank and the arrival of new stock. Such interruptions in vaccine validity are potentially problematic in the case of an outbreak because a vaccine with an expired shelf life is not acceptable for use by regulators, veterinarians, or farmers. The products stored within the vaccine bank should be carefully managed by the owner such that fresh vaccine supplies should arrive prior to the expiry of the current vaccine supply in order to prevent gaps in product availability.

Because bottled vaccines are completely formulated, they have to be discarded and destroyed at the end of their shelf life. Environmental concerns make the destruction of large amounts of bottled vaccines difficult and costly. Destruction also requires highly specialised premises. For these reasons, vaccine banks are almost always owned by governments or maintained by international organisations and only occasionally owned by manufacturers, for whom incorrect sales forecasts could result in the costly destruction of large amounts of expired products. However, rolling stocks of extra quantities of ready-to-use vaccines in countries and regions that carry out routine vaccination is a proven effective tool to respond to outbreaks occurring despite the vaccination programme.

Another disadvantage of manufactured vaccines is their limited use in controlling diseases in which antigenic variation of the pathogens is frequently observed (e.g. FMD, avian influenza), or new combinations of field strains require new combinations of antigens in the composition of the vaccine. The formulation of bottled vaccines is fixed and cannot be adjusted, with the exception of the option to increase the volume of the dose injected if the field strain proves to be different from the vaccine strain; such use could seriously decrease the number of doses available for use as marketed by the commercial supplier.

Banks of inactivated antigens stored in bulk

The technology for storing deep-frozen inactivated bulk antigens over liquid nitrogen has been developed over the past thirty years only for FMD antigens. The reason for this is very likely linked with the necessity for the production of large quantities of FMD vaccines for compulsory vaccination campaigns and for the control of outbreaks in previously free areas. Compulsory FMD vaccination campaigns which are carried out during a fixed and limited period of the year require the delivery of huge amounts of FMD vaccines within a short delay. The control by emergency vaccination of FMD outbreaks in areas where routine vaccination is not carried out, likewise requires the mobilisation of large quantities of vaccines within a short time period that have undergone all required controls prior to use. Freshly manufactured vaccines cannot be produced at a capacity to meet such market demands. Consequently, the solution to this problem was found through the development of a new method for storing stocks of concentrated, inactivated, and often purified antigens that can rapidly be formulated into vaccine for use in vaccination campaigns or in the event of an outbreak. When stored frozen over liquid nitrogen (–130°C), concentrated inactivated FMD antigens have a shelf life of more than five years, which is significantly better than the shelf life of bottled vaccines (Table I).

When required for use, antigens kept frozen above liquid nitrogen are subject to formulation into a registered vaccine and must be manufactured according to the regulatory framework of the final vaccine product (registration dossier, good manufacturing practice [GMP]

Table I
Comparison of the shelf life of foot and mouth disease frozen antigens and of foot and mouth disease vaccines prepared from frozen and fresh antigens

Type of product	Shelf life	Vaccine potency
Frozen antigens in banks	5 years at – 130°C	Equivalent
Vaccine prepared from frozen antigens	12 to 24 months at +4°C *	Equivalent
Vaccine prepared from fresh antigens	12 to 24 months at +4°C *	Equivalent

* Temperatures as indicated in the Marketing Authorisation in force

and requirements for the prevention of the transmission of agents causing spongiform encephalopathy). In the version adopted in May 2006 by the International Committee of the OIE, the FMD Chapter of the *Terrestrial Manual* (available at www.oie.int) describes for the first time the storage and monitoring of antigen concentrates.

The use of vaccine could be the best choice to prevent or control many well-known transboundary diseases, such as highly pathogenic avian influenza, classical swine fever (CSF), African horse sickness (AHS), rinderpest, bluetongue, West Nile fever or Rift Valley fever, etc. Due to a low market demand for such vaccines and, consequently, a low return on investment, vaccine producers have not directed research toward the production of antigens for storage in antigen banks for emergency use. In the early 1990s, in an effort to participate in the control of a severe AHS serotype 4 episode raging in Portugal, Spain and Morocco, a European vaccine manufacturer produced a number of commercial batches of inactivated purified AHS serotype 4 antigen (31, 42) to be stored as frozen antigen in bulk until reformulated into vaccines. Later this manufacturer extended this process, on a small scale, to include several batches of inactivated vaccine against vesicular stomatitis (32). The lack of interest at that time by governments and international organisations to use these vaccines in their disease control policy was responsible for the absence of follow-up studies on the target diseases and for the cancellation of the programme concerning the establishment of vaccine banks for other transboundary diseases.

Technical advantages of antigen banks

As the only operational antigen banks are for FMD antigens, the following sections will deal strictly with FMD antigens; however, all of the technical aspects described can be applied to other frozen antigens, provided they share similar properties.

Compared to the traditional 'in line' production scheme for freshly manufactured antigen, modern FMD vaccine manufacturing processes include an inevitable step before the final formulation of the vaccine is completed: freezing of the antigens in a revolving antigen bank (Fig. 1).

The following specified technical advantages of reconstituting vaccines from antigens stored in antigen banks outweigh any of their disadvantages.

The first technical advantage of using antigen banks is the consistency in the manufacturing of the vaccine batches. Several runs of inactivation of several thousand litres of industrial virus harvests can be pooled as raw antigens. Equally, several pools of raw antigens can be processed to obtain highly concentrated and purified batches of bulk antigens, resulting in up to seven million doses at a potency of 6 PD50 (50% protective dose) in a volume as small as 50 l. A concentration factor of approximately 300 is very common; however, this value is not frequently exceeded due to the increased antigen losses that this entails.

Under such manufacturing conditions, production and testing of blends of several batches of consistently

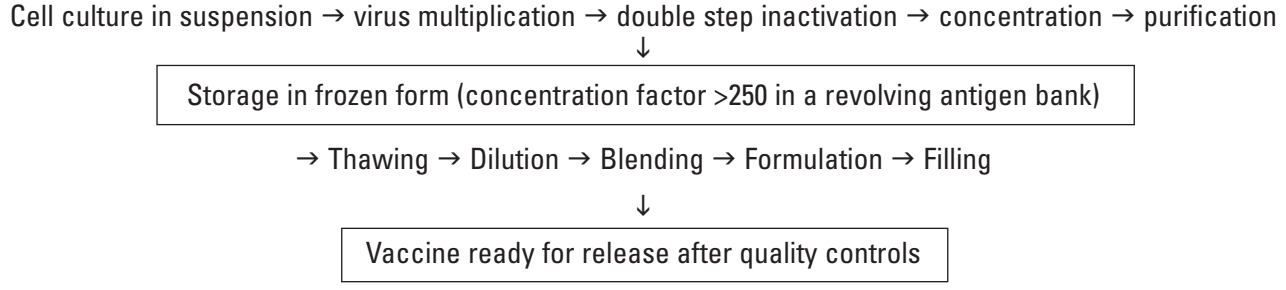


Fig. 1
Modern foot and mouth disease vaccine production scheme, including the storage of frozen antigen (in a revolving antigen bank)

manufactured antigens minimise the number, duration, and cost of quality control tests prescribed in the *Terrestrial Manual* (44) or by the European Pharmacopoeia (28) to assure quality, safety, and efficacy.

The second technical advantage is the possibility to formulate the stored antigens at several different time points, possibly years apart, into the same final vaccine preparation. Additionally, the shelf life of the final product starts from the time the vaccine is formulated without reference to the time that the antigen was produced. Today, between 90% and 95% of FMD vaccines are produced routinely by manufacturers using antigens from antigen stocks, which means that the virus production units and vaccine manufacturing units can operate independently. Thus, at any given time there is a ready-to-use supply of antigens in the antigen bank available to meet the market demand.

The third technical advantage of establishing antigen stocks, applicable to manufacturers of the antigens, is that blends of several batches of monovalent bulk antigens can be formulated into trial vaccines and fully tested before storage. The blends can ensure that any vaccine produced from a given controlled antigen will meet the minimum requirements of the OIE, the European Pharmacopoeia, or other established requirements. During the storage time, periodic tests are conducted to ensure that the antigenic characteristics (antigen content and immunogenicity) of the antigen stocks have not deteriorated (4) (Table II).

The fourth technical advantage is the option to calibrate the final vaccine composition, which is an extension of the third advantage and is commonly used by manufacturers but rarely by bank owners. Starting from the same bulk antigen, several blends made up of different antigen payloads can be tested to adjust the composition of the final vaccine according to the protection level required by the disease situation in the field. Consequently, different compositions of the same bulk antigen can be processed to produce final vaccine preparations with an expected potency ranging from 3 to 10 PD50. This is a true breakthrough for manufacturers who are, therefore, not obliged to wait for the vaccine control results and can adjust the vaccine potency according to the specification required by the contracting party in response to the emergency situation and the immunological relationship of the vaccine strain to the particular field virus. Consequently, the number of doses available in the antigen bank can vary according to the antigen payload selected to produce the final vaccine preparation, and must therefore always be expressed in relation to the expected potency.

The fifth technical advantage lies in the rapidity with which the antigens can be turned into the final vaccine. Because the antigens have been fully tested before storage it is possible to produce the final vaccine product within a few days of the receipt and registration of an official order. The possibility of the emergency release of vaccines formulated from antigen stocks without waiting for the completion of the quality controls, as permitted by the European Pharmacopoeia providing that the formulation unit complies with the EU GMP requirements, is another major advantage of maintaining antigen banks. Vaccines against FMD are an exception in terms of standard authorisation procedures, which have been outlined in the monograph of the European Pharmacopoeia, but not in the *Terrestrial Manual* at the present time. The European Agency for the Evaluation of Medicinal Products (now known as the European Medicines Agency [EMEA]) noted in a Position Paper on Requirements for Vaccines against Foot-and-Mouth Disease, 'The Ph. Eur. monograph "foot-and-mouth disease (ruminants) vaccine (inactivated)" is unique in that it contains a special provision to allow Competent Authorities to release vaccine in the event of urgent need, provided that a trial blend representative of the vaccine to be released has been tested with satisfactory results and provided that the various components of the final blend have passed sterility tests'. Practically, authorisation exception for the early release of emergency vaccine is always used by a client facing an FMD crisis and this explains the very short period of time between the receipt of the order by the manufacturer and the delivery of the vaccine on site (which varies between four and thirteen days according to shipping distance and flight availability).

A sixth technical advantage is associated with the banks that contain highly purified antigen. In-depth purification of bulk antigens has demonstrated the elimination, to a very large extent, of the non-structural proteins (NSP) of the FMD virus (38). Non-structural proteins occur as a result of FMD virus replication and are considered markers

Table II
Quality control scheme currently used for foot and mouth disease antigens in the European antigen bank

Time point	Quality control employed
Prior to storage in bank	Full quality controls according to the marketing authorisation for vaccine release
Each year during storage	Testing of antigen mass (in micrograms) in sample tubes kept with bulk antigens
Mid shelf life and at end of shelf life	Testing of vaccine trial blends from sample vials; vaccine potency is tested on five cattle using a virus neutralisation test

of infection. However, because one copy of the NSP, called 3D or Virus Infection Associated Antigen (VIAA), remains attached to the capsid of a high proportion of virions, complete NSP elimination is not possible. Recently, serological tests have been developed to detect in a vaccinated population those animals that have been infected with replicating FMD virus. These tests rely on the detection of antibodies to the NSP of the FMD virus which are evidence of viral replication in the animal (Table III).

Several authors have published studies on the serology of ruminants after FMD vaccination and infection (5, 35, 36, 37). So far, however, there have only been a few publications on serological investigations following emergency vaccination using vaccines formulated from concentrated inactivated antigens: two of these were presented to the Research Group of the FAO European Commission for the Control of Foot-and-Mouth-Disease (EUFMD) in 1998 (6, 43) and a third to the OIE International Conference on the Control of Infectious Animal Diseases by Vaccination in 2004 (7).

The seventh and last technical advantage of using antigen banks relates to the cooperation between the owners of the banks in assisting each other when outbreaks occur. For example, the EU Antigen Bank (see below) lent several million doses to governments that had made diplomatic requests for vaccine for emergency use in disease outbreaks. The vaccines were used successfully and the vaccine doses were replaced in the EU Antigen Bank a short time later with newly manufactured antigens with a full shelf life.

Such inter-governmental cooperation results in greater efficiency in the global control of FMD using vaccination and allows for greater instant production capacity. Recently, initiatives were launched to create what could be described as a 'global virtual network for antigens from banks' (39) and workshops were organised on the subject by the EU-funded FMD and CSF Coordination Action (a project that will gather and share information relevant to the control of two of the most important OIE listed diseases, both of which have caused devastating outbreaks of disease in Europe and continue to pose a serious threat; further information is available at www.fmd-and-csf-action.org). However, there are limitations to the sharing and dissemination of information because details on the content of strategic antigen reserves are considered classified information (30).

Table III
Differentiating infected from vaccinated animals (DIVA) system applied to cattle herds vaccinated against foot and mouth disease with vaccines produced with purified antigens from the European antigen bank

Cattle herds	Seropositivity to FMD virus	Seropositivity to FMD virus NSP
Infected/carriers	Yes (>2 years)	Yes (>2 years)
Multivaccinated and non- infected	Yes (>2 years)	No
Non-infected/Non-vaccinated	No	No

FMD: foot and mouth disease
NSP: non-specific proteins

Technical disadvantages of antigen banks

Difficulties in producing concentrated and purified antigens are not easily overcome since the integrity of the inactivated virus particles (the antigen) has to be maintained during the freezing stage, the storage stage, and the thawing and dilution processes required for vaccine preparation. If the total antigen losses in the final vaccine product are greater than 50% of the initial quantity of virus particles, the process loses much of its advantage and the cost per vaccine dose prepared in this way is commercially non-viable. Industrial know-how is therefore the most important factor for the manufacturer and the profitability of his operation, and for the bank owner who expects the product quality to be similar to a freshly made product. Presently, virus particle recovery, expressed in micrograms of antigen, after production of the final vaccine product is about 70%, which signifies that 30% or more of the virus particles from the initial cultures are regularly lost during the manufacturing process.

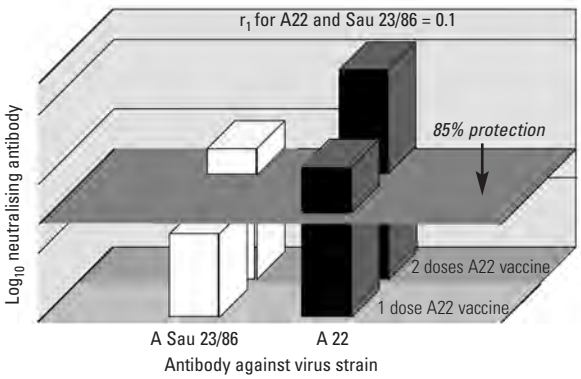
The second technical disadvantage associated with antigen banks is the antigen losses which occur during storage at –130°C. At this ultra-low temperature, virus particles rupture or aggregate over time (3). These phenomena are not well documented: firstly, because stability seems to be strain-dependant and secondly, because the data are proprietary and not readily published by manufacturers (34). It is accepted and considered to be normal by manufacturers that 10% of the initial virus particles will be lost within the first five years of storage of highly purified antigens. A very limited number of studies have demonstrated that after 14 years of storage up to 40% of the antigen mass may be lost (3, 34). Such data clearly indicates that the storage duration for strategic reserves is limited and do not support a 'buy and store indefinitely' policy. Regular monitoring and quality control are necessary during the storage period.

The third technical disadvantage associated with antigen banks is that, as already mentioned, the list of antigens stored is predefined and, thus, the bank may not contain the appropriate antigens to respond to a particular epidemiological need. Like several other animal pathogens, the FMD virus has a range of diverse serotypes and a large number of strains within some of the serotypes to which

there is limited cross-immunity. Consequently, there is a probability that the list of antigens retained in an antigen bank may not match or provide immunity against a new pathogen appearing in the field and may become obsolete over a ten year storage period depending on how much the epidemiological situation has changed.

For example, in 1996 a severe A22 related virus outbreak was observed in Albania, which rapidly contaminated a part of the former Yugoslav Republic of Macedonia. The only suitable type A antigen available in the EUFMD antigen bank at the time of the outbreak was the A22 Iraq 1964 virus which was ranked with a serological relationship of only 30% (r1=0.3) with the newly emerged virus. Despite the low serological relationship, a joint decision was made by the EU Commission and the EUFMD to use the A22 Iraq vaccine against the A22 Albania-96 virus and to inject two doses at one month intervals to achieve the level of immunity necessary to stop the epizootic (a similar observation related to a Saudi outbreak is illustrated in Fig. 2).

Additionally, as demonstrated recently by the UK FMD crisis in 2001, viruses occurring in any region of the world are a potential threat to all other regions, no matter how far away from each they are, and consequently should also be considered for inclusion in national or regional antigen banks. Strain selection is a complex responsibility for manufacturers and bank owners. An antigen collection should strive to reflect the major strains involved in recent epidemiological situations and also the strains expected to be involved in potential epidemiological situations in the next five years.



EUFMD: European Commission for the Control of Foot-and-Mouth-Disease

Fig. 2
Low immunological relationship (10%) between the vaccine strain (A 22 Iraq 1964) and a field strain from Saudi Arabia (A Saudi 1986)
A22 Iraq strain is now present in the EUFMD antigen bank
The second injection of vaccine A 22 Iraq 1964 boosted cross-reactive neutralising antibody levels against the A Saudi 1986 field strain above an expected protection level of 85% (white columns)
Source: C.G. Schermbrucker (unpublished results)

However, this attempt is hampered because the standard sera produced by manufacturers from their vaccines are again proprietary and this prevents governments or international organisations from being able to constantly match the existing antigens against an evolving epidemiological situation.

The fourth technical disadvantage associated with antigen banks, from the point of view of governments and international organisations, is the vulnerability of the reserves. Even when properly stored and monitored carefully by owners or manufacturers, antigen reserves are vulnerable to terrorism, accidents, or other unpredictable destructive events. Strategic reserves are valuable assets and essential materials for governments and international organisations. Consequently, security should be guaranteed in all cases. One of the solutions to minimising risks associated with strategic reserves involves splitting the antigen reserves between two or more storage sites that are situated at a considerable distance from one another (30). Having more than one storage and adjacent formulation facility is also very convenient when different orders requesting different emergency vaccines are submitted at the same time.

Strategic reserves of vaccines and antigens: the European Union viewpoint in 2006

The current 27 Member States of the EU are home to numerous species that are susceptible to FMD, accounting for approximately 300 million domestic animals. The EU is a major producer and exporter of food of animal origin but also imports products of animal origin from a wide range of countries. Following the establishment of the European Single Market, a high animal health status has been maintained despite a number of serious setbacks due to major outbreaks in certain parts of the Community of infectious animal diseases, such as classical swine fever, foot and mouth disease and, more recently, highly pathogenic avian influenza and bluetongue.

The economic and social consequences of these epizootics together with epidemiological and climatic developments have increased consideration of the role of vaccination in controlling animal diseases of major importance to international trade.

Thanks to these developments vaccination against, for example, African horse sickness or bluetongue has never attracted major media attention and a flexible legislation has minimised the implications of such vaccination on trade.

The great success of a recent oral vaccination campaign against classical swine fever in wild boar in certain areas of the Community has stimulated the establishment of a limited reserve of vaccine against this disease. Recently, the Community adopted legislation on the purchase of additional quantities of a marker vaccine against classical swine fever and specified certain conditions on the use of such vaccines.

At the Agriculture Council convened on 21 December 2004, the European Health and Consumer Protection Commissioner, Markos Kyprianou, announced a new EU Animal Health Strategy to improve the prevention and control of animal disease in the EU. According to the strategy, the Commission plans to propose a Communication in 2007 setting out actions for 2007-2013. The Commission intends to develop a new and improved animal health strategy for the EU that will go beyond what has already been achieved with the existing animal health policy. The announcement concluded that animal disease outbreaks are costly and that there are also ethical issues related to the use of mass slaughter as a disease control method. Furthermore, there is growing concern about the potential impact of certain animal diseases on human health, e.g. a disease like avian influenza could lead to a worldwide pandemic. The new EU animal health strategy, therefore, aims to develop a policy on disease prevention, make emergency vaccination a more viable option, simplify the legislation, and make better use of financial resources. The existing EU animal health policy has undergone an external evaluation, the results of which were discussed at the Conference on Community Animal Health Policy on 7 November 2006 in Brussels (26).

With the recent enlargement of the EU, the Community now shares common borders with a geographical area in which certain major epidemic diseases are not yet eradicated. To stabilise and further improve the animal health situation in those countries neighbouring the EU require close cooperation between EU Member States and infected countries, when possible within the framework of international organisations or through regional agreements, as well as a constant high level of disease awareness and preparedness by the EU Member States, including the capacity for emergency vaccination.

Historically, Council Directive 85/511/EEC established Community measures for the control of FMD (9) (repealed by Directive 2003/83/EC) (15) and required Member States that practiced vaccination to carry out vaccination programmes in a more systematic way and in combination with stamping out of infected herds and ring vaccination where necessary. Upon adopting Directive 90/423/EEC (11) (repealed by Directive 2003/83/EC) the Council decided to abandon prophylactic vaccination in eight of the then twelve Member States that practised such vaccination in cattle and, in turn, made provisions for the use of vaccines in emergency situations and established Community reserves of concentrated inactivated antigen (CIA) of the FMD virus. The details on these reserves are contained in Council Decision 91/666/EEC (13) (last amended by Council Regulation (EC) No. 807/2003) (17). To ensure the quality of the vaccines formulated from the stored antigens, Council Decision 91/665/EEC (12) designated a Community Coordinating Institute and described its functions. However, for technical reasons this Institute was dissolved after the Decision expired on 31 December 1996.

Decision 91/666/EEC also outlined procurement procedures through public tender and provided through the veterinary fund regulated by Decision 90/424/EEC for the financing of the supply and storage of the antigen and the formulation and distribution of the vaccines formulated from such antigen (10) (amended by Directive 2003/99/EC) (16).

It is important to note that the arrangements for the Community antigen bank were not only made to ensure independence from manufacturers and a strategic distribution of relevant antigens but also with the prospect of slaughter of the vaccinated animals. Consequently, little attention was paid to acquiring a marketing authorisation for these vaccines as required for veterinary medicinal products administered to food producing animals in accordance with the Community code relating to veterinary medicinal products described in Directive 2001/82/EC (14) (amended by Directive 2004/28/EC) (18).

Legal aspects

At present the Community control measures for FMD are laid down in Council Directive 2003/85/EC (15) (amended by Decision 2006/552/EC) (25). The new Directive formulates a more prominent role for emergency vaccination in controlling FMD. The Directive distinguishes between 'suppressive vaccination' of animals that are intended to be destroyed following vaccination, and 'protective vaccination' of animals that are intended to be kept alive. In either context, emergency vaccination is incorporated in a stamping out policy applied to infected and suspected to be infected animal holdings and contact holdings and is followed by testing on vaccinated animals with subsequent slaughter of animals in holdings that had contact with the field virus. For the most part, the policy follows the recommendations for the re-establishment of FMD-free status without practicing vaccination (Article 2.2.10.7) and the surveillance guidelines (Appendix 3.8.7) in the OIE *Terrestrial Animal Health Code (Terrestrial Code)*.

The relevant provisions for the Community antigen reserves are contained in Articles 80 to 84 of the Directive and in Annex XIV. In order to facilitate the process of deciding whether or not to implement emergency vaccination, the Directive incorporated recommendations from the report of the European Commission's Scientific Committee on Animal Health and Welfare published in 1999 on the 'Strategy for emergency vaccination against foot and mouth disease (FMD)' (21).

The new legal framework places particular emphasis on marketing authorisation for the vaccines and requirements for the purity of the vaccines with regard to inducing antibodies against NSP. Such requirements are in line with the relevant recommendations in the OIE *Terrestrial Manual* (paragraph 4(c) of Chapter 2.1.1).

Following the recommendations of the report of the Scientific Committee on Animal Health and Animal Welfare in April 2003 on 'Diagnostic techniques and vaccines for foot and mouth disease, classical swine fever, avian influenza and some other important OIE List A diseases' (23), the Community supports the validation of appropriate tests for the detection of infected animals in vaccinated herds. It is worth mentioning that the European Parliament has been following the aforementioned recommendations with great interest and supports the development of tools that make emergency vaccination a viable disease control option.

Procurement of antigens

The following procedures are observed when there is an intention to purchase quantities and subtypes of FMD virus antigen:

– the Commission evaluates the recommendations for priority antigens issued at least once a year by the FAO EUFMD Research Group. However, following the designation of a Community Reference Laboratory in 2006 in accordance with Commission Decision 2006/393/EC (24), it will now be an integral part of the duties and functions of the laboratory to advise the Commission on the priority antigens that should be banked for possible emergencies;

– after obtaining the opinion of the Standing Committee on the Food Chain and Animal Health, which takes into account the estimated needs in accordance with the contingency plans of Member States, the Commission adopts a formal Decision on the purchase of antigens;

– following a public tender advertisement published in the 'S series' of the *Official Journal of the European Union*, a special commission selects the best offer and defends its choice to the Advisory Committee for Procurements and Contracts. However, in certain cases a negotiated procedure is recommended when the antigens to be purchased may possibly be formulated together with existing stocks of the same strain, other relevant strains, or other relevant serotypes from the same manufacturer in order to provide a complete vaccination campaign, for example, that would be administered in a neighbouring third country;

– subsequently, two contracts are concluded between the Commission and the manufacturer of choice which include the conditions of supply and storage of antigen and the formulation, production, labelling, and delivery of the ready-to-use vaccines reconstituted from the antigens.

The Community purchased antigens in 1993, 1997, 1999, 2000, 2003, and 2006.

Designation, functions and duties of antigen banks

Over the last decade the application of the rules laid down in Decision 91/666/EEC has been modified to take into account technical developments, changes in the structure of the pharmaceutical industry, and production standards. While Directive 2003/85/EC repealed Decision 91/665/EEC and thereby abandoned the established concept of a Community Coordinating Institute as the quality checking institution for antigens stored in the bank, it maintained Decision 91/666/EEC until new provisions could be put in place.

Decision 91/666/EEC allows the Commission to designate premises as a Community antigen bank for the storage of CIA. Following inspection, two of the three designated institutions storing antigens for the Community were abandoned in 2005, thus, concentrating the antigens at two distinct sites of a single manufacturer to reduce the risks of damage to the antigen.

The relevant provisions for the functions and duties of the antigen bank are described in Annex I of Decision 91/666/EEC. In particular the bank shall:

– store the Community reserves of CIA of the FMD virus in such a way as to maintain the usefulness of the antigens for the production of a safe and potent vaccine for emergency use against FMD. In accordance with the European standards for 'Good Manufacturing Practice' this will include keeping adequate records of the conditions under which the antigen is stored, performing regular checks, and when necessary adjusting the temperature regime. The CIA shall be stored at –70°C or colder;

– deliver CIA to the place of formulation, bottling, and distribution of the vaccine at the request of a Member State when emergency vaccination is applied in accordance with Community rules or at the request of the Commission for use of the vaccines in the EU or a third country.

Although the provisions of Decision 91/666/EEC do not contradict Annex XIV to Directive 2003/85/EC, they should be replaced for legal clarity and in order to take into account the Position Paper on Requirements for Vaccines against Foot-and-Mouth Disease (8), adopted by the Committee for Medicinal Products for Veterinary Use (CVMP) on 16 June 2004, and Article 80(4) of Directive 2003/85/EC which requires that:

'The conditions for the establishment and maintenance of Community reserves of antigen and authorised vaccines at the premises of preferably at least two manufacturing establishments shall be laid down in contracts concluded between the Commission and the manufacturing establishments. Such contracts shall include at least:

a) conditions for supply of quantities and subtypes of concentrated inactivated antigen;

b) conditions for secure storage of antigen and authorised vaccines;

c) guarantees and conditions of rapid formulation, production, bottling, labelling and distribution of vaccines.'

Subtypes and quantities of antigen of the foot and mouth disease virus in the European Union antigen bank

Annex I to Decision 91/666/EEC, as amended by Decision 2001/181/EC (22), requires that the bank maintain antigens in quantities that are sufficient to carry out emergency vaccination, taking into account the estimated risk that the different subtypes present to Community livestock (at least 2 million doses of each subtype). Actual antigen stocks vary between 2 and 5 million doses for individual serotypes and strains depending on the estimated risks and the amounts required to formulate polyvalent vaccines.

The Chief Veterinary Officers of the Member States receive regular updates on the status of the bank in the framework of the Standing Committee on the Food Chain and Animal Health and the secretariat of the EUFMD is informed during the biannual meetings of the Executive Committee.

Technical requirements for the supply of concentrated inactivated antigens and vaccine formulation

Annex II to Decision 91/666/EEC specifies the technical requirements for the supply of CIA and its formulation into vaccines. These requirements are, when applicable, included in the appropriate contracts.

Technical requirements for supply and storage

The storage and supply of vaccines from CIA stored in the European antigen bank are subject to the following technical requirements:

a) The production of antigen and the preparation of finished vaccine shall be carried out in accordance with the principles and guidelines of good manufacturing practice for veterinary medicinal products as laid down in Commission Directive 91/412/EEC of 23 July 1991 (20).

b) In accordance with Article 65 and Annex XII of Directive 2003/85/EC, the establishment which supplies the CIA must comply with the 'Security standards for FMD laboratories' outlined in the report of the 30th Session of the FAO EUFMD (27), and the establishment producing the antigen must be included in the list of establishments authorised to handle live FMD virus in Annex XI (B) to the aforementioned Directive. This list was recently amended by Decision 2006/552/EC in order to take account of certain commercial developments in the sector.

c) Full details should be provided on the tests conducted by the producer on the seed virus, cells, and other materials used in the production process. Samples of each master seed virus must be made available for confirmatory testing of identity, purity, safety and potency.

d) The virus shall be propagated in cell cultures. Cells and other ingredients shall be tested to verify freedom from bacteria, fungi, mycoplasma and extraneous viruses. After culture, the virus shall be separated from the particulate matter by appropriate procedures. No seed virus, cell, or ingredient of animal origin shall be derived from animals infected or suspected to be infected with bovine spongiform encephalopathy (BSE). Account shall be taken of:

– the opinion of the EMEA on the potential risks associated with medicinal products in relation to BSE (16 April 1996) (19)

– the current guidelines administered by the CVMP and the Committee for Medicinal Products for Human Use (CHMP) described in the document entitled 'Minimising the risk of transmission of agents causing spongiform encephalopathy via medicinal products' (29). It must in particular be ensured that bovine tissue originating in countries affected by BSE is not used or is only used under particular conditions. Documentary evidence of the origin of bovine products shall be made available for confirmatory tests of identity and purity.

e) The antigen and the vaccine must comply with the requirements of the European Pharmacopoeia, particularly those concerning safety, innocuity and sterility.

f) The antigen and the vaccine must exceed the requirements of the European Pharmacopoeia with regard

to potency and should have an observed potency of 6 PD50 in cattle

g) Virus inactivation using cyclised binary ethyleneimine (BEI) or an equivalent method must be validated. The fluids from culture shall be transferred into sterile vessels within 24 h after the addition of the inactivating agent. After completion of the inactivation period, samples shall be removed to verify that inactivation was successful. The inactivation test must comply with the FMD vaccine monograph of the European Pharmacopoeia. For each batch of antigen the kinetics of inactivation must be followed and documented by the producer. The range of inactivation must be such that the entire batch is free from infective virus, and the safety margin should be in the range of about 3 $\log_{10}$ (based on extrapolation).

h) Further processing must be carried out in a non-contaminated environment (FMD virus free). The antigen shall be concentrated and purified by a method that will result in a reduction of the original volume by at least 1/100th and preferably by 1/200th or greater. The purification procedure will be sufficient to ensure a long shelf life of the finished vaccine. The antigen content of the CIA shall be determined as 146 S particles. The manufacturer must specify the number of finished vaccine doses corresponding to the volume unit of CIA.

i) The CIA shall be supplied in containers suitable for storage above liquid nitrogen. Each container shall be labelled with the serotype, serial number, date of harvest and volume, and be sequentially numbered to indicate the order in which the containers were filled. The number of vaccine doses corresponding to the volume of concentrated material in the container shall be indicated.

j) The batch of CIA must be tested prior to delivery to the storage facilities for sterility, innocuity, and potency, in accordance with the European Pharmacopoeia. For these tests, samples of CIA must be formulated into the vaccine product by the manufacturer. Delivery of the batch of CIA to the storage facilities of the manufacturer will be authorized after completion of the tests.

k) Representative samples from the batches of CIA (one batch per subtype) must be made available in sufficient quantity by the contracted manufacturer together with complete information on the tests performed and a detailed description of the vaccine formulation protocol to ensure that potency testing can be performed in accordance with the European Pharmacopoeia each year during the five year storage period. Reformulation of the antigen into vaccine for testing will be carried out by the manufacturer who shall inform the Commission of the results of the tests performed. A batch could be considered unsatisfactory if the 146 S particle content is found to be significantly lower that at the time of the challenge test.

l) Each batch of CIA may be tested on behalf of the Commission by an independent institution at any time for 146 S particles and potency within the five year storage period and during the five years after the Contractor's warranty has ended. The testing shall take place on samples of vaccine reconstituted from stored CIA by the manufacturer. For this purpose the manufacturer shall arrange for sufficient representative samples of each batch at the time of delivery of the CIA to the storage facilities and reserve these samples for external testing.

m) The antigen provided by the producer should have an expected stability of at least five years.

Formulation of vaccines

The formulation and production of vaccines from the CIA stored in the European antigen bank are subject to the following requirements:

a) the guarantee provided by the manufacturer that the vaccine supplied fully complies with the European Pharmacopoeia;

b) supply of the vaccine within the following time limits:

– immediate supply, i.e. delivery of a minimum of 300,000 doses and a maximum of 2 million doses of finished vaccine per formulation site within four days following notice by the Commission;

– urgent supply, i.e. delivery of 1.5 million doses in oil emulsion and 5.5 million doses in aqueous formulation within a period of 5 to 14 days following notice by the Commission;

c) formulation of the vaccines according to the prescription of the producer. Vaccines for pigs will be formulated as oil emulsions. For cattle, vaccines adjuvanted with aluminium hydroxide, saponin or oil may be used;

d) disposal and replacement of any batches deposited in the antigen bank that are found to be unsatisfactory when reconstituted and tested. The cost of testing, disposal, and production of the replacement batch will be the responsibility of the producer;

e) delivery in bottles of suitable size (labelled in the language or languages of the country in which the vaccine is to be used) to predefined places as close as possible to the outbreak;

f) formulated vaccines must be stored at cool temperature conditions as specified in the European Pharmacopoeia. The shelf life should be at least four months, but is normally guaranteed by the contractor to be 24 months, subject to compliance with storage conditions.

Access to and operation of the European antigen bank

The use and operation of the antigen bank is embedded in the decision tree that is used when determining if vaccination should be implemented. Such a decision may only be taken by an affected Member State, except under particularly severe circumstances when the Commission may present a proposal to the Standing Committee on the Food Chain and Animal Health in order to protect wider Community interests.

According to the right of initiative for emergency vaccination within the framework of the approved contingency plans, all Member States have equal drawing rights from the bank independent of the existence of a supplementary national bank. In the case of an emergency, coordination between Members would have to be ensured through the Standing Committee on the Food Chain and Animal Health. For Member States that are members of the EUFMD, coordination between the member countries of that organisation is facilitated through annually updated inventories that are kept as classified information at the EUFMD headquarters and can be accessed by the Chief Veterinary Officers of the member countries.

In the case of emergency vaccination in Member States, the formulation of vaccines is triggered by a request by a Member State to the Commission, independent of whether the decision to vaccinate was initiated by the Member State or was based on a Commission Decision.

The Community has concluded agreements with various neighbouring and some distant countries on regulated and limited access to the bank in the case of an emergency. The Commission therefore welcomes the OIE initiative concerning the establishment of guidelines for international standards for vaccine banks, which are described in Chapter I.1.11 of the *Terrestrial Manual* (45).

The Commission is actively participating in OIE led discussions on cooperation between various antigen and vaccine banks in different regions of the world. However, differences in production standards and registration requirements as well as security aspects have impeded the establishment of a global network of antigen banks. To overcome these difficulties, the relevant services in the Commission actively participate in various FMD oriented programmes, such as in the Work Programme No. 4 on Vaccine Reserves, within the framework of the FMD/CSF Coordination Action (http://www.fmd-and-csf-action.org/about/workplan/).

The European antigen bank has been utilised in FMD control measures carried out in third countries: the Balkans in 1996, certain Maghreb states in 1999, the Far East in 2000, and Turkey in 2000 and 2006. When supplying vaccines to countries in the Far East and Turkey the established requirements for immediate supply were met. However, in certain cases it was observed that the timely delivery of the ready-to-use vaccines donated by the Community was delayed due to lack of coordination between different governmental bodies in the beneficiary country involved in the operation.

Testing of antigens

The results of a first round of external testing of antigen stock in the European antigen bank were published in 1996. The Community Coordinating Institute, which is no longer in operation, reported satisfactory results upon testing of four of the antigens in both cattle and pigs (4).

More recently, the Commission adopted Decision 2001/75/EC 'for safety and potency testing of foot-and-mouth disease vaccines and bluetongue vaccines', which included testing of FMD virus antigens banked since 1993. Potency testing carried out in cattle, in accordance with the requirements of the European Pharmacopoeia, confirmed that the tested antigen had a potency significantly above the required 6 PD50, despite the prolonged storage period.

Potency testing in pigs is not described in the European Pharmacopoeia and such testing was not included in the recent review of the FMD monograph of the European Pharmacopoeia due to known problems of overwhelming challenge conditions resulting from unprotected pigs re-challenging other protected vaccinates before isolation. In accordance with Decision 91/666/EEC, antigen must also be suitable for the preparation of oil emulsion vaccines for pigs, in which case 1/6 of the volume of a single dose must protect at least five out of ten pigs when challenged by intrapodal injection of 1,000 ID50. However, when an oil emulsion vaccine formulated from the same antigens was tested in accordance with the relevant guidelines described in the OIE *Terrestrial Manual*, the vaccine failed the test. This failure was most likely due to problems similar to those described previously in comparable tests conducted by Barteling *et al.*, 1996 (4).

Following the designation of a Community Reference Laboratory, plans have been drawn up to proceed with challenge testing in the upcoming years. However, it is important to recognise the difficulties associated with potency testing in the Member States and, thus, to encourage scientists and manufacturers to collaborate in developing suitable alternatives to replace animal experiments, such as seromonitoring of vaccinated animals or the employment of *in vitro* techniques as described by Ahl *et al.*,1990 (2).

The use of tests for the detection of antibodies against non-structural proteins

With the modifications that were first introduced into the FMD chapter in the fourth edition of the OIE *Terrestrial Manual* and the adoption of amendments to the FMD chapter of the OIE *Terrestrial Code* in May 2002, emergency vaccination may become a more attractive option for controlling FMD.

Modern FMD vaccines should not induce antibodies against NSP if used for the purpose of emergency vaccination. Modifications to the FMD Monograph incorporating such purity requirements were not adopted by the European Pharmacopoeia but were supported by the European Commission and have been included in past procurement activities. Following the Position prepared by the Immunological Working Party of the European Medicines Agency and adopted by the CVMP, it is now up to the purchaser to request that the manufacturer provide substantiation of the claim that the vaccine produced is suitable for post-vaccination surveillance in accordance with OIE requirements.

With regard to the stocks currently maintained in the European antigen bank, guarantees have been provided by the manufacturer that any antigen purchased since 1996 will not induce the production of antibodies against NSP even after multiple administrations. This statement is supported by field findings where serosurveillance was carried out following emergency vaccination in third countries with vaccines supplied from the European antigen bank and through a challenge test requested by the Commission.

Security aspects of operating the European antigen bank

The risks of intentional introduction of FMD virus were discussed at a meeting with participants from the OIE, FAO, EUFMD and EC Commission at FAO Headquarters on 6 and 7 February 2002.

The Commission services shared the conclusions that even the worst case scenario of an intentional simultaneous multi-focal outbreak caused by more than one distinct serotype or strain of FMD virus would not be a feasible approach for bioterrorists if emergency vaccination was a viable option of disease control within the framework of national contingency plans.

Subsequently, certain recommendations from the aforementioned meeting have been taken into account in recent Commission legislative activities. In particular, future control measures for FMD should include requirements for contingency plans against such scenarios and, in addition, classification of any information about the quantities and subtypes of CIA in the banks.

Conclusion

The experience gained from the use of antigen banks for the control of FMD outbreaks in countries that had remained free from disease for a long time prior to the outbreak shows that this strategic option works effectively in delivering large quantities of vaccine and controlling the spread of disease in fully susceptible populations. Antigen banks represent the best strategy against the lightning spread of FMD in unvaccinated livestock. The key requirement for the success of emergency vaccination is that experts must select the appropriate strains(s) to be stored in the bank and the appropriate strain to be utilised in emergency vaccination campaigns. If an appropriate strain is not available in the antigen bank then an effective vaccine cannot be reconstituted.

The costs of maintaining an updated antigen bank are very few compared to the cost of FMD epizootics in developed countries. The use of emergency vaccination avoids a potential resort to massive culling, which is costly and is usually associated with considerable public concerns regarding animal welfare.

The recent possibility of banking highly purified antigens consisting of ultra-low levels of FMD virus non-structural proteins offers emergency vaccine users the option to perform serological tests that allow differentiation of infected from vaccinated animals (DIVA strategy). The demonstration that the virus is no longer circulating in the livestock in areas in which the emergency vaccine was administered is a necessary step to regain official recognition by the OIE of FMD-free status (46).

Although, until now, antigen banks have mainly been under the management of FMD-free countries, they have been used successfully in a few infected countries through international collaborations. One of the next steps in the antigen bank programme should be the rapid expansion of this successful model to include antigen banks devoted to transboundary diseases. Attracting the interest of vaccine producers in supplying international antigen banks devoted to the main transboundary scourges is necessary in order to achieve this goal.

With the establishment of the Community antigen bank, the EU has developed an operational and effective system to respond to a possible FMD emergency. Such a response system is expensive and can never secure full protection. It therefore remains a primary objective of national authorities and international bodies to prevent the

introduction and spread of this disease into geographical regions that are disease free as well as the dissemination of new virus strains into endemically infected areas.

In order to improve the efficiency of the existing antigen bank, the authors, taking into account numerous discussions with experts from diagnostic, research, and vaccine production laboratories, as well as epidemiologists and administrators, believe that the following points should be urgently addressed:

– the development and validation of alternative potency testing methods to the currently prescribed challenge test in cattle. This is particularly important in light of the decreasing availability of suitable animal housing space and of animal welfare considerations;

– the development of rapid procedures for the determination of the degree of cross-protection between new field isolates and existing vaccines with the aim of replacing, when possible, the costly development of new vaccines by modulating the composition and potency of currently available vaccines to achieve sufficient cross protection;

– a serious engagement of vaccine manufacturers to facilitate the above objectives and to adhere to minimum standards for the production of vaccines that would allow international cooperation between the banks in the case of an emergency and the exchange of vaccines in the case of shortages;

– compliance of OIE Member Countries with internationally agreed standards for disease notification and information exchange. Such compliance should include the involvement of reference laboratories and the exchange of suitable samples between laboratories for the rapid identification of the virus topotype and the antigenic relationship with existing vaccines, where necessary with the support of international animal health organisations.

■

Banques d'antigène et de vaccins : prescriptions techniques, et rôle de la banque d'antigène de l'Union européenne dans la vaccination d'urgence contre la fièvre aphteuse

M. Lombard & A.-E. Füssel

Résumé

Les banques d'antigène et de vaccins constituent des stocks de matériel immunogène prêt à entrer dans une composition vaccinale (pour l'antigène en vrac) ou prêt à être utilisé (pour les vaccins) si cela s'avérait nécessaire pour les différentes parties contribuant à la banque. Ces stocks ont été mis en place (surtout dans les pays européens indemnes de fièvre aphteuse) afin de maîtriser les épisodes imprévus de fièvre aphteuse survenant après que l'application régulière de la vaccination ait été interdite, dans les années 1990. Pour diverses raisons, y compris le manque d'antigènes adéquats ou de tests discriminatoires à utiliser en cas de vaccination d'urgence, aucune banque de ce type n'a à ce jour été prévue pour contrôler les autres maladies transfrontalières, bien qu'au cours des dernières années des stocks de vaccins aient été constitués par la Communauté européenne pour étayer les mesures de lutte contre la fièvre catarrhale du mouton ou le peste porcine classique.

L'antigène du virus de la fièvre aphteuse stocké dans les banques l'est à très basse température (habituellement –130 °C) afin de garantir une durée de conservation d'au moins cinq ans, par opposition aux deux années de conservation garanties par le stockage à +4 °C. Un volume de 50 litres d'antigène concentré peut contenir jusqu'à 15 millions de doses pour application chez les bovins, en vertu des spécifications de puissance prescrites dans le *Manuel des tests de diagnostic et des vaccins pour les animaux terrestres* de l'OIE. Le choix de l'antigène/souches vaccinales à stocker dans la banque et la sélection des souches à utiliser en cas de vaccination d'urgence sont de la responsabilité des

spécialistes de la fièvre aphteuse. Les auteurs étudient le rôle des tests sérologiques qui permettent de reconnaître les animaux infectés au sein d'une population vaccinée, ce qui est nécessaire pour évaluer le statut au regard de la fièvre aphteuse. Les auteurs soulignent également les avantages et les inconvénients techniques des banques d'antigène et de vaccins en général. Pour finir, l'article rappelle l'expérience de l'Union européenne (UE), qui organise, renouvelle et supervise une importante banque d'antigène du virus de la fièvre aphteuse depuis 1993, ainsi que l'utilisation de cette banque européenne dans le cadre de programmes internationaux en dehors de l'UE.

Mots-clés
Banque d'antigène – Banque de vaccin – Fièvre aphteuse – Méthode DIVA – Protéine non stucturale – Sélection de la souche vaccinale – Stock stratégique – Stratégie de prophylaxie – Union européenne – Vaccination d'urgence.

Bancos de antígenos y vacunas: requisitos técnicos y papel del banco europeo de antígenos en vacunaciones de emergencia contra la fiebre aftosa

M. Lombard & A.-E. Füssel

Resumen
Los bancos de antígenos y vacunas constituyen reservas de material inmunógeno listas para ser formuladas en forma de vacuna (antígenos a granel) o para uso inmediato (vacunas) en caso de necesidad de una de las partes interesadas en el banco. Esas reservas fueron instituidas (básicamente por países europeos libres de fiebre aftosa) con el fin de luchar contra episodios inesperados y graves de fiebre aftosa una vez prohibidas las vacunaciones sistemáticas a partir de los años noventa. Por varias razones, incluyendo la falta de antígenos adecuados o de pruebas discriminatorias que se pueden utilizar en caso de la vacunación de emergencia, tales bancos no han sido hasta ahora desarrollados para controlar otras enfermedades transfronterizas, aunque durante los últimos años la Comunidad Europea ha reservado bancos de vacunas para apoyar las medidas de control para lengua azul o peste porcina clásica.
Los antígenos del virus de la fiebre aftosa de esos bancos se almacenan a temperaturas muy bajas (en general –130°C) para garantizar un tiempo de conservación mínimo de cinco años, frente al año o dos años de vida que presentan las vacunas a +4°C. Un volumen de 50 litros de antígenos a elevada concentración puede contener hasta 15 millones de dosis para ganado vacuno, según las especificaciones normativas sobre potencia farmacológica que figuran en el *Manual de pruebas de diagnóstico y vacunas para animales terrestres* de la OIE. Los especialistas sanitarios en fiebre aftosa tienen la responsabilidad de seleccionar tanto las cepas de origen de los antígenos y vacunas que se conservarán en un banco como las cepas apropiadas para vacunaciones de emergencia. Los autores examinan el uso de pruebas serológicas para distinguir en la población vacunada los animales infectados,

distinción indispensable para el reconocimiento del estatus de "libre de fiebre aftosa". Además, los autores destacan las ventajas y desventajas técnicas de los bancos de antígenos y de vacunas en general. Por último, presentan la experiencia de la Unión Europea (UE) a la hora de organizar, renovar y gestionar desde 1993 un banco de antígenos de la fiebre aftosa de un volumen considerable, y describen su empleo en actuaciones internacionales fuera del territorio de la UE.

Palabras clave
Banco de antígenos – Banco de vacunas – Estrategia de lucha – Fiebre aftosa – Método DIVA – Proteína no estructural – Reserva estratégica – Selección de cepas vacunales – Unión Europea – Vacunación de emergencia.

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Good manufacturing practice for immunological veterinary medicinal products

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Summary

Good manufacturing practice (GMP) is applied to the manufacture of immunological veterinary medicinal products (IVMPs) in a number of regions around the world. Within the European Union (EU) there are well-established requirements for GMP in the manufacture of IVMPs. Maintaining GMP when producing IVMPs is important because there are particular risks associated with their manufacture. These risks concern contamination and cross-contamination, environmental and operator protection, the variability of biological manufacturing processes and the limitations of some IVMP finished product tests. Whilst the general requirements of GMP are applicable to all medicinal products, guidance which addresses the specific concerns for IVMPs is provided by Annex 5 and also Annex 1 in *Medicinal Products for Human and Veterinary Use: Good Manufacturing Practice* (referred to as the GMP Guidelines). Extending and harmonising GMP requirements for IVMP manufacture throughout the world will increase the availability of high quality, safe and efficacious IVMPs.

Keywords

Directive 91/412/EEC – Directive 2001/82/EC – Good manufacturing practice – Immunological veterinary medicinal products – Veterinary vaccine.

Background

This paper addresses the requirements of good manufacturing practice (GMP) for immunological veterinary medicinal products (IVMPs). IVMPs are defined as any veterinary medicinal product administered to animals in order to produce active or passive immunity or to diagnose the state of immunity (3). As such, this category covers a range of veterinary medicinal products including vaccines, immunosera, allergen products and diagnostic products administered to animals (e.g. tuberculin).

Good manufacturing practice requirements are applied to the manufacture of IVMPs in many countries around the world (7). Within the European Union (EU) there are well-established requirements for GMP in the manufacture of these products, with an EU-wide legal framework for GMP. The legislation also provides the legal basis to ensure compliance with the requirements of GMP, by means of inspection of manufacturers by regulators.

Legal basis of good manufacturing practice for immunological veterinary medicinal products within the European Union

The current legal requirements for GMP during the manufacture of IVMPs are embodied in two EU directives which are implemented by national legislation in EU Member States. These directives apply to all veterinary medicinal products including IVMPs. Directive 2001/82/EC (3), as amended by Directive 2004/28/EC (4), sets out wide-ranging requirements for veterinary medicinal products in the EU. Commission Directive 91/412/EEC (2) lays down the principles and guidelines of good manufacturing practice for veterinary medicinal products.

Directive 2001/82/EC

This legislation requires that the manufacture of veterinary medicinal products within the EU be subject to the holding of a manufacturing authorisation for products intended for the EU market and also those intended for export to third countries. A further requirement is that the holder of a Manufacturing Authorisation is obliged to 'comply with the principles and guidelines on good manufacturing practice for medicinal products'. The Directive also requires Member States to ensure 'by means of repeated inspections' that 'the legal requirements relating to veterinary medicinal products are complied with'. This latter requirement also allows for the inspection of manufacturers established in third countries outside of the EU to ensure that the appropriate standards are met.

Commission Directive 91/412/EEC

This Directive reiterates that all veterinary medicinal products manufactured in or imported into the EU, including veterinary medicinal products intended for export, should be manufactured in accordance with the principles and guidelines of GMP. It then sets out these broad principles and guidelines and reiterates that it is the responsibility of Member States to ensure that manufacturers adhere to them. The Directive also refers to detailed guidelines published by the European Commission, these being entitled *Medicinal Products for Human and Veterinary Use: Good Manufacturing Practice* (6) which are referred to hereafter as the GMP Guidelines. These guidelines are contained in volume 4 of the *Rules Governing Medicinal Products in the European Community*.

For adoption of the legislation for GMP, it was agreed by all Member States and the industry that the GMP requirements applicable to the manufacture of veterinary medicinal products were the same as those applicable to the manufacture of medicinal products for human use. However, certain detailed adjustments were set out in annexes to the Guidelines which concerned specific product types; one of these annexes covers the manufacture of IVMPs.

It should be noted that in addition to this legislation applying in EU Member States, provisions of the above directives have been implemented by Norway, Iceland and Lichtenstein. Thus, the requirements for GMP apply to the manufacture of veterinary medicinal products, including IVMPs, throughout the European Economic Area (EEA).

What is good manufacturing practice?

Whilst it is clear that EU legislation requires that veterinary medicinal products are manufactured in accordance with GMP, a concise definition of the term good manufacturing practice is not provided. However, the following definition is provided in Chapter 1 of the GMP Guidelines:

'GMP is that part of Quality Assurance (QA) which ensures that products are consistently produced and controlled to the quality standards appropriate to their intended use and as required by the marketing authorisation or product specifications.'

Thus, for IVMPs, as for other medicinal products, GMP effectively leads to a set of measures that are intended to ensure the manufacture of safe and efficacious products in a consistent manner in accordance with the requirements set out in their marketing authorisations.

Good manufacturing practice is applicable to both production and quality control (QC) aspects for medicinal products and the basic requirements can be summarised as follows:

– manufacturing processes should be defined, reviewed and shown to be capable of consistently manufacturing products of the required quality and in compliance with their specifications

– critical manufacturing steps and process changes should be validated

– necessary facilities for GMP should be provided, including:

i) adequate levels of qualified staff

ii) suitable premises

iii) suitable equipment and services

iv) correct materials, containers and labels

v) appropriate storage facilities

– clear, written instructions and procedures should be available

– operators should be trained to carry out procedures correctly

– full manufacturing records should be kept: deviations from procedures and instructions should be recorded and investigated

– records should be retained

– distribution methods should minimise risk to product quality

– a system should be in place to recall products from sale or supply

– complaints about products should be examined, the cause of quality defects investigated and appropriate measures put in place to prevent reoccurrence.

Importance of good manufacturing practice in the manufacture of immunological veterinary medicinal products

There are a number of potential issues, as considered below, which may be associated with IVMPs and their manufacture. These issues can have a negative impact on product quality and therefore, either directly or indirectly, affect the safety or efficacy of the product. Application of GMP principles to their manufacture plays an important role in reducing this potential negative impact.

Contamination and cross-contamination

A feature of IVMPs is the wide range of animal species for which products may be manufactured. At the current time, IVMPs available on the EU market include those for farm animals (e.g. poultry, cattle, sheep, pigs), aquatic species (e.g. salmon, trout), and companion animals (e.g. dogs, cats, horses, rabbits). As a consequence of this, IVMP manufacture (and in particular veterinary vaccine manufacture) may involve the handling and production of a wide range of pathogens associated with the range of target species to be treated. However, it is often the case that relatively small amounts of materials derived from each pathogen are required, as batches of finished product are often relatively small.

This situation contrasts with that of immunological medicinal products for human use, which may be produced in much larger batch sizes for a narrower range of pathogens. As a consequence of the potential wide range of pathogens handled during production of IVMPs and the smaller batch sizes, it is common for their manufacture to occur in premises where a range of products are manufactured on a campaign basis. Such campaign manufacture leads to an inherent risk of cross-contamination of products due to the handling of different pathogens in the same facilities.

Contamination of IVMPs with environmental or other contaminants such as bacteria, moulds or viruses is as much a concern as cross-contamination with other production organisms. This is a particular issue due to the production methods which are involved in IVMP manufacture: open aseptic processing steps are a frequent part of their production. Whilst these open process steps will normally be performed under a filtered air flow in a 'clean room' there is always a potential risk of products becoming contaminated. Furthermore, unlike the situation for some sterile pharmaceutical products, terminal heat sterilisation of finished IVMPs is not normally an option due to their heat labile nature. Technological solutions to these issues are becoming more common, such as the use of steam-sterilised, closed systems for the culture, transfer and blending of products. However, open aseptic processing steps continue to be a common part of the manufacturing process for IVMPs; the filling and freeze-drying of IVMPs are both open processes.

Another potential route of contamination of IVMPs is via the raw materials that are used in their manufacture. Many raw materials may harbour bacterial or fungal contamination. In addition, there are potential risks that extraneous viruses or transmissible spongiform encephalopathy (TSE) agents could be introduced via animal-derived materials which are often used in IVMP manufacture (1, 5).

If an IVMP does become contaminated during its manufacture, then there is the potential that either the production process (e.g. virus culture), or the product itself might support the growth of the contaminant and allow its numbers to multiply.

The application of GMP principles to the manufacture of IVMPs is intended to reduce the potential risk posed by contamination or cross-contamination. If a contamination or cross-contamination problem is detected prior to the release of a product there will be significant costs involved, due to loss of the batch of product and downtime in the manufacturing facility whilst investigations and remedial action are performed. If such a problem is not detected prior to release, then there could be serious animal health and welfare implications. Although not due to a clear GMP compliance failure, a recent case was reported where a number of inactivated clostridial vaccines which had been released to the market were later found to be contaminated with live *Clostridium sordellii*. Of the 202,525 animals in affected herds, 41,767 animals were infected and 22,189 died (9).

Environmental protection concerns

Due to the virulent nature of some organisms handled during the manufacture of IVMPs, environmental protection measures are required. Accidental release of live biological agents to either the immediate production environment or the outside environment must be prevented. Release to other areas of the site gives rise to the potential for cross-contamination. Release to the outside environment may potentially pose both animal and human health issues. The animal health issues may be of particular importance when manufacture involves the handling of exotic organisms or notifiable disease agents (e.g. foot and mouth disease virus or bluetongue virus).

Operator protection concerns

Due to the zoonotic nature of some of the organisms handled, e.g. rabies virus, *Leptospira* spp. and *Mycobacterium bovis*, systems must be in place to ensure adequate protection of the staff. These systems will involve the use of containment facilities, protective clothing and, where appropriate, vaccination of staff.

Variability of biological manufacturing processes

It is a generally accepted aspect of biological manufacturing processes that the potential variability may be significantly greater than for pharmaceutical product manufacturing processes. As a result there is an inherent risk of inconsistencies arising in IVMPs when compared with their pharmaceutical counterparts and anecdotal evidence suggests that up to 10% of IVMP batches may be subject to minor deviations from the required specifications or details given in their marketing authorisation dossiers. The rigid application of GMP principles to the manufacture of these products plays a role in minimising the potential variability and ensures that any deviations are recorded and their potential impact investigated.

Relative inefficiency of some finished product tests in assuring the quality of immunological veterinary medicinal products

It should be noted that the inherent variability of biological systems (discussed above in relation to manufacturing processes) may also cause problems for the biological assay systems used in QC testing of IVMPs. Both *in vitro* and *in vivo* testing using biological methods are a frequent part of the testing of IVMPs. This aspect, along with issues of sample size, may limit the efficiency of finished product testing of IVMPs. An example of this is the European Pharmacopoeia sterility test: due to the sample size used it is possible that low-level contamination may not be detected. In addition, for tests based on the culture of any contaminants or live agents present (e.g. sterility, purity, inactivation, extraneous agents, etc.), the correct culture conditions are essential (e.g. media, incubation conditions, etc.) to ensure that any live microbial contaminants are detected. With reference to the example given above concerning contaminated clostridial vaccines, it should be noted that the *C. sordellii* contamination was not detected by the finished product sterility test.

Taking the above issues into account it is considered that the application of GMP to the manufacture of IVMPs is of paramount importance in ensuring the availability of IVMPs of suitable quality that are safe and effective. Application of GMP principles to IVMP manufacturing processes effectively builds quality into the product from the outset rather than placing the emphasis solely on testing of the finished product, with the inherent limitations of this approach.

The good manufacturing practice guidelines and their structure

The GMP Guidelines consist of two parts which describe the basic requirements that are applicable to all medicinal products and their raw materials. The requirements are adapted and modified for some specific issues and product types by a set of annexes. Part 1 of the GMP Guidelines describes the basic requirements for the manufacture of medicinal products, whilst Part 2 describes the GMP requirements for active substances used as starting materials for medicinal products.

Part 1 consists of 9 chapters which reflect the key principles of GMP that are set down in Commission Directive 91/412/EEC. The chapter titles, along with some of the broad requirements, are included in Table I.

Part 2 of the GMP Guidelines addresses the GMP requirements for active substances used as raw materials for medicinal products. These requirements were previously voluntary and had been included as Annex 18. However, amendment of Directive 2001/82/EC by Directive 2004/28/EC made it mandatory for active substances for use as raw materials in medicinal products to be manufactured in accordance with GMP. However, it should be noted that the mandatory application of Part 2 of the GMP Guidelines had less impact on the manufacture of IVMPS than it did on the production of veterinary pharmaceuticals. Due to the nature of these materials and the fact that they are normally manufactured by the final product manufacturer, the production and testing of these antigens has routinely been subject to GMP and the requirements for their manufacture are provided in the relevant Annex to the GMP Guidelines.

Annexes 1 to 19 expand on the basic requirements. As indicated above Annex 18 has been changed to Part 2, but Annex 18 has not been reassigned to prevent confusion. The majority of the annexes address the manufacture of certain specific types of medicinal products, the remaining annexes providing more detailed information on a number of specific topics.

Table I
Chapters of Part 1 of the European Union Good Manufacturing Practice Guidelines and some of the key requirements for human and veterinary medicinal products

Chapter	Examples of key requirements
Quality management	An effective pharmaceutical quality assurance (QA) system should be in place Management and staff should be actively involved in the QA system The QA system should incorporate good manufacturing practice (GMP) and quality control (QC) The QA system should be adequately resourced
Personnel	There should be sufficient levels of competent and appropriately trained staff Job descriptions for key staff should be defined All personnel should be aware of the principles of GMP
Premises and equipment	These should be located, designed, constructed, adapted and maintained to suit their purpose Their design and layout should minimise the risk of error and permit effective cleaning and maintenance to prevent cross-contamination, build up of dust or dirt and, in general, any adverse effect on the quality of products
Documentation	Clear, accurate documents such as specifications and instructions should be in place Records should be kept
Production	Clear defined procedures should be followed to ensure that products of the requisite quality are produced in accordance with the relevant manufacturing and marketing authorisations These procedures should comply with the principles of GMP
Quality control	Sampling and testing should be performed as appropriate Release procedures should be in place to ensure that materials are not released for use, or products released for sale or supply, until their quality has been judged as satisfactory QC should be involved in all decisions which may concern the quality of the product
Contract manufacture and analysis	Systems should be in place to ensure that GMP requirements are met when work is contracted out by the manufacturer
Complaints and product recall	Complaints and other information concerning potentially defective products should be reviewed in accordance with written procedures A system should be in place to recall from the market, in an effective and timely manner, products known or suspected to be defective
Self inspection	A system of self-inspection should be in place to monitor compliance with GMP and propose necessary corrective actions

Of the specific product annexes, two are of direct relevance to the manufacture of IVMPs; these are Annex 5 'Manufacture of Immunological Veterinary Medicinal Products' and Annex 1 'Manufacture of Sterile Medicinal Products'. Annex 5 is applicable to all IVMPs; however, all parenteral and most liquid IVMPs are required to be sterile (or pure if they are live vaccines) and thus also fall under the scope of Annex 1. In addition, many other non-parenteral IVMPs including a significant number of freeze dried viral vaccines for use in poultry, although not required to be sterile, are not permitted to contain more than one non-pathogenic organism per dose. In order to comply with this limit it is, in practice, necessary to manufacture such products in accordance with Annex 1 requirements.

A number of other annexes may apply to the manufacture of IVMPs. These include Annex 8 'Sampling of starting and packaging materials', Annex 11 'Computerised systems', Annex 15 'Qualification and validation' and Annex 16 'Certification by a qualified person and batch release'.

The GMP Guidelines are subject to periodic review and update when necessary. Updates arise from initial input by EEA GMP inspectors with further input from industry and other interested parties, via a formal consultation process. Following finalisation, the revised chapter or Annex (or new Annex) is forwarded to the European Commission's Pharmaceutical and Veterinary Pharmaceutical Committees for adoption.

Annex 1 and Annex 5: specific good manufacturing practice guidance for immunological veterinary medicinal products

As highlighted earlier, in addition to the basic requirements in Parts 1 and 2 of the GMP Guidelines, there are specific requirements applicable to the manufacture of IVMPs, which are laid out in Annex 1 and Annex 5. The general requirements of Annex 1 which are applicable to the manufacture of IVMPs and the more specific requirements of Annex 5 are considered in this section.

The requirements of Annex 1 focus on minimising the potential for microbiological, particulate or pyrogen contamination. The skill and training of staff, and the role played by QA, are of particular importance in the manufacture of sterile medicinal products.

The manufacture of sterile products is required to be performed in 'clean areas' with microbial and particulate limits for these areas being set at levels dependent on the activities being performed. The lowest category of clean area (i.e. with the highest permitted particulates and microbial levels) is designated as Grade D, whilst the highest category is designated as Grade A. Grade A conditions are required for operations where the product is most at risk, i.e. during open manipulations such as filling. Frequent monitoring of clean areas should be performed to demonstrate the continued compliance with the stated air cleanliness grades. In addition, detailed requirements concerning clothing for staff working in clean areas are provided. Annex 1 also provides guidance on the requirements for premises and equipment, aseptic preparation, aseptic process validation, sanitation and sterilisation.

The requirements of Annex 5 concentrate on the areas highlighted earlier as being specific issues for the manufacture of IVMPs. These issues concern contamination and cross-contamination, protection of the environment and operators, the variability of biological manufacturing systems and the relative inefficiency of some finished product tests. Some examples of specific concerns and the applicable Annex 5 requirements which address them are provided in Table II.

Whilst the requirements of Annex 1 and Annex 5 generally complement each other there are some occasions where a balance may need to be struck between the requirements of the two annexes. For example, whilst inactivated materials and IVMPs should be handled in classical clean areas as required by Annex 1, live IVMPs and materials prior to inactivation should be handled only in containment facilities. However, as there is also the requirement to keep the live material or IVMPs pure, the air cleanliness grading and monitoring requirements of Annex 1, along with most other Annex 1 requirements, generally apply to the Annex 5 containment conditions (e.g. where there is open processing this should be in a Grade A area with a Grade B background). Some care needs to be taken with this approach though, as there are a small number of cases where Annex 1 requirements are not appropriate for containment areas. An obvious example of this concerns the pressure cascades for clean and contained areas. In a clean area a positive air pressure cascade should be in place to protect the product. However, where live materials are handled, this approach would spread contamination and so a negative cascade (or suitable alternative arrangement designed to prevent the release of live agents) is required. Another Annex 1 requirement which is not appropriate for the operation of containment facilities is the use of continuous particulate monitoring systems in Grade A and B areas; in this case a stream of potentially contaminated air could be drawn into the system, thus causing a breakdown of containment.

Good manufacturing practice in different countries and regions

The manufacture of IVMPs is regulated in many countries around the world and the requirement for manufacture in compliance with GMP is often a key aspect of this regulation. The GMP requirements in place for IVMP manufacture in four key regions in the world (Europe, North America, Japan and Australia/New Zealand) are briefly considered.

Europe

The requirements of GMP as applied to the manufacture of IVMPs within the EEA have been discussed. In addition, manufacturers based in third countries which supply the EU market are required to manufacture in accordance with GMP and are normally subject to routine GMP inspection by EU inspection authorities. Inspections of IVMP manufacturers may be arranged in connection with nationally authorised products or for centrally authorised products. Inspections in relation to this latter group of products are coordinated by the Inspections Section of the European Medicines Evaluation Agency (EMEA) (10).

With expansion of the EU the application of GMP has extended across Europe. New Member States are required to apply EU standards to the manufacture of IVMPs on accession to the EU.

In addition to the application of GMP by EU/EEA Member States, a Mutual Recognition Agreement (MRA) is in place between the EU and Switzerland, this covering veterinary medicinal products.

Table II
Specific concerns applicable to the manufacture of immunological veterinary medicinal products: examples of the requirements contained in Annex 5 of the European Union Good Manufacturing Practice Guidelines

Issue	Concern	Measures to address concern
Personnel	Personnel may be a significant source of contamination or cross-contamination	Appropriate protective clothing should be used at different stages of manufacturing Procedures should be in place governing movement between different manufacturing areas (movement restrictions)
Handling of live biological agents	Accidental release of live biological agents should be prevented	Live agents should only be handled in contained areas Containment facilities should include: – negative pressure work area – no direct venting of air out of the area – entry of staff and equipment via air locks – system for collection and disinfection or sterilisation of effluents and wastes
Handling of sterile and inactivated materials and products	Sterile and inactivated materials and products should be protected from contamination and / or cross-contamination	Sterile and inactivated materials and products should be handled in clean areas Clean areas should meet Annex 1 requirements, including positive air pressure cascade
Potential contamination due to certain manufacturing operations	Certain manufacturing operations may act as a source of contamination	Areas which are likely to be a source of contamination should be separated from other production areas, e.g: – QC laboratories – animal houses – virus culture areas – spore bearing bacteria culture areas – media preparation
Disinfection, decontamination and fumigation procedures	Contamination may be a concern if procedures are ineffective	Procedures should be validated to demonstrate their effectiveness
Potential cross-contamination during storage	One material or product should not pose a cross-contamination risk to another	Live or infected materials should be separated from sterile, non-infected or inactivated materials Separate, dedicated incubators or coolers should be used for the storage of live and inactivated products (although storage of live and inactivated finished filled products in the same area is accepted)
Product consistency	Measures should be taken to prevent or minimise variability between product batches	Seed lot and cell bank systems should be used where appropriate to ensure consistency of the seed material used for scale up Limits to the number of generations between the seed and the finished product should be in place, in accordance with the marketing authorisation dossier
Potential cross-contamination arising from a product	Contamination arising from product during manipulation should be avoided	The formation of aerosols, droplets and foam containing live agents should be prevented or minimised Accidental spillages should be handled in a prompt and safe manner Only one live agent should be handled in an area (or one virus and one cell line) at a time, unless closed systems are in use. An exception to this would be during the blending of live viral vaccines
Inactivation	Procedures should ensure complete inactivation	A double tank inactivation procedure should be followed
Consistency of production	Manufacturing yields should meet expected levels	Yield reconciliation should be performed following manufacture steps Deviations from expected yields should be investigated

United States of America

Immunological veterinary medicinal products fall under the scope of the Virus/Serum/Toxin Act of 1913 (10). A licence is required to manufacture these products at a specified facility, this being issued by the Animal and Plant Health Inspection Service (APHIS) of the United States Department of Agriculture. This licence is required for manufacture for both the domestic and overseas markets. To obtain the licence, blueprints and blueprint legends for the facility must be submitted for approval. The Animal and Plant Health Inspection Service reviews these to ensure that the facility will operate in a manner consistent with GMP. If changes to the facilities occur, revised blueprints must be submitted immediately. Prior to issue of the licence, the applicant's premises are subject to inspection by APHIS examiners. The inspection is intended to ensure that the facility operates in a manner consistent with GMP by confirming that the establishment is configured in accordance with the blueprint and legends, that the production line is set up in accordance with the approved outline of production and that records are adequately kept. Following issue of the licence, APHIS routinely conducts unannounced post-licensing inspections ordinarily within 12 to 18 months of the last inspection. Special inspections may be performed prior to approval of changes to the facility or the production method.

Canada

The manufacture of IVMPs in Canada is subject to licensing and inspections in accordance with the country's Health of Animals Act and Regulations. Inspections of IVMP manufacturers are performed by the Veterinary Biologic Section of the Animal Health and Production Division of the Canadian Food Inspection Agency (www.inspection.gc.ca).

Japan

The regulation of IVMPs within Japan falls under the jurisdiction of the Ministry of Agriculture, Forestry and Fisheries. The Ministry issues licences to manufacture, import, and sell IVMPs. Conformity to GMP is stipulated as one of the conditions for obtaining a licence to manufacture (10).

Australia and New Zealand

Good manufacturing practice requirements for the manufacture of IVMPs are in place in Australia (for further details visit the website of the Australian Pesticides and Veterinary Medicines Authority: www.apvma.gov.au) and in New Zealand (see the New Zealand Food Safety Authority website: www.nzfsa.gov.nz). These requirements along with the legislative basis for them are considered to be equivalent to those in the EU and vice versa. Equivalence was determined prior to the start of the operational phases of MRAs between each of these countries and the EU.

International bodies involved in good manufacturing practice and immunological veterinary medicinal product quality control

Pharmaceutical Inspection Convention and Pharmaceutical Inspection Co-operation Scheme

The Pharmaceutical Inspection Convention and Pharmaceutical Inspection Co-operation Scheme (jointly referred to as PIC/S) is an international body which is primarily involved in the spread and harmonisation of GMP standards throughout the world. Together, the Convention (a formal treaty between countries) and the Scheme (an informal arrangement between health authorities) provide the basis for active and constructive co-operation in the field of GMP.

The PIC/S mission statement is 'to lead the international development, implementation and maintenance of harmonised good manufacturing practice (GMP) standards and quality systems of inspectorates in the field of medicinal products' (www.picscheme.org).

The aim of PIC/S is to achieve this mission by 'developing and promoting harmonised GMP standards and guidance documents; training competent authorities, in particular inspectors; assessing (and reassessing) inspectorates; and facilitating the co-operation and networking for competent authorities and international organisations'. PIC/S has adopted the EU GMP Guidelines in their entirety as their own GMP Guidelines (with suitable modification to remove references to EU Legislation, etc. [8]).

Members of PIC/S include inspectorates from the EU and various countries around the world (e.g. Australia, New Zealand, Malaysia) and membership continues to expand. Among the current membership, the only inspectorate that is dedicated solely to veterinary inspection is that of the Czech Republic; however, a number of members are involved in the inspection of both human and veterinary products. Expansion of PIC/S membership by veterinary

inspectorates around the world would assist in the harmonisation of GMP standards for the manufacture of veterinary vaccines.

Other organisations

Various other organisations are involved in the standardisation and quality of veterinary vaccines and other IVMPs throughout the world. These include the World Organisation for Animal Health (OIE), the Food and Agriculture Organization (FAO) of the United Nations and the Veterinary International Cooperation on Harmonisation (VICH) (10). However, none of these organisations currently play a significant role with regards inspection or GMP for these products, concentrating more on the official testing of IVMPs. One organisation with a slightly wider remit on this issue is the Pan African Veterinary Vaccine Centre (PANVAC) which was set up in 1991 with FAO assistance and European aid. The main function of PANVAC has been the testing of various veterinary vaccines; however, it also provides training for specialists from African countries in the production and testing of veterinary vaccines.

Conclusions

Good manufacturing practice principles are applied to the manufacture of IVMPs in a number of countries and regions of the world. In the EU and other areas, legislation is in place to ensure the stringent application of GMP requirements. It is considered that these requirements are of paramount importance in reducing the potential impact of the inherent risks which apply to the manufacture of IVMPs. These measures thereby ensure that IVMPs are of the appropriate quality, are safe and efficacious. The requirement for manufacture in accordance with GMP complements other regulatory safeguards such as the licensing requirements for these products.

The expansion of GMP to the manufacture of IVMPs in regions where it is currently not applied will bring significant benefits in terms of product quality, safety and efficacy. A key role in the development and promotion of harmonised GMP standards for all types of medicinal products is being played by PIC/S. However, other organisations, such as PANVAC, which already have an important part to play in the improvement of quality for veterinary vaccines may be candidates for a more prominent role in the future promotion of harmonised application of GMP standards for IVMP manufacture, particularly in developing countries. Whilst the application of these standards may have significant cost implications, it is considered that these should be outweighed in the long term by the benefits to animal health, and consequently to human health, in these regions through the availability of safe, efficacious veterinary vaccines, manufactured to high quality standards.

■

Bonnes pratiques de fabrication pour les médicaments vétérinaires immunologiques

J.I. Todd

Résumé

Dans nombre de pays, les médicaments vétérinaires immunologiques (MVI) sont produits en suivant des procédures appelées « bonnes pratiques de fabrication » (BPF). L'Union européenne met en œuvre depuis longtemps les BPF pour la fabrication des MVI. Il est essentiel de respecter ces exigences, compte tenu des risques particuliers associés à la fabrication des MVI, qui portent sur la contamination, la contamination croisée, la protection de l'environnement et des agents chargés de manipuler les médicaments, la variabilité des processus de fabrication de produits biologiques et les limites de certains tests applicables aux produits finis. Les exigences des BPF couvrent tous les médicaments, ceux à usage spécifiquement vétérinaire étant couverts par les Annexes 1 et 5 des Lignes directrices de l'UE relatives aux bonnes pratiques de fabrication des

médicaments à usage vétérinaire et humain. Il convient de développer et d'harmoniser les exigences des BPF partout dans le monde afin d'assurer une disponibilité de MVI de grande qualité, innocuité et efficacité.

Mots-clés
Bonne pratique de fabrication – Directive 91/412/EEC – Directive 2001/82/EC – Médicament vétérinaire immunologique – Vaccin vétérinaire.

Buenas prácticas de fabricación de productos inmunológicos veterinarios

J.I. Todd

Resumen
En varias regiones del mundo, la producción de medicamentos inmunológicos de uso veterinario se rige por una serie de buenas prácticas de fabricación. Dentro de la Unión Europea (UE) existen requisitos bien definidos en la materia. El hecho de atenerse a un conjunto de buenas prácticas en la fabricación de dichos medicamentos es importante por los particulares riesgos que el proceso conlleva, riesgos ligados a la contaminación, la protección del entorno físico y de los trabajadores, la variabilidad propia de los procesos de fabricación de productos biológicos y las limitaciones de que adolecen algunas de las pruebas a que son sometidos los productos finales. Si bien los requisitos generales de las buenas prácticas de fabricación son válidos para todo producto medicinal, en los anexos 5 y 1 de la guía comunitaria de normas de correcta fabricación de productos medicinales de uso humano y veterinario se sientan pautas referidas específicamente a los medicamentos inmunológicos veterinarios. La extensión de las buenas prácticas de fabricación a otras partes del mundo y su armonización acrecentarán la oferta de productos inmunológicos veterinarios seguros, eficaces y de calidad.

Palabras clave
Buena práctica de fabricación – Directiva 91/412/EEC – Directiva 2001/82/EC – Productos inmunológicos veterinarios – Vacuna veterinaria.

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Innate immunity and new adjuvants

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Summary

Vaccination remains the most cost-effective biomedical approach to the control of infectious diseases in livestock. Vaccines based on killed pathogens or subunit antigens are safer but are often ineffective and require coadministration with adjuvants to achieve efficacy. Unfortunately, most conventional adjuvants are poorly defined, complex substances that fail to meet the stringent criteria for safety and efficacy desired in new generation vaccines. A new generation of adjuvants that work by activating innate immunity presents exciting opportunities to develop safer, more potent vaccines. In this review the authors highlight the role of innate immunity in protection against infectious disease and provide some examples of promising new adjuvants that activate innate immunity. They do not review the conventional adjuvants present in many vaccines since they have been reviewed extensively previously.

Keywords

Adjuvant – Infectious disease – Innate immunity – Livestock – Vaccines.

Introduction

Infectious diseases continue to be a major cause of death and economic losses in domestic animals. Today, the most cost-effective strategy for the control of infectious diseases is clearly vaccination. Indeed, vaccination has already greatly improved livestock production and reduced animal suffering. However, there are concerns regarding many of today's vaccines with respect to their safety and efficacy, and therefore there is a need for safer and more efficacious vaccines for livestock. Furthermore, the realisation that the majority of newly emerging diseases not only affect animals but can be transmitted to humans has created an even greater need for effective vaccines for domestic animals. Vaccines based on live and killed pathogens have traditionally been used in the livestock industry, and each of these has its perceived advantages and disadvantages. Live vaccines are often more effective as they tend to stimulate vigorous immune responses, often similar to natural infection, but they can potentially revert to virulence and cause disease especially in immunocompromised hosts. Killed vaccines or their components are generally regarded as safer, but they often fail to induce protective immunity.

The realisation that certain components in killed vaccines may be harmful to the host has led to the evolution of a vaccine development approach that involves the identification of defined molecules (protective antigens) that are associated with induction of protective immunity. With the recent and rapid progress in molecular biology, genomics, proteomics, and immunology it is now possible to identify a myriad of potential targets for vaccine development. Furthermore, combining the advances in molecular biology with those in immunology and pathogenesis, it is now possible to correlate the immune response induced by specific proteins with different levels of protection. Thus, we now know, in most cases, what components of the infectious agent are critical for preventing infection or aiding in recovery from infection as well as which immune responses are desired. Unfortunately, it is not always easy to induce the correct immune response. This is especially the case where

recombinant proteins or killed vaccines are used as immunogens. These killed antigens are generally poor at inducing immune responses and, more importantly, the quality of the immune response induced may not give optimal protection. This could possibly be improved by developing novel adjuvants and formulation technologies. Conventional adjuvants used in commercially available animal vaccines have been extensively reviewed elsewhere and the reader is referred to these excellent reviews for details (10, 48).

A detailed understanding of the requirements for immune activation has provided an explanation for why recombinant vaccines fail to be effective. These vaccines often lack the components of pathogens that trigger 'danger' signals that activate innate immunity leading to enhanced vaccine efficacy. In this regard, the search for new adjuvants has focused on molecules that activate innate immunity. We will briefly discuss innate immunity and highlight some promising new adjuvants that enhance vaccine efficacy primarily by stimulating innate immunity.

Innate immunity

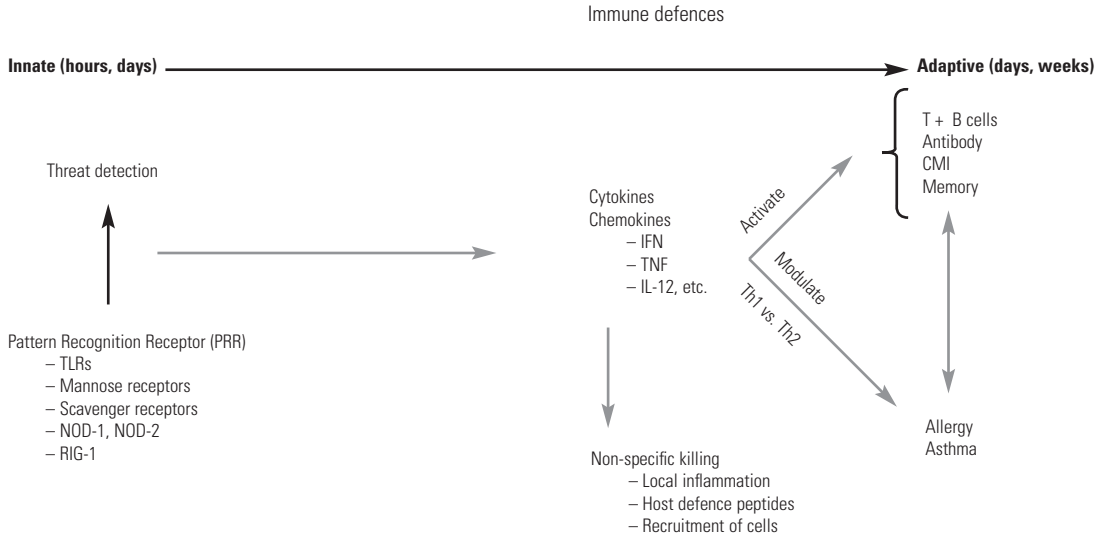
The immune system has evolved two general strategies to protect the host against infectious diseases: the innate and adaptive immune responses (Table I). Innate immunity represents a very effective first response against invading pathogens and consists of a set of conserved mechanisms to recognise and counter the constant threat of microbial infections (3, 27). As such, innate immunity is regulated by a network of complex receptor-ligand interactions which eventually lead to the creation of a pro-inflammatory local environment and thereby set the stage for the development of adaptive immune responses. The adaptive immune system, which is relatively slow to respond, forms the second line of immune defence, a 'back-up' strategy called into action to clear any pathogens that survive or evade the innate immune responses. Indeed, in the case of a rapidly replicating pathogen, this delay affects the success of the naïve host in attacking the invading organism (45). Therefore, the early interplay between innate and adaptive immunity is essential for effective immunity against most invading pathogens (23). By exploiting this link between innate and adaptive immunity, it is possible to develop more potent adjuvants, leading to more effective vaccine formulations.

Table I
Innate versus adaptive immunity

Innate immunity	Adaptive immunity
Very early after exposure to infection (hours, days)	Delayed (days, weeks)
Activated by a wide range of pathogens	Specifically activated by certain components of pathogens (antigen)
Confers broad protection	Vaccines stimulate this type of immunity
Primary functions:	Primary functions:
Controls spread of infection	Clearance of infection
Direct development of adaptive immunity	Development of memory response

Stimulation of innate immunity is initiated by the interaction of pathogen components with receptors present on immune cells. These pattern recognition receptors (PRR) recognise highly conserved components of pathogens called pathogen-associated molecular patterns (PAMP) (51, 53). Pattern recognition receptors represent a large group of conserved receptor molecules including toll-like receptors (TLR), complement receptors, C-type lectins, and nucleotide-binding oligomerisation domain (NOD) receptors NOD 1 or NOD 2 (22, 32, 43). Following recognition of pathogen PAMP, signalling through these receptors leads to activation of the nuclear factor-κB, which in turn increases expression of chemical mediators including cytokines, chemokines (Fig. 1) and co-stimulatory molecules (34). Several of these cytokines induce epithelial cells to express antimicrobial peptides, increasing the antimicrobial capacity of the epithelial barrier (63). In addition, expression of these molecules creates a local pro-inflammatory environment, which helps to recruit and activate phagocytic cells, activate the complement cascade, contain the invading pathogen and chemoattract the effector cells of the adaptive immune response (Fig. 1). However, over-stimulation can also result in septic pro-inflammatory responses such as secretion of tumour necrosis factor, which in severe cases can be detrimental to the animal.

Pattern recognition receptors can be found in large concentrations at the cutaneous and mucosal surfaces of the body and are expressed in various types of immune cells including antigen-presenting cells (APC) and lymphocytes. Of special importance are dendritic cells (DC), highly effective APC that express a wide variety of PRR. These receptors are used by DC as 'sensors' for pathogens and they also sample antigens in their microenvironment. Signalling through PRR leads to activation of APC and expression of several responses. Subsequently, these cells migrate towards the draining lymphoid tissues where the antigen is either directly presented or passed on to resident DC for the induction of an adaptive immune response. Thus, DC represent an important link between innate and specific immunity. Furthermore, the type of initial innate stimulus will impact the ability of DC to link innate and adaptive immune responses with regard to the quality and magnitude of the responses. Thus, DC can 'imprint' the adaptive immune response by shifting the type of effector response to either a T helper (Th)1 type (protects primarily against



CMI: cell-mediated immune responses
IFN: interferon
IL: interleukin
TNF: tumour necrosis factor

Fig. 1
Activation of innate and adaptive immunity through pattern recognition receptors
The innate immune system uses a network of pattern recognition receptors to detect the presence of infectious agents These include toll-like receptors (TLR), nucleotide-binding oligomerisation domain receptors (NOD) and retinoic acid inducible genes (RIG). Engagement of these receptors initiates a signalling cascade that results in production of a variety of mediators (cytokines, chemokines), which mediate the effector responses. These responses serve two primary functions:
a) to control spread of infection via non-specific killing
b) to activate and direct the development of the adaptive immune responses (T helper [Th]1 and Th2)

intracellular pathogens) or a Th2 type (protects primarily against extracellular pathogens) of immune response, and by instructing effector cells to selectively home back to certain compartments of the immune system. Thus, stimulation of DC by vaccine adjuvants represents an important strategy for novel vaccination.

Adjuvants

Adjuvants were first described by Ramon (41) as 'helper' substances which when added to an antigen produce stronger immune responses than can be induced by the antigen alone. Since then many different natural and synthetic substances have been evaluated, primarily by trial and error, and some have been found to have adjuvant activity.

Adjuvants can be classified into two broad categories:

a) delivery systems

b) immunostimulatory adjuvants (48).

Delivery systems include many conventional adjuvants such as alum, liposomes, microparticles and oil/water emulsions. The mechanisms by which these adjuvants work are not well understood, but many of them form a 'depot' at the site of injection, where the antigen is slowly released and stimulates infiltrating cells of the immune system. Furthermore, these are often poorly defined, crude substances that have been associated with severe tissue damage at the site of injection. Ironically, the efficacy of some of these adjuvants is dependent on the degree of tissue damage, i.e. a substance that causes severe tissue damage has more potent adjuvant activity. Therefore, the challenge for vaccinologists is to discover and develop adjuvants that activate protective immunity but do not cause severe tissue damage. This paradigm shift has generated great interest in the second class of adjuvants, the immunostimulatory adjuvants, which tend to stimulate immunity with minimal or no tissue damage. These adjuvants are predominantly microbial components (Table II) and as the name suggests their adjuvant activity is dependent on their ability to stimulate innate immunity. Current understanding of how the body senses infectious threats involves the use of a variety of receptors (see innate immunity, above) which sets the stage for a 'danger' signal that triggers a cascade of innate immune responses, subsequently leading to the recruitment and expansion of cells involved in the development of adaptive immunity.

Indeed, several pathogen-derived components such as bacterial endotoxin (lipopolysaccharide [LPS]), the mycobacterial component of complete Freund's adjuvant, single-stranded ribonucleic acid (ssRNA), and bacterial deoxyribonucleic acid (DNA), including synthetic CpG DNA (sites where cytosine [C] lies next to guanine [G] in the DNA sequence; the p indicates that C and G are connected by a phosphodiester bond), can generate 'danger' signals and thus have adjuvant activity (17, 35, 44, 54). Therefore, molecules that activate innate immunity provide a novel class of adjuvants that not only enhance immune responses but can be selectively used to 'tailor' the quality of the desired response.

Shortly after the discovery that cytokines were critical in inducing immune responses, there was a flurry of activity to use cytokines as adjuvants. Initially, these studies involved interleukin (IL)-2 and interferon gamma (IFN-γ), two potent immune modulators. Some of these studies clearly showed the benefits of incorporating cytokines into vaccines. For example, IL-2 enhanced immune responses to bovine herpesvirus antigens, and other studies have also shown enhanced primary and secondary immune responses in the presence of IFN-γ (24). However, studies also showed that the dose of cytokine was critical and that immune suppression could occur if inappropriate doses were used (24). This is not surprising because the immune system generally is not engineered to respond to a large bolus of a single cytokine. Indeed, a very fine balance between the different cytokines is crucial to ensure appropriate cell signalling. This can be achieved by use of adjuvants that stimulate innate immunity, leading to production of a variety of cytokines and other mediators, resulting in stimulation of well-regulated immune responses.

In addition to their traditional role in preventing infectious diseases, vaccination strategies are also being developed as therapies for other diseases, including cancer and allergies. Development of safe and effective vaccine adjuvants is critical not only for improvement of existing vaccines but also for developing novel vaccines. In the next section the authors discuss some of these new adjuvants.

CpG oligodeoxynucleotides

As early as the 1890s, a surgeon in New York observed that cancer patients injected with crude bacterial preparations had significantly longer remission periods. Subsequently, bacterial DNA was identified as the primary mediator of anti-tumour immunity in mice (50, 58). It has now become clear that bacterial DNA, as well as synthetic oligodeoxynucleotides (ODN) containing CpG motifs (CpG ODN), provides a 'danger' signal that induces vigorous immune responses. To date, numerous investigators have shown that treatment of animals with CpG DNA can protect against a variety of experimental infectious and non-infectious diseases (56). Based on encouraging results from mouse models, human clinical studies are now being undertaken to evaluate the efficacy of CpG ODN therapy against infectious disease, cancer, asthma and allergy (28). In this regard, addition of CpG ODN to a commercial hepatitis B virus (HBV) vaccine resulted in significant increases in HBV surface antigen-specific antibody response in human volunteers (14). Furthermore, immunisation of human immunodeficiency virus (HIV)-infected individuals with an HBV vaccine in the presence of CpG DNA significantly increased the number of seropositive subjects and also increased the HBV-specific lymphocyte proliferative response (15). Thus, CpG DNA is a promising adjuvant for human vaccines.

Synthetic CpG DNA has been evaluated as a vaccine adjuvant in large animals. Unlike conventional oil-based adjuvants, which typically promote Th2 type immune responses that may not be protective against some infections, in these studies, CpG ODN promoted predominantly Th1 type immune responses (13, 28). For example, CpG ODN was shown to be an excellent adjuvant for stimulating immune responses against an experimental vaccine based on a subunit protein (gD antigen) of bovine herpesvirus-1 (BHV-1) in mice, sheep and cattle by producing enhanced serum immunoglobin 2a levels and IFN-γ in splenocytes or peripheral blood lymphocytes, indicating a more balanced, or Th1 type, response (25, 26). Interestingly, the use of CpG ODN in combination with low levels of mineral oil enhanced the

Table II
Examples of adjuvants that stimulate innate immunity

Adjuvant	Evidence for adjuvant activity in:	References
CpG deoxyribonucleic acid	Cattle, sheep, pigs, horses, monkeys, humans, laboratory animals	1, 13, 14, 15, 18, 25, 26, 31, 42
Host defence peptides	Laboratory animals	4, 6, 11, 30, 49
Single stranded ribonucleic acid and imidazoquinolines	Monkeys, laboratory animals	54, 55
Polyphosphazenes	Laboratory animals, sheep	33, 38, 40, 57, unpublished observations

immune response and reduced the amount of tissue damage associated with conventional vaccine adjuvants in sheep (25). In addition, CpG ODN in combination with alum demonstrated protection against BHV-1 (42), and CpG ODN in combination with Emulsigen® (a mineral oil adjuvant) was shown to be a potent adjuvant for stimulating a protective immune response against the gD antigen of BHV-1 in cattle (26). Similarly, incorporation of CpG ODN in a commercial equine influenza virus vaccine resulted in significant enhancement of antibody production against influenza virus (31).

Therefore, CpG ODN is compatible with commercially available vaccines, and in some cases CpG synergises with conventional adjuvants present in these vaccines, resulting in even greater enhancement of immune responses. This should expedite the application of CpG in commercial vaccines because there should be less need to perform all the safety trials required for new vaccines as new adjuvants are simply being added to currently licensed vaccines. Indeed, clinical trials are currently in progress to evaluate the benefits of incorporating CpG DNA in commercial livestock vaccines.

Host defence peptides

Cationic host defence peptides (HDP) are endogenous antibiotics found in virtually every life form. Mammalian HDP are very short peptides that can be grouped into defensins and cathelicidins. Typically, HDP are amphipathic positively charged molecules (20, 39).

Host defence peptides are fundamental components of the innate immune response. Their wide spectrum of functions includes direct antimicrobial activities, immunostimulatory functions of both innate and acquired immunity, and involvement in wound healing, cell trafficking and vascular growth (9, 12, 36). While the antimicrobial activities of HDP have been known for a long time, recent evidence suggests that at physiological concentrations mammalian HDP have a number of immunomodulatory functions, including recruitment of immature DC and T-cells, glucocorticoid production, macrophage phagocytosis, mast cell degranulation, complement activation, and IL-8 production by epithelial cells (59, 61, 62). Other HDP have been shown to up-regulate gene expression in epithelial cells and monocytes, and to neutralise pro-inflammatory cytokine induction and lethality in response to LPS/endotoxin (2, 7, 9, 16, 19, 20, 29, 36, 37, 46, 47).

Evidence for the ability of HDP to enhance adaptive immunity (indicative of adjuvant activity) is based on various observations. For example, human neutrophil peptides (HNP) 1 to 3, human beta-defensins 1 and 2, and murine beta-defensins (mBD) have been described to be chemoattractive for immature DC and lymphocytes (4, 60), and monocytes and macrophages (21). Recognition by immature DC occurs through chemokine receptor 6 (4) and other not yet identified receptors (60). Furthermore, in addition to chemoattraction of immature DC, HDP have also been demonstrated to attract mature DC (4, 6, 16). The immunoenhancing activity of HDP has been demonstrated in several studies. For example, ovalbumin-specific immune responses were enhanced in mice when HNP 1-3 were co-administered intranasally to C57/Bl mice (30). This observation was further supported by other investigators (49) who demonstrated that intraperitoneal injection of HNP 1-3 together with keyhole limpet haemocyanin (KLH) and B-cell lymphoma idiotype antigen into mice enhanced the resistance of immunised mice to subsequent tumour challenge. Brogden *et al.* (11) also confirmed the immunoenhancing activity of various defensins. More evidence for the immunoenhancing activity of HDP is derived from studies using DNA vaccines. When mBD2 and mBD3 were fused with B-cell lymphoma epitope sFv38, strong immune responses and stronger anti-tumour immunity were observed in immunised mice (4, 6). The same researchers also demonstrated that human immunodeficiency virus-1 glycoprotein 120 (HIV gp120) specific mucosal, systemic, and cytotoxic lymphocyte (CTL) immune responses could be achieved after immunisation with a fusion DNA vaccine encoding the murine β-defensin 2 and the HIV gp120 gene (5). Thus, these examples provide evidence that HDP can be used as adjuvants to enhance vaccine-specific immunity.

To co-formulate HDPs into novel vaccines several issues need to be addressed, including reduction of the cost of producing the peptide, co-formulation and possible interaction with the antigen, and the stability and safety of the vaccine formulation. Recent research has already demonstrated that short peptide derivatives of only 7 to 12 amino acids, which include only specific motifs for certain functions, can behave very similarly to the parental HDP (8). These derivatives are much cheaper to produce and potentially have less interaction with other vaccine components. More research is required to better understand the peptide motifs that are responsible for immunomodulatory and antimicrobial functions. In addition, although a large variety of HDP has been described in domestic animals (11) very little information is currently available about the immunomodulatory functions of these peptides. Thus, future research needs to address the immunoenhancing activities of these HDP in their respective host species, analyse their potential cross-species activity and investigate the prophylactic potential for preventing infectious disease in domestic animals. However, preliminary results provide a degree of hope that these molecules will be able to improve vaccine responses with minimal adverse reactions.

Ribonucleic acid oligonucleotides and imidazoquinolines

Synthetic ssRNA and small anti-viral compounds (imidazoquinolines) activate a class of receptors similar to those stimulated by CpG ODN. Imidazoquinolines have adjuvant activity and appear to promote Th1 rather than Th2 immune responses (52). Studies in mice have revealed that appropriately formulated ssRNA is a potent adjuvant and modulator of vaccine-associated immune responses (54). Furthermore, conjugation of imidazoquinoline derivatives to an HIV experimental vaccine dramatically enhanced the magnitude and altered the quality of Th1 immune responses in monkeys (55). Although they have not yet been tested for adjuvant activity in humans and livestock, based on the results from mice and monkeys it is a reasonable expectation that these molecules will have adjuvant activity in livestock. Evidence in support of this notion comes from numerous studies in the authors' laboratory, which have confirmed that ssRNA and imidazoquinolines are highly stimulatory when tested in immune cells from cattle, pigs and sheep (Mutwiri *et al.*, unpublished observations), strongly suggesting that studies testing these molecules as adjuvants in livestock are warranted.

Polyphosphazenes

Polyphosphazenes are synthetic, water-soluble and biodegradable polymers that are inexpensive to produce. One of the most interesting properties of polyphosphazenes is that they are stable at room temperature and can be stored on the bench for several months without loss of activity, eliminating the need for refrigeration. The prototype member of this class of polymers is poly[di(sodium carboxylatophenoxy) phosphazene] (PCPP) which has previously been shown to have adjuvant activity with a variety of viral and bacterial antigens in mice (33, 40, 57). Despite the compelling evidence for adjuvant activity of these polymers in mice, they have not been tested in large animals. In this regard, the authors have shown that PCPP is also a potent adjuvant in sheep when used at only double the dose used in mice (Mutwiri *et al.*, unpublished data). A new polyphosphazene polyelectrolyte, poly[di(sodium carboxylatoethylphenoxy]phosphazene) (PCEP) seems to have even more potent adjuvant activity (38). Evidence from numerous studies in mice demonstrates that PCEP is a potent enhancer of antigen-specific immune responses, and its adjuvant activity is far superior to that of PCPP and the conventional adjuvant alum (38). PCEP not only enhanced the magnitude but modulated the quality of immune responses, resulting in more balanced immunity (38). Even more interesting was the observation that the combination of PCEP with CpG showed strong synergy, resulting in dramatic increase in immune responses. The authors hypothesise that because PCEP induces immune responses that have similarities with those stimulated by CpG DNA, PCEP achieves its adjuvant effects by activating innate immunity. Indeed, they have obtained evidence that PCEP activates immune cells to secrete cytokines that have been associated with the development of Th1 type immune responses (Mutwiri *et al.*, manuscript submitted). Thus, activation of innate immunity may be at least one of the mechanisms by which PCEP mediates its potent adjuvant activity. PCEP has not yet been tested in livestock. Given its success in mice, studies in large animals are certainly warranted.

Conclusion

Looking to the future, many new generation vaccines will consist of purified antigens and well-defined adjuvants, and these vaccines will be expected to meet more stringent safety and efficacy requirements. A few examples of directions in which the field of adjuvant development may be headed in the future have been provided here. The authors anticipate that, in future, adjuvants will be used as high precision tools to activate the desired immune responses. In this regard, the selection of adjuvants will be much more focused on stimulating specific immune responses, and not just enhancing antibody responses. Thus, there will be more emphasis on the quality of the immune response with fewer adverse reactions. The use of stimulators of innate immunity such as CpG or other selective modulators of the innate immune response, combined with better formulations, should dramatically improve vaccine efficacy and reduce economic losses to the livestock industry. Furthermore, these more defined vaccine formulations, together with the understanding of their mode of action, should provide the regulatory agencies with a greater level of confidence in the new vaccines. Those vaccines currently being developed will be safer for use in livestock, which is particularly important for food-producing animals that will eventually be consumed by humans.

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L'immunité innée et les nouveaux adjuvants

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Résumé
De toutes les approches biomédicales visant à contrôler les maladies du bétail, la vaccination est la plus rentable. Les vaccins dits inactivés (à agent pathogène mort) ou les vaccins sous-unitaires (utilisant uniquement les fractions immunogènes du microorganisme) présentent une meilleure innocuité mais leur efficacité laisse à désirer et nécessite souvent la présence d'adjuvant. Malheureusement, la plupart des adjuvants classiques sont des substances complexes et mal définies qui ne répondent pas aux critères rigoureux d'innocuité et d'efficacité exigés pour les vaccins de nouvelle génération. Une nouvelle génération d'adjuvants qui agissent en stimulant l'immunité innée offre de nouvelles perspectives pour la mise au point de vaccins plus sûrs et plus efficaces. Les auteurs soulignent le rôle de l'immunité naturelle pour se protéger contre les maladies infectieuses et citent quelques exemples prometteurs d'adjuvants capables de stimuler l'immunité innée. Les adjuvants classiques ont déjà fait l'objet de revues détaillées et ne sont pas examinés dans cet article.

Mots-clés
Adjuvant – Bétail – Immunité innée – Maladie infectieuse – Vaccin.

Inmunidad innata y nuevos adyuvantes

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Resumen
La vacunación sigue siendo el procedimiento biomédico más rentable para luchar contra las enfermedades infecciosas del ganado. Las vacunas basadas en patógenos muertos o subunidades antigénicas presentan menos riesgos, pero con frecuencia es preciso administrarlas con adyuvantes para que sean eficaces. Lamentablemente, la mayoría de los adyuvantes convencionales son sustancias complejas, mal definidas, que no satisfacen los criterios estrictos en materia de inocuidad y eficacia que las vacunas de nueva generación deben cumplir. La nueva generación de adyuvantes que potencia la inmunidad innata representa una oportunidad muy interesante para obtener vacunas más seguras y potentes. En este artículo los autores destacan el papel de la inmunidad innata en la protección contra enfermedades infecciosas y presentan algunos ejemplos de nuevos adyuvantes prometedores que la potencian. No examinan los adyuvantes convencionales utilizados en muchas vacunas puesto que ya fueron analizados exhaustivamente en el pasado.

Palabras clave
Adyuvante – Enfermedad infecciosa – Ganado – Inmunidad innata – Vacuna.

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Vaccines and animal welfare

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Summary

Vaccination promotes animal welfare by protecting animal health, but it also has other welfare benefits, e.g. recent investigations have looked at the potential of vaccines in immunoneutering such as immunocastration – a humane alternative to the painful traditional methods. Similarly, vaccination can be used during disease outbreaks as a viable alternative to stamping-out, thus avoiding the welfare problems that on-farm mass slaughter can cause. Protecting animal health through vaccination leads to improved animal welfare, and maintaining good welfare ensures that animals can respond successfully to vaccination (as poor welfare can lead to immunosuppression, which can affect the response to vaccination). It is clear that vaccination has tremendous advantages for animal welfare and although the possible side effects of vaccination can have a negative effect on the welfare of some individual animals, the harm caused by these unwanted effects must be weighed against the undoubted benefits for groups of animals.

Keywords

Animal health – Animal welfare – Animal well-being – Immunocastration – Pest control – Vaccination – Vaccination side effects.

Introduction

Vaccination of animals is relatively simple and the welfare of large numbers of animals can easily be protected as a matter of routine (see other chapters in this issue of the *Review*). Vaccination is used primarily to promote animal health by preventing disease outbreaks that can have a devastating effect on animal production, as well as on human and animal health. Animal health is a crucially important factor in modern-day farming, but two other related aspects are often not appreciated. First, poor health in itself is a welfare problem for the animals concerned. And secondly, poor animal welfare (or well-being, the words are used interchangeably here) in the absence of any disease is also important because it too can impact on farm productivity. Most farmers, therefore, want to promote good animal health and good animal welfare to help ensure good productivity and food safety. Furthermore, society demands that animals be treated humanely and stock-keepers themselves want to do the right thing for their animals, i.e. they recognise that they have a duty of care.

Vaccination, therefore, is an extremely effective way in which to promote both good animal health and good animal welfare. This may be especially true in some types of farming, such as organic livestock production (17), where the use of traditional therapies is restricted in order to minimise residues and prevent the development of resistant strains of micro-organisms or parasites (23). Vaccination helps provide for sustainable and economic stability for farmers and the communities they serve (16). However, vaccines have to be affordable and animal stock-keepers have to have the knowledge, ability and inclination to use them (5).

In addition to farm animal productivity and food safety, vaccination plays an important role in human health through the control of some zoonotic diseases in wildlife, such as rabies, where the wild animal reservoirs of infection can be reduced through the use of vaccine baits (19). Other areas where vaccination is being used, or is being developed, is for use in the control of pest populations (2), and in the immunoneutering of farm animals to replace painful routine procedures such as castration.

This article examines some of the disadvantages of vaccination and also its potential role in various areas of husbandry. Other aspects of animal welfare, such as public acceptance of animal research, immunocontraceptives and

immunoneutering, and application of the Three Rs in the development and production of vaccines are dealt with in volume II of this issue (see Audonnet *et al.*, Cussler, and Hardy and Braid), and several articles deal with the unwanted side effects of vaccines that may have welfare implications for the animals.

Animal welfare

Animals that have the ability to experience pain, as well as pleasurable states such as happiness, are known as 'sentient', i.e. they are able to experience negative (poor) and positive (good) physical and psychological well-being. It is generally considered that all vertebrates, and even some invertebrates, are able to experience negative well-being, i.e. to suffer in some way. The neurological capacities of animal species to suffer will vary between different classes of animals (mammals, birds, reptiles, amphibia and fish), and even between individuals according to their stage of development (neonates may suffer more pain than adults as their nervous system is immature [13]), their experiences in life, their ability to remember those experiences, and their capacity to respond (e.g. some individuals may be brain-damaged). Animal welfare is about animals' feelings and emotions, which encompass adverse states such as pain, distress, anxiety, discomfort, grief, fear, boredom, frustration, etc., and, at the other end of the scale, happiness and contentment (8).

A deeper question is whether animals can 'suffer' pain as well as 'feel' pain and there is considerable debate about this issue (7). To put it another way, animals may not suffer (pain and any other adverse state) in the sense that they may not mentally reflect very deeply on their feelings and in this way animals may be different from humans. However, it is also possible that animals do suffer like humans but perhaps not in quite the same way or to the same degree, because they are not as self-aware as humans. This is not surprising as, after all, vertebrates have a similar evolutionary history, and feelings such as pain and fear are protective sensations that enable animals to survive in their respective environments.

Feelings of pain and distress are adverse states that can result in animals having a poor quality of life, especially if these feelings persist for any length of time. Consequently, it is important to develop welfare assessment measures that indicate how an animal is feeling, and to what degree its likes, wants and needs are being met in the husbandry system in which it lives. It is also important to develop indicators of how the welfare of diseased animals is compromised (4). When animals become infected (this can be thought of as being exposed to a stressor) they may then experience mental effects due to fever (feeling hot), malaise (feeling tired), lethargy (feeling of having no energy) and nausea (feeling sick). In addition they may suffer from adverse clinical states such as hyperthermia, vomiting, diarrhoea, salivation, retching, coughing, lameness, ulceration, colic, etc. All these are matters of welfare concern and avoiding contracting the disease through vaccination is extremely beneficial for the welfare of animals.

The well-being of each member of a group of animals (herd, flock, etc.) contributes to the overall assessment of the welfare of the group, as well as the health status of the group. Animal 'groupings' may be at an 'on-farm' level, but may also be at national and international levels. An international approach to the conservation of animal health is particularly important as most nations have common borders with other countries, and disease transmission is not limited by such notional geographical separations. Vaccination is a major method by which national herds/flocks are protected from disease.

However, vaccination is not without its disadvantages as sometimes, the welfare of individual animals may be reduced (often temporarily). For example, vaccinating a group of animals may cause side effects in some, but the overall immunity of the group is raised, thus protecting the large majority of animals while harming a few. Some vaccines commonly cause side effects and so the consequential anticipated benefit (deduced from a risk assessment) has to be substantial and sufficient to outweigh the harms caused.

The relationship between animal health and welfare

In general terms 'animal health' is interpreted as involving disease and forms of physical ill health, whereas 'animal welfare' is seen to be about psychological well-being (4). The two are independent of each other in the sense that one can have healthy animals whose psychological well-being is poor, and unhealthy animals whose well-being may be good, although most of the time poor health leads to poor welfare. For example, some healthy captive or confined animals show stereotypic behaviour – a sign of poor welfare. A good example of this would be primates kept in impoverished conditions in zoos, such as in small cages where they constantly pace. On farms it could include tethered animals, such as veal calves, and sows kept in crates. All these animals have poor mental health and through their stereotypic behaviours or self-mutilation they may even damage their own tissues. In contrast to the poor welfare of these healthy animals, the psychological well-being of some 'unhealthy' animals may remain relatively high if the health problem is of low impact, or has no impact at all, e.g. a benign tumour. However, the welfare of animals will usually be negatively affected if their health is poor. These animals will be suffering in different

ways from the tethered farm animals, with feelings related to the animals' immune responses such as fever, malaise, nausea, vomiting, etc. (3).

Many of the notifiable diseases, such as foot and mouth disease (FMD) and classical swine fever, affect productivity and that is why they are so listed. This lack of productivity is due to the negative impact that the disease has on the animals' health and welfare. For example, cattle with FMD show salivation, as they are unable to eat, drink or swallow due to ulcerated tongues, and are lame as they cannot bear weight on their feet. These conditions are all associated with painful lesions and vaccination can help reduce such adverse effects. Overall, poor health, particularly with infectious diseases, leads to poor animal welfare, and this can be prevented through an effective vaccination programme.

Recognising and measuring welfare

Indicators of poor mental health or poor psychological well-being are more difficult to identify than indicators of poor health or poor productivity. Productivity indices such as weight gain, body temperature, milk yield, normal reproductive behaviour patterns, egg yield, etc., are indicators in a general sense of both good health and good welfare. However, there are many instances in which health and welfare are poor but productivity is not affected, so such indicators are often only affected when the health and welfare is compromised to a substantial degree. Thus, productivity may remain unaffected, or be only marginally affected, even when animals are kept so confined that they cannot carry out many of their normal behaviours (e.g. veal calves tethered in small crates, laying hens in small battery cages, cows stalled in cubicles). Under these conditions productivity may even increase, as animals do not expend energy in moving around and some diseases may be reduced. However, other diseases may increase. Similarly, animals may be subjected to acute severe pain early on in life, e.g. through castration or docking, or having their beak trimmed with a hot blade, but productivity in the long term is unlikely to be affected. Nevertheless, it is now being realised that after these 'minor' operations animals may have prolonged pain for several days or even weeks afterwards (14, 20, 21, 22).

Other more extreme indicators of poor welfare are mortality and morbidity, however, one has to be careful in their interpretation. Mortality as an indicator is likely to reflect considerable suffering before death. But a farmer who kills sick animals for humane reasons rather than let them struggle on in the hope that they will live long enough to get better and be sold to make a profit, may have a higher on-farm mortality but cause less animal suffering.

More subtle indicators of welfare include behavioural diversity, stereotypic behaviours, and corticosteroid and catecholamine levels, but such scientific measures of animal health and welfare need to be carefully defined and recorded in such a way that they give meaningful information about the state of the animals concerned. At present they are really only appropriate in a research setting, but it may be possible to link them with on-farm animal welfare (25). A growing area of animal welfare research is 'asking the animals' what they prefer in terms of their environment, i.e. observing how hard they will work to access or avoid a particular environment. This area of research, known as 'preference testing', provides extra information, from the animals' viewpoint, in addition to the more traditional measures of welfare.

Measures of welfare have to be seen in the context of the farming practices being used, the productivity of the animals and other environmental factors. The overall aim for the stockman is to cause only the minimum amount of animal suffering to meet the farming objectives. It is generally seen as being unethical to cause more suffering than is necessary to achieve those objectives. This 'extra' suffering has been termed 'avoidable' suffering. The levels of on-farm measures of welfare can be benchmarked (used as performance indicators), as is happening in some of the farm and food assurance schemes. These benchmarks form a valuable guide for farmers as they will show how much avoidable suffering is being caused.

Poor welfare and response to vaccination

It is important to appreciate that there is a connection between animal welfare and health, and that a healthy mental state can increase resistance to infectious disease, whereas a state of poor welfare can reduce immune resistance and so predispose animals to disease. A reduced resistance may lead to the development of clinical disease from carrier states, and it may mean that the disease is never completely eliminated and that the animal then remains a carrier. Poor welfare at a critical time may also affect the response to vaccination, e.g. castration without anaesthesia or analgesia. Lessard *et al.* (18) found a decreased antibody response to bovine serum albumen challenge (on day of castration and 14 days later) in 10 to 17 day-old castrated piglets compared with sham-operated controls ($P < 0.0001$). They also found reduced lymphocyte blastogenic responses to concanavalin A, phytohaemagglutinin, and pokeweed mitogen. This immunosuppressive effect of castration is probably due to a stress reaction and the secretion of cortisol, potentially reducing vaccine effectiveness.

In conclusion, it is important that animals are in a state of good welfare throughout their lives to ensure that they are in a fit state to respond successfully to vaccination.

Side effects of vaccination

Many vaccines have side effects, but normally they are trivial and of short duration and are usually associated with live vaccines. Sometimes adjuvants in a vaccine can cause an adverse reaction, sometimes latent infections can be caused (e.g. Herpes virus infections), and sometimes an animal may fail to respond (seen as an unwanted side effect). Some other common side effects include:

– transient swelling at the site of injection and a reaction that may change coat colour in the area

– coughing after nasal administration

– transient pyrexia (fever)

– respiratory distress, salivation, vomiting, diarrhoea, urticaria

– reduced fertility, foetal deformities and abortion

– excretion of vaccine virus, which may affect other animals in the herd that are susceptible, e.g. spread of vaccine virus in pigs from fatteners to breeders.

These are relatively uncommon as clear warnings are given by the manufacturers, and safety testing of vaccines helps prevent their occurrence. Of recent note however, has been the development of fibrosarcomata in cats at the site of injection, and the development of peritonitis in fish that are immunised by the intraperitoneal route, quite likely a response to the adjuvant.

Animal welfare in safety testing of vaccines

The welfare of laboratory animals has not always been well protected in the past, particularly due to the requirement that animals should be allowed to die of infection in the control group, and also in the vaccinated but unprotected groups (e.g. Leptospiral challenge tests). The development of humane endpoints where surrogate markers, i.e. early clinical signs, are used as predictors of death provides a real humane alternative to death as an endpoint when there are no other testing strategies that will achieve the same scientific objective (e.g. assessment of safety and potency) (see article by Cussler in volume II of this issue).

Other uses of vaccination

Immunocontraception and immunocastration

Immunoneutering vaccines against sperm, egg antigens and the hormones of pregnancy have been developed and may form the basis of immunological contraceptives in the future (studies are being carried out in humans [1, 15]). Immunisation through the use of baited vaccines has already been used as a strategy for the control of rabies in the wild animal reservoir (10) and, potentially, a similar strategy could be used for controlling the population of so-called 'pest' species (see below).

Castration is a common procedure in the farming of pigs, sheep and cattle and it is normally carried out on farms with no anaesthetic and no post-operative analgesia, something that is unlikely to happen for companion animals or for humans! Whether castration is done surgically or with a rubber ring there is good scientific evidence that animals are in serious pain at the time of castration and that this persists for varying periods of time afterwards (21, 24). The best alternative to this routine farming intervention is not to do it at all, and in some farming systems that is a practical solution. Another approach is the local destruction of testicular tissue by various chemicals. However, more recently, the possibility of preventing testis development through vaccination has been investigated. This can be done either by treating males with exogenous hormones that down-regulate the hypothalamic/pituitary/gonadal axis or by neutralising these hormones with specific antibodies (see paper on immunocastration by Hardy and Braid in volume II of this issue of the *Review*). Very few measurements of the welfare of treated animals have been carried out, but the behaviour of immunised male pigs was found to be similar to that of surgically castrated ones (6), who show reduced aggressive and mounting behaviours and increased feeding behaviour, compared with entire males. While there has been little reaction on the site of injection using this vaccine (9) – because vaccines are directed against hormones (e.g. GnRH) produced by tissues of the animal – it may induce cellular damage away from the injection site, e.g. in the hypothalamus; but whether this causes pain or discomfort is unknown (24).

As well as on farms, vaccination can be used to manipulate the sexual activities of animals, and thus control populations, in other animal facilities such as animal sanctuaries, zoos and wildlife parks (see the article by Plumb in this issue) and such interventions should improve the welfare of the animals. Vaccination can also be used to control pests in the wild (e.g. foxes, possums); controlling numbers will improve the welfare of the group by avoiding food shortages (starvation) and excessive competition for mates and territory, thus leading to better conservation of competitor species.

Prevention of mass slaughter for disease control

For some diseases it has been policy to stamp out an infection on farms through the mass killing of animals. Vaccination of animals with appropriate measures to differentiate vaccinated animals from infected animals, is a useful adjunct, even a viable alternative to mass killing.

With a stamping-out policy, it is a major welfare problem to kill large numbers of animals humanely on farms, unlike in abattoirs where systems can be easily put in place to ensure a humane death. Several reports have been made on some of the problems involving poor welfare that have occurred during a disease outbreak (12) and various reports have addressed the issue of humane killing of animals for disease control (11).

Conclusion

Vaccination can play an extremely important role in the promotion of the psychological well-being of animals through disease prevention, disease control, population control and the replacement of routine painful procedures such as castration. It is relatively inexpensive, highly effective, and while there are side effects the benefits of vaccination outweigh the harms caused through these unwanted effects.

■

Les vaccins et le bien-être des animaux

D.B. Morton

Résumé

La vaccination assure aux animaux un meilleur bien-être en protégeant leur santé. Elle a également d'autres effets positifs sur le bien-être : des recherches récentes ont ainsi révélé les possibilités offertes par les vaccins de supprimer les fonctions de reproduction chez les animaux par des méthodes immunologiques telles que l'immunocastration – une alternative décente aux douloureuses méthodes traditionnelles. De même, en cas de foyer de maladie, la vaccination peut remplacer les stratégies d'abattage sanitaire, évitant ainsi les problèmes de bien-être que peut susciter l'abattage massif d'animaux dans les exploitations. La protection conférée par la vaccination améliore le bien-être des animaux et, inversement, des animaux bénéficiant de bonnes conditions de bien-être réagissent mieux à la vaccination (par opposition à l'immunosuppression observée chez les animaux en mauvaises conditions, qui altère leur capacité de réagir à la vaccination). Il est évident que la vaccination présente un intérêt considérable du point de vue du bien-être animal, et les effets indésirables parfois constatés au niveau individuel ne doivent pas cacher les bénéfices incontestables au niveau des troupeaux.

Mots-clés

Bien-être des animaux – Contrôle des nuisibles – Effet secondaire du vaccin – Immunocastration – Protection animale – Santé animale – Vaccination.

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Vacunas y bienestar animal

D.B. Morton

Resumen
La vacunación, que favorece el bienestar de los animales porque protege su salud, trae también consigo otros beneficios en ese terreno. En fechas recientes, por ejemplo, se han estudiado las posibilidades de uso de métodos inmunológicos para obtener animales asexuados, con técnicas como la inmunocastración (alternativa clemente a los dolorosos métodos tradicionales). Asimismo, ante un brote zoosanitario existe la posibilidad de utilizar la vacunación como alternativa viable al sacrificio sanitario total, soslayando con ello los problemas de bienestar que pueden derivarse de la práctica de sacrificios masivos en las explotaciones. El hecho de proteger la salud de los animales mediante vacunación propicia un mayor grado de bienestar, lo que a su vez garantiza que los animales respondan adecuadamente a la vacunación (pues un animal que viva en condiciones deficientes puede sufrir inmunodepresión, y ello podría restar eficacia a una vacuna). Está claro que la vacunación presenta enormes ventajas desde el punto de vista del bienestar animal y, aunque sus posibles efectos secundarios puedan influir negativamente en el estado de algunos ejemplares concretos, conviene comparar esos eventuales efectos dañinos con los indudables beneficios que la vacunación reporta a grupos enteros de animales.

Palabras clave
Bienestar animal – Castración inmunológica – Control de plagas – Efecto secundario de la vacunación – Sanidad animal – Vacunación.

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Veterinary vaccines for public health and prevention of viral and bacterial zoonotic diseases

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Summary
To meet with the increasing demand for food, the scale of world food production is increasing, as is the transport of animals and food products. At the same time, the contact of animals with the environment remains unchanged or, in the case of free-ranging animals, is even increasing. A number of microorganisms have established themselves in farmed animals, which although relatively harmless to animals are pathogenic to man. In this article, the options for reducing the risk of transferring zoonotic agents from animals (particularly farm animals) to man using veterinary vaccines against viral and bacterial diseases are described.

Keywords
Avian influenza – Brucella – Campylobacter jejuni – Eastern equine encephalitis – Erysipelothrix – Escherichia coli O157:H7 – Food safety – Japanese encephalitis virus – Leptospira – Methicillin-resistant Staphylococcus aureus – Mycobacterium – Rabies – Salmonella enteritidis – Salmonella typhimurium – Streptococcus suis – Vaccine – Venezuelan equine encephalitis – West Nile virus – Western equine encephalitis – Zoonosis.

Introduction

Vaccination is generally accepted as an adequate tool to control infectious diseases in man and animals. No real alternative exists for viral diseases of animals since there are no antiviral drugs suitable for widespread application in the field; moreover, there might be a restriction of the use of such drugs in humans in the future so as to avoid problems due to resistance. Increasingly widespread antimicrobial resistance among zoonotic bacteria is illustrating the limitations of antibiotic treatments in animals and effective vaccination of animals against zoonotic diseases caused by bacteria may help to solve the problem.

Although parasitic zoonoses are also a public health threat, this paper will focus on a few important bacterial and viral zoonotic diseases, mainly because effective vaccines against relevant parasitic zoonotic diseases are not (yet) on the market. However, future research efforts will have to include the development of antiparasitic vaccines since we can expect that many new and (re-) emerging infectious diseases will also be caused by parasites. Approximately 75% of newly re-emerging infectious diseases are considered to be zoonoses, an assumption which underlines the need for control of infectious diseases in animals by vaccination. The term zoonotic describes an animal pathogen that can move into a human host.

Three categories of zoonotic diseases can be distinguished:

a) those which are rarely transmitted to humans, but which continue in the human population once transmitted, e.g. human immunodeficiency virus (HIV) and severe acute respiratory syndrome (SARS) (it is thought that avian influenza [AI] could be the next pandemic of this type of zoonosis)

b) those which are transmitted to humans directly or via a vector, but which are rarely, if ever, transmitted from

human to human, e.g. Lyme disease, West Nile virus infection, rabies (domestic or wild animal populations are the reservoirs for these pathogens)

c) those which are transmitted by agents that cause little or no harm to the animal populations in which they have established themselves and which spread to humans through the consumption of food products, e.g. *Campylobacter*.

Facts and factors which have favoured the re-emergence of zoonotic pathogens in the last decades are as follows:

– increasing human population expanding into new areas

– a change in the behaviour of humans, including frequent and long-distance travel

– globalisation of trade (for animal products)

– movement of wild and domestic animals over long distances

– climate change that has allowed pathogens and vectors to survive in new areas.

In the past, vaccines for animals have mainly been developed to protect animal health and to increase animal welfare by preventing suffering as a result of infectious disease, but now, the damage inflicted by some highly contagious animal diseases on national and regional economies is an important driving force behind the implementation of vaccination programmes against zoonotic diseases. Slaughter policies are sometimes introduced to limit economic losses, as has been the case for some of the diseases listed by the World Organisation for Animal Health (OIE), but the authors believe that public opinion in most countries will not accept a stamping out policy in the future and it is the task of scientists to present alternatives, such as very efficient marker vaccines, to the decision-makers.

Vaccines against zoonotic diseases should meet high standards so that veterinary authorities can prevent transmission of the disease to humans. Protecting the human population by vaccination of domestic (or wild) animals requires a collaborative effort from veterinarians, epidemiologists, human doctors and politicians. Significant scientific progress has been made by vaccinologists and epidemiologists, such as in designing models to calculate transmission rates (e.g. R-value [36, 37]) in animal populations. Tailor-made vaccination programmes can be designed, based on new technologies and know-how.

Strategies to control zoonoses have been developed in recent years by many organisations. Through various symposia the OIE has published proposals for implementation on a worldwide scale, and in Europe, the European Food Safety Authority has produced guidelines, scientific reports and expert reports in the field of food-borne diseases (65).

This paper does not intend to compete with those comprehensive publications written by experts in the field, but will rather try to highlight a selected number of zoonotic bacterial and viral diseases for which there are real opportunities to develop effective vaccines. The authors consider recent progress in areas such as biotechnology, and assess whether these areas of research can be used to develop improved vaccines that will help meet the target of preventing human disease by vaccination of animals. The paper ends with some recommendations for future research needs and a few general conclusions.

Bacterial diseases

Campylobacter jejuni

Campylobacter jejuni is the major cause of human food-poisoning in most countries (1) and also causes immune-mediated diseases such as the Guillain-Barré (87) and Miller-Fisher syndromes (56). Most human infections with these bacteria are associated with the consumption of poultry, but pets can also be a source of infection (22). The bacterium is very well adapted to chickens, and has a very low infectious dose. The majority of flocks are heavily and persistently colonised with up to 10^{10} colony forming units per gram of faeces without causing any problems for the chickens. Furthermore, *C. jejuni* is naturally competent and incorporates heterologous deoxyribonucleic acid (DNA) and is therefore genetically and antigenically heterogeneous. Although this is a complicating factor, the lack of pathogenic interaction with the host is the main reason why it has proven difficult to develop a reliable vaccine. Many types of live and inactivated vaccines have been tested, but without resulting in a practical solution. Some effect has been demonstrated with intraperitoneal vaccinations with whole cell bacterins and flagellins (82) as well as with a live *Salmonella typhimurium* vector containing a *C. jejuni* antigen (86). Although the bacterium is highly sensitive to specific serum antibodies, colonisation is not prevented by high levels of humoral antibodies as these do not reach the gut. Furthermore, live 'vaccination' with wild-type *Campylobacter* in young chicks (or even in-ovo) does not result in a reaction beyond what is normally found during colonisation and therefore does not result in a reliable reduction of colonisation. So far, the most efficient way to prevent transmission to man is to treat the meat after slaughter (e.g. by freezing) and to implement rigorous kitchen hygiene (30). A vaccine for human use would be another practical solution.

Salmonella

Salmonella typhimurium and *S. enteritidis* are the second most common cause of human food-poisoning, but they

occur mostly asymptomatically in livestock. For poultry, various inactivated (84) and live vaccines based on either or both of the *S. typhimurium* or *S. enteritidis* antigens are commercially available (5, 34). Also, live vaccination with attenuated *S. gallinarum*, which is a strict poultry pathogen has been shown to reduce *S. enteritidis* colonisation levels in chickens (28). *Salmonella typhimurium* infections in swine are mostly subclinical, but are an occupational hazard for pig farmers and can be transferred by meat products. Several vaccination strategies have been shown to be effective, e.g. sow vaccination with inactivated bacterins (62) and live vaccination of piglets with homologous (61) or heterologous serotypes (45).

Vaccination is considered to be one of the cornerstones of the strategy to reduce human *Salmonella* infections. In 2003, the European Union issued Directive 2003/99/EC on the monitoring of zoonoses and zoonotic agents and Regulation (EC) No. 2160/2003 on the control of salmonella and other specified food-borne zoonotic agents. *Salmonella* in poultry is the first priority and from 2008 onwards, vaccination will be mandatory in Member States with an *S. enteritidis* prevalence of above 10% in layers. Further measures are expected for breeders and for swine in the near future.

Escherichia coli

Enterohaemorrhagic shigatoxin-producing *Escherichia coli* (EHEC) O157:H7 is present in the gut and faeces and on the skin of healthy cattle and sheep. The organism is very well adapted to the host and there is no evidence of pathogenic interactions. The organism can survive for several months in the soil (8). Transmission is mostly via food, notably ground/minced beef and raw milk (58), but the bacterium can also persist on lettuce and other produce after dung from infected animals has been used as fertiliser (69). Human outbreaks are often associated with haemolytic uraemic syndrome. Various pre- and post-harvest interventions have been tested in feedlots (9). In the United States of America (USA), the incidence of O157:H7 outbreaks is slowly declining, possibly due to the many hygiene measures which have started to be taken (e.g. washing carcasses after slaughter). Recently, a subunit vaccine containing secreted virulence factors has been tested in field trials in feedlot cattle with variable results (24, 57). In a number of cases, the colonisation level of cattle was reduced but the result was still far from the desired sterile immunity. Clearly, vaccination could be an aid in further reducing the number of outbreaks, but only in combination with hygiene measures.

Bovine tuberculosis

Before pasteurisation of milk was introduced, bovine tuberculosis (TB), due to *Mycobacterium bovis*, caused 2000 deaths per annum in the United Kingdom (UK) alone. In addition, there was considerable economic damage to the cattle industry. The bacterium is still prevalent in cattle and wildlife in many developing countries, and is re-emerging in the UK, Ireland and New Zealand. There is controversy over whether vaccination or culling of wildlife would contribute to a reduction in *M. bovis* infection in cattle (49). Protection from infection can be achieved by vaccinating young animals with bacillus Calmette-Guérin (BCG) vaccine (12); however, this vaccination interferes with the tuberculin test and is therefore not compatible with the current TB surveillance programmes in cattle. Reviews of the efforts to develop TB vaccines and diagnostics have been published recently (33, 78).

Paratuberculosis

Mycobacterium avium subsp. *paratuberculosis* (MAP) is the causative agent of Johne's disease in cattle and sheep, but there is still some controversy as to whether this organism is the causative agent of Crohn's disease in humans (66). There is a long tradition of vaccination against Johne's disease, especially with inactivated BCG, which can both prevent clinical disease and reduce shedding (26). An inactivated ovine vaccine is available commercially in a number of countries (59). However, use of this vaccine interferes with the tuberculin test used in *M. bovis* control programmes. Furthermore, it causes serious injury in humans if there is accidental self-injection (83). Live attenuated MAP vaccines are also available commercially (6), but these also interfere with the diagnostics of the TB control programmes. Recently, promising results have been obtained with a recombinant subunit vaccine (40).

The common theme in (para)tuberculosis control is that the existing vaccines are not fully protective, cause safety problems at the injection site and induce a positive tuberculin test reaction. A lot of effort is therefore being invested into either setting up differential diagnostic tests that would allow the use of BCG-type vaccines in countries with a TB eradication programme or alternatively (and ideally) developing a better and safer subunit vaccine. However, progress is slow since, due to the nature of the infection, vaccine trials in cattle take approximately two years and require a lot of resources. Therefore, such a vaccine will take considerably more time to develop.

Streptococcus suis

Streptococcus suis is a known zoonotic agent and an occupational hazard for workers in the pork industry, mostly occurring as isolated cases of meningitis (4). Recently, 52 people died, mostly from streptococcal toxic shock syndrome during two outbreaks involving

serotype 2 in the People's Republic of China (74). Asymptomatic nasopharyngeal carriage is often found in healthy swine, but can also cause fatal sepsis associated with meningitis and polyarthritis in swine of all ages (70).

A total of 35 serotypes have been described, of which serotypes 2, 7 and 9 are (in that order) the most prevalent. In the pig industry, sows on problem farms with high piglet mortality (and sometimes high mortality in older swine as well) are vaccinated with inactivated auto-vaccines. After vaccination of sows with formalin-inactivated bacterin of serotype 2, high protection of the offspring against experimental challenge with the same serotype is observed (52). The species is genetically diverse and contains a number of putative virulence factors (e.g. suilysin, muramidase-released protein, extra-cellular factor, fibronectin-binding protein), but these neither seem to be absolutely conserved across the species *S. suis* nor necessary for virulence. So far, no cross-protective vaccine based on these antigens is available.

Staphylococcus aureus

Staphylococcus aureus is most known in the veterinary field as the causative agent of mastitis in dairy cattle. Sporadic cases exist where human infections have been linked to cases of bovine mastitis and MRSA [methicillin-resistant *Staphylococcus aureus*] strains have been isolated from cattle (43). However, in general, bovine mastitis strains are genetically different from human isolates (41, 88).

Swine can be a source of *S. aureus* infection and in the Netherlands, pig breeders were identified recently as a group with an increased risk of being MRSA carriers. In cases of hospitalisation, they are kept isolated from other patients until proven negative (76). Furthermore, MRSA strains have also been isolated from chickens (43). Further research is necessary to determine whether poultry and swine are sources, or accidental recipients, of MRSA.

Brucellosis

Brucella infections in livestock result in abortions, weak offspring and long-term fertility problems. The three most pathogenic species for man are *B. abortus, B. melitensis* and *B. suis*, which have a host preference for cattle, smaller ruminants, and swine, respectively. However, they also infect other species of domestic animals as well as wildlife. Human infection usually occurs by direct contact with (aerosols from) amniotic fluids or unpasteurised milk.

Since cellular immunity is required for long-term protection, the best results have been obtained with live-attenuated vaccines (54). Strain 19 has been used for vaccination against *B. abortus* since 1941. Since it is a smooth strain and interferes with *Brucella* serological diagnostic tests, it is mostly given at a young age. By the time the animals enter their reproductive stage the serological response has disappeared but lifelong protection remains. The vaccine also works in herds of adult animals, but lower doses are used to prevent abortion and interference with serological diagnostic tests. More recently, the rough strain RB51 with reduced O-antigen expression was developed and has been used in the USA since 1996. It has a slightly lower protective effect than strain 19, but it does not interfere with serological diagnostics and it can be used at higher doses in pregnant cattle. For vaccination against *B. melitensis*, strain Rev1 is the most successful in small ruminants. For *B. suis* no commercial vaccines are available and none of the other *Brucella* vaccines give significant cross-protection. Generally speaking, none of the available *Brucella* vaccines give sterile protection and successful brucellosis control programmes use hygiene measures and stamping out protocols as well.

Leptospirosis

The most prevalent human leptospiral diseases (e.g. Weil's disease) are caused by contamination of surface water by leptospiruric rodents. This route of infection cannot be controlled by vaccination. However, both *Leptospira borgpetersenii* serovar *hardjo* (type *hardjo*bovis) and *L. interrogans* serovar *hardjo* (type *hardjo*prajitno) infect dairy cattle and can cause milk drop syndrome and fertility problems (25). After renal colonisation, animals shed the bacteria in the urine and can infect farmers by the ocular route in the milking parlour. Commercially available vaccines containing inactivated whole cell bacteria can protect cattle from renal colonisation and urinary shedding and thus protect farmers from this occupational health hazard (7).

Erysipelothrix rhusiopathiae

This bacterium is known to cause erysipelas in pigs and poultry (11). *Erysipelothrix rhusiopathiae* infection is an occupational hazard for pig farmers, veterinarians and slaughterhouse workers. Most human infections are mild and cutaneous, but systemic infections with associated endocarditis have also been reported. The bacterium can be isolated from almost all farms and can survive for a long time in the environment. Vaccination against erysipelas is common practice in the pig industry and many efficacious vaccines are available.

Viral diseases

Among the many animal viral infections known to also infect humans, there is a large variation in the degree of

human suffering ranging, for instance, from the mild conjunctivitis caused by Newcastle disease virus (NDV) of poultry to the inevitable lethal outcome of symptomatic rabies transferred by the bite or scratch of a rabies virus-infected animal. The scope of this section is limited to preventable viral diseases of economic or social importance, as listed in Table I. For newly emerging viral infections, such as hantavirus or SARS, there are as yet no veterinary vaccines.

Table I
Vaccination against viral zoonoses of economic or social importance

Lethal disease in humans	Vaccinated vectors	Unvaccinated vectors
Rabies	Dogs, cats, wildlife	Bats
(Avian) influenza	Poultry, swine	Wild birds
Viral encephalitis	Equines, swine	Wild birds
Severe acute respiratory syndrome	None*	Bat, civet cat
Hantavirus disease	None*	Rodents

*For these diseases there is currently no practical method of administering vaccines to wildlife vectors

Rabies

Rabies virus infection invariably results in a fatal outcome in humans and a great variety of other mammals. That rabies infected animals are a threat for human beings has been known for centuries. Only when, more than a century ago, the concept of vaccination became available through the pioneering work of Pasteur, could this disease be treated or prevented. Even post-infection treatment is possible; rabies being probably the only virus infection where post-exposure vaccination is effective.

Most of the human rabies cases are located in Asia, especially India (22,000 to 30,000 fatalities per annum) (73, 85), and in Africa (24,000 fatalities per annum) (85). Moreover, it is very likely that the numbers are higher than this, as there appears to be substantial underreporting, perhaps as much as tenfold to a hundredfold (19, 20).

In African and Asian countries rabies is a disease of poverty. It particularly affects children under the age of fifteen, because they do not always recognise changes in the behaviour of infected dogs, are more playful and often completely unaware of the danger posed by rabid dogs. Educating and creating awareness are therefore instrumental in the fight against rabies.

Unfortunately, there are a number of reasons why rabies in dogs is not getting the attention that it deserves in these countries:

– the disease has been present for hundreds of years and is considered a fact of life, so it does not have much publicity value or receive political attention

– dogs have no 'governmental status'; there is no clear ministerial responsibility

– a combination of population control and vaccination (parenteral and oral) can substantially decrease the burden of endemic rabies in dogs, but the priority is protecting human health, so greater emphasis is given to controlling, rather than vaccinating, the dog population (73).

Vaccination strategies

In many countries programmes are in place to control rabies in domestic dogs and wildlife. The programmes are enforced by governmental campaigns for the vaccination of all dogs or by the existence of a law that allows border crossing only if it can be shown that a domestic carnivore (dog, cat or ferret) has the minimal antibody titre against rabies, i.e. ≥ 0.5 IU.

A lot of effort has been put into the eradication of rabies from wildlife such as European foxes (21, 63) and programmes are continuing, particularly in Eastern Europe (67). Along the same lines, oral vaccination programmes are in place in Canada and the USA, where raccoons and skunks are the main targets for vaccination (17). Bats cannot be vaccinated and rabid bats cause several fatal human cases each year.

Significant progress has been made as a result of vaccination programmes for companion animals, as can be seen by the data provided on the website of the Rabies Center at the Centers for Disease Control in Atlanta in the USA (17). There has been a tenfold decrease in the number of rabies cases in companion animals from 1955 to 1975, which has been maintained to date. However, the number of wildlife cases per annum has increased since 1975. The use of oral vaccines now plays a key role in diminishing the prevalence of rabies in the field (3).

Parenteral vaccination of companion animals (carnivores)

Initially, mouse or sheep brain vaccines were produced for preventive vaccination. However, these vaccines came with a number of side-reactions that limited their use. It was the development of cell culture vaccines, mainly based on baby hamster kidney (BHK) cells that played a crucial role in the increased parenteral vaccination of companion animals and this led to a dramatic decrease in the number of human exposures. Now, in addition to this parenteral vaccination approach, oral vaccination of dogs is receiving more and more attention (85).

In Latin America, yearly vaccination campaigns have been very effective and have reached up to 90% or more of the

dog population (18). Instrumental in this achievement was the realisation that often dogs are not covered by a ministerial department; they do not fall within the scope of the Ministry of (Human) Health, nor were they considered to be under the remit of the Departments of Agriculture, Wildlife or the Environment: they were nobody's responsibility. Anecdotally, it was therefore decided to confine the Ministers of all three Departments in one room and not allow them to leave until the responsibility was clarified. It was decided that the Department of Human Health should take the responsibility, and, as a result, there is now a very effective yearly vaccination programme in Latin America that involves vaccinating millions of dogs in only a few days.

Oral vaccination of wildlife and stray dogs

The large-scale use of oral vaccine baits started in Switzerland with the pioneering work of Steck and Wandeler (71, 72). Their use of an attenuated strain of rabies (SAD-strain) given as a liquid in a plastic container, hidden in a chicken head obtained from the local slaughterhouse, was tremendously effective in foxes. The baits were spread in rural areas and woods by hunters or dropped from planes and helicopters. By using natural barriers, one area after another was cleared of rabies. After the successful start in Switzerland, many other European countries followed (10, 21, 51, 63, 67).

The situation is remarkably different in developing countries in Asia, the Middle East and Africa, where vaccination reaches only a very small part of the dog population. Here, as in other areas of the world, dogs are the major intermediate in human rabies cases, and free-roaming stray (ownerless) dogs are the endemic reservoir for the virus. Oral vaccination is without doubt the single most effective measure for combating rabies in stray dogs, especially since the implementation of recombinant DNA technology, which has allowed the development of vaccines with greatly improved safety and efficacy (23, 29, 38, 47).

Influenza and avian influenza

Influenza can best be defined as a re-emerging zoonotic infectious disease, and there is a worldwide fear of an upcoming pandemic among the human population caused by transmission of avian viruses belonging to the H5, H7 and H9 subtypes (2, 16).

In a recent review by Webster and Hulse (81) the factors for the evolution of the virus are described. Not surprisingly, these factors include the increasing population densities of poultry, swine, and humans, as mentioned for zoonosis emergence in general in the introductory section of this paper. The importance of wet markets for the increasing risk of avian virus transmission to humans has also recently been investigated (80).

Although initially the reassortment of influenza viruses in pigs was regarded as the major source of new pathogenic human influenza viruses, we now know that the greater risk comes from the mutation or recombination of aquatic poultry viruses.

Intensive (commercial) poultry farming forms an important 'in between' step in the transmission of highly pathogenic avian viruses to humans. It has been postulated that vaccination of domestic poultry could accelerate the antigenic drift of AI viruses (42). However, recent success in the application of the DIVA [Differentiation between Infected and Vaccinated Animals] principle based on a differentiating vaccine with a heterologous neuraminidase, in the control of avian influenza in the field shows the potential of vaccination for the reduction of virus transmission (14).

In another article in this issue of the *Review*, I. Capua gives a detailed report on a relevant field case.

More recent laboratory studies (32) have proven that vaccination of chickens with a conventional inactivated vaccine can completely prevent the spread of a highly pathogenic AI virus to susceptible in-contact birds. The potential of using highly effective live vector vaccines (e.g. the NDV vaccine [77]) as marker vaccines in the field should be tested to evaluate all of the available options to minimise the risk of bird to human transmission of influenza viruses. The impact of vaccination of chickens on animal, particularly poultry, health has been proven and the recent progress means that we can state that an optimal vaccination strategy for domestic poultry will prevent transmission of influenza viruses from poultry to man. Scientists should make use of modern tools like epitope analysis (50) and bioinformatics (44) for rational vaccine design to develop an optimal vaccination strategy for poultry vaccination with the aim of preventing transmission of pathogenic influenza viruses from domestic poultry to humans.

Encephalitides transmitted by mosquitoes

A number of viral zoonoses that cause severe, sometimes lethal, infections in humans are transmitted by mosquitoes. One group of viruses belonging to the family of *Togaviridae* is the so-called Group A arboviruses (arthropod-borne viruses), commonly called alphaviruses. These include Venezuelan equine encephalomyelitis virus (VEE), Western equine encephalomyelitis virus (WEE) and Eastern equine encephalomyelitis virus (EEE). All of them infect the brain and the spinal cord of the host, as is reflected in the names (15, 31).

Two other strains of encephalitis-causing viruses belong to the family *Flaviviridae*. They are also transmitted by mosquitoes and can cause encephalitis in man and equines. They belong to the Group B arboviruses and are generally called flaviviruses. The relevant virus species here are Japanese encephalitis virus (JEV) and West Nile virus (WNV).

Initially, the clinical signs are flu-like symptoms, later on, as the infection affects neuronal cells, behavioural changes similar to rabies infection are seen. Because of its neurological symptoms the disease is also called sleeping sickness. Vaccination of equines is practised for all of the viruses described here and in addition, in the case of JEV, vaccination of swine is performed. Preventive vaccination is principally undertaken for economic reasons, although for people working daily with horses in infected areas, or with swine in the case of JEV, the additional benefit is that the exposure pressure for humans will certainly also be reduced (35). However, the most practical prevention for humans is to avoid mosquito bites, by, for instance, the use of a good insect repellent. See Table II for an overview of the encephalitis viruses and the vaccine types in use.

Venezuelan equine encephalomyelitis virus

The VEE virus caused devastating epidemics in the 1960s and 1970s in Middle and South America where thousands of horses died (79). The outbreaks were finally stopped by the administration of a live attenuated vaccine (39). Today, various inactivated vaccines are also available. There is no direct spread from horse to horse or from horse to man; the transfer of the virus is always by insect. The endemic reservoir is wild birds; no specific species has been clearly identified yet.

The infection of humans is mostly mild; however, more severe cases of encephalitis have been reported. Due to strain differences between the vaccine and the various field virus variants the efficacy of VEE vaccination is not always optimal.

Eastern equine encephalomyelitis virus

As its name indicates EEE is mainly found in the Eastern part of the USA. Of all the encephalitis viruses it is EEE that affects humans most severely; as much as 30% of the infections by EEE virus are lethal. In equines it is even more pronounced and the infection may lead to mortality in as many as 90% of cases (48).

Several brands of inactivated vaccines are available for use in equines, often combined with WEE and VEE as a trivalent vaccine.

Western equine encephalomyelitis virus

The 'western' name is derived from its presence mainly in the western part of the USA. The clinical signs in humans and horses are usually worse than with VEE but less severe than with EEE. Still, some 3% to 10% of the human cases are ultimately fatal (60). Vaccination of horses is practiced mostly using the trivalent vaccine as mentioned above.

Japanese encephalitis virus

Japanese encephalitis virus belongs to the flavivirus group and its greatest economic impact is on the pig industry in Southeast Asia. The mortality both in swine and equines can be as high as 50% in endemic areas. The same is true in humans, and people that recover often suffer from permanent neurological problems (27). Live attenuated and inactivated vaccines are available for swine (53, 75). Horses are also vaccinated to a limited extent. A vaccine developed in Japan for human use is also licensed in the USA; using this vaccine is probably the most effective way to protect humans. As with the other encephalitis viruses, the reservoir is wild birds.

Table II
Vaccination against encephalitis viruses (principally undertaken for economic reasons)
In the 'natural reservoir' column the bird species that is considered to contribute most to the maintenance of the infection is given in brackets

Family Genus	Virus	Affected domestic species	Type of vaccine	Wild reservoir	Insect vector
Togaviridae					
Alphavirus	Venezuelan equine encephalomyelitis virus	Equines	Live attenuated, inactivated	Wild birds	Mosquitoes, black fly
	Western equine encephalomyelitis virus	Equines, pheasants	Inactivated	Wild birds (Passerine)	Mosquitoes, tick
	Eastern equine encephalomyelitis virus	Equines, pheasants	Inactivated	Wild birds (Water fowl)	Mosquitoes
Flaviviridae					
Flavivirus	Japanese encephalitis virus	Swine, equines	Live attenuated, inactivated	Wild birds (Heron, Egret)*	*Culex* species
	West Nile fever virus	Equines	Live, vector, inactivated, DNA	Wild birds	*Culex* species

*In the case of Japanese encephalitis virus swine are also considered to be a reservoir

West Nile fever virus

West Nile fever virus is one of the more recent emerging zoonotic viruses. It was first observed sporadically in Africa, Israel and Eastern Europe, and more recently (in 1999) a large epizootic occurred in the USA (46, 55, 68). A relatively large percentage of infected individuals (approximately 10% of humans and 30% of equines) do not survive the infection (13, 15).

The 1999 outbreak of West Nile fever triggered a lot of developments in modern approaches to vaccines against equine encephalitis virus infections. This has already led to the licensing of a West Nile DNA-based vaccine. In addition, the use of a live vector that expresses a viral glycoprotein essential for protective immunity against the encephalitis virus has been receiving great attention. A canary-pox vector vaccine is available, and chimeric vaccines based on the yellow fever 17D strain are in a very advanced stage of development for both human and veterinary use (64). In addition, subunit vaccines, based on antigens produced by baculo, yeast or *E. coli* expression systems, are being explored.

Conclusion/recommendations

Development of vaccination strategies against zoonotic infectious diseases requires a collaborative effort of human and veterinary vaccinologists if the programme is to serve animal and human welfare. Recent progress in molecular biology and immunology allows the design of tailor-made vaccines which can meet the specific requirements for each vaccine/disease.

In general terms, modern vaccines against infectious animal diseases should be marker vaccines, they should be mass applicable and the relevant protective immune response should be measurable by an *in vitro* test system.

Of the viral diseases reviewed in this article rabies and avian influenza are perhaps the most important in terms of the impact the development of an animal vaccine could have on protecting humans. If based on the available epidemiological knowledge, a vaccination scheme for stray dogs using a safe rabies vaccine strain administered orally could save many lives in Asia, the Middle East and Africa.

For avian influenza, use of a mass applicable vaccine could eventually replace the relatively expensive injectable products if the same efficacy could be achieved. Further research is required by epidemiologists and vaccinologists to design tailor-made vaccination programmes for the various husbandry systems of the poultry industry.

For food-borne diseases, various *Salmonella* vaccines have been shown to be effective in reducing transmission through animal-derived products. Tools and data are available to develop prophylactic programmes. More research is required to develop a complementary vaccination/sanitation programme to protect man from *E. coli* infection.

A marker vaccine which will not interfere with the current diagnostic procedures in vaccine recipients will certainly help in controlling bovine TB. Alternatively, the development of a more specific diagnostic method to replace the current skin test, will allow the development of both live and killed whole-cell based vaccines.

The authors believe that tools and candidate vaccines to develop effective prevention programmes for most diseases are available, but targeted efforts are required to create specific programmes for each disease.

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Les vaccins vétérinaires en santé publique et la prévention des zoonoses virales et bactériennes

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Résumé
Face à une demande toujours accrue, la quantité de denrées alimentaires produites au niveau mondial ne cesse d'augmenter, de même que les capacités de transport d'animaux et de produits alimentaires. En même temps, les contacts entre les animaux et leur environnement restent inchangés, voire, dans le cas des animaux vivant en liberté, se multiplient. Certains microorganismes désormais bien établis chez les animaux d'élevage et relativement inoffensifs pour l'animal sont dangereux pour l'homme. Les auteurs décrivent les différentes options envisageables pour réduire le risque de transfert à l'homme des agents pathogènes zoonotiques d'origine animale (en particulier ceux qui affectent les animaux d'élevage), au moyen de vaccins vétérinaires dirigés contre les maladies virales et bactériennes.

Mots-clés
Brucella – Campylobacter jejuni – Encéphalomyélite équine de l'Est – Encéphalomyélite équine de l'Ouest – Encéphalomyélite équine vénézuélienne – Erysipelothrix – Escherichia coli O157:H7 – Influenza aviaire – Leptospira – Mycobacterium – Rage – Salmonella enteritidis – Salmonella typhimurium – Sécurité sanitaire des aliments – Staphylococcus aureus résistant à la méthicilline – Streptococcus suis – Vaccin – Virus de l'encéphalite japonaise – Virus West Nile – Zoonose.

Vacunas veterinarias para la salud pública y prevención de enfermedades zoonóticas virales y bacterianas

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Resumen
Para satisfacer la creciente demanda de alimentos, el volumen de su producción mundial, así como del transporte de animales y productos alimentarios, se está incrementando. Simultáneamente, el contacto de los animales con su entorno no ha cambiado o, en el caso de animales criados en libertad, incluso aumenta. Algunos microorganismos que se han establecido en los criaderos son relativamente inocuos para los animales, pero tienen una acción patógena en los seres humanos. En este artículo se describen las opciones para reducir el riesgo de la transferencia de agentes zoonóticos de los animales (en particular, los de criadero) a los seres humanos mediante su vacunación contra las enfermedades virales y bacterianas.

Palabras clave
Brucella – Campylobacter jejuni – Encefalomielitis equina del Este – Encefalomielitis equina del Oeste – Encefalomielitis equina venezolana – Erysipelothrix – Escherichia coli O157:H7 – Estafilococo áureo resistente a la meticilina – Influenza aviar – Inocuidad de los alimentos – Leptospira – Mycobacterium – Rabia – Salmonella enteritidis – Salmonella typhimurium – Streptococcus suis – Vacuna – Virus de la encefalitis japonesa – Virus del Nilo occidental – Zoonosis.

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Veterinary vaccines and their use in developing countries

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Summary

The burden of infectious diseases in livestock and other animals continues to be a major constraint to sustained agricultural development, food security, and participation of developing and in-transition countries in the economic benefits of international trade in livestock commodities. Targeted measures must be instituted in those countries to reduce the occurrence of infectious diseases. Quality veterinary vaccines used strategically can and should be part of government sanctioned-programmes. Vaccination campaigns must be part of comprehensive disease control programmes, which, in the case of transboundary animal diseases, require a regional approach if they are to be successful. This paper focuses on the salient transboundary animal diseases and examines current vaccine use, promising vaccine research, innovative technologies that can be applied in countries in some important developing regions of the world, and the role of public/private partnerships.

Keywords

Anthrax – Biotechnology – Bluetongue – Brucellosis – Contagious bovine pleuropneumonia – Foot and mouth disease – Mycoplasma – Rift Valley fever – Vaccination – Vaccine.

Introduction

The growing demand for livestock products (fuelled by population growth, increased urbanisation and greater purchasing power of individuals in developing or middle-income countries) coupled with the necessity of complying with the standards of trade agreements, mean that governments must improve animal health in their countries, particularly as it relates to infectious disease control (27, 28, 40), limits on residues in commodities, and animal welfare (13). Recent assessments show that infectious diseases will continue to be a major constraint to sustained international exports in livestock commodities from developing countries unless targeted sanitary measures are instituted in those countries to reduce the burden of these diseases (68). This paper addresses vaccines for selected epidemic diseases of livestock, examines historical and current trends for prophylaxis to improve animal production in the high-risk and endemic areas, and highlights some opportunities and recent advances in vaccine research. Excellent vaccines used in a less than optimal vaccination strategy will fail to truly curb the incidence of disease. Furthermore, transboundary animal disease containment and control (for eventual eradication) require regional approaches and 'buy-in' from the public and private sector (including smallholders that raise animals to meet their own needs), but developing such regional vaccination strategies – based on quality, effective vaccines – requires well equipped and proficient diagnostic laboratories linked to reliable veterinary epidemiological units. Vaccines and vaccination must complement other aspects of disease prevention and control, namely, enabling legislation, open and risk-based surveillance, diagnostic proficiency, early response, transport and market regulations, compliance, and communication.

Veterinary vaccines for selected transboundary animal diseases

All transboundary animal diseases, including those selected for discussion here, have the following defining characteristics:

– they are of significant economic, trade and/or food security importance for a considerable number of countries

– they can easily spread to other countries and reach epizootic proportions

– their control and management, including exclusion, requires cooperation among neighbours, whether these be local, provincial, national, or regional (44).

Vaccines for livestock offer an important and, at times, an essential tool for progressive control of a given transboundary animal disease, but they require complementary actions:

– enabling legislation

– surveillance

– investment for diagnostic proficiency and capability

– early response

– coordination among several agencies

– management of livestock transport mechanisms

– market inspection and hygiene compliance

– public communication.

Foot and mouth disease

Foot and mouth disease (FMD), a highly contagious viral disease of mammals of the order Artiodactyla, is still considered globally as one of the most economically important diseases and is a threat to livestock production and agricultural development. Despite the fact that there are numerous viruses (serotypes) that cause clinical disease characterised by a variety of lesions and a drop in productivity, the most important aspect of FMD is its impact on trade in animals and animal products (8, 61, 93).

Since the beginning of the 21st Century, FMD has occurred in almost two thirds of the Member Countries of the World Organisation for Animal Health (OIE), either in an epizootic or enzootic form, causing varying degrees of economic losses. However, some of the major livestock-producing regions of the world, including North America, Western Europe, Oceania and some parts of South America and Asia, are recognised as free of the disease at present. Due to increased global trade and movement, FMD has shown great potential in recent years for sudden and unanticipated international spread. The evolution of the pandemic Pan-Asia strain of type O FMD virus in recent years and the introduction of SAT types to the Arabian Peninsula are good illustrations (61, 93).

The epidemiology of FMD is characterised by the relative stability of the virus, its ability to survive outside living animals, the rapid growth of the virus, the small quantities of virus required to initiate the infection, the existence of asymptomatic carriers and, in sub-Saharan Africa, the persistence of the infection in wildlife (61, 93).

The first FMD vaccine, developed in 1938 by Waldmann and Köbe, was based on formaldehyde inactivated virus harvested from tongues of artificially infected cattle, collected at the height of the clinical disease and adsorbed on aluminum hydroxide. The large-scale production of the FMD vaccine started with the Frenkel vaccine in the late 1940s, using bovine tongue epithelium collected from abattoirs as *in vitro* culture system. Although this approach lasted through the early 1990s, one of its major disadvantages was the inability to guarantee freedom from bacteria or yeast or any form of contamination (8), nor guarantee the standardisation of the primary amplification mechanism (i.e. primary culture versus cell culture).

The finding, by Mowat and Chapman in 1962, that FMD virus could multiply efficiently in a baby hamster kidney (BHK) cell line opened the door to the cell-based production of FMD vaccine in suspension and monolayer cultures (8). Vaccines currently used against FMD throughout the world, including Africa and South America, often contain one or more serotypes that have been grown in large volume in BHK cell culture and then inactivated using aziridine compounds (usually binary ethyleneimine) (2, 31, 86). The virus harvest is then concentrated and formulated with an adjuvant (either saponin/aluminum hydroxide gel or various oil emulsions) to potentiate the immune response of the host. Such vaccines have been used successfully for decades to eradicate FMD in different parts of the world.

Vaccination has proven to be a very effective way of controlling and eliminating FMD from certain regions of the world, such as Western Europe and parts of South America (58, 86). Different forms of vaccination programmes are implemented in different regions of the developed world, with varying challenges to their success. One key challenge is the availability and high cost of the vaccine. Because the vaccine needs to contain a large quantity of specific antigen (1 µg per dose or perhaps closer to 5 µg per dose) and the production of large volumes of FMD virus needs to be conducted in a biosecure facility that will prevent virus escape into the

environment, they are expensive to produce. Most production plants are owned by multinational biopharmaceutical companies, usually driven by profit rather than disease control or eradication imperatives. Furthermore, the duration of immunity induced is short and booster inoculations need to be administered at 4 to 6 monthly intervals in most animals, including young cattle. In swine, aqueous-based vaccines are ineffective, and the application of oil-based technology to protect swine and prolong immunity to bovids (i.e. boosters every 6 to 12 months) offered great advantages in the 1980s when first applied widely in South America. Oil-based FMD vaccines have been shown to be very effective as emergency vaccines for pigs (34, 85).

Another challenge in the control of FMD is the debatable situation of 'asymptomatic carriers': ruminants vaccinated against FMD may be protected from developing clinical disease but are not necessarily protected from infection and some vaccinated animals may become persistently infected following challenge (1). However, the precise epidemiological role of the persistently infected animal in the maintenance of the disease and their responsibility for disease outbreaks in susceptible species has been an issue of much debate over the past 80 years. The epidemiology of FMD in endemic regions of sub-Saharan Africa has unique features that render the control of the disease extremely complex: firstly, the prevalence of six of the seven FMD serotypes and secondly, the reservoir role played by wildlife, mainly free-living African buffalo (*Syncerus caffer*) populations infected with the three SAT-types of FMD virus, i.e. SAT1, SAT2 & SAT3 (99). Little is known about the sylvatic maintenance of the virus in Asia and South America.

The significance of viral diversity (and thus antigenic diversity) as a complicating factor in effective vaccination against FMD in Africa is frequently ignored. Immunity is induced only to virus serotypes and subtypes included in the vaccine. In addition to the large number of serotypes prevalent on the African continent, sub-Saharan Africa is the only region of the world where the SAT serotypes of FMD virus are endemic, with widely distributed serotypes O and A, and serotype C being detected in Kenya. SAT2 (102), SAT1 (10) and serotype A (57) have been shown to harbour considerable nucleotide sequence diversity, giving rise to lineages with >20% sequence divergence. These divergences have been shown to be associated with considerable geographically based antigenic variation (98), corresponding to different topotypes within the occurring serotypes. An effective and systematic progressive FMD control programme using vaccination, in Africa or other endemically affected regions, should therefore include vaccine strains that are likely to protect against challenges by field viruses occurring in specific localities. The development of such control programmes is hindered by the fact that most outbreaks of FMD in Africa and Asia are not investigated thoroughly enough with respect to the occurrence of intratypic variants.

The role of the African buffalo and other wildlife species in the persistence of FMD in sub-Saharan Africa has not been well studied beyond southern Africa, despite the fact that in some other regions there are large numbers of wildlife (99). However, for logistical reasons it is still difficult to envisage in the foreseeable future the scenario in which vaccines would be used against FMD in wildlife, except perhaps under special circumstances, e.g. in zoos or game ranches.

International regional collaboration has proven successful in the progressive control of FMD, e.g. the work of the Pan American Foot-and-Mouth Disease Centre in South America and the European Union FMD Commission (47, 86). Sadly, no similar organisations have been operational on the African continent. A number of southern African countries have, however, been successful in instituting control mechanisms that have proven successful in controlling FMD, these include Botswana, Lesotho, Namibia, Swaziland and South Africa. In 2003, a meeting of the National Chief Veterinary Officers of the Southern African Development Community (SADC) agreed on a 20-year framework for the progressive control of FMD in the SADC region and early steps are being taken towards this objective, under the aegis of the SADC Livestock Technical Committee (71). Similarly, there are some encouraging early steps being taken in Asia towards regional cooperation in FMD control, such as the Southeast Asia FMD Campaign, the Indian FMD control project and some projects in the Greater Mekong Delta. So far, however, there is no international mechanism for galvanising national and regional efforts towards coordinated progressive control of FMD in a manner akin to the concerted effort for global rinderpest eradication begun in the late 1980s.

Given the complexity of FMD epidemiology in Africa, broader control approaches will have to be designed, taking into account aspects of movement control, diagnostics, training and effective vaccines and vaccination programmes. Effective vaccine and vaccination strategies of the future should address the following needs:

a) broad spectrum coverage, even within a serotype (such vaccines should protect against all topotypes within a serotype, especially those of the SAT FMD viruses)

b) differentiation between vaccinated and infected animals

c) vaccines that can provide durable protective immunity (beyond 12 months in a developing country setting)

d) vaccines and a vaccination strategy for wildlife, if feasible (i.e. oral vaccination with proven efficacy in a controlled challenge setting)

e) genetic typing and geographical overlay maps for all possible serotype and topotype variants to better design appropriate vaccines, while developing effective and appropriately financed vaccination programmes.

Lubroth and Brown concluded that differentiation between vaccinated and infected animals, through post-vaccination serological monitoring and analysis of virus circulation, would depend upon improved quality control of FMD vaccines to ensure the elimination of non-structural proteins (NSP) during vaccine production and formulation (62), recently established as a standard by the OIE. Subunit FMD vaccines that lack any of the FMD NSP, including the highly immunogenic 3D protein, could be produced as a spin-off from conventional production. Such vaccines would only contain antigenic portions of the viral genome required for virus neutralisation and elimination, so clear distinctions could be made between vaccinated and infected animals using complimentary NSP-based assays (7, 67). Given the considerable impact of FMD on trade in animals and animal products (and sometimes also on trade in other products such as straw or alfalfa), the speedy recovery of disease-free status in many developing countries becomes an imperative. It is critical to differentiate vaccinated from infected animals. The exclusion of NSP from FMD vaccines means that vaccination, in combination with post-vaccination serological surveys and appropriate measures such as improved animal management, has become a feasible way of combating the disease in developing countries. Such vaccines could be obtained either through improved antigen purification during the production of inactivated FMD vaccines (7) or through the use of live vectored vaccines expressing only the empty FMDV capsid (67). The same research groups that are working on developing such vaccines, in an attempt to generate an early protection or prophylactic antiviral treatment, have successfully used expressed porcine type 1 interferon (IFNa/b) in swine to stimulate early protection prior to the vaccine-induced adaptive immune response (50, 66). With the advent of new technologies in vaccine development, it is expected that some of the genetic diversity and virus persistence problems associated with FMD might be addressed. Developments in adjuvant technology have already resulted in more effective vaccines against FMD. Recombinant deoxyribonucleic acid (DNA) technology, recombinant protein and/or DNA-based vaccines are being used in various heterologous systems to test different new generation vaccines (2, 3, 30).

Rift Valley fever

Rift Valley fever (RVF) is an insect-borne, multi-species zoonotic viral disease of livestock whose causative agent was first isolated in the 1930s. It had been exclusively confined to the African continent, but RVF spread to the Middle East in 2000. It is considered a threat to other countries in the region such as Iran and Iraq, and possibly Pakistan and India (25). The disease also features on most lists of potential biological warfare agents due to its severe zoonotic nature.

The occurrence of the disease is usually reliant on the presence of susceptible animals, a build-up of the mosquito vector population (usually associated with heavy rains) and the presence of the virus. Since the development of the live attenuated Smithburn vaccine, vaccination has been used for the control of RVF in southern and East Africa.

There are currently two types of vaccines used for the control of RVF in domestic animals: a live attenuated vaccine and an inactivated vaccine. All currently used live attenuated vaccines are based on the Smithburn isolate, which was derived from mosquitoes in Western Uganda in 1944 and passaged 79-85 times by intracerebral inoculation of mice (this resulted in loss of hepatotropism, acquisition of neurotropism and the capacity to immunise sheep safely when administered parenterally) (90). The 103 and 106 mouse brain passage levels of the virus are used to produce the vaccine in cell culture in South Africa and Kenya respectively, using BHK cells. Millions of doses of this vaccine have been produced by Onderstepoort Biological Products (OBP) in South Africa since 1952 and by the Kenya Veterinary Vaccines Production Institute since 1960 and have been widely used in Africa (54, 94).

Rift Valley fever vaccines based on the Smithburn virus have several disadvantages: they may induce abortions, teratology in the foetuses of vaccinated animals, hydrops amnii, and prolonged gestation in a proportion of vaccinated dams. Being a live vaccine, the vaccine cannot be used during an outbreak. Even in endemic areas vaccination is often not sustained during years in which there have been no outbreaks. To address these problems, and also the poor antibody response to the Smithburn vaccine in cattle (5), an inactivated vaccine was developed and has been used for years in South Africa; it is suitable for use in all livestock species (including pregnant animals) and can be used during outbreaks. The inactivated RVF vaccine makes it possible to vaccinate cows that can then confer colostral immunity to their offspring. Given the poor immunogenicity of this vaccine in cattle, it requires a booster three to six months after initial vaccination, followed by annual inoculations (5).

Table 1 summarises the advantages and disadvantages of the different types of RVF vaccines.

The shortcomings of these inactivated and live attenuated vaccines have led to research into alternative new generation vaccines. A lumpy skin virus expressing the two immunogenic glycoproteins of RVF virus has been tested

Table 1
Comparative evaluation of different Rift Valley fever vaccines

Vaccine	Strain	Advantages	Disadvantages
Inactivated	Pathogenic field strain	Safe in pregnant animals	Short-term immunity
		Can be used in outbreaks	Multiple vaccinations required
			Risk of handling virulent strain during production
			Colostral immunity is poor
			Sheep better protected than cattle
			100 × more antigen required than for live attenuated
			Long lead time for production and limited shelf life
Live attenuated	Smithburn	Highly immunogenic	Only partially attenuated
		Single dose	Teratogenic for foetus
		Good immunity (within 21 days)	Potential risk of reversion to virulence
		Effective and easy to produce	Not advisable for use in outbreaks
		Safer production	Theoretical possibility of transmission by mosquitoes?
Live attenuated	MP12	Effective and easy to produce	Teratogenic for foetus
		Safe production	Abortion in early pregnancy
			Not available commercially
Avirulent natural mutant	Clone 13	Safe in pregnant animals	No registered vaccine yet available
		Safe in outbreaks	No large-scale field data yet available
		Produced as standard freeze-dried live vaccine	
		Safe, effective and easy to produce	

in the laboratory and, to a limited extent, in target animals (103).

A live attenuated candidate vaccine strain, the MP12 (developed by mutations of a human isolate in the presence of the mutagen 5-fluorouracil) has been tested extensively and shown to be safer than the Smithburn vaccine (69). However, despite showing good immunogenicity in late pregnant ewes and young lambs, when tested in a more extensive vaccination trial the MP12-based vaccine resulted in abortions and/or severe teratogenicity when administered between day 35 and 50 in gestating ewes (54, 55). Earlier, an avirulent RVF virus isolated from a non-fatal case of RVF in the Central African Republic had been passaged in mice and Vero cells, and then plaque purified in order to study the homogeneity of virus subpopulations. A clone designated 13 did not react with specific monoclonal antibodies and when further investigated was found to be avirulent in mice yet immunogenic. The attenuation appeared to be the result of a large internal deletion in the NSs gene (70).

Clone 13 has been used to produce a vaccine that has been extensively tested in South Africa, with very good safety and efficacy results in cattle and sheep. The safety was shown in trials conducted in sheep synchronised for oestrus and artificially inseminated. After confirming pregnancy on day 30, all the ewes were vaccinated with a high dose of the Clone 13 vaccine (10^6 $MICLD_{50}$ [mouse intracerebral lethal dose]): 7 on day 50 and 4 on day 100 post vaccination. Four pregnant cows were also vaccinated with the same dose of vaccine. None of the ewes or cows showed clinical signs of disease and no abortion occurred, with all dams giving birth to healthy offspring. While all unvaccinated control ewes aborted after virulent challenge, all ewes vaccinated with Clone 13 vaccine containing at least 10^4 $MICLD_{50}$ virus antigen gave birth to healthy lambs and did not show any clinical signs that could be associated with RVF (54).

Ongoing trials are being conducted in Africa with Clone 13-based RVF vaccine. Though still preliminary, the novel master seed for vaccine production appears to be a better alternative to the Smithburn-based vaccines, since a non-teratogenic vaccine will make it possible to envisage vaccination programmes in endemic regions of RVF where the unknown pregnancy status of animals will not be a constraint.

Bluetongue

Bluetongue is an arthropod-borne viral disease of sheep and cattle, caused by one or many of the 24 known serotypes of the bluetongue virus (BTV). The virus has been recognised as an important aetiological agent of disease in sheep in South Africa, and until 1943 was

believed to be restricted to Africa, south of the Sahara. The disease has since been identified in several countries outside Africa, such as Cyprus, Israel, the United States of America (USA), Portugal, Pakistan, India, Italy, France, Spain, the People's Republic of China, Malaysia, Bulgaria, Australia, Argentina, and, most recently Kazakhstan, as well as North African countries including Morocco and Tunisia. In 2006, the disease was reported for the first time in some northern European countries (Germany, Belgium, and the Netherlands). Bluetongue virus commonly occurs between latitudes 35° S and 40° N, but the virus has also been detected further north at beyond 48° N in Xinjiang, China, western North America and in Kazakhstan (35).

The factors contributing to the spread of BTV include animal migration and importation, extension in the distribution of its major vector, *Culicoides imicola*, involvement of novel *Culicoides* spp. vector(s), the ability of the virus to overwinter in the absence of adult vectors, and its persistence in healthy reservoir hosts such as cattle and some wild ruminants. The eradication of BTV from endemic regions of Africa is virtually impossible due to the role played by the widely distributed *Culicoides* spp. midge vectors, the multiplicity of serotypes that may circulate at any point in time, and the presence and ubiquitous distribution of reservoir species, both known and unknown. However, most indigenous breeds of sheep in sub-Saharan Africa are resistant to the disease.

Strategies for the control of BT depend on whether they are aimed at outbreaks of the disease in endemic areas or in areas where the disease is not usually present. In the latter case, the aim is usually eradication, whereas in endemic areas attempts can only be made to limit the occurrence of the disease and its economic impact, through vaccination. The initial BT vaccine was developed more than 50 years ago in South Africa and has been improved over that time to currently include 15 of the 24 serotypes known to occur in southern Africa (101). The current vaccine consists of live attenuated, cell-adapted, plaque-purified viruses in three pentavalent vaccines, which are administered separately at 3-week intervals. After two to three annual immunisations, most sheep are immune to all serotypes in the vaccine. An average of 8 million doses is used annually in South Africa, while a limited number of sheep are vaccinated in other southern African countries. Some concerns about the current vaccine have been raised in recent years. These include:

– the teratogenicity of attenuated BT vaccine strains resulting in brain defects in the foetus when administered during the first half of gestation (hence the recommendation not to administer the vaccine during the first half of pregnancy in ewes)

– the risk of reassortment and recombination between attenuated and virulent strains in the field

– the risk of transmission of attenuated viruses by vector midges or their release in the environment.

The risk of reassortment in the field is minimised by the long interval between the recommended vaccination period (i.e. late winter, early spring) and the BT season (summer), which would make the co-circulation of vaccine and virulent wild-type viruses unlikely.

Since the isolation of VP2 protein and the demonstration of its ability to induce protective immunity (53), and the subsequent discovery that other BT-viral proteins could also elicit protective immunity, different expression systems and combinations have been explored and tested in sheep with varying results. These have included baculovirus expression systems which use insect cell cultures and produce BTV-like particles (VLPs [viruse-like particles] and CLPs [core-like particles]) (80). While eliciting protection against virulent challenges and having the advantage of easy discrimination between infected and vaccinated animals, the big challenge to date of new generation BT vaccine candidates is the difficulty of scaling up the production of these different vaccine antigens at affordable costs. The difficulty in achieving simultaneously protection against multiple serotypes, as is the case of BT in southern Africa, is another challenge to overcome.

During recent outbreaks of BT in the Mediterranean region, monovalent and various multivalent BT vaccines were custom-made and used as emergency vaccines by some affected countries (29). Concern about the importation of exotic virus vaccines – though attenuated – for use in a novel environment with unknown residual virulence, and differences in the genetics of sheep and their susceptibility to BTV have lead countries to opt for the inactivated or other forms of non-replicating vaccines. While inactivated BT serotype 2 and 4 vaccines have been developed (12, 29), produced and used, no recombinant-derived vaccine has yet been produced commercially.

African horse sickness

African horse sickness (AHS) is a vector-borne viral disease affecting all equidae, and resulting in high mortality in susceptible horses. Several of the nine known serotypes of the virus can occur during the outbreak season in southern Africa, the only region of the world where all the serotypes have been isolated. While AHS causes severe clinical disease in horses, with high morbidity and mortality, donkeys, zebras and mules usually suffer milder forms of the disease but may act as amplifiers of infection as they serve as sources of blood meals for haematophagous vectors. Probably on account of its larger number of horses and the rather temperate climate as compared to other countries in the region, South Africa has definite seasonal occurrence of AHS, with the first cases usually noticed

towards midsummer, and the disease disappearing abruptly after the onset of cold weather in autumn (23).

African horse sickness appears to be endemic in the tropical regions of Africa. The Sahara Desert forms a formidable barrier against northward spread, but on occasion, AHS outbreaks in northern African countries have occurred in regions with high concentrations of horses such as Morocco, the Horn of Africa (particularly Ethiopia and Eritrea) and across the Sahel of West Africa (in countries such as Senegal). The disease has also occurred outside Africa on a few occasions, the most notable being the major outbreak in the Near and Middle East from 1959 to 1963 and in Spain (1966 and 1987 to 1990) with subsequent extension into Portugal and Morocco.

After its first reported occurrence in South Africa in 1719, and the subsequent repeated outbreaks, including the most serious 1854 to 1855 outbreak in the Cape Colony that resulted in the death of 40% of the horse population, it became clear that vaccination would be the only way to protect horses in South Africa. The quest to study the causative agent of AHS and develop a vaccine was one of the reasons behind the establishment of the Veterinary Research Institute at Onderstepoort (north of Pretoria, South Africa) in 1908. The earliest attenuated vaccine was derived from subpassaging the virus some 100 times in embryonated eggs. Subsequently, additional serotypes of AHS virus have been identified and similarly included in the vaccine. Further changes were made to the attenuation process, by reducing the number of egg passages with subpassaging using suckling mice brains or through a cell line.

However, in the early 1980s problems were encountered, with some of the mice brain attenuated strains causing neurological problems in vaccinated horses. This led to the withdrawal of serotype 4 from the vaccine for a period, and a decision that all strains should be cell culture attenuated. Serotype 4 was re-introduced into the multivalent two-dose vaccine, but serotype 5 was withdrawn, and to date has remained absent from the current live attenuated vaccine. Several attempts were made to further attenuate serotype 5 by adaptation to a cell line, but the strain remains neurotropic when administered in combination with the other vaccine strains to horses.

In South Africa, around 150,000 doses of AHS live attenuated vaccine are used annually for a horse population of 350,000. Most horses and other equidae in rural disadvantaged communities do not receive vaccines. An increase in the occurrence of outbreaks in recent years, which have involved horses in these communities has called for the need to expand vaccination into such communities in order to break the cycle of spread of the infection. The recent outbreaks of AHS in South Africa have also been characterised by either the re-emergence of serotypes that had been dormant for a few years, such as AHS serotype 5, or the appearance of virulent strains of serotypes that had previously been considered to be mild, such as AHS serotype 9. These events highlight the need for alternative and more comprehensive vaccines. Non-replicating, inactivated or recombinant polyvalent AHS vaccines with proven safety and efficacy are therefore required.

Inactivated AHS 4 vaccines have been extensively studied since the first commercial vaccine was produced in the early 1990s (52). Besides limited use in Spain, no large-scale use has since taken place. No work has been conducted in a multiserotype environment to determine the level of protection that could be achieved against all serotypes or different strains.

Through DNA recombinant technology, different subunit and CLPs have been tested as candidate vaccines. Indications from different studies are that such vaccines might require the incorporation of multiple viral proteins in order to stimulate protective immunity (37, 81). One such study was conducted by Martinez-Torrecuarda *et al.*; results indicated that VP7 was required for the baculovirus-expressed VP2, VP5 and VP7 combination to induce neutralising antibodies and protection (64).

Contagious bovine pleuropneumonia

Contagious bovine pleuropneumonia (CBPP) is an insidious pneumonic disease of cattle caused by *Mycoplasma mycoides* subspecies *mycoides* small colony variant (MmmSC). Phylogenetically, the organism is a member of the *Mycoplasma mycoides* cluster, which are pathogens of ruminants. Contagious bovine pleuropneumonia is primarily a disease of cattle with *Bos taurus* and *B. indicus* breeds being fully susceptible. The water buffalo *(Bubalus bubalis)* has a lower level of susceptibility and the African buffalo *(Syncerus caffer)* is not affected by the disease (97). The disease is characterised by the presence of sero-fibrinous interlobular oedema and hepatisation giving a marbled appearance to the lung in acute to subacute cases, and capsulated lesions (sequestra) in the lungs of some chronically infected cattle. Joint infections are common in calves. The occurrence of sub-acute/sub-clinical infections and chronic carriers after the clinical phase of the disease, creates major problems in the control of CBPP. With the exception of South America and Madagascar, the disease has occurred in most parts of the world at some point in time (97).

Contagious bovine pleuropneumonia is endemic in numerous areas of Africa, and is suspected to occur occasionally in the Middle East and possibly in some parts of Asia. North America, Europe, Australia and most parts

of Asia have eradicated the disease through slaughterhouse inspection and traceback methods, test and slaughter, and animal movement control. In the case of Australia, the adjunct, judicious use of vaccines was employed. In Africa, CBPP is found in an area south of the Sahara desert, from the Tropic of Cancer to the Tropic of Capricorn and from the Atlantic to the Indian Ocean. In the early seventies, the CBPP disease situation appeared to be under control. However, after almost 20 years of respite, CBPP made a spectacular comeback on two major fronts – one in the east of the African continent and the other in the south. Almost at the same time, there was a resurgence of the disease in previously known endemic areas of West Africa. It is endemic in the pastoral herds of much of western, central and eastern Africa, Angola, northern Namibia and Zambia. Botswana, Lesotho, Madagascar, Malawi, Mauritius, Mozambique, southern Namibia, South Africa, Swaziland and Zimbabwe are currently (2006) free from the disease (42, 96). Contagious bovine pleuropneumonia represents a major constraint to cattle production in Africa and is regarded as the most serious infectious animal disease affecting cattle, now that rinderpest is almost eradicated (with the possible exception of the Somali ecosystem) from the continent.

In the 1960s and 1970s, sustained research on CBPP vaccines in Kenya (Muguga), Chad (Farcha), Nigeria (Vom) and other African countries, coupled with a large multi-donor funded international campaign – known as Joint Project 16 – resulted in the disappearance of clinical disease from most parts of Africa. Various strategies have been used in the continent to control CBPP and these include the stamping-out of infected herds and targeted/mass vaccinations coupled with the use of antibiotics (although official policy on using antibiotics to control CBPP suggests the contrary). In view of the epidemiological situation of the disease, most CBPP endemic countries control the disease by vaccination, which is usually carried out by the official Veterinary Services. In the past, vaccination was practised in some parts of Africa by using traditional methods such as placing a piece of diseased lung tissue under the skin on the bridge of the nose of susceptible cattle (96). In modern times, immunisation has been refined by attenuation of the immunising agent. Attenuated vaccines against CBPP have been developed by multiplying the CBPP agent in heterologous host such as embryonated chicken eggs and later by serial subculture on artificial *Mycoplasma* growth media (51, 88). The efficacy of live vaccines is directly related to the virulence of the original strain of MmmSC used for their production. Attenuated virulent strains of MmmSC stimulate the best immunity (65) but they also induce the most severe and undesirable local and systemic reactions, which may even result in the death of the animal. The live attenuated vaccines currently in use are a compromise between virulence, immunogenicity and safety. In the past, it was thought that if vaccination were to be effective, a local lesion had to be produced at the site of inoculation. This led to strong resistance from farmers, who feared their cattle would die as a result of post-vaccinal reactions. Among the many vaccinal strains developed during the CBPP outbreaks in Australia and Africa almost 40 years ago, those currently in use in Africa are the T1 vaccine strain, isolated in Tanzania and passaged 10 times in embryonated eggs and then 44 times in broth cultures (T1/44), and its streptomycin-resistant derivative, T1/SR (15, 76). Broth culture vaccines have been replaced by lyophilised vaccines. The combined rinderpest/CBPP vaccine (Bisec) was actively used in many parts of West Africa to control the two diseases. The discontinuation of rinderpest vaccination and the disuse of Bisec have contributed in large part to the resurgence of CBPP in parts of West Africa, because many countries were no longer able to pay the recurrent costs involved in getting vaccine teams into the field to continue vaccination against CBPP. Vaccination failures with T1/SR vaccine in Botswana and parts of East Africa prompted concerns about the efficacy and/or identity of the strain being used for vaccine production. The question of identity was resolved by the use of molecular epidemiological tools which allowed its characterisation and distinction from the KH3J vaccine strain (60). The two predominant vaccine strains of T1/44 and T1/SR were re-evaluated for efficacy and immunogenicity (95). In these studies naïve cattle were vaccinated with vaccine containing the minimum dose of 10^7 viable mycoplasma organisms per dose, as recommended by the OIE (104), in three locations in Africa (Cameroon, Kenya, Namibia) and challenge studies were carried out. Primary vaccination induced a protection rate of 40% to 60% wherever it was used. The duration of immunity was found to be longer with T1/44 than with T1/SR and revaccination after one year enhanced the level of protection (80% to 90%).

Mycoplasmas are prone to frequent genetic variations, so to prevent varying levels of immunogenicity in the final product great care should be exercised in CBPP vaccine production so that the cloning procedures do not cause antigenic drifts (22, 63). Simple modifications to current accepted protocols include:

a) buffering the growth medium so that neutral pH is maintained thereby attaining the minimum of 10^7 viable mycoplasma organisms per dose;

b) including a pH buffer in the vaccine so that deleterious acidification would be visibly recognised and ineffective or damaged vaccine discarded;

c) changing the reconstitution method for freeze-dried vaccine to exclude 1 M $MgSO_4$ which could be substituted with phosphate buffered saline. It has also been argued that improving the quality of the existing CBPP vaccines is more likely to deliver significant beneficial effects than

developing a new generation of vaccines, which could be an expensive and time-consuming process.

The thermostability of CBPP bacterins has been an area of great concern considering that the vaccines have to be transported over long distances to remote areas where cold chain maintenance is difficult. A new live vaccine dehydration and preservation technology, called *xerovac*, was developed at the Pan African Vaccine Centre (PANVAC) in Ethiopia (107). This method, which mimics the survival strategies of cryptobiotic organisms, could be applied to vaccine production to yield thermotolerant vaccines. Litamoi *et al*. demonstrated that rapid dehydration of CBPP vaccine in an excipient composed of a high concentration of trehalose (25%) following the *xerovac* method rendered the CBPP vaccine product more heat tolerant than similar vaccines prepared by conventional methods (59). Trehalose is one of the most chemically unreactive and stable sugars and is very stable to hydrolysis. They also demonstrated that the addition of chitosan as a mycoplasma precipitating agent, conferred additional heat resistance to the vaccine, although the titre of mycoplasma dropped (due perhaps to the greater need to homogenise chitosan and mycoplasma cultures) and there was a consequent titre loss due to the fragility of mycoplasmas. These findings require further studies to establish a more suitable MmmSC culture medium with low solute concentration, and substitution of glucose with trehalose in the culture medium for CBPP vaccine production. The establishment of an independent laboratory, such as PANVAC under the auspices of the African Union, would be in a position to certify CBPP and other animal disease control vaccines and contribute to quality control and a better CBPP vaccine product (59, 107).

Haemorrhagic septicaemia

Haemorrhagic septicaemia (HS) is a peracute to acute highly fatal bacterial disease, principally of cattle and water buffalo *(B. bubalis)*, caused by specific types of *Pasteurella multocida*. The disease is endemic throughout southern and south-eastern areas of Asia and many regions of Africa. Haemorrhagic septicaemia was traditionally regarded as being caused only by serotype B or E, but other serotypes are recognised as causing the disease, particularly serotypes B:1 and B:3,4 (9). The two common serotypes of *P. multocida* associated with the disease are types B:2 (in Asia) and E:2 (in Africa). The Asian B:2 serotype has also been associated with sporadic septicaemic disease in pigs. In Egypt and Sudan, the presence of both B and E serotypes has been reported (89). Outbreaks of HS are usually limited in extent and tend to be associated with conditions of stress. The Asian form of HS occurs in countries with high seasonal rainfall and is usually endemic in marshy zones or along river deltas. The diagnosis of HS depends on the isolation of the causative organism, *P. multocida*, from the blood or bone marrow of a dead animal by culture and biological methods, and the identification of the organism by biochemical, serological and molecular techniques. Since HS is primarily a bacterial disease, it should theoretically lend itself to effective antibiotic therapy. However, treatment is constrained by a number of factors. The acute nature of most cases of the disease limits the efficacy of antimicrobial therapy of sick animals, but it can be effective if they are detected and treated in the early stages of the disease. As the disease occurs in places with substandard husbandry practices, most cases escape early detection. Vaccination, therefore, appears to be an alternative effective control option. A solid, long-lasting immunity is conferred on animals that recover from the natural disease, which persists longer than that induced by vaccination (26).

Haemorrhagic septicaemia is preventable using vaccines containing the causative bacterial agent. However, since *Pasteurella* is a poor immunogen, a large amount of antigen (whole bacterial cell) has to be administered. This procedure occasionally leads to endotoxic shock (26). There are three types of bacterins used against HS: alum-precipitated vaccine, oil-adjuvanted vaccines and formalinised bacterins. Some significant success in the control of HS has been achieved in Asian countries by the immunisation of buffaloes and cattle with alum-precipitated or oil-adjuvant bacterins (19). Immunity is, however, of short duration, lasting from six to nine months on primary vaccination and 12 months after secondary vaccination. Large-scale vaccination of cattle against HS is not practised in many countries of Africa. An outbreak of HS in Zambia in 1979 was largely controlled by using formalinised bacterin obtained from Sudan (45). It is advisable to use bivalent vaccines (B and E) in Eastern and Central Africa because of the presence of both B and E serotypes. Despite the fact that the alum-precipitated vaccine is known to provide immunity of short duration, it is still the most common vaccine in use, since it is the easiest vaccine to inject. The oil-adjuvant vaccines, though known to be more potent, are difficult to inject on account of their high viscosity. During the past decade, a considerable amount of research has been done in South Asia aimed at producing oil-adjuvant vaccines of low viscosity. It is known by one of the authors (W. Amanfu) that Sri Lanka and Indonesia have successfully used lower levels of lanoline as the emulsifying agent in an effort to reduce viscosity. Malaysia has modified the use of the alum-precipitation technique to concentrate broth cultures in order to reduce the dose volume of the oil adjuvant in the vaccine formulation, which they believe will facilitate injection (unpublished data). In India, at least one vaccine producer is marketing a combined FMD-HS-Blackquarter oil-adjuvant vaccine.

A live heterotypic vaccine made with *P. multocida* serotype B:3,4 isolated from a fallow deer *(Dama dama)* in the

UK (56) protected cattle against a serotype B:2 challenge and conferred immunity against HS for one year in cattle vaccinated subcutaneously (72). The intranasal vaccine has been recommended by the Food and Agriculture Organization of the United Nations (FAO) as a safe and potent vaccine for use in Asian countries based on trials in Myanmar. However, there is no report of its wide-scale use in other countries and inactivated bacterin preparations are preferred. The safety, efficacy and cross-protection of a live intranasal HS bacterin containing *P. multocida* serotype B:3,4 were further tested in young cattle and buffaloes in Myanmar (73). In this study, the administration of 100 times the recommended dose to 50 cattle and 39 buffalo calves was innocuous. Seven months after vaccination, all 39 buffaloes were protected and 12 months after vaccination, three out of four buffaloes were protected against a subcutaneous challenge with serotype B:2, with vaccinated cattle developing serum antibodies detectable by the passive mouse protection test. The serum of vaccinated cattle cross-protected passively immunised mice against infection with *P. multocida* serotypes E:2, F:3,4 and A:3,4. This finding could be studied further in parts of Africa where both serotypes B:2 and E:2 occur. The recent adoption (2005) of standards by the OIE on the requirements for vaccines and diagnostic biologicals for HS should provide the necessary technical platform for the production and quality assurance of HS vaccines (105).

Brucellosis

Brucellosis is caused by bacteria of the genus *Brucella* which are Gram-negative non-spore-forming non-encapsulated coccobacilli or short rods with rounded ends. The disease is characterised by abortion, retained placenta, orchitis and infection of accessory sex organs in males. Arthritis and hygromas may be seen, especially in cattle. The disease is prevalent in most countries of the world, and primarily affects cattle, buffalo, pigs, sheep, goats, dogs and some wild terrestrial and marine mammals. As a zoonotic disease, brucellosis is of serious public health significance, particularly those infections caused by *Brucella melitensis*, which in man has the most virulent characteristics of all the brucellae. In cattle, brucellosis is primarily caused by *B. abortus*, but in some countries, particularly in southern Europe and in the Middle East, *B. melitensis* has been implicated as a cause of abortions especially when cattle are kept in close contact with infected sheep or goats. Occasionally, *B. suis* may infect the mammary gland of cattle, but this has not been associated with abortions in cattle. *Brucella melitensis* commonly causes caprine and ovine brucellosis, whilst *B. ovis* causes ram epididymitis. *Brucella suis*, which causes porcine brucellosis, consists of five biovars. Brucellosis in pigs is characterised by an initial bacteraemia followed by the development of chronic lesions in the bones and reproductive organs. Other brucellae organisms of significance are *B. canis*, which causes epididymitis and orchitis in male dogs and metritis in bitches, and *B. neotome*, which was isolated in rodent species in the USA. Most of the serological tests for isolation of smooth *Brucella* spp. infections (*B. abortus*, *B. melitensis*, and *B. suis*) have been developed to detect antibodies directed against antigens associated with the smooth lipopolysaccharide (S-LPS) and are shared by all the naturally occurring biovars of *B. abortus*, *B. melitensis*, and *B. suis* (24). Since *B. abortus* antibodies may cross-react with those against *Escherichia coli* O157 and *Yersinia enterocolitica* serovar 0:9, false positive reactions may be important during the final stages of an eradication programme. In rough brucellae such as *B. canis* and *B. ovis*, specific antigens associated with the rough lipopolysaccharide (R-LPS) have to be used for the diagnosis of infections caused by these organisms.

Cattle vaccines

The most widely used vaccine for the prevention of brucellosis in cattle is the *B. abortus* Strain 19 (S19), which remains the reference vaccine to which other vaccines are compared. The standards and administration of S19 vaccine in cattle, have been well described (106). Briefly, the S19 vaccine is used as a live bacterin and is normally given to female calves between 3 and 6 months of age as a single subcutaneous dose of 5-8 $\times$ 10^{10} viable organisms. A reduced dose of from 3 $\times$ 10^{8} to 3 $\times$ 10^{9} organisms can be administered subcutaneously to adult cattle. Some animals may develop persistent antibody titres, may abort and excrete the vaccine strain in the milk. Alternatively, the vaccine may be given at any age as two doses of 5-10 $\times$ 10^{9} viable organisms, given by the conjunctival route. This produces protection without a persistent antibody response and reduces the risks of abortion and excretion of live vaccine strain *B. abortus* in the milk. Vaccination with S19 bacterin increases resistance to *B. abortus* but does not induce sterilising immunity. If an animal is infected, vaccination will not cure the infection. The increase in resistance following vaccination has been termed *relative immunity* since it is estimated to be only about 70% effective against field challenge by preventing unrestricted multiplication of *B. abortus* in the uterus and mammary gland (74). The main disadvantage of S19 vaccination is the induction of post-vaccinal antibodies that are detected in serological tests. Currently, there is no single fully validated test that can be used to distinguish between antibodies due to infection and those due to vaccination, although newer tests and combinations of tests have been developed to attempt to overcome this problem (49).

Brucella abortus rough strain vaccine RB51 has been the official vaccine used in the USA since 1996 for the prevention of brucellosis in cattle (87). Protocols for use of this bacterin in the USA have been reviewed (49). However, some countries in Latin America have officially

adopted the use of RB51, but use different regimens. The vaccine strain is a rough rifampicin-resistant mutant of *B. abortus* strain 2308, of the virulent *B. abortus* biovar 1 strain. This mutation has been described as very stable, with no reversion to smoothness *in vitro* or *in vivo*. Some studies have been conducted in cattle to compare the vaccine potency of RB51 with that of S19, with a general conclusion that RB51 does not significantly induce a higher degree of protection than S19 and its superiority to S19 still remains controversial (21). An advantage of using RB51 vaccine, however, is that antibodies induced by its administration are not detected by the currently prescribed serological methods. *Brucella abortus* S19 and RB51, although attenuated strains, are still capable of causing disease in humans, and in the case of RB51, the organism is resistant to rifampicin, one of the drugs of choice for treating human brucellosis.

Strain 45/0 of *B. abortus* was isolated in 1922 and after 20 passages in guinea pigs a rough derivative isolate named 45/20 was obtained. Strain 45/20 has been used as an inactivated vaccine incorporating an oil adjuvant, but this is not as protective as S19. In addition, large unsightly granulomas developed after use in some instances and its use has been discontinued (49).

Small ruminant vaccines

In small ruminants, the *B. melitensis* Rev. 1 Elberg strain has been recognised as a superior bacterin, compared to *B. abortus* S19 and *B. suis* S2 (11). The Rev. 1 bacterin not only protects against *B. melitensis* but it also protects other animal species against *B. abortus* or *B. suis*. In spite of this, the traditional approach is still dominant: homologous vaccines, e.g. *B. abortus*, *B. melitensis*, and *B. suis*, are administered to each of their principal hosts, respectively. Due to the ability of Rev. 1 to induce abortion and the fact that the organism can be excreted in the milk, it is suggested that the vaccine be administered subcutaneously prior to the first gestation at 3 to 7 months of age. This regimen could lead to long-lasting persistence of specific antibodies, which could create serious problems in the serological diagnosis of the disease (43). When used as a flock vaccination programme, inoculation of *B. melitensis* Rev. 1 greatly decreases the prevalence of brucellosis in goats and sheep, and hence reduces brucellosis in human populations. When Rev. 1 is administered by the conjunctival route, the immunity conferred is similar to that induced by the standard method, but the serological response evoked is significantly reduced (4). Production of *B. melitensis* Rev. 1 vaccine is based on a seed lot system where master seed cultures must be obtained from OIE/FAO Reference Laboratories, and must conform to minimal standards for viability, smoothness, residual infectivity and immunogenicity.

Future outlook: novel biotechnological vaccines

Vaccination is one of the most important and cost-effective methods of preventing infectious diseases in animals (reviewed in 79). Currently, the majority of licensed bacterial and viral vaccines are either live attenuated or inactivated. Live attenuated vaccines are very efficient in inducing long-lasting immunity via cell-mediated and humoral immune responses. These vaccines, however, do have disadvantages when given to pregnant and immuno-compromised animals, and some have the potential to revert back to virulence (i.e. RVF). Inactivated vaccines cannot replicate and are thus non-infectious but also lack the ability to induce a long-lasting and comprehensive, especially cell-mediated, immune response. They are thus often regarded as inferior in stimulating immunity in comparison with live attenuated vaccines, although their negative effects are less severe.

Because of the globally increasing qualitative and quantitative demands for livestock and their products, vaccine producers are increasingly being required to fulfil a set of prescribed specifications. These include ensuring that the protective antigens used during the production of validated attenuated vaccines are free from pathogen-associated toxins and immunosuppressive components and are capable of eliciting long-lasting immunity. Recombinant subunit, DNA and non-pathogenic virus-vectored vaccines are currently the most cost-effective methods of producing antigens that are free from the exogenous materials that are associated with conventional vaccines.

Live vaccines

Live vaccines can be regarded as the most successful category of vaccines available and include not only conventional attenuated vaccines, but also gene-deletion attenuated and recombinant virus-vectored vaccines. The advantages of attenuated vaccines include their low reactivity and their ability to induce protective systemic, as well as mucosal, immune responses. In addition, there are low manufacturing costs due to minimal downstream processing and the fact that adjuvants are not required for their formulation. The overall safety profiles of conventional attenuated live vaccines fall short of what is desired from an ideal vaccine. Conventional live bacterial and viral vaccines are produced by selecting attenuated mutants which have the capacity to induce infection but have a reduced or non-existent ability to induce disease. In most attenuated vaccines, attenuation is achieved by blind serial passage in heterologous tissues (cell cultures, eggs, laboratory animals or broth cultures). But also mutation is induced spontaneously either by chemical treatment,

heating or spontaneous mutagenesis (and clonal selection). It is important to realise that mutational attenuation is an uncontrollable process and the induced mutations are rarely characterised at the genomic level. Therefore, it is very difficult to control the degree of attenuation. Reversion to virulence is the greatest potential risk of attenuated live vaccines, causing not only potential disease, but also possible shedding of organisms into the environment. Despite these risks, there are many examples of safe and effective attenuated veterinary vaccines in current use. The global eradication of rinderpest is about to be achieved through the wide use of such live attenuated vaccines of undetermined genetic definition. Recent advances in genetic engineering have not only enabled the identification of genes associated with pathogenicity or virulence in infectious organisms, but have also allowed for the deletion or inactivation of selected target genes, thereby increasing the safety profile of candidate vectored vaccines. The first commercial live gene-deletion attenuated vaccine was a glycoprotein E deleted (gE-) pseudorabies (Aujeszky's disease) vaccine that is currently used for eradication programmes in Europe and the USA. More recently, a gE–gG–TK– attenuated pseudorabies vaccine has been developed. It seems highly likely that these vaccines will become more prevalent in the future once they are perceived as being safer than conventional attenuated vaccines, as their degree of attenuation can be more effectively managed. The delivery of heterologous antigens via a recombinant live vector offers significant advantages as part of a comprehensive vaccination strategy, in that the recombinant organism would only induce a mild infection and subsequently induce immune responses to the gene derived from the pathogenic organism (91). Regardless of the vector organism used, expression of the heterologous (protective) antigen by the recombinant vector is the key to effective antigen presentation and induction of an adequate immune response. Currently, it is possible to use viruses, bacteria and even parasites as live recombinant vectors. The first recombinant live viral vector evaluated was vaccinia virus, a poxvirus that has since been used to express viral, bacterial and parasitic antigens that have been reported to elicit protective immunity in several animal disease models. Vaccinia virus demonstrated efficacy as a recombinant live viral vector in experimental trials when virus expressing rabies gG as a surface antigen was orally delivered to foxes and other wild animals. Vaccinia virus expressing pseudorabies gD as a surface antigen delivered intramuscularly induced an effective immunological response very similar to a conventional inactivated vaccine. Currently, a number of pox-based vectored vaccines are being marketed. Viruses with large genomes, such as vaccinia and adenovirus, are better candidates for recombinant viral vectors than smaller genome viruses, due to the fact that they can accommodate substantially larger inserts of foreign DNA while retaining their infectivity. These viruses present a cost-effective option for vaccination strategies since their genomes have been sequenced and because commercial expression vectors are available and can be cultured to very high titres resulting in reduced production costs. An additional advantage of using recombinant live vaccines is the possibility of producing multivalent vaccine, which means that more than one disease can be addressed at the same time and the cost of vaccination campaigns can be reduced. This is important for developing countries where infrastructures are poor.

Despite very promising results with live vectored vaccines there are also some concerns. The major concern is that live vector vaccines will not induce adequate immunological responses in animals that have pre-existing antibodies against the vector, with some exceptions. Genes of interest or their fragments, can not only be added but also deleted from a genome. The first gene-deleted live attenuated pseudorabies vaccine was a naturally occurring mutant, lacking glycoprotein E (gE–), known as the Bartha vaccine, which has now been used for decades in controlling pseudorabies virus in pigs. The Bartha vaccine was subsequently recognised for its ability to differentiate vaccinated animals from naturally infected animals, effectively making it the world's first marker vaccine. Indeed, in order to evaluate the effectiveness of a disease eradication programme, an insertion, mutation, or detection method is required in order to differentiate vaccinated (immune) animals from naturally infected animals (DIVA). This is achieved by developing vaccines that lack one or more antigens, or that have one or more extra antigens, or that have one or more detectable changed antigens, thereby inducing an antibody response (or lack thereof) in vaccinated animals that could subsequently be used to serologically differentiate between infection that was induced by a wild-type infection and infection induced by vaccination. These DIVA vaccines are important tools in assessing the effectiveness of vaccination programmes, without the time-consuming and near impossible task of individually evaluating infectivity, transmissibility and susceptibility within a vaccination programme. With the availability of recombinant technologies and sequenced pathogenic genomes the identification of potential markers is more feasible than ever before. It seems logical that vaccines developed in the future will increasingly embrace DIVA principles in order to provide a much needed tool for livestock disease prevention or eradication programmes. Currently, numerous national animal disease eradication programmes based on the serological confirmation of infection alone require the destruction of herds to limit the spread of disease. Due to a reliance on serological confirmation for absence of disease or infection, a vaccination programme is actually incompatible with surveillance. Thus, vaccination programmes are often banned, along with animals originating from countries vaccinating for certain diseases. However, the application of the DIVA marker technology creates a compatibility between surveillance and

vaccination programmes, allowing vaccination to play a large and extremely significant role in the eradication of these diseases. Several marker vaccines are already commercially available, and their role and contribution in disease eradication appears promising. For them to be embraced by the industry, however, requires support from governments and the livestock industry to ensure that the biopharmaceutical industry develops these vaccines. The move to eradicate diseases is not only driven by the financial considerations of the producer, but is often politically motivated: once a country is declared disease-free, the country can use this disease-free status as an effective trade barrier, making such vaccines even more attractive. The upcoming technology of reverse genetics, which relies on full-length genetic sequences created *de novo*, offers great promise in vaccinology, as precise and stable manipulations can be made, with control of the transcription and translation processes.

Inactivated vaccines

Molecular biology and genetic engineering have had an enormous impact on vaccine development by providing the tools and techniques to produce a single protein in a prokaryotic or eukaryotic system. Furthermore, if the protein is produced in prokaryotic systems, it can be tailored in such a way that the protein of interest is either expressed on the surface of the bacteria, in the periplasm, as insoluble inclusion bodies or secreted into the media. The recombinant approach to subunit vaccines is to clone the gene that encodes the protective antigen into a secondary, preferably non-pathogenic, organism that is capable of expressing the immunogen in its native form or with minimal alterations. This protein can then be expressed and harvested using traditional bacterial antigen production methods, or delivered by a live non-pathogenic vector. Recombinant subunit vaccines eliminate the risks associated with handling pathogenic organisms as well as the risks associated with live or inactivated products either reverting or still possessing a pathogenic or virulent state due to incomplete inactivation or attenuation. In all subunit vaccine approaches, the identification of proteins or epitopes involved in eliciting a protective immune response is crucial. Enormous advances in computer modelling and bioinformatics have made the rapid identification of protective or critical epitopes possible, including cross-species identification of functionally similar proteins. The power of recombinant technology lies not only in single protein or epitope subunit vaccines, but also in generating fusion epitopes. The possibility even exists that multiple protective epitopes could be cloned from a variety of pathogens to create a single protein. This 'string of beads' vaccine should be capable of inducing protective immunity to a wide range of viruses in a single subunit. The combination of genomics, bioinformatics and recombinant technology has even allowed for the development of vaccine candidates before the pathogen could even be cultured (78). As an example, it is still not possible to culture (human) hepatitis B virus, yet a human vaccine has been available for over a decade.

Commercial production of a recombinant subunit vaccine requires the selection of an appropriate expression system based on the nature of the protein being expressed. Critical factors in the selection of a biopharmaceutical expression system include the production of an immunologically protective epitope, affordable protein production, affordable extraction and cleanup, minimal immunological interference from host proteins and minimal pyrogen production. For the production of non-glycosylated proteins, bacterial expression systems are excellent candidates. Organisms such as *E. coli* and *Salmonella typhimurium* have been used extensively for the expression of a wide variety of foreign genes and as a result many production, stabilisation and optimisation strategies have been described. While prokaryotic expression is efficient and affordable for the production of a broad range of immunogens, including a few natively glycosylated proteins, production of many viral glycoproteins in prokaryotic systems does not result in protective immunity due to the lack of glycosylation, despite producing significant immune responses. Additionally, the presence of lipopolysaccharides and other pyrogens leads to various complications, including interference and possible injection-site reactions. Thus, for the expression of glycoproteins and other modified proteins, eukaryotic expression systems are far more suitable. Examples of such systems include those that use yeast, insect cells, plants and mammalian cells, which have been systems of choice for producing many immunogens. Although expression of proteins in mammalian cells is generally expensive, for some viral glycoproteins availability of such expression systems is critical. This is especially true where post-translational modification such as glycosylation of nascent proteins is important for proper folding and generation of specific epitopes. Viral expression systems remain the preferred method of commercial production of native glycoproteins. This technology was originally demonstrated with vaccinia virus as the vector, but almost any virus can be used as an expression system for producing either whole proteins or epitopes.

The molecular breakthroughs in cell transformation and gene therapy have contributed to the development of the new field of DNA vaccinology, with its enormous potential for providing safe, inexpensive and effective DNA-based vaccines. The basic concept of DNA vaccine use is the delivery of plasmid DNA, which encodes for immune stimulating and protective proteins, into the cells of the host animal, where direct transcription and translation occurs – effectively transforming the vaccinated animal into a bioreactor for the production of its own vaccine (36). The fact that the protein is produced within the host means the vaccine should be correctly modified post-

translationally and as such should possess the authentic conformational and chemical structure (i.e. glycosilation) and, thereby, functional and immunostimulatory epitopes. Despite the fact that the antigen is produced by the host's cellular machinery, the immune system recognises the protein as being foreign and mounts both a cellular and humoral immune response very similar to one induced by live vaccines or in animals that have recovered from natural infection. Although CpG motifs, which are commonly present in DNA vaccines, induce a wide range of cytokines, it seems that Th1-like or Th2-like responses are not equally proportional, with Th1 induction exceeding Th2-like responses.

The type of immunological response that follows vaccination depends not only on the gene being introduced but also on the site of administration and the method of delivery. One of the major problems of current vaccines is their inability to elicit active immunity in neonates possessing passively acquired maternal antibodies. DNA vaccines have been shown to effectively circumvent maternal antibody interference. Furthermore, since the mucosal immunological system functions as an inter-related system, the delivery of a DNA vaccine at one mucosal site should induce at least partial immunity at other mucosal sites and may in fact serve to prime the immune system prior to vaccination with a traditionally delivered, inactivated or subunit vaccine. A large number of experimental DNA-based veterinary vaccines have been evaluated in a number of species with varying degrees of immunological response and efficacy against challenge. Despite promising results, some questions have been raised regarding the safety of DNA vaccination. Possible integration of plasmid DNA into the host genome and potential risk of malignancy and integration of foreign genes into germ cells could potentially lead to vertical gene transmission and expression. Additionally, the potential exists for the development of antibodies against the DNA vaccine itself and thus for the induction of autoimmunity as a consequence, although no evidence exists for such an event occurring, even when milligram quantities are successively injected. Despite the lack of scientific evidence to substantiate these plausible risks from DNA vaccination, it would be unwise to rule them out entirely and it is reasonable to expect regulatory authorities to impose cautionary measures in order to avoid these situations.

Vaccine formulation and delivery and stimulation of mucosal immunity

Subunit or inactivated vaccines require specific adjuvants in order to elicit an immunological response tailored to mimic responses induced by natural infection. It has been generally accepted that the optimal protective response is achieved when the vaccine is administered via the same route by which the infection enters the body (32, 33). The correct formulation is therefore essential in the development of an effective vaccine, as the adjuvant must be compatible with the route of administration and complementary to the antigen. Today's highly efficacious and safe vaccines, be they recombinant or conventional inactivated vaccines, would not be available if it were not for the adjuvants and delivery systems developed over the past three decades. A variety of chemical and biologically derived compounds have been added to vaccines in order to increase the elicited immunological response, including aluminium salts, mineral oil, cholera toxin and *E. coli* labile toxin. More recently, several classes of compounds, including immunostimulatory complexes (ISCOMs), liposomes, virosomes and microparticles have been employed to act as antigen delivery vehicles and they are proving to be potent adjuvants, greatly enhancing the magnitude and the duration of the immunological response to the formulation. An efficacious response to non-replicating vaccines (subunit and inactivated) is entirely dependent on the adjuvant. Vaccines have traditionally been administered via intramuscular or subcutaneous injection. However, both intramuscular and subcutaneous injection routes share a disadvantage in that although they can induce comprehensive systemic responses, only poor mucosal immunity is elicited. Mucosal immunity and the production of local IgA antibodies are central to prevention of pathogen penetration of mucosal surfaces, the major route of infection for numerous diseases (e.g. FMD, classical swine fever, brucellae, influenzas, etc.). Administration of vaccine onto mucosal surfaces such as those in nasal passages, eyes, lungs and the gastrointestinal tract is an effective way of inducing mucosal immunity. It is important to emphasise that all mucosal sites are interconnected by a common mucosal immune system and that the administration of protective antigens at one primary site will stimulate antigen-specific lymphocytes which migrate and provide immunity at other mucosal sites, regardless of the site of induction. In veterinary vaccinology, the delivery of live attenuated organisms to mucosal surfaces has proven to be very effective.

Public/private partnerships

Private industry/Government partnerships

Since 1999 several studies have predicted a growing demand for livestock products due to population growth, increased urbanisation and economic growth, primarily in what are now developing or middle income countries (27, 28, 40). A combination of growth in animal densities, intensification of production methods, the globalisation of trade, climate change and the movement of people and animals is increasing the risk of international spread of infectious animal diseases. Recent assessments point to the

burden of infectious diseases continuing to be a major constraint to sustained international trade in livestock commodities from developing countries unless targeted sanitary measures are instituted in those countries to reduce the burden of infectious diseases (16, 38, 39, 75, 82, 84).

Varying levels of disease risk in different countries mean that there are likely to be differences between the specifications and requirements for vaccine use in the Organisation for Economic Co-operation and Development (OECD) countries – where major infectious diseases (e.g. FMD) are generally absent – and those required by developing countries for progressive disease control from an endemic status. For example, in the case of FMD vaccines, the requirement for OECD countries is likely to be for vaccines prepared from highly purified and concentrated antigens with highly purified or synthetic adjuvants or immodulators. Such vaccines would be required to be powerful enough to block infection and induce a rapid onset of immunity or immediate (but not necessarily long-lasting) protection (50, 61, 62, 67, 93). By contrast, the vaccines needed for controlling endemic FMD in developing countries are similar to the type of moderate potency vaccines that were successfully used in Europe and South America some 20 years ago to control FMD and which induce long-lasting immunity, i.e. 12 months or longer (6, 7, 30, 48). A second example is the growing tendency for OECD countries to move away from the use of live attenuated vaccines, especially those derived from RNA viruses, for fear of mutation/recombination and reversion to virulence, whereas these have been the bedrock for disease control programmes and are likely to continue to be the most cost-effective tools for disease control in developing countries in which the disease has endemic status. Such differences in intrinsic specifications are likely to weigh heavily in considerations for technology transfer from OECD countries, or from Middle Income Countries such as those in South America, to vaccine manufacturing units in the Low-Income-Food-Deficit-Countries (LIFDCs). The technology transfers are likely to be facilitated through judicious public/private sector partnerships in which individual livestock owners may have to pay for the vaccine, but also have access to a better future through the establishment of associations or cooperatives, methods to increase commodity manufacture, and improved production and animal hygiene methods. This means that livestock owners can therefore afford more from the service industries (i.e. veterinary care, purchase of preventive medicine) and will experience a general improvement in their livelihoods. Technology offers opportunities for the commercial sector in the LIFDCs as long as stakeholders or shareholders (investment) are patient and hold a medium to long-term vision. Should commercial vaccine selling interests be based on immediate purchases – profits are likely to be short-lived.

An additional factor in vaccine demand, and therefore public/private partnership opportunity, is likely to be in the range of vaccine requirements. For example, the requirement for vaccines in OECD countries is likely to be for a limited range of vaccine types, targeting only those highly contagious diseases, such as FMD, that pose a high risk of spread either through globalised trade or factors associated with climate change (e.g. RVF, bluetongue, AHS). Countries of sub-Saharan Africa are likely to have a requirement for a much wider range of vaccines than even in other developing regions of the world for the simple reason that Africa has the heaviest burden of infectious and protozoal diseases in the world (84). For this reason, the future demand for vaccines in developing countries is not likely to be one that can be addressed simply by importing vaccines produced in, and for the needs of, OECD countries or by relocating vaccine production plants from OECD to developing countries. Future vaccine requirements in developing countries are likely to be met in a variety of ways, including technology transfer, development of vaccines that are specifically tailored for the disease epidemiology in developing countries, and investment at the local level, as has been the case in Latin America – though at times this investment has focused on making profits in the short-term rather than on building longer-term relationships between farmers and the vaccine-production industry. What would be beneficial are public/private partnerships which take into account the socio-economic dynamics and recognise the requirement for public service commitment in a commercially profitable environment.

Public/private partnerships for vaccine manufacture

The premise is that vaccine production and marketing are most sustainable when undertaken in some form of commercial environment. In developing countries, experience has shown that neither relying on purely market forces nor on governmental production and free distribution of vaccines in total disregard of commercial practices is sustainable. Vaccine production and distribution in a conventional governmental department setting tends to focus on the number of doses; it often does not adhere closely to good manufacturing processes or quality assurance programmes and there is little focus on return on investment and/or technology renewal. Consequently, there is a tendency to persist with out-dated technology, and little effort is made to ensure purity, efficacy, or potency of the product. Almost invariably, government-led production does not target the export market because of the need to meet demanding quality standards, so all that remains is the non-lucrative domestic market. By contrast, the private sector will only concentrate on those vaccines that guarantee high profitability. So there is an increasing need in developing

countries for public/private partnerships for vaccines. Similarly, in the OECD countries public/private partnerships would be valuable in aspects of research and development, though in recent history production, marketing and delivery have been relegated fully to the private sector, with some exceptions such as exotic or foreign transboundary animal diseases.

For any partnership to flourish there needs to be either a convergence or complementarity of interests and objectives. Government objectives for vaccines revolve around national animal health programmes and the need for quality assured vaccines that are affordable, efficacious and safe. The objective for vaccine production companies is to generate profit from the sale of licensed vaccine products. Where there is an assured high volume demand for particular vaccines (e.g. FMD vaccines in South America) there is good incentive for the private sector to invest in vaccine manufacturing plants, since it is reasonably certain that they will recoup their investment. It would then be the government's role to regulate and ensure the quality control of the end product and monitor the efficiency and efficacy of vaccine use, rather than invest in production themselves. In this case, the government/private sector partnership is complementary, as the government requires a sustained supply of efficacious and affordable vaccines from the private sector, while the private sector is satisfied to have a government-guaranteed demand within the competitive environment of different vaccine suppliers.

However, increasingly, the private sector does not have sufficient confidence of a guaranteed high volume market, either because of market uncertainty or because the market is simply too narrow, and although it may be of strategic importance to the country it is unlikely to be very profitable. Therefore, the increasing tendency in developing countries has been for governments or other sections of the quasi-public sector (e.g. livestock farmers' cooperatives or associations) to either set up parastatal companies with commercial autonomy or to seek some form of partnership with commercial enterprises for technology transfer and/or for the management of vaccine production. Haigh has described the different forms of partnerships (51), they include the following:

a) A self-contained parastatal local company that is empowered to seek targeted alliances with private enterprise for the purpose of acquiring some specific technology and, in addition, to either manufacture under licence or to act as a distribution agent for a foreign company. An example of this format is Onderstepoort Biological Products in South Africa.

b) A second category of partnership is one in which either a government department or a farmers' cooperative or association sets out to produce quality vaccines for its members and seeks an agreement with a foreign established vaccine manufacturing firm to provide a comprehensive technology transfer service that would include design, construction and procurement of the necessary equipment and engineering services, training of staff, commissioning of the new vaccine plant and a post-launch consultancy service for a limited period. Examples of this category include the Indian Dairy Development Corporation and VECOL in Colombia, both of which manufacture FMD vaccines.

c) A third category is one by which the recipient government sets up a vaccine trading company and then seeks a technology transfer arrangement with a reputable vaccine manufacturing firm. The arrangement will include an agreement for long-term technical management support, with options for periodic up-dating of the technology by the contracted firm. The Botswana Vaccine Institute is such an example.

Whatever format is selected, there must be guidelines which the technology purchasing government and the technology vendor need to take into account in order to ensure a successful and financially sustainable partnership (51).

It must also be stressed that the partnership may be involved in the manufacture of non-profitable vaccines. It is important that the government partner does not force the commercial partner to produce vaccine at a loss. The most appropriate point for the government to intervene is at the point of sale, i.e. to provide subsidies to the vaccine users (the livestock farming community) so that they can afford vaccines whose prices reflect the true cost of production.

Public/private partnerships for vaccine technology development

Traditionally, the discovery work for vaccines was often undertaken in governmental research establishments. Private industry picked up promising lines of research to develop them into a vaccine product. Foot and mouth disease vaccines provide a prime example of government/private industry development of vaccine technologies. The culture of bovine tongue tissues, the culture of FMD virus in suspended bovine tongue tissues, the inactivation of the virus with formalin and subsequent formulation into an immunogenic vaccine were all done in a government research establishment, in Amsterdam in the Netherlands (46). However, the scaling up of this technology to industrial proportions for the production of millions of doses of vaccines became possible when the technology was taken up by commercial enterprises in Europe and Argentina (7). Furthermore, modern FMD vaccines arise from the culture of the virus in BHK cells.

The technology evolution for this type of vaccine also followed the same pathway, i.e. discovery research being done in government establishments and process development in industry. Thus, the establishment of BHK cells as a continuous culture, the demonstration of their susceptibility to FMD virus, the adaptation of BHK cells to continuous culture in suspension, plus a reconfirmation of their susceptibility to FMD virus and the demonstration of first order kinetics inactivation of FMDV by aziridine compounds were all first discovered in governmental or inter-governmental research establishments (2, 14, 17, 18, 20, 92). The volume of FMD vaccine production is now larger than that of any other vaccine (human or veterinary), and this was achieved because government discoveries were developed and industrialised by private companies (initially Wellcome and later other commercial enterprises in Europe and South America [77]).

As already shown, the demand for conventional vaccines is likely to be predominantly in developing countries, especially for those diseases that are transboundary in nature and considered 'exotic' by most OECD countries. Controlling these epidemic diseases in their endemic area would decrease the risk of their extension. Therefore, there is likely to be a need for governments in developing or middle income countries to invest in and import vaccine technology development with a view to licensing such technologies to the private sector of public/private partnerships. Other tendencies are likely to be the so-called 'south-to-south' technology transfer (cooperation between developing countries with similar levels of development) and partnerships in vaccine production and licensing.

Recently, there has been a sharp drop in the amount of research being carried out on vaccines for tropical animal diseases, whether based on conventional or recombinant DNA technology. The net result is that there are no vaccines against such critical diseases to human and animal welfare as African swine fever, malignant catarrhal fever, trypanosomosis and other blood parasite diseases. Moreover, some of the available vaccines, such as those for CBPP, are far from optimal for the purpose. At the same time, there is only very limited international motivation to create the animal disease equivalent of the Global Research Forum for Vaccines. This alliance of governments, the private sector and international development agencies, which has been spearheaded by the World Health Organisation (WHO) since 1996, was established to coordinate research on otherwise non-commercially attractive vaccines for human infectious tropical diseases, including some zoonoses. It should be noted, nonetheless, that recent intiatives such as GALVmed (Global Alliance for Livestock Veterinary Medicines) or the European Technology Platform for Global Animal Health (ETPGAH) have been set up as global not-for-profit pharmaceutical outreach programmes funded through private/public partnership principles. The objectives of these initiatives are to improve access to pharmaceuticals, vaccines and diagnostic products and applied research.

Public/private partnerships for vaccine distribution and accessibility

Access to vaccines and other veterinary products for local communities of the developing world, initially under government control, has almost disappeared in most countries, often on account of dwindling resources, general deterioration of infrastructure and reforms such as the decentralisation of Veterinary Services and the cost-recovery approach. This problem is compounded by the cold chain requirement for most vaccines and the limited extension activities in sub-Saharan Africa and South Asia. Public/private partnerships could facilitate access to vaccines for rural livestock owners, either through the support of big corporations, or through funding that encourages the establishment of local distributors. The most notable example is the work of the National Dairy Development Board of India, which not only produces vaccines through its subsidiary, Indian Immunologicals, but also ensures wide distribution of vaccines, including FMD vaccine, to all members of the national dairy cooperative throughout India.

In sub-Saharan Africa some promising initiatives are beginning to emerge. For example, the initiative of the International Rural Poultry Centre, an Australian non-governmental organisation (NGO), working together with a mining company in Chibuto, Mozambique, is an example where the private interest, the mining company, through facilitation by an NGO, is assisting the government and the communities to control poultry diseases and distribute Newcastle Disease vaccine produced by the National Veterinary laboratory, thus creating a market for the latter. The establishment of local distribution networks is one of the approaches that is being developed by GALVmed to improve access to veterinary pharmaceuticals, including vaccines, in developing countries.

Conclusions

Several groups and authorities have proposed that the global management of high impact animal diseases is best tackled through programmes which focus on controlling diseases at source, i.e. in developing countries (39, 44, 83). The approach to disease control/management in developing countries is one that is likely to be driven by risk-based surveillance and risk management principles, whether this is for the progressive control of diseases identified at the national or regional level as of strategic

importance or whether it is for tactical intervention to halt or prevent the spread of infectious diseases beyond pre-determined acceptable levels of risk. Whether the objective is to solely to protect animal health or to protect human health via the control of zoonoses and food-borne infections, the dominant motivating factor for disease control is likely to be market access. Several approaches have been advocated to promote animal health management that enhance access to livestock commodities for developing countries, without undue risk of disease transmission. These have included bench-marking progress in animal health so that developing countries can progressively access market opportunities (local, regional to international) as their animal health status improves and in-country processing of animal products to reduce the risk of distant transmission of serious infections, particularly where the slaughter animals are sourced from low-disease-risk zones within exporting countries (41, 84, 100). The net effect is that there is likely to be an increasing demand for vaccines of assured efficacy and safety.

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Les vaccins vétérinaires et leur utilisation dans les pays en développement

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Résumé

Les maladies animales infectieuses, notamment celles qui affectent les animaux d'élevage constituent, encore aujourd'hui, une entrave au développement durable de l'agriculture, à la sécurité sanitaire des aliments et à l'accès des pays en développement et en transition aux bénéfices économiques générés par le commerce international des produits de l'élevage. Ces pays doivent donc prendre les mesures ciblées appropriées pour limiter les risques d'apparition de maladies infectieuses sur leur territoire. L'utilisation stratégique de vaccins vétérinaires de qualité peut et doit faire partie des programmes approuvés par les gouvernements. Les campagnes de vaccination doivent s'inscrire dans le cadre de programmes planifiés de lutte contre les maladies ; s'agissant de maladies animales transfrontalières, ces programmes ne réussiront que s'ils ont une envergure régionale. En centrant leur propos sur les principales maladies animales transfrontalières, les auteurs décrivent l'utilisation actuelle des vaccins, les travaux de recherche prometteurs et les technologies innovantes qui pourront être appliquées dans les pays appartenant aux grandes régions en développement de la planète. Ils étudient également le rôle des partenariats public/privé dans ce domaine.

Mots-clés

Biotechnologie – Brucellose – Fièvre aphteuse – Fièvre catarrhale du mouton – Fièvre charbonneuse – Fièvre de la Vallée du Rift – Mycoplasme – Pleuropneumonie contagieuse bovine – Vaccin – Vaccination.

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Las vacunas veterinarias y su utilización en los países en desarrollo

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Resumen

Las enfermedades infecciosas del ganado y otros animales siguen constituyendo un pesado lastre para lograr la seguridad alimentaria y un desarrollo agrícola duradero, y para que los países en desarrollo o en transición participen de los beneficios económicos que reporta el comercio internacional de productos ganaderos. En dichos países deben aplicarse medidas destinadas específicamente a reducir la aparición de enfermedades infecciosas. Empleadas de modo estratégico, las vacunas veterinarias de calidad pueden y deben formar parte de programas avalados por el gobierno de cada país. Las campañas de vacunación deben estar inscritas en programas generales de control zoosanitario, cuyo éxito, en el caso de enfermedades animales transfronterizas, exige trabajar a escala regional. Los autores, centrándose en determinadas enfermedades animales transfronterizas de especial relevancia, pasan revista al uso actual de las vacunas, así como a las investigaciones prometedoras en la materia, a tecnologías novedosas que pueden aplicarse en importantes regiones en desarrollo del mundo y a la función de las alianzas publico-privadas.

Palabras clave

Biotecnología – Brucelosis – Carbunco bacteridiano – Fiebre aftosa – Fiebre del Valle del Rift – Lengua azul – Micoplasma – Perineumonía contagiosa bovina – Vacunación – Vacuna.

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Vaccines for emerging infections

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Summary

Emerging infectious diseases represent a grave threat to animal and human populations in terms of their impact on global health, agriculture and the economy. Vaccines developed for emerging infections in animals can protect animal health and prevent transmission of zoonotic diseases to humans. Examples in this paper illustrate how industry and public health can collaborate to develop a vaccine to prevent an emerging disease in horses (West Nile virus vaccine), how poultry vaccination can protect animals and prevent transmission to people (avian influenza vaccine), how regulatory changes can pave the way for vaccines that will control the carrier state in animals and thus prevent infection in humans (*Bartonella henselae* vaccine in cats) and how novel technologies could be applied to vaccinate wildlife reservoir species for rabies. Stemming from the realisation that zoonotic diseases are the predominant source of human emerging infectious diseases, it behoves academic, public health, and animal health agencies to consider creative constructive approaches to combat serious public health challenges. Vaccination of vector/reservoir species, when efficacious vaccines are available, offers significant advantages to combating zoonotic human disease.

Keywords

Animal vaccination – Avian influenza vaccine – Emerging infection – Public health – Rabies vaccine in wildlife – West Nile virus vaccine – Zoonotic disease.

Introduction

In its 1992 report 'Emerging infections: microbial threats to health in the United States', the Institute of Medicine (IOM) in the United States of America (USA) defined the causes of emergence as follows: 'emergence may be due to the introduction of a new agent, or the reappearance of a known disease after a decline in incidence, recognition of an existing disease that has gone undetected, or to a change in the environment that provides an epidemiologic "bridge"'(31). In its follow-on report in 2003, the IOM's Convergence Model described factors leading to the emergence of an infectious disease as 'the combination of biological, environmental and host-related risk factors that create the opportunity for microbial pathogens to wreak havoc on their human and animal hosts' and as a 'microbial perfect storm' that is, 'a tempest that may happen only once in a century, created by so rare a combination of factors that it could not possibly have been worse' (32).

Many factors contribute to the emergence of infectious diseases. Increasing human populations have multiplied the global demand for animal protein as a food source. As larger numbers of animals are reared in situations of high confinement, as is often done in modern methods of food production, crowding and stress on the animals can increase the likelihood of bacterial and viral shedding of zoonotic pathogens (e.g. *Salmonella* in cattle and swine). The increase in demand for animal protein also leads to methods of animal rearing that preclude the proper biosecurity precautions needed to prevent introduction of pathogens that have health significance for both animals and humans (e.g. H5N1 avian influenza in free-ranging

ducks and geese in Southeast Asia). Our changing global climate leads to extended periods of flooding and drought, which impact the vectors of transmission (e.g. the floodwater *Aedes* spp. of mosquitoes which serve as the vector of Rift Valley fever virus to animals). International travel can transport infected animals, infected humans and vectors much farther and more quickly (e.g. this may be the explanation for the emergence of West Nile virus [WNV] in North America in 1999). War and political unrest destroy the fabric of society and disrupt governmental infrastructures that serve to protect animal health. Encroachment by humans and agricultural species into wildlife habitats increases the likelihood that all three types of species could be infected with novel pathogens. The microbes themselves are very adept at finding ways to perpetuate themselves and bypass the animal immune system to infect animal cells.

Outbreaks of infectious diseases in animals come at great cost to society. In 2001, the foot and mouth disease outbreak in the United Kingdom (UK) led to the depopulation of 4.2 billion head of cattle, sheep, pigs, and goats at an estimated cost to the UK economy of over £6 billion (70). An inestimable cost was the mental stress to producers, their families, and their communities caused by the mass slaughter of healthy animals to control this outbreak. In light of the UK experience, the impact of consumers' attitudes towards euthanasia of healthy animals as opposed to animal-sparing approaches to outbreak control may impact future policy.

Therefore, the concept of animal vaccination to prevent emerging infections becomes of key importance. However, when a vaccine is deployed as a control measure for emerging infections, unique considerations must be given to its use. Will the vaccine be used only in an emergency? Is it fully licensed for use, or are special authorisations required for its use? What storage conditions are required to maintain the product safely in the field setting? Is it stored fully prepared or does it need to be reconstituted? What is the goal of vaccination – to prevent infection or disease? If vaccine is to be used in an emergency, does it have sufficiently early onset of protection to warrant its use? Are several doses required to establish sufficient immunity to prevent infection? Are sufficient quantities of vaccine available to vaccinate the entire herd or flock? Is it necessary to vaccinate the entire herd or flock to produce adequate immunity to interrupt transmission? What is the impact on human health, if the pathogen is zoonotic? Does the vaccine reduce pathogen shedding sufficiently to prevent morbidity and mortality in the animal, but still allow low levels of pathogen excretion, sufficient for humans in contact with the animals to be at risk of infection? What is the public opinion of vaccination as compared to their view of alternative control measures such as depopulation? Is there a marker in the vaccine such that a diagnostic test can differentiate vaccinated from infected animals to permit trade? The lack of ability to distinguish between vaccinated and infected animals has historically been perceived as a barrier to the use of vaccination and should be continually reevaluated through scientific research and policy development.

The existence of animal mediators of zoonotic disease (both as vectors or reservoirs) offers significant potential for the development of control strategies for zoonotic disease that are not necessarily available for the control of disease transmitted directly from human to human or directly from the 'environment' to humans. Vaccination for emerging infections in animals is an important potential tool for control of zoonotic disease and may provide an alternative to much more drastic control measures, such as depopulation. In addition to prevention of human disease, vaccination of reservoir species may also protect important nonhuman species from disease as well.

In the following case studies, we chose four examples of emerging infections in companion animals, domestic poultry, and wildlife to highlight the important role that vaccination can play in disease prevention in the animal host or reservoir, and how these vaccines directly or indirectly impact prevention or control of emerging infectious diseases in humans.

Case study 1: companion animals

Vaccination of animals to protect animal health: West Nile virus vaccine in horses

West Nile virus (WNV), a flavivirus related to Japanese encephalitis, St. Louis encephalitis and Murray Valley encephalitis viruses, was responsible for outbreaks of encephalomyelitis in humans and horses in New York in 1999 (13). West Nile virus had previously been identified as a cause of infection and encephalomyelitis in horses in Egypt and France in the early 1960s (53, 63). Risk factors that may have contributed to the emergence of WNV in North America included international transportation of the infected mosquito vector, bird or human, and a climate favourable to the maintenance of the mosquito vector in nature. Thus, WNV is now considered to be endemic in North America.

Mosquito vectors become infected with WNV by feeding on infected wild birds. Occasionally infected mosquitoes, when biting to consume blood, can transmit the virus to people and horses. Humans and horses are thought to be incidental hosts, that is, once infected they cannot be a source of infection for mosquitoes or other animals. The only vector proven to be involved in transmission to birds

and humans is the mosquito. Scientists have identified at least 59 mosquito species infected with WNV, but species from the genus *Culex*, including the common house mosquito *Cx. pipiens*, seem to be the most common carriers (30).

Clinical signs of WNV in horses are associated with central and/or peripheral nervous system dysfunction. Most horses exhibit secondary central nervous system-derived neurologic manifestations such as ataxia, including stumbling, staggering, wobbly gait or incoordination, or at least two of the following: circling, hind limb weakness, inability to stand, multiple limb paralysis, muscle fasciculation, proprioceptive deficits, altered mental status, blindness, lip droop/paralysis, and teeth grinding. Fever is not a consistent finding (52).

Since the emergence of WNV in the USA, there has been concern among the scientific community about whether horses could serve as amplifying hosts for WNV, exacerbating the challenges faced by public health and veterinary sectors in controlling its spread. In a study conducted in 2002, Bunning *et al.* demonstrated that horses experimentally infected with WNV strains known to be pathogenic for birds and humans developed low levels of viraemia of short duration. Furthermore, mosquitoes that fed upon experimentally infected horses were negative for the virus, leading investigators to the conclusion that horses were unlikely to serve as important amplifying hosts for WNV in nature (7).

However, the impact on equine health has been profound. From 1999 to 2005, the United States Department of Agriculture (USDA) Animal and Plant Health Inspection Service (APHIS) National Animal Health Surveillance System reported WNV cases in horses as follows: 1999, 25 cases; 2000, 66 cases; 2001, 738 cases; 2002, 15,257 cases; 2003, 5,181 cases; 2004, 1,406 cases and 2005, 1,075 cases (73). Among the 15,257 equine cases in horses reported in 2002, approximately 33% died from the infection (72).

The high mortality rate observed in horses formed the impetus for vaccine development. Fort Dodge Animal Health manufactured the first WNV vaccine in the USA, produced from a killed virus. Because of the emerging nature of WNV and its impact on horses, in an effort to find an intervention for this devastating disease, field safety trials of the killed vaccine were conducted among approximately 650 horses in eight states. Preliminary product purity and field safety data submitted to the USDA Center for Veterinary Biologics (CVB) led to the issuance of a conditional license in 2001. Subsequent efficacy data yielded from experimental challenge studies led to the issuance of full licensure of the killed vaccine in 2003. In addition to the inactivated monovalent product, three other WNV vaccines are licensed by CVB: a live canarypox virus-vectored vaccine manufactured by Merial and licensed in 2003; a deoxyribonucleic acid (DNA) vaccine developed in collaboration with the US Centers for Disease Control and Prevention (CDC) and manufactured by Fort Dodge Animal Health, licensed in 2005 (further described below), and a live flavivirus chimeric vaccine manufactured by Intervet and licensed in 2006 (personal communication, M.B. Evans, USDA).

In 2001 in collaboration with Fort Dodge Animal Health, CDC began efforts to develop the world's first DNA WNV vaccine for horses (19). DNA vaccines use specific fragments of a pathogen's unique genetic material to stimulate a targeted immune response from the host, unlike traditional inactivated vaccine development that involves killing the virus or bacteria in such a manner that allows the pathogen to produce immunity but no disease (via replication) in the recipient. Studies to determine duration of immunity conferred by vaccination are ongoing. The vaccine label contains a caution that vaccinated horses may not be eligible for export, as current commercially used tests may not be able to differentiate among the DNA vaccine, conventional vaccines, and horses that have been exposed to the actual virus.

In areas of high vector activity, control of WNV in horses can present a challenge to their owners and veterinarians. A research study of immunologic responses in horses vaccinated with the killed vaccine demonstrated that a portion of horses may respond poorly to the vaccine and that WNV antibody titres conferred after two doses of vaccine have been administered can decline to low levels within 5 to 7 months, leading to the recommendation that vaccination every 6 months may be indicated (18).

Although not specifically recommended by the vaccine manufacturers, the American Association of Equine Practitioners has suggested that horses that are stressed, such as show and race horses, should have two boosters annually, in April and late July. Owners should recognise that horses vaccinated against Eastern, Western, and Venezuelan equine encephalitis are not protected against WNV. Control measures for WNV other than vaccination include reduction of mosquito vectors through use of topical repellents approved for horses and removal of standing water and old tyres from areas proximal to where horses are kept (3).

West Nile virus continues to emerge in horses in new parts of the world. In 2003, WNV outbreaks were reported in horses in Mexico and Morocco (44, 64). This case study is an example of the use of new technological approaches to devising useful interventions for the prevention of disease in humans; it also demonstrates how rapidly the research community and industry can respond to an emerging

infection, identify needs and collaborate to discover, evaluate and continually improve an intervention for an emerging infectious disease in animals. The DNA technology used to develop the equine WNV vaccine is serving as the foundation for an experimental human WNV vaccine (11).

Case study 2: domestic poultry

Vaccination of the animal reservoir to prevent disease in the animal, prevent slaughter of the animal, and reduce likelihood of transmission to people: avian influenza vaccine

Perhaps the best example of an emerging infectious disease affecting animals and humans today is the epizootic of highly pathogenic avian influenza A H5N1 (HPAI H5N1) that began in 1997 (21). Outbreaks of HPAI H5N1 have spread through three continents at an unprecedented rate, resulting in devastating health and economic consequences. From 1999 to 2003, poultry outbreak control measures in the European Union alone resulted in the depopulation of 50 million birds, at a significant cost to the global economy (9). From 18 March 2004 to 16 October 2006 the world has witnessed the occurrence of 256 laboratory confirmed human cases of HPAI with 151 fatalities associated with the epizootic of H5N1 in birds (82). In almost all the human cases, the primary risk factor for infection was close contact with infected domestic poultry or poultry products.

In the case of HPAI H5N1, the circumstances have indeed combined to create the perfect microbial storm. Agricultural practices, increasing human population with an associated increase in demand for animal poultry as a nutrient source, legal and illegal movement and transport of poultry, and risk behaviours of humans in their interaction with birds have all contributed to the emergence of a complex eco-epidemiologic picture that will not disappear in the near future. It is imperative that the veterinary sector seek opportunities to control HPAI in the animal reservoir, both to protect agriculture and peoples' livelihoods, but even more importantly to prevent the onset of a pandemic that could occur once the virus acquires the ability to transmit in a sustained and efficient manner from person to person.

Much effort has been placed into addressing control of HPAI H5N1 in the animal reservoir through the combined efforts of organisations such as the World Organisation for Animal Health (OIE), Food and Agriculture Organization (FAO), and World Health Organization (WHO). These organisations have jointly sponsored several international meetings to address this problem (24, 25, 26).

Each of these meetings highlighted the importance of poultry vaccination as an effective control measure. Information on the types of avian influenza (AI) vaccines that are available for use in poultry and on the technological advancements for vaccines developed for compatibility with laboratory diagnostic systems that can distinguish between vaccinated and infected poultry to allow trade is beyond the scope of this particular article and is discussed elsewhere in this volume.

However, a brief review of the advantages and limitations of poultry vaccination merits discussion, so the reader will appreciate the factors that should be taken into consideration in the decision to use vaccination as part of the containment strategy for the control of HPAI H5N1. Vaccination can play an important role in protecting poultry from development of clinical illness and from mortality, as well as reducing virus shedding and increasing resistance to infection. Vaccination is useful in situations where there is risk of major disease spread and where stamping out is not a viable option (i.e. in countries where the depopulation of birds will deprive the community of an important protein source). Vaccination strategies that include distinguishing infected from uninfected, vaccinated birds (DIVA) are critically important for surveillance, to show that disease control programmes are working. DIVA also represents a major advantage to a country's economy, since it can remove barriers to trade caused by restrictions on movement and sale of birds if they can be demonstrated to be free of infection (10).

In an intercountry consultation in Manila in 2005, experts from WHO observed that vaccination, if properly used, can have a positive impact on human health as well. It was noted that in places in Southeast Asia where infection in poultry has been controlled or eliminated, human cases no longer occurred. The WHO acknowledged that prevention of HPAI H5N1 avian influenza in humans is best achieved by controlling infection in poultry. The WHO supported FAO and OIE recommendations that control strategies for HPAI H5N1 should consider vaccination of poultry (81).

However, vaccination does have limitations that should also be considered. Even with vaccination, the virus is still able to replicate in clinically healthy vaccinated birds (10). This reduces the likelihood that disease eradication will be achieved and can elevate the risk to human health by leading to increased antigenic pressure for virus mutation in the poultry population. A recent publication by Savill *et al.* modelled impact of vaccination on silent spread of HPAI H5N1 between poultry flocks (62). The authors determined that 90% of birds needed to be successfully vaccinated to reduce the probability of an outbreak by 50%, but this could result in undetected outbreaks in birds. As the proportion of birds vaccinated rose, fewer

birds became infected but outbreaks became harder to detect. The use of sentinel birds could increase the likelihood of detection of outbreaks, especially at the end of a production cycle.

In the Savill model, elements of a successful vaccination programme for HPAI H5N1 include an effective vaccine, a high proportion of birds vaccinated, use of unvaccinated sentinel birds to ensure rapid detection of virus, good biosecurity practices and rapid removal of infected flocks once infection is detected. In the overall scheme of control of HPAI, vaccination should not be used as the sole control measure but as part of the overall control strategy. It should be carefully managed to ensure protection of poultry and human health.

Case study 3: companion animals

Vaccination of the carrier state in animals to prevent transmission to people: *Bartonella henselae* vaccine in cats

In March 2005, the CVB, in response to requests from industry, came to an agreement with the Department of Health and Human Services Food and Drug Administration in the USA to assume jurisdiction for vaccines intended to control the carrier state in animals. Notice No. 05-07 of the CVB, 'Biologics for Reduction of Colonisation and/or Shedding in Animals' informed stakeholders that USDA had changed its policy to permit licensing of veterinary biological products that claim to reduce colonisation or shedding of pathogens that may not cause significant clinical disease in animals, but may cause the animal to be a disease carrier (12).

The Notice stated that the jurisdiction for animal vaccines targeted at the reduction or elimination of a carrier state of organisms would lie with APHIS as long as certain criteria were met. Those criteria included the following:

– products must be indicated for administration to animals only and must act primarily through the direct stimulation of the immune system

– label claims must contain statements supported by data to show reduction of colonisation or shedding in the animal; no food safety or human health claims can be made

– products are required to show significant and clinically relevant efficacy as defined by APHIS

– products must demonstrate the ability to cause a substantial decrease in number of animals colonised and/or numbers of organisms shed by vaccinated animals.

The significance of this change should foster development and licensure of vaccines that will provide a novel approach for controlling significant zoonotic public health problems, for example, *Bartonella henselae* infections (cat-scratch disease).

An estimated 22,000 cases of cat-scratch disease (CSD) occur annually in the USA, ranking CSD as one of the most common zoonotic, non-foodborne infectious diseases and the leading cause of subacute unilateral adenopathy of children (83). *Bartonella* spp. infections are now recognised to be associated with several distinct clinical syndromes in people, including bacillary angiomatosis, bacillary peliosis, relapsing fever with bacteraemia, endocarditis, granulomatous hepatosplenic syndrome, retinitis and swelling of the optic nerve, arthritis, osteolytic lesions, and pulmonary granulomas (38). *Bartonella henselae* is frequently implicated in the onset of otherwise unexplained encephalopathy, including AIDS-associated encephalopathy (36, 37). Cats can be asymptomatically bacteraemic for many weeks and develop detectable antibodies concurrently with bacteraemia (57, 58). The seroprevalence of *B. henselae* in cats varies throughout the USA and appears to be influenced by climate, with some areas showing in excess of 50% of cats having had prior infections (33). The recognition that human CSD is epidemiologically correlated with having been scratched by a cat predated the recognition of the causative agent by 50 years. Cat fleas are believed to play an important role in the transmission of *B. henselae* between cats (14, 33).

Because of the zoonotic origin of human CSD infections and the evidence that indicates that *B. henselae* infections are quite common in pet cats, vaccination of feline reservoirs of disease offers the opportunity to interrupt disease transmission and make a positive impact on public health, without negatively impacting the status of cats as companion animals. Both CDC and industry data show that vaccination can prevent *B. henselae* bacteraemia in cats challenged with infectious *B. henselae*. Future potential feline vaccines should therefore reduce the subsequent risk of transmission of *B. henselae* to humans, which is especially important among immunocompromised individuals.

Practical considerations may inhibit vaccine development and use for vector/reservoir species. These considerations might include:

– uncertain commercial profitability after the considerable costs for product development and licensure

– avoidance of vaccines for companion animals that produce unwanted side effects (e.g. use of adjuvants that might induce local reactions)

– competing commercial products that may reduce the perceived need for vaccination (e.g. effective flea treatment for cats).

In addition, if immunologically distinct microbes are responsible for what is clinically recognised as a single syndrome, either polyvalent vaccines may be required to prevent complete protection from multiple agents, or the public must be educated to understand that a vaccine based on a single agent, however efficacious for a target organism, may not prevent all disease associated with a clinically defined syndrome.

Case study 4: wildlife

Vaccination of reservoirs to prevent disease in other hosts: lyssaviruses and other emerging infections

The existence of known or suspected animal reservoirs handicaps disease eradication efforts, especially related to emerging human and animal pathogens (17). The major recent human medical successes in defeating smallpox, polio, measles, mumps, and most common paediatric diseases may owe much of their success to the fact that eradication efforts are unhampered by complications related directly to vector-borne or zoonotic issues (54). Historically, combinations of quarantine and importation policies, diagnostic test-and-slaughter programmes, proper use of antibiotics and parasiticides, and rational vaccine administration have been effective veterinary management practices.

These techniques are especially amenable to domestic species for the interruption of infectious disease cycles, as demonstrable in foot and mouth disease, hog cholera, brucellosis, tuberculosis, trichinosis, and many other diseases throughout the world (27, 65, 71, 74). However, strategies such as vaccination are often impractical for direct application to free-ranging wildlife because they are impacted by a multiple host-agent complex (22, 56, 80).

Although wildlife may pose substantial hurdles to disease eradication, concerted, multidisciplinary, international efforts have been responding gradually to this challenge. Perhaps one of the best modern paradigms concerning wildife vaccination is also one of the oldest recognised zoonoses. Rabies is an acute, progressive encephalitis caused by ribonucleic acid (RNA) viruses in the family *Rhabdoviridae*, genus *Lyssavirus* (50). All mammals are believed to be susceptible. Nevertheless, members of the Carnivora, especially domestic dogs, represent the major global public health burden, with tens of thousands of humans dying annually and millions of people bitten by suspect animals each year, primarily in developing countries (35). Oddly, the historical application of the original human vaccination experiments performed by Pasteur and his colleagues at the end of the 19th century were rather slow in practical extension to animals (76). In the 1920s, Japan became the first country to successfully apply mass rabies vaccination to domestic dogs. More routine veterinary use of rabies vaccination ensued, especially after World War II, and canine rabies control had progressed throughout developed countries by the mid-20th century. The concept and success of wildlife vaccination was borne out by several factors: the realisation that canine rabies could be eliminated by achieving herd immunity; the appreciation of the role of wild mammals such as foxes, raccoons, and other carnivores in dissemination of rabies; the recognition that oral vaccine administration is effective as a means of delivery; and continued progress in development of safe and effective biologicals and attractive baits (5). Beginning in the late 1970s, the tactical field application of rabies vaccine-laden baits over substantial regions in Europe and North America has led to the significant control, and in some cases selective elimination, of the disease among wild mammalian carnivores (15, 45, 67). In addition to self-replicating modified-live and recombinant viruses for oral use, inactivated rabies vaccines for parenteral administration have been used in trap-vaccinate-release programmes, particularly in urban areas (59). Such enterprises could be extended to other terrestrial mammals and diseases.

While the successes realised by wild carnivore vaccination against rabies have provided an important adjunct to traditional veterinary control techniques focused on domestic animals, any true disease elimination may be overshadowed in part by other relevant major reservoirs. For example, members of the Chiroptera represent an important source of rabies throughout the Americas, and a reservoir for emerging lyssaviruses in Africa, Australia, and Eurasia, some with considerable antigenic variation from conventional rabies vaccines (29, 39, 43, 50, 75). Moreover, bats are implicated in a number of other emerging viral diseases, including Ebola, severe acute respiratory syndrome (SARS), and Henipah virus infections (6, 8, 41, 42, 46, 48, 51). Could bat vaccination be considered for disease control and prevention, as implemented for wildlife rabies control in carnivores?

While this suggestion is intriguing, several barriers exist to immediate utilisation of similar vaccination strategies for bats. Whereas only a single species each of fox (*Vulpes vulpes*) and raccoon (*Procyon lotor*) are involved significantly for rabies in western Europe and eastern North America, respectively, bat species are highly diverse. As opposed to fewer than 300 described carnivore species (and few new additions), more than a thousand bat species are described, and nearly 50 new taxa have been suggested (77). Additionally, the relative comparative abundance of bats is high, with some local colonies estimated to consist of thousands to millions of individuals. Because bats are not terrestrial and can migrate over long distances, their dispersal ability creates challenges for vaccine

administration (16). Unlike the situation for domestic or wild carnivores, there are no currently licensed biologicals for the Chiroptera (49).

Given these difficulties, at first inspection, those searching for solutions to bat-related disease issues should perhaps initially consider other available prevention strategies (23). Viable approaches include public education to avoid direct contact with such wildlife; use of existing medical interventions if exposures occur; when available, pre-exposure vaccinations if warranted, and management techniques to minimise bat-human-veterinary conflicts. Traditional practices such as population reduction are not indicated because the extremely low turnover of bat populations may lower the population to a level from which it cannot recover. For example, vampire bats, which prey upon livestock and humans from Mexico to Argentina, have been targeted by specific applications of anti-coagulants, which exploit their hematophagous and social grooming activities (4). However, despite this particular scenario, significant other points related to efficacy, economics, ecology, and ethics argue against lethal means of disease control (61). Moreover, while vampire bat control may be perceived as useful, species eradication is not, because these bats are not only unique biologically, but can offer otherwise unrealised biomedical benefits (28). Today, in nearly all indications, lethal control of bat populations is not espoused by public health nor agricultural agencies in the USA (49). Thus, management techniques for bat-related diseases range from doing nothing, based upon risk assessment and practicality, to focusing only upon humans or affected domestic species, in an attempt to interrupt infectious chains of transmission.

Alternatively, some useful experience with bat vaccination already exists. Vaccination is a consideration because some bats that are brought into captivity for exhibition, applied research or conservation purposes may be incubating disease (66). As with other mammals, bats will respond to inactivated vaccines by parenteral administration (55). Commercial rabies biologicals have been applied to bats, not only for pre-exposure use, but also during outbreaks in captive colonies, in which CDC assistance was requested and provided in both public zoo and private research settings. Intramuscular or subcutaneous vaccination of bats may be quite safe in captivity, but is largely impractical for free-ranging animals.

Other techniques will be needed for field applications of vaccines to bats. In very large bat aggregations, such as in maternity colonies, it has been shown that the aerosol route of infection may be effective (16). This aerosol delivery system may be useful for the potential application of vaccines in bats (34, 78, 79). Oral recombinant vaccines have been tried in captivity for vampire bats (1). Application to one individual of a colony may be spread to others via social grooming (2). For remote delivery, extended baiting may be envisioned, e.g. by adding vaccine to flavored liquids (as done for certain frugivorous or nectivorous bats), by including vaccine-laden bait in backyard feeders (as done for hummingbirds on a small scale), or by developing plant-based vaccines for consumption (69). As to potential vaccine candidates, given the revolution in reverse genetics, rabies virus itself can be used as an expression vector, for incorporation of foreign genes (20). Other vaccines 'on the wing' could be constructed for selected insectivorous bat species, by the creation of transgenic insects, expressing in appropriate context the immunogen of interest, such as the rabies virus glycoprotein (60). Extrapolating from the concept of both remote delivery and natural hypodermics, bat ectoparasites may be designed to harbour and administer vaccine vectors of interest. One area for potential focus is rhabdoviruses, as many are shared between invertebrates and vertebrates; one could imagine reverse-engineered rhabdovirus vaccines opening new arenas for discovery (40).

Conclusions

In this article, we have explored vaccination of the animal host or reservoir to protect animals against emerging diseases and how such vaccination can serve as a barrier to protect human health. We have reviewed the development of vaccines for a few emerging infections based on established and new technologies and examined their advantages and limitations; we have also looked at the concept of 'altruistic' vaccination of animals for emerging diseases which may not cause ill effect in the animal but which have an adverse effect on human health. Stemming from the realisation that zoonotic diseases are the predominant source of human emerging infectious diseases, it behoves academic, public health, and animal health agencies to consider creative constructive approaches to combat serious public health challenges (68). Veterinary vaccination remains a significant option for zoonotic disease control. Vaccination of vector/reservoir species, when efficacious vaccines are available (e.g. the currently licensed rabies vaccine), offers significant advantages for combating zoonotic human disease.

The concept of zoonotic vaccines is relevant not only for *B. henselae*, but also for other zoonotic pathogens of public health concern. Most notably, studies on the origins of food pathogens have identified that carriage of *Escherichia coli* O157:H7, *Salmonella enterica* and *Campylobacter jejuni* in food ruminants and poultry, as well as pet animals, is relatively common and serves as the ultimate source for human illness from these pathogens. These infections are a huge public health burden, estimated to be responsible for millions of human illnesses and hundreds of deaths each

year in the USA (47). Though much progress has been made in limiting the food contamination during processing through programmes such as hazard analysis and critical control point (HACCP) systems, control will also need to be applied at the farm level. Vaccines, probiotics and other biologics aimed at eliminating or limiting carriage of human pathogens in food animals are central to the next generation of zoonotic foodborne disease control programmes.

We have explored the exciting possibilities of vaccination of the wildlife reservoir for rabies, which ultimately could hold the key to prevention of other emerging diseases such as Ebola, SARS, and Henipah virus. However, for any practical application of the vaccine concept to bats for infectious disease control, multiple research needs abound. Intensive studies into the applied ecology, population biology and sociobiology of selected bat species are a prerequisite. Renewed pathogen discovery, community modelling, and host-agent dynamics must be appreciated. Basic investigations are needed to understand the relationship between bat physiology and immune response, akin to the investigations that have already been carried out for other mammals. There is a clear need for development of specific biotechnological applications and methodology relevant to free-ranging bats. Such suggestions are not mere idle academic speculations, especially if one considers the alternative consequences of a lack of innovation, the pressures for an integrated response after the eventual establishment of a crisis, and the timeline for practical research and development within the modern regulatory framework. All must be balanced against the substantive public health, veterinary, and economic benefits that have been achieved to date from the oral rabies vaccination of wild carnivores, from original concept to tangible reality.

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Les vaccins contre les maladies infectieuses émergentes

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Les résultats et les conclusions exprimés dans cet article sont ceux des auteurs et ne reflètent pas nécessairement la position du Centers for Disease Control and Prevention.

Résumé

Les maladies infectieuses émergentes font peser une grave menace sur les populations humaines et animales, du fait de leur impact sur la santé mondiale, sur l'agriculture et sur l'économie. Les vaccins vétérinaires mis au point pour lutter contre ces maladies permettent de protéger la santé animale et d'empêcher la transmission à l'homme des maladies zoonotiques. A travers plusieurs exemples, cet article montre successivement comment la coopération entre la santé publique et le secteur pharmaceutique a permis de mettre au point un vaccin contre une maladie émergente des équidés (vaccin contre la fièvre du Nil occidental), comment la vaccination des volailles protège les animaux et prévient la transmission du virus à l'homme (vaccin contre l'influenza aviaire), comment les nouvelles réglementations ouvrent la voie à des vaccins qui contrôleront l'état de porteur des animaux et permettront ainsi de prévenir l'infection chez l'homme (vaccins contre *Bartonella henselae* chez le chat), et enfin comment des technologies innovantes permettent de vacciner des espèces sauvages servant de réservoir animal au virus de la rage. Sachant que les maladies zoonotiques sont la principale source des maladies infectieuses émergentes chez l'homme, il incombe aux institutions de recherche et aux instances de santé publique et de santé animale de concevoir des approches

créatives et constructives afin de lutter contre ces défis majeurs pour la santé publique. Lorsque des vaccins efficaces sont disponibles, la vaccination des vecteurs et des espèces qui servent de réservoir offre des avantages non négligeables pour lutter contre les zoonoses transmissibles à l'homme.

Mots-clés
Maladie infectieuse émergente – Santé publique – Vaccin antirabique pour les animaux sauvages – Vaccin contre l'influenza aviaire – Vaccin contre la fièvre du Nil occidental – Vaccination des animaux – Zoonose.

Vacunas contra infecciones emergentes

N. Marano, C. Rupprecht & R. Regnery

Los resultados y conclusiones expuestos en este artículo son responsabilidad de los autores y no corresponden necesariamente a los puntos de vista de los Centros de Control y Prevención de Enfermedades.

Resumen
Por sus efectos sobre la salud en el mundo, la agricultura y la economía, las enfermedades infecciosas emergentes representan una grave amenaza para las poblaciones tanto animales como humanas. Las vacunas fabricadas para luchar contra esas dolencias en los animales pueden no sólo proteger la salud de éstos, sino también prevenir la transmisión al ser humano de enfermedades zoonóticas. Valiéndose de una serie de ejemplos, los autores explican cómo la industria y la salud pública pueden trabajar concertadamente para obtener una vacuna capaz de prevenir una enfermedad emergente en el caballo (la provocada por el virus West Nile), cómo la vacunación de aves de corral puede proteger a los animales y prevenir el contagio de personas (en el caso de la influenza aviar), cómo los cambios reglamentarios pueden preparar el terreno para vacunas que permitan controlar a los animales portadores y prevenir así la transmisión al ser humano (vacunación de gatos contra *Bartonella henselae*) y cómo las nuevas tecnologías podrían aplicarse a la vacunación de especies salvajes que actúan como reservorio de la rabia. Una vez ha quedado claro que las enfermedades zoonóticas son la fuente básica de infecciones emergentes en el hombre, corresponde a los expertos y organismos de salud pública y sanidad animal encontrar soluciones creativas y constructivas para combatir estas graves amenazas que pesan sobre la salud pública. Cuando existen vacunas eficaces, su administración a las especies que sirven de vector o reservorio presenta considerables ventajas a la hora de combatir enfermedades zoonóticas.

Palabras clave
Enfermedad zoonótica – Infección emergente – Salud pública – Vacuna de la influenza aviar – Vacuna de la rabia en animales salvajes – Vacuna contra el virus West Nile – Vacunación animal.

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Vaccination for notifiable avian influenza in poultry

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Summary
Notifiable avian influenza (NAI) is a listed disease of the World Organisation for Animal Health (OIE) that has become a disease of great importance both for animal and human health. Prior to 2000, vaccination against NAI was discouraged and used to aid control of only a limited number of outbreaks, without reaching the goal of eradication. Pivotal work on the application of a vaccination programme aimed at, and resulting in, eradication was carried out in Italy, and was followed by other research, e.g. in Hong Kong and the United States of America. Given the spread of Asian lineage highly pathogenic avian influenza (HPAI) H5N1 to three continents, vaccination is now being used on a wide scale under different conditions, which in most cases are not ideal. Although in some countries, a lack of infrastructure and resources can greatly limit the overall success of control programmes that encompass vaccination, it is imperative that international organisations set guidelines to 'accredit' control strategies. These guidelines should include recommendations on seed strains to be used in vaccine preparations, the characteristics of the vaccine, the most appropriate field strategy to apply in the different phases of a control/eradication programme, and models of exit strategies. The availability of harmonised protocols would greatly facilitate the achievement of tangible results and would save time and avoid unnecessary wastage of resources.

Keywords
Avian influenza – Biosecurity – Control – Epidemiology – Eradication – Monitoring – Stamping out – Vaccination.

Introduction

Avian influenza (AI) is one of the greatest public health concerns to have emerged from the animal reservoir in recent times. Over the past five years there has been a sharp increase in the number of outbreaks of AI in poultry. It has been calculated that the impact of AI on the poultry industry has increased 100-fold, with 23 million birds affected in the forty year period between 1959 and 1998 and over 200 million from 1999 to 2006 (2). In fact, from the late 1990s, AI infections have assumed a completely different profile both in the veterinary and medical scientific communities. In recent times some outbreaks have continued to be of only minor relevance while others, such as the ongoing Eurasian-African H5N1 epidemic and outbreaks that occurred in Italy (1999-2000), the Netherlands (2003) and Canada (2004) have led to devastating consequences for the poultry industry, negative repercussions on public opinion and in some cases have created significant human health issues, including the risk of generating a new pandemic virus for humans via the avian-human link.

The increased relevance of AI in the fields of animal and human health has highlighted the lack of scientific

information on several aspects of the disease. This has hampered the adequate management of some of the recent crises, thus resulting in millions of dead animals and concern over loss of human lives and over management of the pandemic potential.

Before 1999, AI did not have a high-profile, so the information and the specific tools necessary to manage AI epidemics adequately were not developed. Moreover, until recent times vaccination for avian influenza in its notifiable form had always been discouraged. As a result of this, studies and investments in the field of AI vaccinology have not been a priority in the past, and currently we are facing an emergency with only limited resources and experience.

Definition of avian influenza

The revised chapter on AI in the World Organisation for Animal Health (OIE) *Terrestrial Animal Health Code (Terrestrial Code)*, which came into force in January 2006 (44), is the first document that approaches AI in a more modern manner, taking into account the new scientific data that is available on this disease, and making use of it for regulating trade. This revised definition logically follows the scientific evidence that low pathogenic avian influenza (LPAI) viruses of H5 and H7 subtypes are the progenitors of highly pathogenic avian influenza (HPAI) 1998 (19, 25, 29); recognising this is crucial for prevention and control of future outbreaks. In addition, the provisions in the revised chapter aim to limit the circulation of AI viruses, as this is one of the primary risk factors for the generation of reassortant viruses. The latter occurrence could represent the basis for the initiation of a human pandemic.

It is therefore imperative that official veterinary services identify surveillance and early detection measures for AI in poultry as a priority, and manage LPAI outbreaks caused by viruses of the H5 and H7 subtypes in an appropriate manner.

The definition adopted by the OIE during its 73rd General Session is as follows (44):

'For the purposes of this *Terrestrial Code*, avian influenza in its notifiable form (NAI) is defined as an infection of poultry caused by any influenza A virus of the H5 or H7 subtypes or by any AI virus with an intravenous pathogenicity index (IVPI) greater than 1.2 (or as an alternative at least 75% mortality) as described below. NAI viruses can be divided into highly pathogenic notifiable avian influenza (HPNAI) and low pathogenicity notifiable avian influenza (LPNAI):

– HPNAI viruses have an IVPI in 6-week-old chickens greater than 1.2 or, as an alternative, cause at least 75% mortality in 4- to 8-week-old chickens infected intravenously. H5 and H7 viruses which do not have an IVPI of greater than 1.2 or cause less than 75% mortality in an intravenous lethality test should be sequenced to determine whether multiple basic amino acids are present at the cleavage site of the precursor haemagglutinin molecule (HA0); if the amino acid motif is similar to that observed for other HPNAI isolates, the isolate being tested should be considered as HPNAI;

– LPNAI are all influenza A viruses of H5 and H7 subtype that are not HPNAI viruses'.

The term low pathogenicity avian influenza (LPAI) is then used to define all infections caused by AI viruses that are not NAI viruses. Following the application of this revised definition there are significant changes in the notification obligations and in trading regulations with reference to AI. The main difference compared to the past is that in order to trade, countries/zones/compartments must demonstrate freedom from NAI infection. In the past, when only HPNAI was notifiable, freedom from infection relied primarily on the absence of clinical cases. With LPNAI being included in the definition, it follows that it is not possible to rely on clinical evidence only, but that freedom must be demonstrated through appropriate surveillance programmes.

The revision of the definition of AI has thus resulted in modified trade requirements, as these now also apply to LPAI of H5 and H7 subtypes (44).

Another major change in the OIE *Terrestrial Code* is that it introduces the concept of allowing the use of vaccination in avian populations whose commodities are destined for international trade. In fact, 'infection' is defined as:

'The following defines the occurrence of infection with NAI virus:

a) HPNAI virus has been isolated and identified as such or viral RNA specific for HPNAI has been detected in poultry or a product derived from poultry; or

b) LPNAI virus has been isolated and identified as such or viral RNA specific for LPNAI has been detected in poultry or a product derived from poultry; or

c) antibodies to H5 or H7 subtype of NAI virus that **are not a consequence of vaccination have been detected in poultry** [author's emphasis]. In the case of isolated serological positive results, NAI infection may be ruled out on the basis of a thorough epidemiological investigation

that does not demonstrate further evidence of NAI infection.'
When these two articles are combined, it appears that although infection with any virus of the H5 and H7 subtype, or any other virus fulfilling the virulence criteria must be notified, the presence of antibodies as a result of vaccination does not limit international trade in the commodities originating from those birds. With this in mind, the use of vaccination for NAI viruses can be considered in a wider range of circumstances.

In this paper, vaccination for NAI viruses of the H5 and H7 subtypes will be addressed with particular reference to the field experiences gathered to date.

The use of vaccination

Currently available vaccines

At the time of writing there are two different types of vaccines available on the market: inactivated vaccines based on adjuvanted whole virions and live recombinant vaccines. The former can be prepared using master seed viruses isolated from natural outbreaks or generated by reverse genetics. Recombinant vector-based vaccines are engineered products, based on a vector virus expressing the haemagglutinin (H) protein of avian influenza. Only a fowlpox vectored vaccine and a Newcastle disease virus (NDV) vaccine are commercially available.

Rationale behind the use of vaccination for control and eradication purposes

It is proven that any product containing an antigenic fraction derived from the haemagglutinin of AI viruses is cross-protective against challenge, provided the H component of the vaccine is of the same subtype as the H component of the challenge virus, e.g. any H5 haemagglutinin will protect against any H5 challenge virus, even if they belong to different lineages (30).

In order to fully understand the potential use of vaccination to combat AI infections, the term cross-protection should be explained. Vaccines defined as cross-protective are those that prevent the onset of clinical signs. In order to achieve a reduction or prevention of clinical symptoms, viral replication in the host must be reduced. However, in most cases, a certain degree of viral replication occurs even in the vaccinated host, and the extent of this replication is assessed and measured by evaluating shedding (42). The latter is achieved in the laboratory by collecting tracheal and cloacal swabs at fixed day intervals and the amount of viable virus eliminated by natural routes into the environment is assessed by means of appropriate tests such as the inoculation of specific-pathogen-free embryos. Most vaccines prevent clinical signs and mortality and significantly reduce, but do not completely suppress, shedding. In experimental trials only a limited number of vaccine candidates have completely suppressed shedding (34). Another effect of vaccination is that it increases the resistance to challenge with infectious virus. For example, in one experiment, naïve turkeys were susceptible to an infectious dose containing 10^4 EID_{50} (50% embryo infective dose) of virus, while vaccinated birds were resistant to this infectious dose and susceptible to a challenge suspension containing 10^6 EID_{50} (6).

It appears, therefore, that if vaccinated birds are placed in the field and they are subsequently challenged by a field virus of the same subtype, they will be more resistant to infection and shed less infectious virus. If the farms at risk of exposure are also vaccinated, it is possible that the infectious cycle may be blocked because not enough virus is shed in the environment to infect vaccinated birds, as the latter are more resistant to challenge. This has been shown experimentally (37) and in the field (10).

However, in order to exploit the advantages of vaccination, it is imperative that vaccination is carried out in an adequate manner (healthy population, full dose, and appropriately stored and administered product) in the framework of a control programme. Such a programme must be based on the application of biosecurity and restriction measures developed to prevent spread from a vaccinated/exposed to an unexposed flock. The failure to implement such a system may result in the circulation of virus in a vaccinated population, with the subsequent undesirable consequences. The most worrisome is probably the emergence of antigenic drift within the viral population. Antigenic drift is a well-known effect of extensive immunological pressure on antigenic properties of influenza viruses. It has been reported for mammalian (human, equine and swine) influenza viruses, but to date, there has only been one instance for avian influenza viruses (21). The result of such occurrence is an antigenic modification of the H protein, which 'escapes' the immune response generated by the vaccine strain. For this reason, influenza vaccines designed for mammalian viruses need to be frequently updated to contain a new H component, more similar to the drifted field virus.

There is no logical reason to exclude the possibility that antigenic drift for avian influenza viruses could occur under the above-mentioned circumstances. The consequences of the emergence of multiple antigenic variants from extensive immunological pressure, generated by a variety of different vaccines are unpredictable, but certainly inauspicious.

Emergency vaccination

Examples of emergency vaccination for low pathogenicity notifiable avian influenza viruses of the H5 and H7 subtypes

Mexico

An LPNAI virus of the H5N2 subtype was first diagnosed in Mexican poultry in May 1994. This virus mutated to a highly pathogenic variant in December of the same year in two different states, Puebla and Querétaro. Both HPNAI outbreaks were extinguished by stamping out (40). Despite the application of this control policy the LP virus continued to circulate in the country and a vaccination programme under official control was implemented. The programme consisted of improved biosecurity, monitoring and vaccination. Two types of vaccines, an oil emulsion inactivated vaccine and a fowlpox recombinant vaccine were licensed for use (40). Monitoring of field exposure within the vaccinated population was not a part of the strategy. Most probably as a result of this, infection is still present eleven years after the implementation of the vaccination campaign (40). The extensive use of vaccination has generated – for the first time with reference to avian influenza – an antigenic drift. A study by Lee *et al.*, 2004 (21) indicated a reduced antigenic cross-reactivity between the vaccine seed and the currently circulating field viruses.

Italy

Between 1999 and 2001 Italy was affected by four successive epidemic waves of AI caused by viruses of the H7N1 subtype. The first epidemic wave was caused by an LPAI virus of H7N1 subtype that subsequently mutated into an HPAI virus after having circulated in the industrial poultry population for approximately nine months. Following the emergence of the HPAI virus, a complete depopulation of the infected area was carried out in accordance with the measures indicated in Council Directive 92/40/CE (12). Approximately 16 million birds either died or were culled. Emergency vaccination was not applied during this epidemic. Four months later, in late summer, the H7N1 LPAI virus re-emerged following the repopulation of poultry farms (4).

From August to November 2000, the H7N1 LPAI strain infected 51 meat-type turkey farms, and 4 quail farms. All birds were stamped out. In order to intervene against a possible re-emergence of the H7N1 LPAI virus, a coordinated set of measures, including strict biosecurity, a serological monitoring programme and a DIVA (Differentiating Infected from Vaccinated Animals) vaccination strategy were enforced. The DIVA strategy, which was implemented as from December 2000, was based on the use of an inactivated oil emulsion vaccine containing the same H subtype as the field virus, but a different N subtype, in this case an H7N3 strain. A serological test based on the detection of specific anti-N1 antibodies was developed as a way of using the diverse N group to differentiate between vaccinated and naturally infected birds (5).

The control of the field situation was ensured through an intensive serosurveillance programme aimed at the detection of the LPAI virus, through the regular testing of sentinel birds in vaccinated flocks and through the application of the anti-N1 antibody detection test. Serological monitoring was also enforced in unvaccinated flocks, located both inside and outside the vaccination area. In addition, the efficacy of the vaccination schemes was evaluated in the field through regular testing of selected flocks. The vaccination programme did not include broilers, but only longer-living birds such as meat turkeys and layers and a very limited number of chicken and turkey breeders.

Notwithstanding the depopulation of the infected premises, the serological monitoring programme revealed an additional incursion of the LPAI H7N1 virus shortly after the beginning of the vaccination programme (December 2000 to March 2001). The H7N1 LPAI virus infected 3 meat-type turkey farms in the vaccination area and 20 poultry holdings (19 turkey farms and 1 layer farm) located in a contiguous unvaccinated area. Only one vaccinated flock was affected and the virus did not spread from this to other vaccinated farms. All infected flocks were culled; the last H7N1 LPAI infected poultry flock was stamped out on the 26 March 2001. The results of the serological surveillance carried out both within and outside the vaccinated area to assess the possible presence of AI infection, demonstrated that the H7N1 AI virus strain was not circulating any longer. In addition, the DIVA discriminatory test was performed and the results were negative; consequently, on 30 November 2001, Commission Decision 2001/847/EC (13) authorised the marketing of fresh poultry meat obtained from vaccinated birds for intra-community trade. The emergency vaccination programme was discontinued in May 2002.

In 2002 and 2003 Italy once again experienced outbreaks of AI involving an H7N3 subtype influenza A virus of low pathogenicity. In October 2002, an H7N3 LPAI strain was introduced from the wild reservoir into the domestic poultry population located in the densely populated poultry area which had previously been affected by the H7N1 epidemic in 1999-2001 (2).

Since the infection rapidly spread among poultry flocks, it was decided to implement a vaccination campaign. The vaccination programme designed was based once again on a DIVA strategy and was carried out using an AI inactivated

heterologous vaccine (strain A/ck/IT/1999-H7N1), which was administered only to layers, capons and meat turkeys. The implementation of the DIVA vaccination campaign was delayed until 31 December 2002, due to unavailability of an appropriate vaccine. From 10 October 2002 to 10 October 2003, the H7N3 LPAI virus was able to spread and infect a total of 388 poultry holdings. Stamping out measures or controlled marketing were enforced in all infected flocks.

On the 13th January 2004, based on the field data generated on the reliability of the DIVA strategy in detecting any LPAI virus infected flock (7), Commission Decision 2004/159/EC (14) authorised the marketing of fresh meat and of table eggs originating from vaccinated turkeys and layers for intra-community trade (13, 14).

United States of America

Although vaccination has been used for many years to combat LPAI viruses (20), only very recently was it applied to combat LPNAI – and it has never been used against HPAI. In 2003, a large layer operation, consisting of three farms all owned by the same company, was infected with an H7N2 virus. This virus was phylogenetically related to the H7N2 virus which had been circulating in the live bird market system in the United States of America (USA) and that had spilled over to the industrial poultry rearing systems on more than one occasion. Given the size of the outbreak, it was decided to vaccinate the animals, rather than apply a stamping out policy (28).

Both naïve and infected birds were vaccinated with two doses of a conventional inactivated vaccine containing a seed virus of the same subtype as the field virus. The reason for this choice was the degree of homology with the field virus and the immediate availability of 3.2 million doses. Subsequently, a vaccine containing an H7N3 strain was used. In order to complement vaccination efforts biosecurity was upgraded and the farmer agreed to comply with certain rules laid down by the health authorities. In order to monitor the evolution of infection, individually identified sentinels were introduced on to the premises and tested serologically every two weeks, and virus isolation or reverse transcription polymerase chain reaction (RT-PCR) was performed daily on the animals that died. Once the vaccination process began, there was no evidence of further viral circulation in the flock. H7N2 was eradicated and the effort was considered a success (28).

Examples of emergency vaccination for highly pathogenic avian influenza

Pakistan

Since 1995 Pakistan has suffered several incursions of H7N3 viruses, and recently of H5N1. In 1995 an HPAI virus was introduced in the northern part of the country, primarily affecting the broiler industry, and an LPNAI virus of the same subtype affected the same area in 2000. Both infections were controlled, although not eradicated, through the application of biosecurity measures and the implementation of a vaccination programme based on an inactivated preparation containing a field isolate (24).

In 2003 and 2004 the southern part of the country, which specialises in the layer industry, was affected by a novel incursion of an LPNAI H7N3 that subsequently mutated to HPNAI H7N3. It appears that an LPAI virus of the H9N2 subtype was also co-circulating at the time. Infection rapidly spread throughout the area causing severe economic damage to the poultry industry.

A control policy, based on voluntary depopulation (without compensation), establishment of a vaccination policy and active surveillance, was implemented by the veterinary authorities. Two months after the peak mortality in the field, the severity and incidence of the disease started to decline. The vaccination campaign covered 80% of the commercial layers and was extended to the breeding stock in the north of the country.

The surveillance efforts showed a reduced circulation of the H7N3 HPAI virus following the implementation of the national control programme (24).

Hong Kong

In 1997 in Hong Kong, a severe outbreak of HPAI H5N1 in poultry spilled over to human beings, infecting 18 people and causing the death of 6 of them. This outbreak was controlled by stamping out approximately 1.3 million poultry. Despite the implementation of restriction measures on the introduction of waterfowl into the territories, further H5N1 outbreaks occurred in chickens and other birds between 2001 and 2003 (11).

Between February 2002 and March 2003, a field trial including vaccination, biosecurity and monitoring was implemented in the Pak Sha district of the Northern Territories, considered at high risk of exposure. The trial was carried out using a heterologous H5N2 virus, included 22 chicken farms and was based on a double vaccine administration (with 4 weeks between the first and second shot) and restocking with vaccinated birds. Thirty unvaccinated birds were left in each farm as sentinels. Surveillance was based on serology and on virus detection in sentinels or dead birds using RT-PCR.

The control programme was extended to 53 farms in December 2002 and during an outbreak between December 2002 and January 2003 vaccination was used to interrupt transmission. In June 2003, the Administration

approved a universal vaccination programme of chickens entering the live-bird market system.

Despite the high infectious environmental pressure in Southeast Asia, and the significant numbers of introductions of live poultry from the mainland, since the implementation of the first vaccination trial no H5N1 virus has been detected in the vaccinated populations. Similarly, no active circulation of H5N1 was detected following the extension of the vaccination programme, and the attempt to interrupt transmission was successful. The vaccination programme will continue to be implemented as long as the high-risk of introduction of H5N1 remains.

Other Asian countries

Several Asian countries are combating avian influenza infection with the aid of vaccination. These include China, Indonesia and Vietnam – which have implemented official programmes encompassing vaccination. Although there is some information on the products that are being used in these campaigns details of the precise conditions under which these products are used are currently unavailable (27).

Certainly the infrastructure and resources necessary to manage a vaccination campaign, following guidelines which have been successfully used in developed countries, are mostly unavailable in these countries. In addition, the logistics of implementing a harmonised programme in a big and populated country such as China, which rears over five billion poultry (27), or in Indonesia where the cultural diversity and various different languages represent an additional problem to the logistics of organising a campaign across approximately 17,000 islands, cannot even be compared to the difficulties that may be encountered in the European Union (EU) or in the USA.

However, one substantiated piece of encouraging information concerning public health has emerged following the implementation of a 'blanket' vaccination campaign in Vietnam: as a result of mass vaccination in poultry, no human cases have been detected since December 2005 (43).

Prophylactic vaccination for notifiable avian influenza

Prophylactic vaccination for viruses of the H5 and H7 subtypes is a completely innovative concept. This is primarily due to the fact that it is only recently that situations for which this policy may be cost-effective have been pinpointed and identified.

The rationale behind the use of prophylactic vaccination is that it should be able to generate a level of protective immunity in the target population. The immune response may be boosted if there is evidence of the introduction of a field virus.

Prophylactic vaccination should increase the resistance of birds and, in case of virus introduction, reduce levels of viral shedding. It should be perceived as an additional tool to maximise biosecurity measures when a high risk of exposure exists. Ultimately, it should result in preventing the index case, or alternatively in reducing the number of secondary outbreaks, thus minimising the negative aspects of animal welfare and the potential economic losses in areas where the density of the poultry population would otherwise result in uncontrollable spread unless there was pre-emptive culling.

Prophylactic vaccination should only be considered when there is circumstantial evidence that a country/area/compartment is at risk of infection. Risk of infection may be subdivided into two categories:

a) risk of infection with an unknown subtype, either H5 or H7, (e.g. from migratory birds)

b) risk of infection with a known subtype (e.g. H5N1, which is present in Asian and African countries, or subtypes that are known to circulate in live bird markets in the USA).

In the first case, a bivalent (H5 and H7) vaccination programme could be implemented. In the second case, a monovalent (either H5 or H7) programme would be sufficient.

The choice of the vaccine is crucial to the outcome of prophylactic vaccination campaigns. Ideally, vaccines that do not interfere with diagnosis in the case of field exposure with any AI virus should be used and vaccination should be carried out as long as the risk of infection exists; some countries are using it in a targeted manner in the face of an increased risk of H5N1 introduction. Appropriate and detailed exit strategies should be formulated before prophylactic vaccination is undertaken.

What appear to be lacking in some situations are guidelines that define an appropriate approach to field surveillance. These guidelines may be derived from general guidelines on surveillance for epizootic diseases, but must be adapted to the local situations and must be targeted towards a well-defined and pursuable objective. In addition, due to recent exposure of a vast variety of avian species to HPAI, it is imperative that specific research programmes are developed to evaluate the efficacy of vaccination in these species and to develop and validate novel vaccination concepts that enable the DIVA system.

European Union

Prophylactic vaccination has been recently approved in several countries in the EU, and its application is now foreseen in EU Directive 2005/94/EC (18). Italy started a prophylactic vaccination programme in October 2004 following the EU Commission Decision on introducing vaccination to supplement control measures to control LPAI in Italy (15). At risk categories in an area at high risk of exposure were vaccinated with a bivalent (H5/H7) inactivated vaccine containing seed strains supplied by the Italian National Reference Laboratory. The vaccination programme was part of a wider control strategy encompassing monitoring, restriction and biosecurity.

In 2006, the Netherlands and France were also authorised to implement a prophylactic vaccination programme to combat the possible introduction of HPAI H5N1 from the wild bird reservoir (16, 17). The Dutch programme was developed as a voluntary effort concerning hobby flocks and free-range farms. Vaccinated farms or flocks were monitored for field exposure under official control. The French programme was implemented in free-range duck and geese farms in defined areas at risk and was coupled with a monitoring effort. At the time of writing, monitoring efforts in all three countries have been able to exclude the circulation of any HPNAI.

Conclusions

The epidemiology of AI infections has changed over the last decade due to the persistence and the continuous spread of HPAI H5N1 in domestic poultry populations in vast geographical areas of three continents, and to the marked increase of AI outbreaks worldwide. Furthermore, H5N1 has been able to spread to previously unaffected areas and its recent isolation from apparently healthy migratory birds in southern China suggests that they can contribute to virus transmission over long distances during migration (8). This unprecedented situation could be the result of reintroductions from the wild or of a re-emergence in domestic poultry populations worldwide, with inauspicious and unpredictable consequences.

To combat this global threat, the international veterinary community has designed a strategy and identified a set of coordinated measures that can be enforced to prevent, control and eventually eradicate AI infections (3, 45, 46).

The application of the different control options, which may include vaccination, should be used in different ways on the basis of the characteristics of the poultry producing sector in its entirety, the eco-epidemiological situation, the response capacity of the veterinary infrastructure and the availability of adequate resources. Previous experiences have indicated that in order to succeed in controlling and ultimately eradicating the infection, vaccination programmes must be part of a wider field strategy that includes ongoing monitoring and feedback inputs as the situation evolves. Essentially, this strategy must include monitoring the evolution of infection (DIVA approach), early detection of any possible outbreaks, and enforcement of adequate biosecurity, restriction and eradication measures. Whenever such a strategy cannot be implemented, the establishment of an endemic status due to sub-clinical virus circulation in the vaccinated poultry population cannot be ruled out.

Research in the field of AI vaccinology has greatly increased in the last few years with a variety of novel preparations ranging from recombinants (e.g. vaccines for fowlpox virus, NDV and infectious laryngotracheitis virus) (1, 9, 23, 26, 31, 32, 33, 36, 38, 39, 41) to products based on reverse genetics (22, 35) that are being developed to complement existing conventional inactivated products. Most of these have undergone experimental evaluation and some of them appear to perform very well, and will certainly be valid options in the future. However, the concerns regarding vaccinating against AI should not be limited to the vaccine itself, as there are other aspects, which must be taken into account and that are crucial to the success of a vaccination programme both in the short and in the long term.

One issue which will certainly be a concern in the coming years will be the occurrence and extent of antigenic drift in avian influenza viruses. At the time of writing this occurrence has been identified with certainty only in the Mexican lineage of H5N2 viruses, following a 10-year vaccination programme with two different vaccines containing diverse haemagglutinin proteins (21). The antibody response to the plethora of seed viruses that are currently being proposed in vaccines will certainly impact the antigenicity of circulating viruses and should be closely monitored. Under these conditions it would seem appropriate that only a very limited number of the most cross-protective AI strains be selected as vaccine seed strains.

As no country can consider itself not at risk for AI, it is imperative that contingency plans are prepared and discussed in advance. These contingency plans should also contain control strategies that can be applied on a country-by-country basis, bearing in mind the realistic possibilities of each country. If vaccination is considered a possible control option, vaccination programmes should be laid down in the framework of national AI contingency plans. In fact, the probability of implementing an efficacious emergency vaccination programme is mainly related to the level of preparedness and the capacity of the Veterinary Services in the affected countries or in those at risk.

Vaccination enabling the DIVA system in conjunction with a strategy that includes monitoring and appropriate management of field exposed flocks can be successful in controlling and eradicating AI infections in poultry and is compatible with the continuation of international trade. However, it is complicated to manage and may include failures such as the spread of infection within the vaccinated population. It is therefore essential that the vaccination programme be implemented using a suitable product that enables the detection of field exposed flocks and that the monitoring system in place is developed to guarantee early detection of and rapid response to AI introduction. For this reason, international organisations that govern trade regulations and animal disease control should establish a set of guidelines so that control programmes may be 'accredited' and consequently internationally recognised. Such a policy would appear to have several practical advantages which would result in improved crisis management. These include harmonisation of seed viruses to be used in vaccines, rapid approval of established control programmes, constant updating on the field situation, feedback on successes and failures, harmonisation of protocols and systems and increased public awareness of control and eradication programmes. In this way, even developing countries that are experiencing notifiable avian influenza infections – and that have no experience with AI management – can maximise the outcome of other experiences to combat this infection in an educated manner, thus avoiding wastage of resources and time.

The harmonisation of programmes and the sharing of information would also improve our knowledge on the epidemiology of NAI infections and this would benefit our management skills, including the identification of reservoir species.

Vaccination is now being used extensively to aid the prevention of introduction or to control widespread HPAI H5N1. Overall, the veterinary community has limited first-hand experience with managing this disease aided by vaccination. It is most likely that if managed appropriately positive results will be achieved. However, errors will be made, and it is imperative that control and eradication processes can benefit from the learning process generated by such errors. In addition, control programmes need to be managed in a flexible and transparent manner to respond to the continuous challenges that AI infections pose.

■

La vaccination des volailles contre l'influenza aviaire à déclaration obligatoire

I. Capua

Résumé

L'influenza aviaire à déclaration obligatoire figure sur la liste de l'Organisation mondiale de la santé animale (OIE) et revêt une importance désormais capitale tant pour la santé animale que pour la santé publique. Jusqu'en 2000, la vaccination contre l'influenza aviaire à déclaration obligatoire était déconseillée et n'a été appliquée que dans de très rares foyers, en appui aux mesures de lutte et sans objectif d'éradication. Des études décisives portant sur des programmes de vaccination visant l'éradication ont été conduites en Italie, puis dans d'autres pays tels que Hong Kong et les États-Unis d'Amérique. Compte tenu de la propagation sur trois continents de la souche asiatique H5N1 du virus de l'influenza aviaire hautement pathogène, la vaccination est maintenant appliquée à grande échelle, dans des conditions qui ne sont généralement pas idéales. En dépit de l'obstacle que constitue, dans certains pays, le manque de ressources et d'infrastructures pour la réussite d'ensemble des programmes de contrôle appliquant la vaccination, il est impératif que les

organisations internationales fixent des lignes directrices pour « accréditer » les stratégies de lutte, en prévoyant des recommandations sur les souches à utiliser pour la préparation de vaccin, sur les caractéristiques de ces vaccins, sur les meilleures stratégies à appliquer sur le terrain à chaque étape des programmes de lutte et d'éradication, et sur le modèle à suivre pour sortir de l'application des mesures. L'application concertée de protocoles harmonisés permettrait d'obtenir des résultats concrets, de gagner du temps et de rationaliser l'utilisation des ressources.

Mots-clés
Abattage sanitaire – Biosécurité – Épidémiologie – Éradication – Influenza aviaire – Lutte contre les maladies – Suivi – Vaccination.

Vacunación de aves de corral contra la influenza aviar de notificación obligatoria

I. Capua

Resumen
La influenza aviar de notificación obligatoria es una enfermedad inscrita en la lista de la Organización Mundial de Sanidad Animal (OIE), que últimamente ha cobrado gran relieve desde el punto de vista de la salud tanto humana como animal. Antes de 2000 se desaconsejaba la vacunación, utilizada sólo esporádicamente como instrumento auxiliar de lucha contra algún brote, pero no como medio de erradicación. En Italia se llevó a cabo un trabajo de gran trascendencia sobre la aplicación de un programa de vacunaciones que perseguía el objetivo, a la postre cumplido, de erradicar la enfermedad. Después siguieron otras investigaciones en países como Hong Kong y los Estados Unidos de América. Ante la extensión a tres continentes del linaje asiático H5N1 de la influenza aviar altamente patógena, la vacunación se está utilizando ahora a gran escala en diversas circunstancias, que a menudo distan de ser idóneas. Aunque en algunos países la falta de infraestructuras y recursos pueda reducir considerablemente el éxito global de programas de lucha que incluyen la vacunación, resulta imperativo que las organizaciones internacionales establezcan pautas para 'homologar' las estrategias de control. Tales pautas deberían incluir recomendaciones sobre las cepas originales que deben usarse para preparar vacunas, las características de éstas y la estrategia que conviene aplicar sobre el terreno en las distintas fases de un programa de control o erradicación, junto con modelos para finalizar la aplicación de las medidas. La existencia de protocolos armonizados facilitaría considerablemente la obtención de resultados tangibles, ahorraría tiempo y evitaría un inútil despilfarro de recursos.

Palabras clave
Bioseguridad – Control – Epidemiología – Erradicación – Influenza aviar – Sacrificio sanitario – Vacunación – Vigilancia.

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Vaccination in conservation medicine

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Summary

Unprecedented human population growth and anthropogenic environmental changes have resulted in increased numbers of people living in closer contact with more animals (wild, domestic, and peridomestic) than at any other time in history. Intimate linkage of human and animal health is not a new phenomenon. However, the global scope of contemporary zoonoses has no historical precedent. Indeed, most human infectious diseases classed as emerging are zoonotic, and many of these have spilled over from natural wildlife reservoirs into humans either directly or via domestic or peridomestic animals. Conservation medicine has recently emerged as a meaningful discipline to address the intersection of animal, human, and ecosystem health. Interest in the development of novel vaccines for wildlife encounters important challenges that may prevent progress beyond the conceptual phase. Although notable examples of successful wildlife immunisation programmes exist, depending upon key considerations, vaccination may or may not prove to be effective in the field. When implemented, wildlife vaccination requires a combination of novel zoonosis pathogen management strategies and public education to balance conservation, economic, and public health issues.

Keywords

Brucellosis – Conservation medicine – Emerging disease – Endangered species – Public health – Rabies – Vaccination – Vaccine delivery – Wildlife – Zoonosis.

Introduction

Zoonotic infections (i.e. those that can be transmitted from animals to people and vice versa) can have major impacts on wild and domestic animal and human health and can result in serious damage to the economies of developing and developed countries (79, 81). Yet, for ethical, ecological and economic reasons, it is no longer optimal to control and eliminate zoonoses mainly by mass slaughter of animals. Vaccination is without doubt one of the most useful measures to prevent animal diseases (50). Since its inception, veterinary science has been strongly linked with the development of vaccinology (82). Veterinary public health (VPH) has been defined as 'the contributions to the physical, mental, and social well-being of humans through an understanding, and application of veterinary science' (29). Despite the laudable contributions of VPH to the human condition, good intentions combined with ecological ignorance, are a blueprint for iatrogenic disaster (66). In response to these realities, conservation medicine has arisen as a relatively new field situated at the crossroads of VPH, wildlife diseases, and ecological health. The persistence of zoonotic diseases in domestic livestock, companion animals, or wildlife continues to pose significant risks to human health. For many of these diseases, veterinary vaccines or regulatory programmes have been developed to prevent transmission to humans,

protect companion animal health, and prevent economic losses. In addition to the zoonotic diseases for which control measures have been implemented, new zoonotic diseases continue to emerge worldwide. In a global society, known or unknown zoonotic diseases can be rapidly transported to naïve populations on other continents by animal reservoirs or infected humans. Development of efficacious vaccines and rapid diagnostics continues to be needed to protect human health, control zoonotic diseases identified in animal reservoirs, and prevent both domestic and international transmission of zoonotic diseases. Our ability to meet the VPH and conservation medicine challenges of the 21st Century will be greatly influenced by our ability to expand relevant, cost-effective, problem-oriented basic and applied zoonotic vaccinology research, while coordinating international research efforts and communicating research output to end-users. This paper reviews key issues, challenges, and opportunities in vaccinology for conservation medicine.

Emerging zoonoses, conservation medicine and vaccination

Animal-borne pathogens are important, not only because of the disease they can cause, but because new human diseases can arise from unsuspected animal reservoirs (79). Indeed, nearly 75% of all human infectious diseases classed as emerging are zoonotic, and many of these have spilled over from natural wildlife reservoirs into humans either directly or via domestic or peridomestic animals (80). Unprecedented human population growth and anthropogenic environmental changes have resulted in greater numbers of humans living in closer contact with more animals (wild, domestic, and peridomestic) than at any other time in history. Intimate linkage of human and animal health is not a new phenomenon. However, the scope, scale, and worldwide impacts of contemporary zoonoses have no historical precedent (81).

Historically, zoonotic diseases have been suggested or described for millennia, e.g. the Eshnunna Code (2300 BC), an ancient legal text from Mesopotamia, lists the penalties to be paid by animal owners should a man die from the bite of a 'mad' dog; vedic medical texts from India (1800-1200 BC) describe anthrax in cattle; and the Roman writer Columella (~AD 100), who wrote mainly on agricultural topics, recommends methods to decrease the disease now recognised as rabies in goats, swine, and dogs. More recent events such as Jenner's work with cowpox and Pasteur's research on anthrax and rabies established the concept of vaccination with live organisms as a mechanism to prevent zoonotic diseases (65).

Pathogen emergence continues, with more than 35 new infectious diseases in humans recognised since 1980 (34). Causes for pathogen emergence are many, but most likely include changes in human demography and behaviour, loss of wildlife habitats, wildlife consumption, the global wildlife trade, and transmission of pathogens to naïve reservoirs via close association of species that do not normally interact (19, 20, 46, 78). As the world population increases and interactions between human and wildlife increase, it is unlikely that the emergence of new zoonotic agents will decline. Recent disease events have focused global attention on how the interrelated factors of land-use changes, natural resource management, and the demands of human population growth alter the inherent ecological balance between zoonotic pathogens and their human and animal hosts. Zoonotic pathogens, such as avian influenza, are the most significant cause of emerging infectious diseases in people, especially from the standpoint of conditions that appear in a population for the first time or are increasing in prevalence or geographic distribution (68). Accordingly, wildlife and domestic animals are an important part of the public health picture, as they provide a 'zoonotic pool' from which diseases may emerge (19).

Notably, in the autumn of 2002, an influenza-like illness was described in Guangdong province, the People's Republic of China, which was eventually designated as severe acute respiratory syndrome (SARS) (40). Data suggest that the causative agent of SARS, a coronavirus, was maintained in Himalayan palm civets (*Paguma larvata*) or raccoon dog (*Nyctereutes procyonoides*) reservoirs in markets (31). However, the primary host species for SARS may reside in Chiroptera, such as horseshoe bats (*Rhinolophus* sp.) (40). Transmission of SARS to humans may have been via exposure to live animals, or association with restaurants in which palm civet meat was prepared and consumed. The 2003 outbreak was associated with a 10% mortality rate in humans (54). Although vaccine candidates are in the process of evaluation (6, 61), outbreaks in the near future would most likely be controlled by quarantine and isolation procedures for humans, and limited culling of wildlife reservoirs, as utilised in 2003.

Bats (*Pteropus* sp.) have also been implicated as reservoirs in the emergence of the Hendra and Nipah paramyxoviruses, highly pathogenic viruses associated with disease outbreaks in humans and livestock (25). Hendra virus was the cause of human and horse mortalities in Australia in 1994, 1999, and 2004 and Nipah virus caused encephalitis in humans in Malaysia in 1998 and 1999, and Bangladesh beginning in 2001. Both viruses are highly pathogenic in humans with fatality rates of 40% to 70% (26). In regards to the emergence of Nipah virus in Malaysia, climate changes, habitat loss caused by deforestation, and movement of the reservoir host into areas where domestic swine were raised have allowed

transmission to domestic livestock and humans (14). Although human vaccines are under development, and an efficacious Nipah virus vaccine for pigs has been reported (73), effective vaccines to prevent zoonotic infections or address the reservoir of this disease are lacking.

The recent emergence of H5N1 influenza virus in domestic and wild birds has caused economic losses and anxiety across the globe. An intermediate host, such as swine, has been proposed as a likely remixing vessel in which co-infection with avian and human influenza viruses leads to emergence of antigenically different strains (2). The emergence of antigenically different strains of influenza has led to human pandemics in 1918 (H1N1), 1957 (H2N2), 1968 (H3N2), and 1977 (H1N1) (2). The rapid dissemination of H5N1 via the domestic fowl trade and migrating wild birds and the transmission to humans through contact with domestic poultry have led to human mortalities and significant economic costs in a number of countries.

As a logical outgrowth of these historic and recent experiences, conservation medicine has emerged as a meaningful discipline to address the intersection of animal health, human health, and ecosystem health (67). This intersection occurs between physicians, veterinarians, wildlife biologists, ecologists, epidemiologists, anthropologists, sociologists, and economists and builds upon integrating reliable knowledge derived from conservation biology (ecology of fragmented habitats, global change, invasive species); studies on the evolution of host–parasite relations (invasion, competition, and virulence); and the principles and practices of public health (20). The principal goals of conservation medicine are to develop scientific understanding of the connections between environmental crises and human and non-human health and to develop solutions to problems at this interface between environmental and health sciences (49).

Although primarily designed to address the disease in the animal host, veterinary programmes have a positive contribution to make in preventing human infection and clinical illness. The most common tool for disease control in veterinary medicine has been vaccination, with success influenced by vaccine efficacy and the proportion of the population inoculated (16, 32, 59). Veterinary vaccines have historically been produced from attenuated strains, although molecular techniques are facilitating development of safer and more efficacious vaccines which facilitate diagnostic investigation and enhanced surveillance. Wildlife disease reservoirs offer unique challenges for control programmes and vaccine development. In wildlife populations, capture of all animals is usually not practical, and remote vaccine delivery will then be required. Species differences in immunologic responses have been noted in addition to differences in responses to vaccines delivered via routes other than injection (48, 55). In a global society, veterinary medicine can be the front line for detection of many zoonoses, and thus veterinary vaccination must continue to be considered within the realm of conservation medicine.

There are many reasons to consider vaccination programmes aimed at free-ranging wildlife, not the least of which is protection of public health. Just as wildlife vaccination programmes may be designed to protect public health, they may also be aimed at protecting economic interests when the disease is transmissible between wildlife and domestic animals. Wildlife vaccination programmes may also be aimed at reducing the impacts of disease on susceptible wildlife. Conservation of endangered species threatened by infectious disease can also be a goal of wildlife vaccination. Vaccination programmes may be employed to reduce the signs of disease that negatively affect wildlife viewing and therefore reduce the potential for tourism. Protection of livestock health and productivity is also a major concern and can result in dramatic conflicts between those working in wildlife conservation, agriculture, and land-use planning.

Novel vaccine development for wildlife: the example of brucellosis

At Yellowstone National Park (8,987 km^2), the only free-ranging North American plains bison (*Bison bison*) population that survived the 19th Century *in situ* was infected with brucellosis (*Brucella abortus*) from transmission by livestock early in the 20th Century (45). Following this, Rocky Mountain elk (*Cervus elaphus*) living in the 90,000 km^2 greater Yellowstone area (GYA) surrounding the park also acquired *B. abortus*. The potential for 'spillback' transmission of brucellosis from wildlife to livestock across the GYA has led to intensive local, regional, and national concerns including extremely divisive legal and policy conflicts. Remote brucellosis vaccination of the Yellowstone bison population is increasingly being viewed as a component of adaptive risk management strategies which aim to eventually eliminate the disease (52). However, novel vaccines will need to be developed because the extant bovine brucellosis vaccines S19 and RB51 have not proved very effective in limiting bison or elk maternal and foetal infection or reducing shedding of *B. abortus* into the environment (70).

Development of novel vaccines or improvement of existing vaccine platforms is impaired by a number of issues, including, but not limited to: funding, differences in

wildlife species immunology, remote delivery, and economic issues. Some zoonotic agents such as tuberculosis or brucellosis require higher levels of biosafety containment facilities for vaccine research, which are expensive and not always readily available. Biosafety facilities that are available may not be suitable for housing or handling certain wildlife species due to their size, behaviour or social activity, or because of other factors. Immunologic characterisation of responses to vaccination may be limited by lack of reagents for individual wildlife species. Species differences in immunologic responses may influence the sensitivity and specificity of diagnostic tests and the protective immunity induced by vaccination. Therefore, vaccine development may need to target each reservoir separately and may require development of species-specific vaccines. Public concerns regarding environmental issues or infection of non-target species may also limit the ability to deliver live vaccines to free-ranging wildlife. This creates problems for developing vaccines for some zoonotic agents, such as brucellosis, in which efficacious vaccines are currently limited to live attenuated strains.

Moreover, development of a novel vaccine for wildlife will require basic research to be simultaneously initiated along with applied research to explore novel approaches, establish basic science practices which yield incremental discoveries, and develop information which will facilitate advances in diagnostics and vaccine development (70). In the event that all vaccines evaluated under an empirical approach prove unacceptable, knowledge gained through a basic research approach should then offer alternative vaccines that might be successful. Novel vaccine development will necessarily be underpinned by new reliable knowledge in the areas of reproducible disease models, correlates of protective immunity, host-specific immunologic responses, antigen discovery, adjuvant/formulation/delivery optimisation, genetically-engineered vaccines, and durability of immunogenicity (70). For example, T-cell epitope mapping has emerged as a potentially powerful discovery tool with a range of biomedical applications extending from reengineering protein therapeutics (such as toxins for medical use) to vaccine discovery and design (22). Application of this technology to wildlife vaccines has potential for pathogens for which traditional 'shake and bake' vaccine development approaches have failed.

Illustrative of these novel vaccine development issues are the reviews of brucellosis in bison and elk in the GYA, which have identified a need for sustainable, innovative, basic and applied vaccine research and discovery programmes (30, 45, 70). The three main areas of emphasis for addressing vaccine research needs are:

– consideration of interrelated issues which will influence research progress, implementation, and efficiency

– empirical approaches to rapidly screen new vaccine candidates for efficacy in bison and elk

– discovery or basic research approaches to expand knowledge on pathogenesis, protective antigens, and immunologic responses to *B. abortus* in bison and elk to facilitate development of new second and third generation vaccines.

Several interrelated issues at local, state, national, international, and/or regulatory levels will influence efforts to facilitate brucellosis vaccine development for bison and elk, they include:

– maximising and prioritising productivity and efficiency

– coordinating the work of multidisciplinary teams

– securing funding

– amending regulatory policies which hinder *Brucella vaccine* research

– ensuring that there are sufficient biosafety facilities to conduct the identified research.

To address these issues, it has been suggested that an oversight consortium of industry, academic, and government representatives be formed to oversee efforts in the area of bison and elk brucellosis vaccine research (70). This consortium would identify funding; prioritise research needs; coordinate multidisciplinary or consortium research teams that would integrate vaccine, diagnostic, and delivery expertise; and disseminate progress from empirical and discovery approaches to ensure integrated and coordinated technology transfer towards applied solutions.

As free-ranging bison and elk cannot be readily caught or restrained like domestic livestock or companion animals, development of novel approaches for remote delivery of vaccines will also be necessary, potentially including oral delivery (baits, spiking natural or artificial water sources, incorporating vaccines into salt attractants, engineered recombinant forage, innovative encapsulation technologies), injection (dart delivery, biocompatible bullets, application to antlers/horns taking advantage of evolved fighting behaviours), transdermal delivery (ballistic and contact), delivery via biological vectors (biting arthropods, phages, nematodes, other virus or bacteria parasites), and mucosal delivery (aerosol, bioengineered venereal disease, ocular delivery) (70). It may also be that with vaccines, immunologic responses and protective immunity may differ between parenteral inoculation and other routes of delivery (10, 48, 60).

The difficulties involved in combating wildlife brucellosis in the GYA are considerable and they include several challenges which will increase the cost and time required

to develop novel vaccines to address zoonotic agents in wildlife reservoirs. Yet, the success of the oral rabies baits in free-ranging wildlife described below suggests that challenges are not insurmountable (43).

Vaccine delivery to wildlife: the example of rabies

Vaccination to prevent infectious disease is a cornerstone of modern human and veterinary medicine. Regardless of the patient, strategies for delivery (whether direct or remote) of any biologic have two major barriers to overcome: the skin or mucosal surfaces. Still, both of these basic portals are not mutually exclusive for vaccine delivery consideration. Vaccination remains a prime procedure in zoological medicine because some animals brought into captivity for exhibition, applied research or conservation purposes are incubating disease, or may be surrounded by others in an infectious state. However, delivery beyond restrained animals offers many unique challenges. Wildlife poses substantial hurdles to disease control, not only because of basic species diversity and the limited knowledge of how these different species will react to vaccination, but also due to their motility and distribution; such animals are rarely an obvious or captive audience. As stated clearly by Wandeler (72), wildlife '...does not follow an invitation to visit a veterinarian, and there is no owner to bring it there. It has to be lured by some trick into vaccinating itself'. In this regard, specific vaccine formulations, and applied immunological, administrative, environmental, and regulatory issues will vary greatly dependent upon the choice of vaccine and the favoured method of delivery.

Rabies, a progressive encephalitis, is an ancient zoonosis, but it is one of the best modern paradigms for wildlife vaccination (59). A brief review of the history of rabies vaccination offers a wealth of insight for potential application to other wildlife diseases (74). The disease is global in distribution, with the exception of Antarctica, and the etiological agents are RNA viruses in the family *Rhabdoviridae*, genus *Lyssavirus* (38, 47). All mammals are believed to be susceptible, but members of the *Carnivora*, especially domestic dogs, are the most affected species (36). After initial developments in the laboratory, early canine rabies prevention offered insights that helped related efforts in the control of other diseases, which continues to be the case today (16). During the 1920s, Japan became one of the first countries to successfully apply the idea of mass vaccination to domestic dogs in a practical fashion. Routine veterinary use of rabies vaccination advanced gradually in other countries, especially after World War II. Thereafter, the extension of vaccination to non-traditional species occurred as a result of the combination of several, critical, inter-related factors:

– the basic overarching realisation that dog rabies could be eliminated by achieving herd immunity

– the appreciation of the fundamental role of wildlife reservoirs in disease dissemination

– the demonstration that population reduction was not the ultimate solution to disease control

– experimental recognition that oral vaccine administration was effective as an alternative means of delivery to the parenteral route

– eventual progress in development of remote delivery methods (4).

Parenteral administration of biologicals via needle and syringe is a straightforward method for individual animals. As with domestic species, even more exotic wildlife such as the Egyptian fruit bat (*Rousettus aegyptiacus*) can respond to potent inactivated vaccines by parenteral administration (51). While no vaccines are licensed specifically for parenteral use in wildlife, commercial biologicals can be administered off-label (i.e. prescribed for purposes not listed on the product label) not only for pre-exposure use, but also for post-exposure management during outbreaks in or near captive or managed groups. Such use may work when the products are administered in accordance with the guidelines in use for domestic species (13). Although direct intramuscular or subcutaneous vaccination may be very safe in captivity and quite effective under certain circumstances, it is generally believed to be largely impractical for most free-ranging animals that are spread out over large and inaccessible areas. Still, while less than 30% of Swiss foxes were estimated as reachable by trapping and hand vaccination, this technique was successfully applied to control rabies in urban Toronto (55, 72). Historically, automatic devices have been developed, such as vaccine-loaded syringes designed to spring out of the ground, or revamped 'coyote-getters' armed with explosive charges to deliver product in the mouth, but they were never widely deployed (77).

Ultimately, progress in the use of oral rabies vaccination (ORV) depended upon the development of attractive baits (5, 8, 21, 41, 76). Early prototypes were based on eggs or meat and were placed around a sterile vaccine container, usually made by hand. For example, the original Swiss campaigns against fox rabies utilised chicken head baits (72). These 'cottage industry' beginnings eventually gave rise to mass-produced, factory compiled baits of fishmeal, pet food meal, or other derivates. Throughout the late 1970s and up to the present day, field applications of rabies vaccine-laden baits over substantial regions of Europe and North America led to the widespread control, and in some cases the elimination, of the disease among wild mammalian carnivores (9, 17, 42, 43, 64). To date, only self-replicating modified-live or recombinant viruses have

been employed in ORV; inactivated vaccines do not work by the oral route. The use of vaccine-laden bait could be extended to other terrestrial mammals and diseases.

The use of rabies vaccination in wildlife has proved to be a successful additional use of a veterinary control technique that was traditionally only implemented among domestic animals. However, long-term disease elimination may be hampered by the existence of other relevant major reservoirs. Obviously, alternative techniques will be needed for field applications for a wider variety of scenarios related to different taxa, diseases, and circumstances (18). Some tactics may exploit the behavioural ecology of a given species. In large aggregations, colonies, herds, flocks or packs, the aerosol route of delivery may be useful, especially after critical new insights into agent acquisition via this under-exploited route are further explored (33). Besides mists, fine liquid sprays could be envisioned for joint respiratory or mucosal use, and depending upon the vaccine, application to one individual could result in the spread of vaccine to others in a group setting via social grooming (3). For remote delivery, in addition to the use of solid bait, stable liquid products could be added to critical water sources such as troughs; this is already done for livestock on remote ranges and the practice could also be used on a small scale in birds at backyard sites, or development of special crops expressing vaccine antigen for consumption (69). Considering future vaccine candidates, given the revolution in genetics, various viruses could be used as expression vectors, for incorporation of foreign genes. Some agents could be constructed by the creation of transgenic vectors, expressing the immunogen of interest in an appropriate context (56). Extrapolating from the concepts of remote delivery and natural hypodermics, ectoparasites may be designed to harbour and administer vaccine vectors of interest. For potential focus, one could imagine reverse-engineered viruses (as many are shared between invertebrates and vertebrates) opening new arenas for discovery, as is underway for lyssaviruses and other rhabdoviruses (24, 39).

With this background in mind, a 'Programmatic Environmental Assessment' was developed to evaluate animal rabies management alternatives in the United States of America (USA) (71). Impacts on the biological, physical, economic and social environments, as well as risks and mitigation associated with each alternative, were assessed in relation to selecting the preferred alternative of coordinated ORV. Among the salient issues considered were potential impacts on humans, such as vaccine exposure and infection (57), and programme impacts on non-target species, including those species classified at state or national level as threatened and endangered. At the completion of the ORV programmes each year, an annual evaluation is performed. This includes a review of the effectiveness of the projects and the proposal of any necessary mitigation, including recommendations for improvement in subsequent actions. This process is designed to ensure sound multidisciplinary programme planning and implementation and critical public involvement (71). Such a model should be considered and developed well in advance of any intended vaccination of wildlife to minimise unintended consequences and provide a mechanism for ongoing stakeholder engagements. Development of integrated frameworks involving public health, veterinary, wildlife conservation and animal welfare agencies and regulatory authorities is crucial to the control of wildlife diseases by vaccination.

To vaccinate or not to vaccinate?

Although primarily designed to address the disease in the animal host, veterinary control programmes have a positive contribution in preventing human infection and clinical illness. The most common tool for disease control in veterinary medicine has been vaccination, with success influenced by vaccine efficacy and the proportion of the population inoculated (32). Veterinary vaccines have historically been produced from attenuated strains, although molecular techniques are facilitating development of safer and more efficacious vaccines which make diagnosis easier. Vaccination of a particular host protects not only key target populations, but can also serve as a barrier to protect human and veterinary health. Clearly, lessons learned from rabies vaccination of carnivores (43, 58) provide key insights and models for the prevention of other emerging diseases in a variety of species, especially when combined with additional applied advances in veterinary vaccinology, regardless of whether the target sprints, crawls, swims, or flies (1, 11, 28, 35, 44, 62).

The heightened public attention on zoonotic diseases has fanned the flames of debate on how best to manage diseases in free-ranging wildlife. When the wildlife host is also the focus of conservation efforts, such as endangered species recovery, the management issues take on a whole new level of complexity. Should the wildlife species, which serve as a reservoir for the pathogen, be strongly reduced or eliminated to protect people and domestic animals or should the pathogen be eliminated from the wildlife? If pathogen elimination is possible through vaccination, are there negative consequences of eliminating pathogens from ecosystems?

When considering development of a vaccination programme in wildlife, it is important to carefully define the goals. Disease eradication requires an efficacious vaccine that can be delivered safely to the target species.

For pathogen elimination across larger landscapes, restricted host susceptibility is also likely to be required, as identification and vaccination of wildlife disease has proven to be near impossible in systems with multiple alternative hosts (15, 45, 70). The only success in worldwide disease eradication is presently smallpox, for which an effective and deliverable vaccine has been employed and the host susceptibility is limited to humans (27), but the eradication of rinderpest is also foreseen in the near future (7).

The many different challenges that can present themselves when designing a vaccine programme with conservation implications become apparent when considering the programme's goals. Is the primary goal to eliminate disease in an endangered species; to limit the transmission of disease from wildlife to domestic animals; or to protect people from exposure to wildlife diseases? Each of these goals presents difficulties related to scale. For example, a programme to protect an endangered species will be on a small scale, as there is probably a small population to be vaccinated; unfortunately, vaccines have not been developed for most wildlife species, and it is problematic to secure the necessary investment of resources to develop vaccines with such a finite market. Therefore, vaccines developed for domestic animals are the next best logical choice, but questions of safety and efficacy then become paramount. For example, wildlife managers tasked with recovery of the endangered North American black-footed ferret (*Mustela nigripes*) were severely hampered by the inadequate attenuation of canine distemper virus in the commercially available modified live virus vaccine, when the vaccine caused mortality in this sensitive species (12). Nearly three decades later, safety concerns were alleviated by the development of a canine distemper virus recombinant vaccine for domestic animals and wildlife; however, the question of efficacy for the black-footed ferret and the many other wildlife species susceptible to canine distemper virus remains (75).

Scale continues to be a fundamental programme design challenge when the vaccine must be aimed at a wildlife reservoir that jeopardises threatened species, domestic animals, or people. Consider for example, attempting to design a vaccine programme aimed at migrating wild fowl for the prevention of human exposure to highly pathogenic avian influenza. Even if one focused on a finite area like California's Central Valley (USA), the scale of the programme would be immense. California's Central Valley is a major stop for migrating waterfowl, receiving 60% to 70% of ducks and geese traversing the Pacific Americas Flyway. Therefore, the vaccine programme would be aimed at immunising approximately six million waterfowl to protect the five million people who reside in the same area. In the end, this programme would most likely be ineffective, as a waterfowl vaccination programme would probably only be able to target a small range of potential reservoirs; delivery would be an enormous problem; and vaccine efficacy would be difficult to evaluate.

Vaccination of domestic animal reservoirs has also been attempted to protect endangered species. The Ethiopian wolf, the world's rarest canid, occupies a small range in the Ethiopian Highlands and has suffered severe impacts from rabies virus, presumably introduced from the local domestic dog population in which rabies is prevalent (63). Despite the substantial investment of resources and the successful vaccination of approximately 70% of domestic dogs surrounding the Ethiopian wolf populations in the Bale Mountains, the endangered wolf was not protected, and a subsequent rabies outbreak resulted in significant mortality (53). Fortunately, the outbreak was halted by emergency intervention through direct vaccination of the Ethiopian wolves at the front wave of the emerging epidemic. Why did this vaccine programme targeting the reservoir fail? It is likely that coverage of 70% of the domestic dog population was not enough; migrant populations of people and domestic dogs could not be reached by the community-based vaccine programme; or rabies was introduced by one of the many other possible rabies reservoirs occupying the same habitat – in short: scale.

If programmes targeting density-dependent wildlife diseases are successful, unintended consequences in ecological communities may occur. For example, predation by red foxes (*Vulpes vulpes*) was identified as one major factor limiting the annual numbers of coastal water bird breeding pairs on two islands in the Wismar Bay of the western German Baltic Sea coast (37). Increased predation upon birds after the 1990s was attributed to an increase in overall fox density after the regional introduction of ORV led to a fall in the number of rabies mortalities among foxes (37). The decrease in the bird population was believed to be exacerbated by other concomitant effects, such as seasonal food shortages for waterfowl. Similarly, improved survivorship of certain taxa (e.g. carnivores) could be associated with an enhanced parasite burden (for example, *Echinococcus*) at the local population level, with consequent public health, veterinary, or environmental repercussions (23).

Vaccination of free-ranging animals is also likely to involve considerable regulatory oversight, particularly with any modified-live or recombinant organisms that are delivered to the environment. For example, in the USA, compliance with the National Environmental Policy Act of 1969 (NEPA) is a legal requirement for 'federal actions' (i.e. actions involving federal funding or personnel) such as ORV within the USA and its territories and possessions. The legal authorisation to conduct ORV can also require compliance with additional state or federal environmental statutes (e.g. the United States Endangered Species Act of 1973). After projects have been initiated, NEPA requires

annual monitoring to determine the current status of programme impacts on the environment and the effectiveness of mitigation measures to reduce adverse impacts.

There are specific circumstances in which vaccine usage in wildlife could be practical and effective, e.g. small populations can be protected from extinction risk due to infectious disease when a safe and efficacious vaccine is available. The use of such a vaccine is most successful in animals in captivity, such as those in zoological institutions and captive breeding facilities. In addition, endangered species recovery programmes in the wild may employ vaccines to attempt to protect all individuals, a critical mass of a population, or a portion of a population residing in a high-risk area (32). Even in these situations, careful consideration should be given to the attempted elimination of an organism from an ecosystem. Is the pathogen playing a role that will prove critical to the functioning of the ecosystem? Usually the desire to recover an endangered vertebrate population outweighs concerns for biodiversity at the microorganismal level, but should it? In most cases the point is moot since total pathogen elimination is unlikely given imperfect vaccines and delivery systems, but what will happen to the wildlife population once a vaccination programme is terminated? The goal of endangered species recovery programmes is the return of populations to viable levels. This return to viability usually coincides with reduction in intervention and intensive management. If a vaccine programme has aided recovery but also helped to develop a wildlife population naïve to the pathogen that put it at risk, the population may ultimately decline again if management by vaccination is ceased.

Since citizens and policy-makers demand action on public health and economic issues, vaccination programmes should be considered as a potential line of defence for infectious diseases. In these situations it is important to carefully consider the scale of the vaccine programme to be developed, feasibility, costs, and likely outcomes and benefits (17). While vaccination programmes may be successful if carefully planned, especially in restricted areas and small populations of wildlife, a more common outcome may be the improvement of the public's perception of the handling of the problem. Many times, the most scientifically justifiable and cost-effective vaccine programmes will be aimed at the people or domestic animals which are in need of protection, but social concerns may make these targets unpalatable. In these situations, a combination of pathogen management strategies and public education will be necessary to balance conservation, economic, and public health issues.

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La vaccination et la médecine environnementale

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Résumé

Du fait de la croissance démographique et de changements environnementaux d'origine anthropique sans précédent, un nombre toujours plus grand d'individus vit aujourd'hui en contact étroit avec des animaux (sauvages, domestiques ou péri-domestiques) que par le passé. L'étroite interconnexion entre la santé humaine et la santé animale n'est pas un phénomène nouveau. En revanche, la dimension mondiale des zoonoses contemporaines n'a pas de précédent dans l'histoire. De fait, la plupart des maladies infectieuses classées comme émergentes sont des zoonoses et un grand nombre d'entre elles se sont propagées à l'homme à partir d'un réservoir naturel sauvage, soit directement, soit par l'intermédiaire d'animaux domestiques ou péri-domestiques. La médecine environnementale (*conservation medicine*) a récemment vu le jour en

tant que véritable discipline à l'interface de la santé animale, de la santé publique et de la santé de l'écosystème. L'innovation en matière de vaccins destinés à la faune sauvage se heurte à des enjeux importants susceptibles de freiner le développement de ces vaccins au-delà de la phase de conception. Malgré des exemples remarquables de programmes d'immunisation appliqués avec succès à la faune sauvage, sur le terrain la vaccination s'avère plus ou moins efficace suivant les situations. Une fois mise en route, la vaccination des animaux sauvages doit s'accompagner de stratégies nouvelles de gestion des agents de zoonoses et de mesures pédagogiques destinées au public, afin de trouver un équilibre entre les exigences de la protection de la nature, celles de l'économie et celles de la santé publique.

Mots-clés
Brucellose – Distribution de vaccin – Espèce menacée d'extinction – Faune sauvage – Maladie émergente – Médecine environnementale – Rage – Santé publique – Vaccination – Zoonose.

La vacunación en medicina de la conservación

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Resumen
El crecimiento sin precedentes de la población humana, aunado a los cambios ambientales de origen antrópico, ha hecho que un mayor número de personas vivan en más estrecho contacto con más animales (salvajes, domésticos o peridomésticos) que en ningún otro periodo de la historia. La íntima relación entre la salud humana y la animal dista de ser un nuevo fenómeno. Sin embargo, lo que no tiene precedente histórico es el alcance planetario de las zoonosis contemporáneas. La mayoría de las enfermedades infecciosas del hombre catalogadas como emergentes son en efecto zoonóticas, y muchas de ellas han saltado al ser humano desde un reservorio salvaje natural, ya sea directamente o a través de animales domésticos o peridomésticos. En este sentido, la medicina de la conservación, aparecida en fechas recientes, constituye una disciplina muy apropiada para trabajar en la intersección entre la salud humana, la animal y la ecosistémica. El interés por obtener nuevas vacunas para la fauna salvaje tropieza con una serie de notables dificultades que quizá impidan pasar de la fase meramente teórica. Aunque no faltan ejemplos de programas de inmunización de animales salvajes que han dado buenos resultados, hay una serie de consideraciones básicas de las que depende la eficacia de una vacunación sobre el terreno. Para llevar a cabo una campaña de vacunación de animales salvajes es indispensable combinar estrategias de control del agente etiológico de una nueva zoonosis con una labor de pedagogía pública, a fin de alcanzar un correcto equilibrio entre las cuestiones de conservación, las económicas y las de salud pública.

Palabras clave
Administración de vacunas – Brucelosis – Enfermedad emergente – Especie amenazada – Fauna salvaje – Medicina de la conservación – Rabia – Salud pública – Vacunación – Zoonosis.

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Is vaccination against transmissible spongiform encephalopathy feasible?

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Summary

Prion diseases are a unique category of illness, affecting both animals and humans, where the underlying pathogenesis is related to a conformation change of the cellular form of a normal, self-protein called a prion protein (PrP^{C} [C for cellular]) to a pathological and infectious conformation known as scrapie form (PrP^{Sc} [Sc for scrapie]). Currently, all prion diseases are without effective treatment and are universally fatal. The emergence of bovine spongiform encephalopathy and variant Creutzfeldt-Jakob disease has highlighted the need to develop possible therapies. In Alzheimer's disease (AD), which has similarities to prion diseases, both passive and active immunisation have been shown to be highly effective at preventing disease and cognitive deficits in model animals. In a human trial of active vaccination in AD, despite indications of cognitive benefits in patients with an adequate humoral response, 6% of patients developed significant complications related to excessive cell-mediated immunity. This experience highlights that immunotherapies designed to be directed against a self-antigen have to finely balance an effective humoral immune response with potential autoimmune toxicity. Many prion diseases have the gut as a portal of infectious agent entry. This makes mucosal immunisation a potentially very attractive method to partially or completely prevent prion entry across the gut barrier and to also produce a modulated immune response that is unlikely to be associated with any toxicity. The authors' recent results using an attenuated *Salmonella* vaccine strain expressing the prion protein show that mucosal vaccination can partially protect against prion infection from a peripheral source, suggesting the feasibility of this approach.

Keywords

Bovine spongiform encephalopathy – Chronic wasting disease – Conformational disorder – Mucosal vaccine – Prion – Salmonella – Transmissible spongiform encephalopathy – Variant Creutzfeldt-Jakob disease.

Introduction

Prion disease occurs both in humans and in various animals such as cows, sheep, goats, mink, deer and elk. These diseases are also known as transmissible spongiform encephalopathies or prionoses. They are a unique category of illness in that they can be infectious or transmitted genetically and are sporadic in occurrence. Abundant evidence has made it clear that these slow infections are neither caused by a virus nor any nucleic acid containing particle. A comprehensive body of evidence has presented compelling data that the transmissible pathogen for these diseases is a proteinaceous infectious particle (hence the term 'prion') (37, 38). All prion diseases result from a conformational alteration of the same host-derived prion protein (PrP^{C} [C for cellular]) to a disease-associated conformer called PrP^{Sc} (Sc for scrapie). This conversion can be precipitated by an exogenous, infectious source of PrP^{Sc}, a mutation in the prion protein that predisposes to such a conformational change, or a spontaneous conformational change, as occurs in sporadic prion disease.

The human forms are kuru, Creutzfeldt-Jakob disease (CJD), Gerstmann-Sträussler-Scheinker disease (GSS) and fatal familial insomnia. In animals these diseases include bovine spongiform encephalopathy (BSE) in cattle, scrapie in sheep and goats, chronic wasting disease (CWD) in deer and elk and transmissible mink encephalopathy (42). Neuropathologically, these different forms of the disease are all characterised by spongiform change, neuronal loss and astrocytosis; in addition amyloid deposition may occur. However, the regional pattern of brain lesions and the extent of prion amyloid deposition vary within and between species. Within species, these differences depend on the strain of prion causing the infection. A barrier exists limiting transmission of prions across species, but once this barrier is overcome a new, stable and distinct pattern of infection can develop in the new host species.

Bovine spongiform encephalopathy, variant Creutzfeldt-Jakob disease and chronic wasting disease

Interest in prion disease has greatly increased since the emergence of BSE in the United Kingdom (UK) and the resulting appearance of variant CJD (vCJD) in human populations. Bovine spongiform encephalopathy arose from the feeding of cattle with prion-contaminated meat and bone meal products, while vCJD developed following entry of BSE into the human food chain (8). Since the original report in 1996 (60) a total of 182 confirmed cases of vCJD have been diagnosed, 156 in the UK, 17 in France, 3 cases in Ireland and one each in Italy, Canada, Japan, the Netherlands, Saudi Arabia and the United States of America (USA). The patients from these countries resided in the UK during a key exposure period of the population to the BSE agent. It has been difficult to predict the expected future numbers of vCJD. Mathematical analysis has predicted that between 1,000 and 136,000 individuals will eventually develop the disease. This broad range reflects a lack of knowledge regarding the time of incubation and the number of patients who could be infected from a given dosage of BSE agent. Because the vCJD agent is present at high levels in the lymphatic tissue, screening for PrP^{Sc} was performed on sections from lymph nodes, tonsils, and appendices taken from archives in the UK. Three out of 12,674 randomly selected samples showed evidence of subclinical infection, leading to a prediction that about 4,000 further cases of vCJD may occur in the UK. However, there is much uncertainty about such a predication, as it is not known if all subclinical infections will progress or whether such screening of lymphoid tissue would capture all subclinical cases. The initially predicted epidemic of vCJD does not seem to be materializing, as the number of cases in the UK has declined from a peak of 28 in 2000 to 17 in 2002, with only 5 cases in 2005 (8). A complicating factor for estimating future numbers of vCJD is the occurrence of several transfusion-associated cases. These occurred after incubation periods of 6 to 8 years. One of these disease-associated donations was made more than 3 years before the donor became symptomatic, suggesting that vCJD can be transmitted from silently infected individuals (11). The estimated risk for new cases of vCJD in other European countries is much lower. In the UK, 200,000 cases of BSE were reported (it is estimated that four times this number entered the food chain), compared to a combined total of approximately 500 BSE cases in other European countries. This suggests a significantly lower exposure of these populations to BSE prions. A few cases of BSE have also been reported in other parts of the world, such as Japan, the USA and Canada.

Of greater concern in North America is CWD. This disease is now endemic in Colorado, Wyoming and Nebraska and continues to spread to other parts of the USA. Cases have been reported in the Midwest and it has now been detected as far east as New York State (61). Most vulnerable to CWD infection are white-tailed deer, and the disease is now found in areas with large populations of these animals, which indicates that its prevalence can be expected to increase substantially in the future. Occurrence of CJD among three young deer hunters raised speculation that CWD could be transmitted to humans (7), but autopsy of these three subjects did not show the extensive amyloidosis characteristic of vCJD and CWD (25). However, like BSE, CWD is transmissible to non-human

primates and transgenic mice expressing human PrP^C (41, 54, 58). Therefore, the possibility of such transmission needs to be closely monitored. Chronic wasting disease is similar to BSE in that the peripheral titres of the prion agent are high. PrP^{Sc} has been detected in both the muscle and saliva of CWD-infected deer (1, 30).

Biology of the prion protein

PrP^C is expressed in many types of cells; however, the highest level of expression is found in central nervous system (CNS) neurons (21, 24). A knowledge of the molecular anatomy of PrP^C is crucial for understanding its malfunction in prion diseases. The whole protein is located on the outer surface of the cell anchored to the cell membrane by phosphotidylinositol glycolipid (GPI) attached to its C-terminus. The central portion of the peptide contains one short α-helical segment (α-helix A) flanked by two short β-strands. The N-terminus is unstructured and extends into the intracellular space. The N-terminus harbours five octapeptide repeats. Histidines located within the octapeptides bind copper ions (9). It has been postulated recently that the possible function of PrP^C is to capture, store, and present copper to the neuron (9, 39, 40). The copper binding state of PrP^C influences its conformation and copper chelation has been shown to inhibit PrP^{Sc} infection (48). The exact function of PrP^C remains to be elucidated. The protein is not essential since Prnp knock-out mice (12) did not show a significant disease phenotype. Minor abnormalities in synaptic physiology (14) and in circadian rhythm (55) have been described in these knock-out mice.

Prion diseases and other conformational disorders

The prion diseases belong to a broader category of conformational diseases (43). The etiology of each of the conformational diseases is related to a specific protein that can exist in at least two distinct forms associated with either health or disease. The most common conformational disorder is Alzheimer's disease (AD), in which the disease state is associated with the accumulation of an endogenously expressed peptide, the amyloid-β peptide, in a β-sheet structure within neuritic plaques. Other conformational disorders include Parkinson's and Huntington's diseases. The pathological conformer of PrP^C is PrP^{Sc}, which due to its increased β-sheet content demonstrates increased resistance to proteolysis and the ability to aggregate and polymerize. Although the insolubility of PrP^{Sc} has prevented crystallographic conformational studies, less exact structural methods such as circular dichroism and Fourier transform infrared spectroscopy indicate a β-sheet content as high as 45% (compared with 3% in PrP^C) and a α-helix content of 30% (40% in PrP^C) (3).

Understanding the mechanism that converts PrP^C into PrP^{Sc} is another intriguing aspect of prion diseases. One of the most crucial features of PrP^{Sc} is its ability to bind to PrP^C: this initiates a self-perpetuating vicious cycle and enables prion diseases to be transmitted (38). It has been demonstrated in cellular models that the PrP is transported to the membrane in the PrP^C form and that the conversion of PrP^C to PrP^{Sc} occurs at the cell surface. Neurons produce native PrP^C (24) and transport it to the cellular surface where it can encounter PrP^{Sc}, leading to its conformational change into a high β-sheet content state. During progression of the disease, the amount of PrP^C produced remains stable, whereas the amount of PrP^{Sc} increases. The homozygosity of PrP^C facilitates prion replication. This has been observed in humans with respect to the codon 129 polymorphism, as well as in sheep with respect to the VRQ/VRQ polymorphisms. Evidence from transgenic animals expressing various segments of PrP^C indicates that residues 90-150 are required for the interaction with PrP^{Sc} leading to conversion of PrP^C into PrP^{Sc}. The spontaneous conversion of PrP^C into PrP^{Sc} has been demonstrated in sheep and probably is the major cause of scrapie and sporadic CJD.

The immune system and prion infection

The prion protein is a self-antigen; hence, prion infection is not known to elicit a classical immune response. In fact, the immune system is involved in the peripheral replication of the prion agent and its ultimate access to the CNS (4, 50). Paradoxically, immune suppression with, for example, splenectomy or immunosuppressive drugs, increases the incubation period. This incubation period, during which time the prion agent replicates peripherally without producing any symptoms, is quite long, lasting many months in experimental animals and up to 56 years in documented human cases associated with cannibalistic exposure to the prion agent (15). Lymphatic organs such as the spleen, tonsil, lymph nodes or gut-associated lymphoid tissue (GALT) contain high concentrations of PrP^{Sc} long before PrP^{Sc} replication starts in the brain (10, 26). Cells found to be particularly important for peripheral PrP^{Sc} replication are the follicular dendritic cells and the migratory bone-marrow derived dendritic cells (5, 26). Dendritic cells from infected animals are capable of spreading the disease (5). An emerging therapeutic approach for prion infection is immunomodulation (44, 50).

Vaccination for prion infection

Currently there is no treatment that would arrest and/or reverse progression of prion disease in non-experimental settings, although many approaches have been tried (56). In AD model mice it has been definitively shown that immunotherapy can prevent the onset of cognitive deficits and the development of amyloid lesions (31, 63). Significantly, this method of treatment is associated with consistent cognitive benefits in the mice (2, 20, 32, 49). An antibody-mediated response is probably critical for a therapeutic response, since similar results have been obtained with passive immunisation (6). Active immunisation for AD has recently been tried in humans by Elan Pharmaceuticals, with significant toxicity resulting from the vaccine (18, 62, 63). In the human phase 2A clinical trial of the vaccine (called AN-1792) 18 out of 372 patients worldwide developed symptoms of meningitis or meningoencephalitis, with symptoms apparently responding to immunosuppression in most patients (12 patients out of the 18 responded fully) (18). Recent evidence suggests that patients who developed anti-Aβ titres benefited cognitively from vaccination, including patients among the 12 that initially had complications (18, 19) and that vaccination resulted in amyloid clearance as judged by three autopsies performed in vaccinated patients (two autopsies from patients with encephalitis and one without complications) (17, 28, 33). Hence, it appears that if safety issues can be addressed, a vaccine approach will prove to have important therapeutic value in patients (58, 63) and it is the subject of new ongoing trials.

In part because of this success in AD models, similar experiments with anti-PrP antibodies were initiated in prion infectivity culture models and active and passive immunisation studies were carried out in rodent models. Earlier *in vivo* studies had shown that infection with a slow strain of PrP^{Sc} blocked expression of a more virulent fast strain of PrP, mimicking vaccination with a live attenuated organism (27). In tissue culture studies anti-PrP antibodies and antigen binding fragments directed against PrP have been shown to inhibit prion replication (16, 34, 35). One study demonstrated that active immunisation with recombinant PrP delayed the onset of prion disease in mice, but the therapeutic effect was relatively modest and eventually all the mice succumbed to the disease (46). This limited therapeutic effect may be explained by the observation that antibodies generated against prokaryotic PrP often do not have a high affinity towards PrP^{C} (36), although in studies carried out by the authors the increase in the incubation period correlated well with the antibody titres against PrP^{C}. The follow-up passive anti-PrP immunisation study confirmed the importance of the humoral response, showing that anti-PrP antibodies are able to prolong the incubation period (47). Subsequently, other investigators, using a much higher antibody dosage, were able to completely prevent disease onset in mice exposed to PrP^{Sc}, provided passive immunisation was initiated within a month of exposure (59). This type of approach could be used immediately following accidental exposure in humans to prevent future infection. However, passive immunisation has not been found to be effective closer to the clinically symptomatic stages of prion infection. Moreover, passive immunisation would be too costly an approach for animal prion diseases.

In the development of immunotherapeutic approaches targeting a self-antigen, designing a vaccine avoiding autoimmune related toxicity is a major concern. The emerging data from AD-targeting immunisation is that toxicity is due to excessive cell-mediated immunity within the CNS, while the therapeutic response is linked to humoral immunity. In addition, toxicity could be partially related to the immunogen and/or to the adjuvant used; in the human AD vaccination trial fibrillar Aβ1-42 was used as an immunogen. This peptide is well known to be toxic. Hence, the authors have been promoting the use of nonamyloidogenic derivatives as immunogens for protein conformational disorders, including AD and prion diseases (45, 49, 63) and interestingly a recent study indicated that α-helical PrP elicited an antibody response whereas an amyloidgenic β-sheet form of PrP favored a cytotoxic T-cell response (51). How significant an issue direct toxicity of the immunogen may be for prion vaccination remains unclear. Unlike the amyloid β peptide used for vaccination in AD models, direct application of recombinant PrP has not been shown to be toxic. However, this issue has not been investigated as thoroughly as in the Alzheimer's field. One study has shown that cytosolic accumulation of PrP was toxic (52), whereas other investigators observed that PrP was neuroprotective in another cell culture model (22).

A potential ideal means of using immunomodulation to prevent prion infection is mucosal immunisation. One important reason for this is that the gut is the major route of entry for many prion diseases such as CWD, BSE and vCJD. Furthermore, mucosal immunisation can be designed to induce primarily a humoral immune response, avoiding the cell-mediated toxicity that was seen in the human AD vaccine trail. Recently, the authors have been developing prion vaccines that target gut-associated tissue, the main site of entry of the prion agent. One of their approaches is to express PrP in attenuated *Salmonella* strains as a live vector for oral vaccination. Live attenuated strains of *Salmonella enterica* have been used for many years as vaccines against salmonellosis and as a delivery system for the construction of multivalent vaccines, with broad applications in human and veterinary medicine (29). One of the main advantages of this system is that the safety of administering live attenuated *Salmonella* has been extensively confirmed in humans and animals (23, 53).

Ruminants and other veterinary species can be effectively immunised by the oral route using live *Salmonella*, to induce humoral mucosal responses (13, 57). The authors are currently exploring ways to increase the efficacy even further. In these studies, the mucosal IgA anti-PrP titre correlates well with the delay or prevention of prion infection, further supporting the importance of the humoral response for the therapeutic effect. *Salmonella* target M-cells, antigen sampling cells in the intestines, which may also be important for uptake of PrP^{Sc} (26, 50). Hence, this approach is more targeted than prior vaccination studies, which probably explains the improved efficacy. By exploring other strains of attenuated *Salmonella*, using different bacteria or oral adjuvants, and/or by altering the expression levels or sequence of the PrP antigen, it is likely that the percentage of uninfected animals can be improved. The authors' recent work utilising this approach indicates that complete protection to clinical prion infection via an oral route is possible. Overall, this approach holds great promise as an inexpensive prophylactic immunotherapy to prevent the spread of prion disease, particularly in animals at risk and perhaps eventually in certain high-risk human populations.

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La vaccination contre l'encéphalopathie spongiforme transmissible est-elle une option réaliste ?

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Résumé

Les maladies à prion constituent une catégorie unique de pathologies affectant aussi bien les animaux que l'homme et dont la pathogénèse est associée à une conversion de la protéine de l'hôte, appelée protéine prion, de sa forme cellulaire normale PrP^{C} (C pour cellulaire) en une conformation pathogène et infectieuse appelée PrP^{Sc} (Sc pour *scrapie*, tremblante en anglais). À l'heure actuelle, il n'existe aucun traitement efficace contre les maladies à prion, dont l'issue est toujours fatale. L'émergence de l'encéphalopathie spongiforme bovine et de la variante de la maladie de Creutzfeldt-Jakob exige la mise au point de nouveaux traitements. Dans des expérimentations portant sur la maladie d'Alzheimer (qui présente des similitudes avec les maladies à prion), l'immunisation passive et active s'est révélée efficace pour prévenir la maladie chez les animaux de laboratoire et pour limiter les troubles cognitifs qui en résultent. Lors d'une série d'essais de vaccination active contre la maladie d'Alzheimer chez l'homme, une amélioration des fonctions cognitives a été obtenue chez des patients présentant une bonne réponse humorale, mais 6 % des patients ont souffert de complications graves, liées à une réponse à médiation cellulaire trop importante. Cette expérience met en exergue la nécessité, dans le domaine des immunothérapies dirigées contre un antigène autologue, de parvenir à un difficile équilibre entre la recherche d'une immunité humorale et le souci d'éviter toute toxicité auto-immune. Pour de nombreuses maladies à prion, l'intestin est l'organe par où l'agent pathogène pénètre dans l'organisme. De ce fait, l'immunisation muqueuse est une méthode particulièrement prometteuse qui vise à empêcher totalement ou partiellement le prion de franchir la paroi intestinale tout en produisant une réponse immunitaire ciblée et exempte de toxicité. Les résultats obtenus par les auteurs

en utilisant une souche vaccinale atténuée de *Salmonella* exprimant la protéine prion montrent que la vaccination muqueuse confère une protection partielle contre l'infection à prion à partir d'une source périphérique, ce qui paraît confirmer la faisabilité de cette démarche.

Mots-clés
Cachexie chronique – Encéphalopathie spongiforme bovine – Encéphalopathie spongiforme transmissible – Immunisation mucosale – Prion – Salmonella – Trouble de la conformation – Variante de la maladie de Creutzfeldt-Jakob.

¿Es factible la vacunación contra la encefalopatía espongiforme transmisible?

T. Wisniewski, J.A. Chabalgoity & F. Goni

Resumen
Las enfermedades priónicas constituyen una singular categoría de dolencias que afectan tanto a los animales como al hombre y cuya patogénesis guarda relación con el cambio de conformación de una proteína del propio organismo, que pasa de la llamada forma celular (PrP^{C} [proteína priónica celular]) a una conformación patológica e infecciosa denominada forma priónica (PrP^{Sc} [en inglés, "*scrapie form*"]). En la actualidad no hay tratamiento eficaz para ninguna de esas enfermedades, que resultan invariablemente fatales. La aparición de la encefalopatía espongiforme bovina y de la variante de la enfermedad de Creutzfeldt-Jakob ha hecho más necesario que nunca encontrar posibles terapias. En el caso de la enfermedad de Alzheimer, que presenta similitudes con las afecciones priónicas, se ha demostrado que en modelos animales la inmunización tanto pasiva como activa resulta muy eficaz para prevenir la enfermedad y las consecuentes deficiencias cognitivas. En el curso de un ensayo de vacunación activa contra la enfermedad realizado en seres humanos, y pese a ciertos signos que indicaban beneficios cognitivos en pacientes con una buena respuesta humoral, se observaron importantes complicaciones ligadas a una respuesta excesiva de inmunidad celular en un 6% de los pacientes. Esa experiencia pone de manifiesto que las terapias inmunológicas dirigidas contra un autoantígeno deben hallar un delicado equilibrio entre la búsqueda de eficacia de la respuesta inmunitaria humoral y el riesgo de toxicidad autoinmune. En muchas enfermedades priónicas el intestino es la vía de entrada del agente infeccioso, lo que hace de la inmunización de las mucosas un método en potencia muy atractivo para prevenir, parcial o totalmente, la penetración de un prión a través de la barrera intestinal y también para inducir una respuesta inmunitaria modulada poco susceptible de generar toxicidad. Los resultados obtenidos recientemente por los autores (con una cepa vacunal de salmonelas atenuadas que expresan la proteína priónica) demuestran que la inmunización de las mucosas puede conferir protección parcial contra las infecciones priónicas procedentes de una fuente periférica, lo que lleva a suponer que se trata de un método viable.

Palabras clave
Anomalía de conformación – Caquexia crónica – Encefalopatía espongiforme bovina – Encefalopatía espongiforme transmisible – Inmunización de mucosas – Prión – Salmonella – Variante de la enfermedad de Creutzfeldt-Jakob.

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Mass vaccination and herd immunity: cattle and buffalo

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Summary
The design of effective programmes for emergency response to incursion of epizootic diseases of cattle, for exclusion of such diseases and for implementation of progressive control in enzootic situations leading to eventual virus elimination, is currently largely empirical. This needs to be remedied to provide more cost-effective use of vaccines and more effective control. At population level, protective effects of immunisation can extend well beyond the individual, influencing the dynamics of viral propagation within the whole population, non-vaccinated as well as vaccinated. This concept of herd immunity and application of the resulting epidemiological principles, combined with experience gained from disease control programmes such as the Global Rinderpest Eradication Programme has much to offer in designing effective science-based control programmes. This paper explores practical exploitation of the herd immunity principle by considering some of the factors which militate against mass vaccination achieving effective levels of herd immunity and, with these in mind, suggesting ways to optimise the efficiency of mass vaccination programmes.

Keywords
Cattle – Epizootic disease – Herd immunity – Vaccination.

Introduction

For centuries, even millennia, livestock keepers have conducted immunisation programmes, with some degree of success in protecting their livestock against the contagious diseases which periodically confounded the livestock production systems essential for their well-being. Examples include the use of infectious urine from calves and lesser kudus (*Tragelaphus imberbis*) infected with mild strains to immunise cattle against rinderpest in Ethiopia and eastern Africa, respectively (16); and the subcutaneous implantation of infected lung tissue to immunise cattle against contagious bovine pleuropneumonia. With the increasing availability of a broad spectrum of vaccines in the 20th Century there arose an understanding that the occurrence of disease epizootics needs to be matched by mass vaccination programmes, as if infectious disease epizootics result solely from a failure to vaccinate enough animals. The use of mass vaccination campaigns has come to be seen as virtually synonymous with infectious disease control, particularly in developing countries, even though some of the major gains in disease elimination were achieved in Europe and Asia by the stringent application of zoosanitary procedures, including culling, before vaccines became available; contagious bovine pleuropneumonia (CBPP) and rinderpest are notable examples. However, non-vaccine control methods call for a measure of self-discipline that may not always be forthcoming.

It is manifest that vaccines exert their protective effect primarily by inducing an immune response in the vaccinated animal, yet it has been observed that protective effects of immunisation can extend well beyond the individual, influencing the dynamics of viral propagation within the whole population, non-vaccinated as well as vaccinated. The resulting 'herd immunity' concept was

explored in detail by mathematical modelling in seminal work of the 1980s (2, 3). The fundamental issue is that it is not necessary to immunise every individual within a population to be able to eliminate an infectious agent from, or prevent its entry into, that population; the level of herd immunity must simply be sufficient to reduce the susceptible sector of the population below a critical point of population density.

This paper sets out to explore the practical exploitation of the herd immunity principle by considering some of the factors which militate against mass vaccination achieving effective levels of herd immunity, and with these in mind suggesting means of optimising the efficacy of mass vaccination programmes.

The application of mass vaccination

In attempting to control disease, whether enzootic or epizootic, caused by an infectious agent we are essentially attempting to interrupt the sustained transmission chain from infected host to susceptible host. Logically we can do this by removing either the excretor or the recipient. This principle applies equally to the human and veterinary fields, although the methods of implementation often differ. In dealing with the excretor side of the relationship, old-fashioned fever hospitals were designed to limit the number of contacts available to an infected person; in modern medicine barrier nursing achieves the same result. In the farming world we cannot support such measures and we are prepared at times to destroy our virus excretors, together with many uninfected contacts, in order to break the chain; pictures of piles of burning cattle slaughtered in attempts to limit the excretion of foot and mouth disease-virus are etched on many minds. The emotive welfare issue is increasingly making slaughter-based control socially unacceptable, thus removing from the veterinary armament one of its most effective tools. Inevitably, greater reliance is being placed on the use of vaccines. An economic dimension is also evident as farming is about profitability, either to the individual or to the nation, and as slaughtering can incur massive costs which include the payment of compensation, it follows that a benefit–cost analysis must lie behind decisions made, not just emotive public debate.

Dealing with the recipient side, mass vaccination is also the main tool applied, but with varying degrees of success, depending on the extent to which zoosanitary procedures are also implemented (e.g. movement management and quarantine). One scenario to look at is what might be termed the long, slow approach. This stems from the classic situation in which, at the outset, the weight of infection is so great that a slaughter policy (i) might not work, and (ii) would be too costly to contemplate. Vaccination is then utilised to gradually reduce the weight of infection until either eradication becomes an inevitable consequence, or a terminal slaughter policy becomes an economically attractive alternative. The eradication of FMD from post-war Europe is a vindication of this gradualist approach, and after 30 years' vaccination the way was opened for a declaration of freedom from infection. Success is also testimony to the existence of a public/private sector Veterinary Services alliance able to mount and sustain an efficient, systematic vaccination programme.

Vaccines are also used where insect vectors rule out the use of zoosanitary measures and where short campaigns are expected to be successful because of climatic constraints on vector activity. The limitation and eradication of bluetongue virus type 4 from western Turkey in 1979 and 1980 by two rounds of mass vaccination is a case in point. Vaccine has been less successfully applied to the recent upsurge of multi-serotype bluetongue virus infections in the Mediterranean basin and extending northwards from it. Climate change may be profoundly altering vector–virus–host interactions by extending the season of vector transmission and facilitating overwintering. Inevitably this will impact on vaccination strategies. It merits emphasis that in South Africa, where bluetongue is endemic, the sheep industry (which uses improved breeds) can only exist under a permanent bluetongue vaccine umbrella.

Factors influencing the effectiveness of mass vaccination programmes and optimising their efficiency

'Blitz' vaccination or 'immunosterilisation'

'Blitz' vaccination, whereby a whole population of animals is vaccinated within a very short space of time, can be dramatically successful. Applied to all cattle and buffalo in dairy colonies around Baghdad and the Southern and Central Governorates of Iraq in 1994, three campaigns over a three-month period totally eliminated rinderpest infection, which had persisted for several years despite more casual immunisation programmes. Similar results were achieved in northern Tanzania in 1997-1998, where intense mass vaccination, termed 'immunosterilisation' (22), rapidly eliminated an incursion of rinderpest into the Maasai steppe. Most recently, a single round of rinderpest vaccination of the herds belonging to the Murle and Jie

peoples of southern Sudan eliminated the last reservoir of lineage 1 rinderpest virus in Africa (16). Intensive focal vaccination proved highly effective in eliminating an incursion of type A FMD into Albania in 1996 (8). In all these cases perhaps the decisive factor underpinning success was that these were highly risk-focused vaccination campaigns.

Vaccine quality assurance

It goes without saying that only fully efficacious vaccines should be used in vaccination programmes, yet, in order to attempt to spare the inadequate resources available for vaccination programmes to control serious diseases, it is not uncommon for developing countries to accept vaccines, knowingly or unwittingly, from suppliers who do not have a high reputation for sustaining quality. This highlights the need for independent quality assurance laboratories. The effect that the availability of such a laboratory can have has been very clearly demonstrated in the case of rinderpest, where the work of the Food and Agriculture Organization/Interafrican Bureau for Animal Resources Pan African Veterinary Vaccine Centre was accompanied by a doubling of the acceptability rate of vaccine batches to virtually 100% over five years (15, 23). When combined with the enforcement of internationally coordinated national regulations, which stated that only accredited vaccines should be used for the Pan African Rinderpest Control Programme, this service undoubtedly contributed to the success of rinderpest control programmes. This was so not only in Africa: it is now clear that the existence of an independent quality assurance laboratory was a decisive factor in elimination of rinderpest from Pakistan, where transfer of improved rinderpest vaccine production technology and quality assurance processes improved performance of control programmes there as from 1995; within five years rinderpest virus had been eliminated (16). Vaccination programmes benefited not only directly from the provision of quality-assured vaccines with ensured immunogenicity but also from the renewed faith of farmers in the protective effect of rinderpest vaccines. International and national efforts to bring about progressive control of FMD in Asia lack an independent quality assurance mechanism and this constrains acceptance of vaccine from local suppliers who might produce effective products but lack a means of demonstrating their efficacy.

Arguably the appropriateness of vaccines selected for use should be considered here. It is not uncommon for FMD vaccines not to be matched adequately to the antigenic determinants of the field strains addressed; in extreme cases they might not even be of the serotype required. Clearly, vaccination programmes using such vaccines will be compromised.

Vaccine formulation

Robustness of vaccines can significantly impact on the efficiency of vaccination programmes, especially in tropical developing countries. Ideally one requires a vaccine with enhanced thermostability to reduce dependence on cold chains. The thermostable rinderpest vaccine formulation used in pastoral areas of Africa is believed to have been an important factor in achieving success. The lack of thermostability after reconstitution, however, is probably one factor responsible for reducing the effectiveness of vaccine programmes; discarding vaccine within a working day rarely seems to be an acceptable procedure for vaccination teams or administrators.

Vaccination procedures

Provision of written standard operating procedures and training in their use is essential. It is common to detect serious malpractices when monitoring vaccination practice in the field, which result from inadequate training of vaccination staff. These include the use of uncooled boiled water for reconstituting rinderpest and CBPP vaccines; the use of water rather than saline to reconstitute rinderpest vaccine; the use of hot syringes to draw up vaccine; the use of incorrectly calibrated syringes; retaining reconstituted vaccine for much longer than its effective retention time; transporting vaccine at ambient temperatures or even in sun-heated cars from office refrigerator to field; and lack of cold chain during importation and from central storage to field units.

Many factors interact to reduce the immunising efficiency of vaccination programmes. Even a single effective round of vaccination can be expected to result in an overall immunity level of only 70%, with another round being necessary to achieve 90% (18, 22).

Vaccine delivery systems and the veterinarian–farmer interface

In the extensive pastoral context, such as prevails in much of Africa and Asia, disease control and eradication vaccination programmes are frequently implemented by standing armies of government animal health technicians. The results may be far from optimal for many reasons. One of these is a lack of appreciation of the needs of the livestock owners in terms of seasonal migrations and demands on their time for activities such as ploughing and harvesting of crops. A poor veterinarian–farmer interface can easily result in the poor timing of vaccination campaigns and inappropriate placement of vaccination teams. The result is poor performance and low herd immunity. Community-based approaches have been highly effective in correcting these problems in Africa and in accessing remote and even war-torn areas of countries (9).

Another much neglected resource is that of the private practitioner who can be activated through national veterinary associations and contracted to perform services for the animal health authorities. This can be one way to extend the time period of vaccine availability rather than running only strictly confined pulsed vaccination campaigns.

The participation of livestock in control programmes and campaigns is constrained in developing countries by government monopolies in terms of supplies, delivery systems and logistical arrangements. More flexible arrangements in which farmer, private sector veterinarian and trader organisations participate and contribute, even financially, are called for – an approach which has yielded dividends in FMD control in Latin America.

In developing countries, livestock owners, though possessing considerable knowledge of the diseases affecting their livestock, are often not sufficiently informed of the reasons why they are requested by government authorities to present their cattle for vaccination. For example, in Cambodia in the late 1990s FMD vaccination was discredited when buffalo later died from haemorrhagic septicaemia; their owners had not been made aware that their buffalo had been vaccinated specifically for FMD rather than generically for 'serious disease'. Subsequently they were reluctant to participate in FMD vaccination programmes, a reluctance contributed to by changing policies of cost recovery. In some years vaccines were given free of charge by non-governmental organisations (NGOs) replete with funds, and in other years other NGOs and government tried to implement a more realistic cost-sharing programme. Livestock owners did not object *per se* to paying for vaccination but were confused by changing policy; once accustomed to receiving vaccine free of charge, they were understandably reluctant to see why they should start paying. A consistent and area-coordinated policy is a clear prerequisite as is sincere dialogue with livestock owners which reflects reality.

In the traditional pastoralist area of the Afar region of Ethiopia, under the Joint Project 15 rinderpest control programme of the 1970s, teams used to attempt to vaccinate all cattle in a herd annually despite the fact that livestock owners were reluctant to vaccinate cattle over two years of age because they knew that they would be protected by earlier campaigns (and probably from field infection). In addition, attempting to restrain older cattle for vaccination in extreme conditions wasted enormous effort and alienated cattle owners. Insistence on vaccinating all cattle irrespective of age led to poor cooperation and a failed vaccination programme.

Compromised immune responsiveness to vaccines

For a number of reasons it is not safe to assume that animals given an appropriate course of an efficacious vaccine will be rendered immune. Immune competence is an increasingly important issue that can be compromised by a number of factors. Just as in poultry where it is widely recognised that immune responses are severely compromised by a plethora of physiological, genetic, infectious and toxic agencies in industrialised production systems (5), similar factors might now be compromising immune responses of cattle in feedlots (Fig. 1) and dairy farming systems and swine in intensive fattening units (20, 21). This is not occurring just in developed countries; highly stressful industrialised production systems are on the rise in developing countries as well. In Pakistan, for example, the mixed buffalo and cattle dairy colonies around Karachi, where throughput exceeds 500,000 lactating cows per year, combine high density with poor hygiene, climatic stress and extensive use of bovine growth hormone. One result is a high prevalence of pneumonic pasteurellosis (not typical haemorrhagic septicaemia) which is not controlled by vaccines that are normally reliable (1). In comparison with the poultry industry, little is known of intensive cattle production systems in this respect, but the reduction of immune competence which accompanies selection for production traits could well be an important factor in disease occurrence in cattle production systems in future (5).

A related issue is that industrial-type intensive production systems developed for the production of milk in the Kingdom of Saudi Arabia, combined with an increasing exposure to a multiplicity of FMD virus serotypes, have created a situation where it no longer seems possible to achieve exclusion of FMD by vaccination (26).

Even in extensive production systems, such as the transhumant cattle systems of sub-Saharan Africa, immunosuppression can play a significant role in reducing the efficiency of vaccination programmes. For example, chronic trypanosomosis has been shown experimentally to suppress the immune response of cattle to bacterial and viral vaccines (6, 19). Although the extent to which this impacts at field level has not been defined, it is more than a hypothetical possibility. Chronic trypanosomosis is prevalent in sub-Saharan Africa and, at least in certain localities, a significant proportion of cattle could be expected to be immunosuppressed for this reason – possibly another factor contributing to poor performance of vaccination campaigns. Elsewhere in Asia, prevalent, but largely unrecognised, *Trypanosoma evansi* infections of buffalo and cattle might exert the same effect. Malnutrition, if only seasonal, must also surely contribute to sub-optimal response to vaccines.

Fig. 1
A cattle feedlot for imported cattle in the Philippines (1994)

Maternally derived antibodies can significantly reduce the efficacy of control by mass vaccination. In the case of rinderpest, where such antibodies persist for up to a year, annual vaccination programmes leave a significant proportion of calves vaccinated under one year of age vulnerable to infection for up to a year. Conversely, the absence of colostrum feeding and, therefore, the lack of protection by maternal antibodies, rendered Holstein calves from dairy units entering feedlots in the Kingdom of Saudi Arabia in the early 1990s highly susceptible to rinderpest virus infection, temporarily enzootic at that time. A sustained transmission chain, which showed signs of persisting, was established within contiguous fattening units. Withholding colostrum completely, whatever other detrimental effects resulted, enabled vaccination in the first week of life to render calves immune by 10 days of age when moved to the new premises. The transmission chain was broken and rinderpest was eliminated.

Dangers associated with mass vaccination

Apart from the dangers associated with sub-optimal population immunity aiding viral persistence, as discussed elsewhere, the actual process of mounting a vaccination programme carries with it inherent dangers. There are many reasons why vaccination should be used only when there is no other choice; these include:

a) Vaccines can be a source of adventitious agents and their use can have serious effects. Rinderpest vaccines have been known to be contaminated with virulent rinderpest virus and FMD virus, and CBPP vaccines have contained virulent *Mycoplasma mycoides*. Although usually not documented for commercial reasons, examples abound.

b) Used without a full understanding of epidemiology, attenuated vaccines can have serious and unexpected effects. One of the best examples is that of bovine virus diarrhoea (BVD) virus vaccines in cattle, where so-called attenuated vaccines actually contain 'normal' BVD virus. While causing no observable effects in calves, as is normal for BVD virus, when injected into pregnant cattle this virus can cause the full panoply of fetopathic effects (14).

c) The use of live virus vaccines is attended by risk because they may retain the capacity to cause the disease they are designed to prevent, as documented for live attenuated FMD vaccines in Latin America in the 1970s (13) or they can revert to virulence. This was almost certainly the cause of rinderpest outbreaks in the vaccination buffer zone that was maintained along the borders of Russia until a decade ago (16).

d) The process of assembling large numbers of cattle at vaccination points, as commonly occurs in developing countries, provides an opportunity for transmission of an agent should it be present within the population. Foot and mouth disease, and indeed rinderpest itself, have occasionally been spread in this way during rinderpest vaccination programmes.

e) Attending and investigating veterinarians, traditional healers and vaccinators can and do spread infectious agents by moving from farm to farm. There are many descriptions of such events in pigs and poultry but far fewer in cattle, although anaplasmosis and enzootic bovine leucosis are well-known examples of this phenomenon.

The design and assessment of vaccination programmes

Some epidemiological principles relating to vaccination

Seminal work by Anderson and May (2, 3, 4) initiated development of a body of knowledge relating mathematical theory and field epidemiological information, in the process creating a set of powerful tools for use in designing and evaluating disease control and eradication programmes. Describing the dynamics of infectious diseases in vaccinated populations greatly increases our understanding of how vaccine influences the epidemiology of infectious disease (25).

Theory, borne out in practice, points to the importance of the optimal age for vaccination to achieve eradication (after the loss of maternally derived protection but before the natural acquisition of infection) and the proportion of animals needing to be vaccinated to achieve elimination (the vaccination threshold for exclusion). Such critical points can be calculated by reference to the age at first infection, the proportion of susceptibles remaining in the population at equilibrium, the seropositivity rate at equilibrium, the birth rate, life expectancy, duration of maternally derived immunity and the reproductive rate of the agent (4).

The twin concepts of 'invasion' and 'exclusion' thresholds are crucial to understanding the likely performance of vaccination programmes and the meaning of seromonitoring results. For successful elimination or exclusion of a contagious microbe from a population it is necessary that the sum of all actions taken, including vaccination, should force the effective reproductive rate of the agent below unity; the disease will then die out and be unable to invade again if herd immunity is maintained. Thus, an understanding of the basic reproductive number, R_0, and the effective reproductive number, R_e, derived from it, is critical from both theoretical and practical perspectives. The basic reproduction number is the number of secondary infections resulting from a primary case introduced into a totally susceptible population (4) and is a feature of both the infectious agent and the host population in which transmission is occurring. However, it must be understood that most considerations assume homogeneously-mixing populations and that 'Susceptible–Infected–Recovered' (SIR) models generally assume that each infected individual interacts with an infinite set of other individuals, ignoring the discrete nature of populations in which the few individuals infected rapidly use up all neighbouring susceptibles, thus reducing the value of R_e. This in turn leads to underestimation of the vaccination threshold needed to eradicate a disease.

In the case of sedentary farming systems overall populations are fragmented into holdings which may each contain one or more of a number of species of differing susceptibility to a particular virus. The rinderpest eradication programme was contributed to, at least in its early days, by vaccination campaigns in extensive pastoral herds, yet even these cannot be considered truly to represent one or more homogeneously-mixing populations, for they too are fragmented by discontinuities of population distribution, the ethnic relationships (and antagonisms) of the livestock owners, and by geographical features such as mountain ranges. The varying transmission rates between groups are influenced by annual migrations and grouping/regrouping of animals.

Practical and logistical issues

Homogeneously-mixing models are sufficiently accurate to be of practical use but, for the reasons given above, it is essential to have good quantitative measurements for both the global dynamics and the local behaviour of a disease (7). To succeed, vaccination programmes must include a high proportion of the population but must also achieve uniform coverage, because pockets of susceptible individuals can allow a disease to persist or re-invade (7).

The level of vaccination which needs to be attained to achieve elimination of an infectious agent from a population is often stated didactically, yet rarely is the selected figure determined by science. For rinderpest the level is variously quoted to be from 70% to 90%, usually at the higher end. However, this belies evidence that rinderpest was eliminated from areas such as West Africa when herd immunity levels rarely exceeded 60%. Modelling studies (10) have started to provide a deeper understanding of the interaction between herd immunity and the force of infection exerted by strains of differing virulence. With less virulent strains herd immunity of 50% or so might be sufficient to bring about elimination, whereas virulent strains require far higher seroprevalence levels. This perhaps goes some way to explaining how rinderpest was eliminated from West Africa with relative ease on two occasions, when one understands that the virus west of Nigeria was derived from the Mauritania/Mali focus of rinderpest, which was relatively mild (16).

Another important consideration is for how long immunity must be maintained for elimination to be achieved. For a short-lived infection with a pathogen which does not persist in the environment or an alternative host, and for which there is no reactivatable latent stage, once a suitably high level of herd immunity is achieved, assured by seromonitoring and revaccination if required, the pathogen should very quickly be eliminated and vaccination be withdrawn. It is essential that a statistically significant measure of the immunity induced, usually achieved by serological testing, be included in the assessment of vaccination campaign performance.

Herd immunity generated to a level below the critical exclusion threshold can actually perpetuate the circulation of infection by creating a partially immune population in which either the virus exists in a cryptic fashion, or in which its destructive effects are so limited as to be tolerated by the farmers and hence by politicians – provided no one looks at what it is costing. Rinderpest was perpetuated in India for over 30 years by such a system and similar factors have operated in the Somali ecosystem for 15 years or more (10).

When starting on the progressive control of an epizootic disease it is often difficult to discern relationships between outbreaks and to distinguish reservoirs of infection (i.e. areas of true endemicity) from epidemic indicator areas. It is at this stage that mass vaccination is most valuable. Vaccination over a two- to three-year period can suppress infection to a point where such epidemiological discrimination becomes possible once vaccine is withdrawn. Unless this is done, enabling the use of vaccine to be focused in the light of epidemiological understanding, the tendency is for unfocused mass vaccination programmes to become institutionalised and fail to do more than just suppress disease. Even this outcome may suffer as campaigns lose drive and effectiveness with time. Using mass vaccination in this way is an expensive process that can easily consume a large proportion of the recurrent budget for Veterinary Services in a developing country; we experienced this many times during the eradication phase of the Global Rinderpest Eradication Programme. Arguably one of the major benefits of rinderpest eradication has been the freeing up of resources which were previously tied up in rinderpest control by vaccination.

Often the trick is to recognise when the job has been completed and devise methods of disengaging from the campaigns. Many countries in Africa and Asia continued to vaccinate for decades after rinderpest was eliminated. It seems that it is a lot easier to commence vaccination than to stop, and that 'vaccine addiction' may be a disease in its own right. The temptation to find another disease to which the mass vaccination approach can be applied uncritically must be resisted; CBPP is an example in post-rinderpest sub-Saharan Africa.

Mass vaccination need not, and indeed should not, be conceived as just area-wide, pulsed, 'blanket' vaccination whereby all cattle and/or buffalo in a population are repeatedly vaccinated, usually annually. Focusing vaccination can greatly increase the impact of control programmes, whether that focusing is directed by addressing age cohorts, geographically defined discrete sub-populations, demographic groupings or other factors. Focusing can simplify vaccination programme logistics and reduce costs. Most importantly, directing vaccination to points where transmission is occurring significantly enhances effectiveness of control. Mathematical models (12) have suggested that eradication can be achieved with fewer overall vaccination doses if they are distributed primarily to high contact-rate groups rather than distributed uniformly to the overall population.

A different example of focusing vaccination to good effect is that of the very successful containment of FMD of the SAT serotypes within the wildlife reservoir in the east of South Africa by maintaining, over many years, a surrounding vaccination buffer zone in cattle maintained on fenced ranches (24).

Conclusion

As Anderson and May (3) noted: 'The development of a safe, effective and cheap vaccine [...] is only a first step (albeit an essential one) towards community-wide control.' Undeniably, consideration of issues relating to vaccine quality is of great importance, yet, leaving aside cost issues, this needs to be balanced by the use of sound epidemiological principles and lessons learnt from field experience to ensure the efficacy of vaccination programmes.

Many lessons can be learnt from an analysis of experience gained in rinderpest control and eradication (16, 23). The following points can be stressed:

– where mass vaccination is to be used, the more intensively it is applied, the more rapidly it achieves the objective desired;

– vaccination campaigns require seromonitoring as an integral component to provide quality assurance of vaccination efficacy and so that the results are used to generate remedial action; results must be available within two months at most if they are to provide a basis for action;

– eradication programmes require careful management and work best when they are conceptualised within time-

bound frameworks and managers are permitted to take risks;

– eradication programmes require clear initial objectives and clear exit strategies;

– eradication programmes should be designed around an understanding of the epidemiology of the pathogen involved; in India the epidemiology of rinderpest in small ruminants was not well understood and elucidating the role of peste des petits ruminants was an added difficulty.

Almost invariably the current approach to disease control is empirical, with vaccination programmes being embarked on without consideration of the epidemiological basis; this needs to be remedied to provide more cost-effective use of vaccines and more effective control. Stochastic mathematical modelling has started to demonstrate how this approach can be of value in the veterinary field with rinderpest and CBPP (10, 11, 17) but much remains to be done. Mass vaccination programmes need to be planned, monitored and managed. Modelling can provide quantitative criteria with which the performance of vaccination programmes can be judged, whether in terms of preventing the introduction of an agent and the likelihood of generating a fresh epizootic, or progress of a control programme leading towards elimination of an infection. To do this, surveillance systems need to be tuned to provide the data required and combined with livestock population and performance data to provide the analytical basis essential for science-based decision making in infectious disease control and monitoring the progress of interventions.

Alternatives to vaccination should always be the first resort but, undoubtedly, mass vaccination is, and will continue to be, one of the main tools used for emergency and progressive control of epizootic diseases. However, peculiarities of the immune responses of livestock under different physiological and intercurrent disease states, combined with the epidemiological intricacies of different infectious agents and differences in the composition and efficacy of vaccines, mean that the effective application of vaccines is not as simple and straightforward a matter as we would perhaps wish. Mass vaccination programmes must be designed while respecting epidemiological principles and managed effectively with a defined time-bound objective and an exit strategy in place. They must be energetically implemented with assured funding. Unless budgets are available from the outset and adaptive project management is ensured, campaigns should not be started.

In an early review article on the modelling approach to designing disease control programmes (3) a telling statement was made: 'Many difficulties surround the attainment of sufficient levels of herd immunity to eradicate common infections in developed and developing countries. Theory can define the level of vaccination coverage required for elimination, but success in practice depends on economic and motivational issues.' Cost assessments and likely benefits can be calculated but community motivation requires enlightened professional management and active community involvement.

■

La vaccination de masse et l'immunité de troupeau : bovins et buffles

P.L. Roeder & W.P. Taylor

Résumé

De nos jours, les programmes efficaces visant à préparer une réaction d'urgence en cas d'incursion épizootique chez les bovins, à éliminer ces maladies et à mettre en œuvre des mesures progressives de contrôle en cas d'enzootie pour tenter d'éliminer le virus causal sont conçus de manière essentiellement empirique. Il convient d'y remédier afin d'améliorer le rapport coût-efficacité de la vaccination et d'assurer une prophylaxie plus efficace. Au niveau des populations, les effets protecteurs de l'immunisation ne se limitent pas aux individus mais jouent sur la dynamique de la propagation virale au sein de la population globale (sujets vaccinés et non vaccinés). Le concept

d'immunité de troupeau et l'application des principes épidémiologiques qui en résultent, associés à l'expérience acquise grâce aux programmes de prophylaxie tels que le Programme mondial d'éradication de la peste bovine (GREP) offrent d'intéressantes perspectives pour concevoir des programmes de prophylaxie efficaces et fondés scientifiquement. Les auteurs examinent les possibilités pratiques du principe d'immunité de troupeau en élucidant un certain nombre d'arguments parmi ceux qui mettent en cause la capacité de la vaccination de masse d'atteindre des taux acceptables d'immunité de troupeau ; en s'appuyant sur ces observations, ils avancent des propositions visant à améliorer l'efficacité des programmes de vaccination de masse.

Mots-clés
Bovin – Épizootie – Immunité de troupeau – Vaccination.

Vacunación masiva e inmunización de los rebaños de vacunos y búfalos

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Resumen
La preparación de programas eficaces para luchar en situaciones de emergencia contra brotes masivos de enfermedades epizoóticas, para eliminar esas enfermedades y para instaurar un control progresivo que conduzca a la posterior eliminación de los virus causantes de episodios enzoóticos, que actualmente es muy empírica, debe corregirse a fin de rentabilizar la utilización de las vacunas y mejorar el control de las infecciones. En las poblaciones animales, el efecto protector de la vacunación puede extenderse mucho más allá de cada animal individual e influenciar la dinámica de propagación de los virus en todo el rebaño, esté o no vacunado. Esta noción de la inmunización de los rebaños y la aplicación de los principios epidemiológicos consiguientes, sumados a la experiencia adquirida con programas de lucha contra enfermedades, como el Programa Mundial de Erradicación de la Peste Bovina (PMEPB), pueden ser de gran utilidad para preparar programas de control eficientes basados en datos científicos. En este artículo los autores analizan el aprovechamiento práctico del principio de la inmunización de los rebaños mediante el examen de algunos de los factores que se oponen a la vacunación masiva para lograr niveles de inmunidad satisfactorios y, teniendo en cuenta esos factores, proponen medidas para que los programas de vacunación de rebaños alcancen la mayor eficiencia posible.

Palabras clave
Epizootia – Ganado – Inmunidad de los rebaños – Vacunación.

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The use of vaccination in poultry production

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Summary

Poultry vaccines are widely applied to prevent and control contagious poultry diseases. Their use in poultry production is aimed at avoiding or minimising the emergence of clinical disease at farm level, thus increasing production. Vaccines and vaccination programmes vary broadly in regard to several local factors (e.g. type of production, local pattern of disease, costs and potential losses) and are generally managed by the poultry industry. In the last decade, the financial losses caused by the major epidemic diseases of poultry (avian influenza and Newcastle disease) have been enormous for both the commercial and the public sectors. Thus, vaccination should also be applied in the framework of poultry disease eradication programmes at national or regional levels under the official supervision of public Veterinary Services. This paper provides insight on the use of vaccination for the control of poultry infections, with particular emphasis on the control of transboundary poultry diseases.

Keywords

Avian influenza – Disease control – Newcastle disease – Poultry – Vaccination strategy – Vaccine – Vaccine efficacy.

Introduction

Poultry are kept as a source of animal protein throughout the world. Moreover, poultry are able to adapt to most geographical areas and conditions, they are not expensive to buy, they have rapid generation time and a high rate of productivity, and they do not require large areas of land. Poultry production systems differ, ranging from rural farming to highly industrialised and vertically integrated systems. Backyard poultry production is distributed in most rural and peri-urban areas of the world, and is mainly based on the rearing of domestic poultry, both terrestrial and aquatic. Intensive poultry production is most common in developed countries, but in the last few decades, many developing countries have also adopted this system in order to meet the increasing demand for animal proteins. In recent times, the risk of transmission of certain transboundary poultry diseases to previously unaffected areas has increased as a result of globalisation and the possible persistence and spread of disease agents through domestic and wild reservoirs. The widespread distribution of Newcastle disease (ND) and the epidemics of avian influenza (AI) that have occurred over the last ten years provide examples of the negative impact of such diseases on the poultry producing sector and on society as a whole (8, 9, 12). Different strategies can be implemented to effectively prevent and control the spread of animal diseases at international, national and farm levels and poultry disease control plans often include the use of vaccination. Vaccines are, in fact, an important component of poultry disease prevention and control worldwide. Their use in poultry production is traditionally aimed at avoiding or minimising the emergence of clinical disease at farm level and thus increasing production. Vaccines and vaccination programmes vary widely, depending on several local factors (e.g. type of production, level of biosecurity, local pattern of disease, status of maternal immunity, vaccines available, costs and potential losses). Although poultry vaccination is generally managed by the poultry

industry, it has only rarely been applied in the framework of a disease eradication programme at national or regional level to control a few major poultry diseases (e.g. AI and ND) (1, 16). In this paper, the authors provide insight on the use of vaccination for the control of poultry infections in any given country/area/compartment, with particular emphasis on the control of transboundary poultry diseases.

The control of poultry diseases

This paper does not cover all the detailed control measures that can be implemented to contain and eradicate poultry diseases in various farming systems, and only attempts to summarise and illustrate a few fundamental concepts on the use of poultry vaccines. It should be emphasised, however, that under no circumstances must vaccination be regarded as an alternative to good management practice and biosecurity or to the adoption of adequate control policies for the prevention of the introduction and spread of a contagious disease in any given country/area/compartment (10). Vaccines cannot realistically be expected to provide 100% protection for birds/flocks vaccinated under field conditions. Strict application of disease-prevention management techniques and hygienic practices at the farm level are of fundamental importance in minimising the risk of disease introduction and the related economic impact. The poultry industry involves the trade of poultry products and genetic stock between widespread localities and markets, frequently under the management of multinational companies. The regular reporting of World Organisation for Animal Health (OIE)-listed diseases to international bodies and the definition and application of international and national control policies are the prerequisites to minimising disease impact on human health and poultry production and avoiding unjustified barriers to the trade of live poultry and products.

The use of vaccines for the control of poultry diseases

Vaccination should generally be tailored and adjusted according to local factors that may influence the strategy, the design and the effectiveness of the vaccination programme once it has been implemented. Several different factors should be taken into account, including:

– the type of poultry production (e.g. commercial or rural)

– the organisation of the industry (e.g. vertical integration)

– the densities of different bird species

– the prevailing disease situation

– vaccine availability

– the use of other vaccines

– the prevalence of other diseases

– the resources available (e.g. manpower and equipment)

– the costs involved.

The first expected outcome of the administration of a poultry vaccine is that birds will develop immunity to pathogens and thus be protected against disease. The results that may be achieved through the use of vaccination can be summarised as follows:

– protection against the clinical form of the disease

– reduction of susceptibility to infection (a higher infectious dose is required to trigger infection in vaccinated birds than in those unvaccinated)

– reduction of infectivity (e.g. shedding) in case of infection.

Herd immunity

Protection against the clinical form of the disease is effective at an individual level, whereas the reduction of both susceptibility and infectivity also benefits the entire poultry population in the vaccinated flock/area. The positive effect on a vaccinated population known as 'herd immunity' may be defined as the reduced probability of an individual (bird or flock) becoming infected whenever it is part of a vaccinated population (6, 7). Herd immunity is important at two levels:

– flock level: if a single bird in a vaccinated flock is not immunised, it has a chance of becoming infected which is inversely proportional to the level of protection achieved by the other vaccinated and immunised birds in the same flock;

– country/region/compartment level: the higher the prevalence of vaccinated flocks in the vaccination area, the lower the probability of infection in unvaccinated flocks located in the same country/area/compartment.

In order to optimise the 'herd immunity' effect in a vaccination area, it is of the utmost importance to target the bird species with the highest susceptibility to any given infection (e.g. turkeys with regard to low pathogenic AI viruses) (16). The protection of the most susceptible poultry species serves to lower both the risk of disease introduction and the infectious pressure in the environment, thus reducing the risk of a massive spread of the infection to unvaccinated poultry farms situated in the vaccination zone.

Factors which can affect the outcome of a vaccination programme

The most important aspects to be considered in improving the organisation of a vaccination programme and achieving the expected outcomes will be briefly illustrated below.

Poultry sector involved

The practical application of poultry vaccines is highly influenced by the characteristics of the poultry producing system in question. Generally speaking, there are two main types of poultry production: industrially reared poultry and rural poultry. The spread of an infectious poultry disease and the measures to be applied for its control, including vaccination, are clearly related to the structure and organisation of the local poultry sector.

The poultry industry has substantially grown in an often uncontrolled way, particularly since the system has developed through vertical integration (e.g. poultry house owned by the farmer and day-old chicks and feed supplied by private companies) with a concentration of the productive units in certain territorial areas. In these areas, the high density of poultry farms, hatcheries, abattoirs, feed mills, litter processing plants and other establishments – although convenient from an organisational point of view – poses a series of drawbacks in terms of increased risk of major epidemics (11). These characteristics of the commercial poultry sector have a significant effect on disease prevention and control measures, and also on the use of vaccination. The selection of vaccines and proper administration protocols, together with the use of the right antigen combinations and, for live vaccines in particular, the optimal antigen virulence, have all become essential elements in managing risks and optimising costs. Poultry vaccines and vaccination methods have become a fundamental part of the prevention measures applied in industrially reared poultry in order to maximise the biosecurity level of any given poultry compartment or establishment.

Village poultry are an important component of the rural economy, particularly in developing countries. In order to control infections in rural poultry, the awareness of major poultry diseases and the losses they pose should be increased. This implies the education of rural communities and poultry farmers in the basic concepts of biosecurity, farming hygiene, prevention and vaccination techniques, since basic hygienic standards are rarely respected. Vaccination of village poultry should be carried out using appropriate hygienic and logistic/management practices. The basic quality of vaccines must be guaranteed and vaccines must be administered to each group of birds in an appropriate manner. Vaccine delivery is crucial, and the cold chain must be respected in order for the characteristics of the product to be maintained and efficacy ensured. Adequately planned and managed rural poultry vaccination programmes (e.g. against ND and Gumboro disease) can significantly reduce mortality and increase poultry production (3).

The structure, the organisation and the level of biosecurity in the various poultry producing systems all directly influence the risk of introduction and spread of a given disease in each system, and ultimately the measures that must be applied for its control.

Prevailing disease situation

The application of the different vaccination options should be adjusted in diverse conditions according to the local pattern of disease, the level of biosecurity practised in different types of poultry production systems, and the level of challenge for each type of poultry operation. This overall risk assessment should allow for the correct identification of the area/compartment that is to be subjected to vaccination and the optimal vaccination protocol. An ongoing surveillance programme based on reliable diagnostic testing should be implemented in order to adapt the vaccination programme to any possible change in the epidemiological situation and to monitor vaccine efficacy. Furthermore, it is fundamental to monitor the prevalence of infectious agents capable of producing immunosuppression (e.g. infectious bursal disease, infectious anaemia, and Marek's disease in chickens, and haemorrhagic enteritis in turkeys) and to implement specific vaccination programmes for their control. For example, since the immunosuppressive effect of infectious bursal disease virus is extremely relevant at an early age, eliciting a high level of maternal immunity can be very useful in preventing and controlling this disease (13).

Vaccination strategy

Generally speaking, there are three vaccination strategies: routine, emergency and preventive vaccination.

Routine vaccination can be the tool of choice in territorial areas where an infectious disease is endemic. Used properly, routine vaccination is effective in reducing mortality and production losses. In the longer term, it could also lower the prevalence of infection to a level where eradication measures might be applied, if the eradication of the disease is a feasible option. The continued use of routine vaccination can be rendered unnecessary, provided that effective preventive measures are maintained in order to deal with the potential re-emergence of the disease.

Emergency vaccination is an option whenever a new infectious disease is introduced in a previously unaffected country/area/compartment, and the epidemiological situation indicates that there could be massive and rapid spread of infection. The efficacy of a vaccination

programme depends on the availability of adequate resources and the prompt deployment of effective vaccines. If the disease becomes endemic, the option of applying vaccination on a routine basis can be considered. This choice should be based on a careful evaluation of the epidemiology of the infection, the economic impact of the disease on poultry production compared to the costs of vaccination, and the effectiveness and cost of other preventive and control measures that might be applied to contain the disease.

Preventive vaccination is a measure that may be applied wherever a high risk of introduction and further spread of a contagious poultry disease has been identified. The scientific basis for the use of this strategy is the generation of a level of protective immunity in the target population that can be boosted in case of immediate risk or evidence of introduction of a field virus. The use of vaccination in the absence of any outbreak of disease, together with the application of effective biosecurity measures, could maximise poultry protection whenever a risk of exposure exists. Preventive vaccination is generally carried out for the prevention of poultry diseases that have a clear impact on the industry. For example, as regards ND control, some countries require the preventive vaccination of all poultry even in the absence of outbreaks due to the perceived threat of the disease. The wide use of ND vaccines throughout the world, in fact, makes assessment of the real geographic distribution of the disease almost impossible (1). Generally speaking, prophylactic vaccination should be applied as long as the risk of infection exists, and could also be used in a targeted manner for limited periods of time. In any case, a clearly defined exit strategy should be formulated before preventive vaccination is undertaken.

Cost/benefit analysis

Before implementing a vaccination programme, an overall cost/benefit analysis should be performed by taking into account the costs of vaccines, vaccine delivery (e.g. labour, equipment), monitoring, laboratory testing, and all other related activities. Vaccination campaigns to control a notifiable poultry disease (e.g. AI) require careful previous consideration of the implications on trade and the impact of both the movement restrictions and biosecurity measures applied inside the vaccination area. The decision to use vaccination in fighting certain avian infections (zoonotic diseases) should also consider the potential implications of these diseases to human health.

Availability of different types of vaccines

Vaccines used in poultry production are classically described as live or inactivated. Table I illustrates the general characteristics of live and killed poultry vaccines (2). The availability of different types of vaccines could be one of the major limits to the implementation of effective vaccination programmes. Different types of poultry production (or bird species) or diverse levels of risk require the application of more than one type of vaccine to obtain a high and long-lasting immunological response. As regards ND control, the immune response induced by live ND vaccines increases as their pathogenicity increases. Vaccination programmes using vaccine strains of different pathogenicity and immunogenicity should be applied in relation to the degree of virulence of the virus in circulation. In order to achieve an optimal level of protection without severe adverse reactions, vaccination programmes should include the sequential use of progressively more virulent live vaccine strains or live vaccines followed by inactivated vaccines (1). Generally, inactivated vaccines induce high and uniform levels of protection after administration of a live vaccine. This type of programme should be considered in the implementation of vaccination programmes for breeder and layer flocks due to the fact that they require high and long-lasting immunity for protection during the entire laying period.

Administration of vaccines

After establishing the type of vaccine to be used, the route, method and frequency of administration must be defined, as well as the proper way to combine all these components in the vaccination programme. Vaccine delivery systems significantly influence the outcome of vaccination. An improper vaccine application is considered one of the most common reasons for vaccination programme failure. Various methods of administration can be applied as required by different types of poultry operations (at the hatchery or farm). The choice of method will also depend upon other factors such as the type of production, bird species, size of the flock, length of the production cycle, general health status, maternal immunity, vaccines to be applied, and costs. The vaccination techniques most commonly used in the poultry sector and their main advantages and disadvantages are illustrated in Table II (2).

Factors affecting vaccine efficacy

Several factors can jeopardise the optimal immunisation of vaccinated poultry. Table III summarises these negative factors, classifying them into three main categories: those linked to the vaccine itself, those regarding vaccine delivery, and those endogenous to the bird (14, 17). Management conditions are also relevant and should be considered the fourth factor. As a consequence of inadequate cleansing and disinfection of poultry premises over successive production cycles, the challenge dose could either be high enough to overcome the level of protection induced by vaccination or infection might occur before vaccination is performed. This series of events can also occur in large multi-flock layer complexes in which the simultaneous presence of multi-age layer flocks has reduced the possibility of applying an effective all-in, all-out system.

Table I
General characteristics of live and inactivated vaccines for poultry (2, modified)

Live vaccines	Inactivated vaccines
Smaller quantity of antigen. Vaccination response relies on multiplication within the bird	Large amount of antigen. No multiplication after administration
Easily killed by chemicals and heat	Easier to store
Relatively inexpensive, easy to administer, and can be mass administered: drinking water, spray	Expensive to produce and to apply, since almost always individually administered
Adjuvanting live vaccines is not common	Adjuvanting killed vaccines is frequently necessary
Susceptible to existing antibody present in birds (e.g. maternal immunity)	More capable of eliciting an immune response in the face of existing antibody
In immune birds, booster vaccination is ineffective	In immune birds, additional immune response is frequently seen
Local immunity stimulated (i.e. trachea or gut)	Local immunity may be restimulated if used as a booster but secondary response is poor or absent
Danger of vaccine contamination (e.g. EDS)	No danger of vaccine contamination
Tissue reactions (commonly referred to as a 'vaccine reaction') are possible and frequently visible in a variety of tissues	No microbe replication; therefore, no tissue reaction outside that which is adjuvant dependent
Relatively limited combinations, due to interference of multiple microbes given at the same time (e.g. IB, ND and LT)	Combinations are less likely to interfere
Rapid onset of immunity	Generally slower onset of immunity

EDS: egg drop syndrome
IB: infectious bronchitis
LT: laryngotracheitis
ND: Newcastle disease

General immune system organisation and mechanisms in avian species are similar to those of mammals; both are extremely complex, with a variety of cells and soluble factors working to produce a protective response (19). The protective efficacy of a vaccine depends on its capability to induce a vigorous and long-lasting response in the immune system. The chicken is the most widely studied avian species, and although vaccines developed primarily for this species can be effectively applied to other birds, some differences in immunological response may appear. Therefore, a number of factors (e.g. vaccine doses, routes of administration and protocols) must be adapted to different species in order to optimise vaccine efficacy. The turkey, for example, generally provides a lower response to AI and ND vaccines, thus creating the need to apply specifically designed vaccination programmes (1, 4, 21).

Vaccination programme monitoring

An evaluation of the efficacy of a vaccination programme essentially involves the overall assessment of the health conditions of the flocks vaccinated. The results of the evaluation should indicate when changes in the programme must be made based on the facts. Many poultry flock health status and performance parameters can be compared to existing standards or comparative histories (e.g. feed conversion efficiency, rate of gain, average weight at the time of slaughter, mortality rates, serological profiles, etc.). Such standards have been established in various geographical areas through the collection and analysis of data obtained during the production cycles for different poultry species and types of production. A vaccination programme can be evaluated by taking these parameters as reference points during the consideration of the aspects discussed below.

Vaccination programme effectiveness

An effective vaccination plan should result in a general improvement of the health status and the productive performance of the vaccinated population. Useful measurable and comparable indicators to judge the overall health status of a flock are the morbidity and mortality rates, and other performance parameters, such as feed conversion, egg production and egg quality. The efficacy of vaccine administration and the level of immunological response in vaccinated birds can be serologically monitored (5, 20). If vaccination is routinely applied, data on the antibody response elicited in vaccinated birds should be collected and analysed in order to define the baseline of the antibody titre in different bird species and types of production. This serological monitoring can provide useful information whenever adequate samples have been analysed over time for each vaccination programme. The serological baseline obtained should be

Table II
Vaccine delivery systems commonly used in the poultry industry: main advantages and disadvantages

Type of operation	Vaccination route	Disease	Type of vaccine	Advantages	Disadvantages
Hatchery	In ovo	Marek's disease, infectious bursal disease	Live and live cells mediated vaccines	Early protection; both the innate and adaptive immune responses are stimulated, 20,000-30,000 eggs per hour	Expensive equipment; training needed; poor early liveability due to possible fungal or bacterial contamination through the open hole in the egg
	Spray	IB, ND, coccidiosis	Live vaccines	Minimised handling, good mucosal immunity, inexpensive	Possible respiratory reaction (very small particles), particle size depends on relative humidity, temperature and hygiene
	Subcutaneous/ intramuscular	Marek's disease	Live cell-mediated vaccines	Absence of respiratory reaction, uniform level of immunity, 1,600-2,000 chicks per hour	Regular equipment sanitisation required; possible localised tissue damage; birds are stressed
On-farm	Drinking water	Infectious bursal disease, IB, ND	Most common route for live vaccines	Labour-saving, easy administration in drinking water	Improper/unequal distribution; inconsistency and variability of water quality; inactivation by impurities or residues; birds are stressed by water starvation
	Spray	Infectious bursal disease, IB, infectious LT, ND	Live vaccines	Good mucosal immunity, mass application, minimised bird stress, inexpensive	Possible inconsistencies of vaccine dosage; possible respiratory reaction (in relation to particle size); need to target tissues that stimulate immunity
	Intraocular/ nasal drop	Infectious LT, ND, infectious bursal disease	Live vaccines	Effective and accurate vaccination type for live vaccines, uniform humoral and local immunity	Labour-intensive (individual handling); need to verify vaccine coverage
	Wing web	Fowl pox, avian encephalomyelitis, fowl cholera	Live vaccines	May result in 95%-100% protection	Labour-intensive (individual handling); need to verify the 'vaccine take'; possible contamination at the injection site
	Subcutaneous/ intramuscular	Avian influenza, Marek's disease, ND, salmonellosis	Most common route for inactivated vaccines	Use of inactivated vaccines (no spread of virus, no risk of residual virulence, stable), uniform levels of immunity, low level of adverse reactions	Labour-intensive (individual handling), possible localised tissue damage; use of inactivated vaccine (high costs); regular equipment sanitisation required

IB: infectious bronchitis
LT: laryngotracheitis
ND: Newcastle disease

used only to compare similar species and production types. Deviation above or below the established baseline permits the identification of flocks with possible field exposure or poor protection, respectively.

Field exposure: differentiating infected from vaccinated animals

In order to eradicate major infectious poultry diseases like AI, which have such a negative impact on poultry production and human health, the vaccination system must permit the detection of field exposure in vaccinated flocks. The differentiation between exposed/unexposed vaccinated birds and flocks requires the application of a suitable 'marker' vaccine and a companion discriminatory test. Since this condition is not always fulfilled, a monitoring programme that includes the use of (unvaccinated) sentinel birds could also be set up. In order to assess the possible exposure to other infections not included in the vaccination programme, a regular monitoring programme targeted to the detection of other diseases (e.g. immunosuppressive infections) might be implemented. This could also allow for the detection of new or re-emergent pathogens.

Table III
Factors which interfere with vaccine efficacy in poultry

Type of factor	Impact on vaccine efficacy
Factors associated with the vaccine itself	
Virus serotype	Many infectious agents (e.g. infectious bronchitis virus) have different serotypes, and vaccine antigens do not provide protection against all field strains
Level of protection	Field strain of very high virulence, and/or highly attenuated vaccine strains
Factors associated with vaccine administration	
Handling	Certain live vaccines (e.g. live cell-mediated Marek's disease vaccines) are easily killed if mishandled
Diluent used	Viable vaccines administered in drinking water are destroyed if water sanitisers are not removed
Route	Vaccines administered by injection fail if vaccinators do not deliver the vaccine to the appropriate vaccination site
	Mass vaccination (drinking water and aerosol) tends towards lower uniformity than individual administration
Associations	Administration of certain combinations of live virus vaccines affects the single virus response if they have the same target tissues
Factors associated with the bird/flock	
Maternal immunity	In presence of high levels of maternal antibodies, live vaccines administered during the first two weeks of life may be neutralised
Immunosuppression	Stress, certain infectious agents (e.g. infectious bursal disease, infectious anaemia and Marek's disease in chickens, haemorrhagic enteritis in turkeys), mycotoxins (in particular aflatoxins) impair immune response
Sanitary status	The birds are already infected (incubation period) with the pathogen against which the vaccination is directed
Genetic factors	Different vaccine responses with respect to species or commercial hybrids
Management conditions	
Hygienic practices	Without clean-out and disinfection over successive flocks, the challenge dose might be too high or infection might occur too soon

It is more difficult to assess the efficacy of a vaccination programme conducted in a rural poultry farm because reference data or standards are often unavailable. In this case, evaluation should be based on disease reporting, and a comparison of the situation in the vaccination area before and after the implementation of the vaccination plan. This implies the presence of a surveillance system capable of detecting the disease and providing comparable historical information on its frequency.

Controlling major poultry diseases: mass vaccination versus stamping out

The major poultry epidemic diseases (e.g. AI and ND) have caused enormous financial losses in both the private and public sectors (8, 12, 15). These diseases are difficult to control and the enforcement of eradication measures based on the depopulation of affected and at-risk farms could make poultry farming unsustainable in the long term. Furthermore, the killing of large numbers of birds and the destruction of carcasses is increasingly perceived as being unacceptable by the public on ethical, social, environmental and economic grounds. In developing countries, where adequate compensation measures are often lacking, the use of stamping out measures to control major poultry diseases has had a clearly negative social effect on smallholder livelihood (18). In these countries, in fact, village poultry represent a significant part of the population's intake of dietary protein, particularly for women and children. In order to identify the appropriate strategy to adopt, an accurate cost/benefit evaluation of all the control options available should be conducted while considering different scenarios. This cost/benefit analysis should take into account a number of factors: the pathogenicity/virulence of the virus strain involved, poultry densities, bird species, type of poultry production, organisation of veterinary services, and the impact on trade. In this context, vaccination should be considered as an additional means of increasing the capacity to control the major poultry diseases and should be implemented along with other disease control and eradication measures.

Conclusions

Vaccines are widely applied in all the various poultry producing systems. The global biologics market for these species accounted for total sales of US$ 585 million in 2002, which were almost equally divided between live (45%) and inactivated (55%) vaccines (Wood and

MacKenzie, unpublished data). Vaccination programmes can be successfully implemented in diverse conditions if they are tailored to the local conditions and take into account factors such as the characteristics of the poultry producing sector, the eco-epidemiological situation, and the availability of adequate resources. Although the application of poultry vaccines is a well-established practice at the farm/flock level, vaccination programmes for the control and eventual eradication of poultry diseases are not always properly implemented at the national level. This can be problematic, particularly during the implementation of emergency vaccination programmes, the effectiveness of which depends mainly on the level of preparedness, the capacity of the veterinary infrastructure, and the level of cooperation with poultry farmers and the other stakeholders. Vaccination is more effective to the extent that the target population (bird species and type of production) is homogeneous. Unfortunately, field conditions are often dissimilar and characterised by many different bird species, various rearing practices, and different levels of disease risk. Effective vaccination and monitoring programmes therefore demand considerable effort and high levels of organisation.

La vaccination dans les élevages de volailles

S. Marangon & L. Busani

Résumé

Les vaccins aviaires sont couramment utilisés pour prévenir et maîtriser les maladies infectieuses qui affectent les volailles. Leur utilisation dans les élevages de volailles vise à prévenir ou à limiter l'émergence d'infection clinique dans les exploitations, ce qui favorise une meilleure productivité des élevages. La production de vaccins et les programmes de vaccination sont généralement assurés par la filière avicole et varient d'un endroit à l'autre en fonction de facteurs locaux, notamment le type de production, les caractéristiques de la maladie sur le terrain et les prévisions en termes de coûts et de pertes. Depuis une dizaine d'années, les pertes financières imputables aux principales épizooties affectant les volailles (à savoir l'influenza aviaire et la maladie de Newcastle) ont été extrêmement lourdes pour le secteur privé comme pour le secteur public. Il serait donc souhaitable que la vaccination soit appliquée dans le cadre de programmes d'éradication des maladies aviaires à l'échelle nationale ou régionale, sous la tutelle des Services vétérinaires officiels. Les auteurs donnent quelques éclaircissements sur la vaccination visant à contrôler les maladies aviaires, en mettant un accent particulier sur la prophylaxie des maladies aviaires transfrontalières.

Mots-clés

Efficacité vaccinale – Influenza aviaire – Maladie de Newcastle – Prophylaxie – Stratégie de vaccination – Vaccin – Volaille.

Vacunación en establecimientos avícolas

S. Marangon & L. Busani

Resumen

Las vacunas para aves de corral se utilizan comúnmente para prevenir y controlar las enfermedades contagiosas. Los productores avícolas las emplean para evitar o reducir al mínimo la aparición de enfermedades clínicas en las granjas y, de ese modo, incrementar la producción. Las vacunas y programas de vacunación varían mucho en función de distintos factores locales (tipo de producción, comportamiento de la enfermedad, costos y pérdidas potenciales, etc.) y, por lo general, son los representantes de la industria avícola quienes deciden su administración y aplicación. En la última década, las grandes epidemias que afectaron a las aves de corral (influenza aviar y enfermedad de Newcastle) causaron enormes pérdidas económicas, tanto en el sector privado, como en el público. Por ello, la vacunación también debería administrarse en el marco de programas nacionales o regionales de erradicación de las enfermedades, bajo la supervisión oficial de los Servicios Veterinarios públicos. En este artículo se analiza la utilización de la vacunación para luchar contra las infecciones de las aves de corral, haciendo un particular hincapié en el control de las enfermedades transfronterizas.

Palabras clave

Ave de corral – Control de enfermedades – Eficacia de la vacunación – Enfermedad de Newcastle – Estrategia de vacunación – Influenza aviar – Vacunación.

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Instructions to Authors

Aims and scope of the *Review*

The *Review* is the principal scientific and technical publication of the OIE, fulfilling two of the statutory functions of the Organisation, namely:

– to promote and co-ordinate experimental or other research work concerning contagious diseases of livestock for which international collaboration is deemed desirable

– to publish all facts and documents likely to be of interest to Veterinary Services worldwide.

The *Review* presents information on veterinary activities which may involve international co-operation in the fields of both animal and public health.

Another objective of the *Review* is to inform readers of the activities of OIE Member Countries and of the Organisation in both of the above-mentioned fields.

The *Review* is indexed in the databases *Agris* (FAO, Italy) and *Littérature vétérinaire francophone* (Canada), in the abstract journals *Index Veterinarius* and *Veterinary Bulletin* (CABI databases, United Kingdom), *BIOSIS, Capsule Report, Current Contents® / Agriculture, Biology and Environmental Sciences, Fish and Wildlife Worldwide, Focus On®: Veterinary Science & Medicine, Index Medicus, Medline, SciSearch®* (United States of America), *Zoological Record* (United Kingdom), and Electre (France).

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At least two of the three issues published in each volume are devoted to a specific theme. For these issues, an internationally-renowned expert is designated as co-ordinator, and specialists in the field are invited to contribute papers, thereby providing readers with a comprehensive overview of the topic under discussion.

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4. Acknowledgements (if applicable)
5. References
6. Tables
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The family names of authors should be preceded by their initials and followed by a superscript bracketed Arabic number. The position and full address of each author should be given below the list of names, as follows:

H. Jones [1], M.L. Smith [2] & M. Webber [2]

(1) Department of Animal Studies, Centre for Environmental Research, 12 Wellbeck Street, London WI 6AB, United Kingdom

(2) Institute of Veterinary Research, 4 Portsmouth Road, Southampton 4GY 6NW, United Kingdom

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Summaries should provide an outline of the entire text, including the principal findings and conclusions. It should be written in the original language and not exceed 150 words. The OIE will have the summaries translated into the other two official languages of the Organisation. Eight to ten keywords should be provided after the summary.

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Veterinary drugs, reagents and laboratory materials should be referred to in the text by the generic name (and, only if necessary, the commercial name).

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Mammal Species of the World, Third Edition, 2005, Johns Hopkins University Press

Distribution and Taxonomy of Birds of the World, 1991

Virus Taxonomy – Classification and Nomenclature of Viruses – Eighth Report of the International Committee on Taxonomy of Viruses, 2005, Elsevier

List of Prokaryotic names with Standing in Nomenclature – Available at: http://www.bacterio.net or http://www.bacterio.cict.fr/

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– Article in press:

Capua I. (2007). – Vaccination for notifiable avian influenza in poulty. *In* Animal Vaccination Part 1: development, production and use of vaccines. *Rev. sci. tech. Off. int. Epiz.* (in press).

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J.-P. Dupont [(1)], R.L. Calvey [(2)] & M. Sansom [(2)]

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2. Résumé et mots-clés

Le résumé, rédigé dans la langue originale, ne doit pas dépasser 150 mots. Il présentera la méthodologie, les principaux résultats et les conclusions de l'étude, en reflétant l'essentiel du contenu de l'article. Il sera traduit dans les deux autres langues officielles par les soins de l'OIE. Le résumé sera suivi de huit à dix mots-clés.

3. Texte

La longueur d'un manuscrit ne doit pas dépasser 4 000 mots (14 à 16 pages dactylographiées). Les auteurs souhaitant publier un article plus long doivent obtenir l'accord préalable de la Rédaction. Dans la mesure du possible, les paragraphes comporteront, au plus, une vingtaine de lignes (200 mots environ). Les auteurs rechercheront avant tout dans leur rédaction la clarté et la concision. Les travaux expérimentaux et les enquêtes épidémiologiques seront présentés selon le plan standard suivant : introduction, matériels et méthodes, résultats, discussion, conclusions, bibliographie.

Les unités de mesure seront exprimées en utilisant le système métrique et, si nécessaire, les unités SI. Les nouvelles méthodes de diagnostic seront décrites avec des détails suffisants (par exemple : standard de référence, nature de l'antisérum ou de l'antigène, spécificité, sensibilité, etc.). Les méthodes connues ou déjà décrites dans un journal ou une revue d'audience internationale seront simplement mentionnées avec leurs références.

Les médicaments vétérinaires, réactifs et matériels de laboratoire seront désignés dans le texte par leur nom générique (et, éventuellement, leur nom commercial).

Les abréviations et les acronymes seront définis lors de leur première citation. Le texte ne doit pas comporter de notes de bas de page. Les précisions souhaitées peuvent être incorporées dans le texte.

Les tableaux et les figures seront mentionnés dans le texte à l'emplacement souhaité par l'auteur pour leur insertion.

Les auteurs sont invités à se référer aux nomenclatures internationales les plus récentes publiées par les sociétés scientifiques internationales reconnues. Les noms d'espèces

(animales, bactériens, virales, etc.) doivent être obligatoirement suivis de leur dénomination latine entre parenthèse et en italique.

Nomenclature : quelques ouvrages de références :

Mammal Species of the World, 3e édition, 2005, Johns Hopkins University Press

Distribution and Taxonomy of Birds of the World, 1991

Virus Taxonomy – Classification and Nomenclature of Viruses – Eighth Report of the International Committee on Taxonomy of Viruses, 2005, Elsevier

List of Prokaryotic names with Standing in Nomenclature – Sites Web : http://www.bacterio.net ou http://www.bacterio.cict.fr/

Yeast Nomenclatural Changes, 1992

4. Remerciements

Les auteurs peuvent adresser des remerciements aux personnes ayant apporté une contribution substantielle à l'article. Il incombe aux auteurs d'obtenir des personnes dont ils citent le nom l'autorisation de le faire.

5. Bibliographie

Toutes les références bibliographiques citées dans le texte doivent figurer dans cette section. Dans la bibliographie, les références seront classées dans l'ordre alphabétique des auteurs et numérotées dans cet ordre. Les références bibliographiques citées dans le texte doivent être signalées par un numéro entre parenthèses. Pour un article de recherche, il est recommandé de limiter à 50 le nombre des références ; ce nombre pourra être doublé pour un article de synthèse.

Avant de soumettre leur article, les auteurs sont priés de contrôler l'exactitude de toutes les références et de vérifier que toutes sont citées dans le texte. Les noms des journaux et revues seront abrégés sans ambiguïté. En cas d'équivoque possible, ils seront retranscrits intégralement. Des exemples de titres abrégés et de présentation des références selon les normes de la *Revue* peuvent être trouvés dans les bibliographies de numéros récents.

Les données non publiées et les communications personnelles seront citées dans le corps du texte et non dans la bibliographie. Avant de soumettre leur article, les auteurs sont priés d'obtenir auprès des personnes ou organismes concernés l'autorisation de citer les sources non publiées ou les communications personnelles.

Chaque référence doit indiquer les noms suivis des initiales de tous les auteurs, l'année de publication, le titre complet, le nom du périodique, le volume, le numéro et les pages, conformément aux exemples ci-après.

– Article de journal ou de revue :

Duval B., Martin L., Roussel V. & Clément P. (1982). – Étude de la persistance des anticorps aphteux chez les veaux issus de mères vaccinées. *Rev. sci. tech. Off. int. Epiz.*, **1** (2), 875-892.

– Article sous presse :

Capua I. (2007). – La vaccination des volailles contre l'influenza aviaire à déclaration obligatoire. *In* Vaccination animale – Partie 1 : développement, production et utilisation des vaccins. *Rev. sci. tech. Off. int. Epiz.* (sous presse).

– Chapitre de livre ou rapport de conférence (pour les actes de conférence, il convient d'indiquer également l'éditeur et le lieu de publication, ainsi que le lieu et les dates de la conférence) :

Raimond P., Cousin C. & Mouthon R. (1992). – Évaluation du pouvoir immunogène de diverses souches de *Bacteroides nodosus*. In Comptes rendus du 4e Symposium sur les maladies ovines (P. Morice & P. Raimond, édit.) Paris, 12-14 février 1991. Vigier, Paris, 894-897.

– Les références à des documents disponibles sur Internet doivent comporter la mention de la date à laquelle ces documents ont été consultés. Les pages d'accueil des sites Internet ne sont pas des références bibliographiques. Lorsqu'une page web présente une information non datée, l'année de publication sera celle de la dernière mise à jour figurant en bas de la page web:

Union européenne (UE) (2004). – Revision of Directive 86/609/EEC on the protection of animals used for experimental and other scientific purposes. Page web : www.europa.eu.int/comm/environment/chemicals/lab_animals/revision_en.htm (consultée le 25 avril 2005).

– Lorsqu'un document existe en version électronique et en version papier, il convient de fournir les données bibliographiques complètes de la version papier, en ajoutant, à titre indicatif, la voie d'accès à la version électronique :

Scientists' Working Group on Biosafety (1998). – Manual for assessing ecological and human health effects of genetically engineered organisms. Part one: introductory text and supporting text for flowcharts. Part two: flowcharts and worksheets. The Edmonds Institute, Edmonds, WA. (Page web : www.edmonds-institute.org/manual.html, consultée le 25 avril 2005).

6. Tableaux

Chaque tableau doit porter un titre et être numéroté avec un chiffre romain. Les tableaux seront présentés en double interligne sur des pages séparées à la fin du texte. Chaque colonne sera désignée par un intitulé. Les valeurs individuelles seront autant que possible remplacées par leurs moyennes et leurs écarts types. Les notes, commentaires ou précisions sur les données numériques seront annoncés par de petites lettres en exposant (par exemple : $^{(a)}$, $^{(b)}$, $^{(c)}$, $^{(d)}$) et leur texte donné en note sous le tableau. Les abréviations d'usage peu courant seront explicitées. Les tableaux doivent illustrer les informations contenues dans le texte et non faire double emploi avec celles-ci.

7. Légendes des figures

Les figures seront présentées à la fin du texte, avec la légende correspondante sur une page séparée. Le titre doit être suffisamment explicite pour éviter au lecteur de se reporter au texte. L'objet, le lieu et la date seront mentionnés si possible. Ces informations peuvent être complétées par l'indication des unités de mesures et des sources et par des notes explicatives.

8. Figures

Les auteurs sont vivement encouragés à proposer des figures pour illustrer leur article sous réserve cependant que la figure apporte un complément d'information. Les photographies, diagrammes, graphiques, schémas et cartes géographiques sont considérés comme des figures. Les figures seront numérotées en chiffres arabes dans l'ordre de leur citation dans le texte. La Rédaction accepte pour publication les figures réalisées en formats Excel® et Microsoft® PowerPoint et comportant les données numériques pertinentes.

Les diagrammes, les cartes et les dessins devront être enregistrés dans des formats acceptant les retouches : par exemple, fichiers .eps, .ai (Illustrator®) ou .fr (Freehand®). Sont également acceptés tous documents numériques de qualité photographique. Les photographies numériques devront être enregistrées sous un format .jpg, .tiff ou .eps, largeur 455-2 055 pixels (soit 8,35 cm – 17,4 cm), résolution minimale 250 dpi (pixels par pouce). Les photographies traditionnelles et reproductions photographiques de documents originaux, au format maximum de 8 × 10 cm, sont également acceptées et devront porter au dos, écrits au crayon, leur numéro, le nom du premier auteur et une flèche indiquant le haut de la figure.

Tirés-à-part

Cinquante tirés-à-part seront envoyés gratuitement au premier auteur de l'article. Les commandes de tirés-à-part supplémentaires doivent être adressées à la Rédaction après acceptation de l'article.

Les auteurs et co-auteurs recevront chacun un exemplaire du numéro de la *Revue* où est parue leur contribution.

Instrucciones para los Autores

Objetivos de la *Revista*

La *Revista*, principal publicación científica y técnica de la OIE, cumple con dos de las funciones fijadas por sus Estatutos, a saber:

– promover y coordinar investigaciones y experiencias acerca de enfermedades infecciosas del ganado para las que cabe llamar a la colaboración internacional, y

– poner en conocimiento de los Servicios Veterinarios del mundo entero todos los hechos o textos y documentos que pudieran interesarles.

La *Revista* difunde informaciones relacionadas con las actividades veterinarias que pueden implicar una cooperación internacional tanto en materia de sanidad animal como de salud pública.

Otro de sus objetivos es dar a conocer a sus lectores las actividades de la OIE y de sus Países miembros en estos dos ámbitos.

La *Revista* está repertoriada en las bases de datos *Agris* (FAO, Italia) y *Littérature vétérinaire francophone* (Canadá), las fichas descriptivas *Index Veterinarius* y *Veterinary Bulletin* (bases de datos del CABI, Reino Unido), en *Biosis, Capsule Report, Current Contents® / Agriculture, Biology and Environmental Sciences, Fish and Wildlife Worldwide, Focus On®: Veterinary Science & Medicine, Index Medicus, Medline, SciSearch®* (Estados Unidos de América), en *Zoological Record* (Reino Unido) y en *Electre* (Francia).

Contenido

Cada volumen de la *Revista* incluye como mínimo dos números especiales, consagrados a un tema específico, para los cuales se designa a un experto de renombre mundial y se solicita la contribución de especialistas en el campo considerado con objeto de ofrecer el panorama más completo posible sobre el tema tratado.

Los números de la *Revista* que no son temáticos comprenden generalmente cuatro secciones y cada número reserva especial importancia a las síntesis y los artículos originales. A continuación se describen brevemente dichas secciones.

Síntesis

Las síntesis presentan estudios completos sobre un tema específico de actualidad, como, por ejemplo, la epizootiología, el diagnóstico, el tratamiento y el control de las zoonosis y demás enfermedades animales de mayor trascendencia para la comunidad internacional. Además, esta sección puede abordar también otros temas como la administración de Servicios Veterinarios, la legislación, los sistemas de información o la economía en sanidad animal.

Artículos originales

Estos artículos pueden tratar de investigación, técnicas de diagnóstico, experiencias y resultados en los campos del tratamiento y del control de enfermedades animales y deben ser de interés internacional, pero también pueden referirse a otros temas vinculados con la cooperación internacional de los Servicios Veterinarios.

Comunicaciones

Los temas tratados en esta sección coinciden con el desarrollado en los artículos originales, pero las comunicaciones son de menor longitud o, en todo caso, abordan un aspecto más limitado del asunto. Por otra parte, su contenido puede no ser original y referirse a trabajos ya publicados.

Informes

Se trata de breves reseñas de reuniones científicas y técnicas de la OIE o de otros organismos.

Condiciones para la aceptación de manuscritos

Los autores se comprometen a entregar a la *Revista* artículos que no hayan sido publicados antes, ni parcialmente ni en su totalidad, y cuya publicación por la OIE no requiera autorización previa. Al someter su manuscrito, y únicamente en caso de ser aceptado para publicación, los autores aceptan que el copyright de su artículo sea transferido a la OIE. No obstante, la Redacción considerará todas las solicitudes de autorización por parte de los autores con fines de reproducción de sus artículos.

Los manuscritos pueden ser presentados en cualquiera de los tres idiomas oficiales de la OIE: español, francés o inglés. Se recomienda a los autores que no escriben en su lengua materna que acudan a un relector profesional antes de enviar su artículo a la OIE.

El primer autor (o el autor encargado de la correspondencia con la OIE), recibe de inmediato un acuse de recibo de su manuscrito, el cual es sometido luego a la apreciación del Consejo Asesor de la *Revista*, cuya decisión se comunica posteriormente al autor.

Así mismo, el primer autor o el autor corresponsal son informados de los cambios de estilo que puedan aportarse al manuscrito con objeto de respetar las normas de la *Revista*. El manuscrito modificado se remite al primer autor o al autor corresponsal para su aprobación; resulta esencial que éstos respondan en un plazo de una semana.

Se ruega al primer autor que informe a los demás autores sobre los cambios efectuados en el texto antes de su publicación.

El Consejo Editorial se reserva el derecho de publicar los artículos aceptados en los tres idiomas oficiales de la OIE.

Presentación de manuscritos

Los autores deben enviar una versión electrónica del manuscrito original a la dirección electrónica a.souyri@oie.int o un fichero grabado en un disquete o CD a:

Jefe de Redacción
Revista científica y técnica
Organización Mundial de Sanidad Animal (OIE)
12, rue de Prony
75017 PARÍS, Francia.

Los manuscritos deben estar mecanografiados a doble interlínea, con márgenes anchos, en papel de tamaño A4 (21 × 29,7 cm). Las palabras no deben cortarse en final de línea. Todas las páginas deben ir numeradas y la presentación ha de respetar el siguiente orden:

1. Título, nombre y dirección de los autores
2. Resumen y palabras clave
3. Texto
4. Agradecimientos (si procede)
5. Bibliografía
6. Cuadros
7. Leyendas de las figuras
8. Figuras.

A continuación, se presentan algunas directivas para la preparación de los manuscritos. Para quienes deseen ejemplos concretos, se sugiere consultar un número reciente de la *Revista*.

1. Título, nombre y dirección de los autores

El título del artículo debe ser corto (máximo 70 caracteres) y no incluir abreviaturas. Para facilitar la búsqueda de información, debe utilizarse una terminología estándar. Por ejemplo: "Encuesta epidemiológica sobre el carbunco sintomático de los bovinos en Francia" (tema, enfermedad, especie, país).

Los apellidos de los autores irán precedidos de las iniciales de sus nombres y seguidos de uno o más números de llamada. El lugar de trabajo de cada autor con su dirección completa deberá indicarse a continuación, en el orden de los autores. Por ejemplo:

M.L. Bastos [(1)], J.C. Esteban [(2)] & D. Tamborenea [(2)]

(1) Laboratorio de Inmunopatología, Centro Nacional de Sanidad Animal, Mansilla 2923, 4025 Buenos Aires, Argentina
(2) Centro de Inmunopatología, Facultad de Agronomía y Veterinaria, Avda. Centenario 203, Montevideo, Uruguay

2. Resumen y palabras clave

El resumen, redactado en el idioma original y de 150 palabras como máximo, debe reflejar la metodología, los resultados principales y las conclusiones del estudio y reflejar lo esencial de su contenido. A continuación del resumen, el autor incluirá entre ocho y diez palabras clave. La OIE se encargará de traducir el resumen en los dos otros idiomas oficiales de la Organización.

3. Texto

La extensión de los manuscritos no debe ser superior a 4.000 palabras (14-16 páginas mecanografiadas). Si desea publicar un artículo más largo, el autor deberá solicitar la aprobación de la Redacción. Los párrafos no deberán ser demasiado largos; en general, no sobrepasarán las 20 líneas (200 palabras).

Los autores deberán esforzarse en redactar de manera clara y concisa. Los trabajos experimentales y estudios epidemiológicos se presentarán según la siguiente estructura: introducción, materiales y métodos, resultados, discusión, conclusiones, bibliografía.

Las unidades de medida se expresarán en el sistema métrico y, cuando sea necesario, en unidades SI.

Las técnicas de diagnóstico nuevas se describirán con detalle suficiente (por ejemplo: estándar de referencia, tipo de antisuero o antígeno, especificidad, sensibilidad, etc.). Las técnicas conocidas o ya descritas en un periódico o revista de audiencia internacional no se describirán, sino que se mencionarán con las referencias bibliográficas correspondientes.

Los medicamentos veterinarios, reactivos y materiales de laboratorio se designarán en el texto por su nombre genérico (y, ocasionalmente, su nombre comercial).

Las abreviaturas y acrónimos deberán explicarse la primera vez que se utilicen. En la medida de lo posible, las notas se incorporarán al texto.

Los autores deberán indicar en qué parte del texto desean que se incluyan los cuadros y figuras.

Se recomienda a los autores consultar las nomenclaturas internacionales recientes publicadas por instituciones científicas reconocidas. Los nombres de especies vendrán seguidos por su nombre latín entre paréntesis y en letra cursilla. Los autores podrán consultar al respecto las siguientes obras de referencia:

Mammal Species of the World, 3ª edición, 2005, Johns Hopkins University Press

Distribution and Taxonomy of Birds of the World, 1991

Virus Taxonomy – Classification and Nomenclature of Viruses – Eighth Report of the International Committee on Taxonomy of Viruses, 2005, Elsevier

List of Prokaryotic names with Standing in Nomenclature, sitios web: http://www.bacterio.net o http://www.bacterio.cict.fr/

Yeast Nomenclatural Changes, 1992

4. Agradecimientos

Se podrán incluir agradecimientos a las personas cuya contribución para la realización del artículo haya sido fundamental. Cada autor se encargará de obtener la correspondiente autorización para citar a dichas personas.

5. Bibliografía

Todas las referencias bibliográficas mencionadas en el texto deben incluirse en esta sección. En la bibliografía, las referencias se numerarán siguiendo el orden alfabético de autores. En el texto, las referencias bibliográficas se indicarán mediante el respectivo número entre paréntesis. Para un artículo de investigación, se recomienda limitarse a cincuenta referencias. Tratándose de artículos de síntesis, este número podrá duplicarse.

Antes de entregar su artículo, se ruega a los autores que comprueben la exactitud de las referencias y verifiquen que todas vengan citadas en el texto. Los nombres de periódicos y revistas deberán abreviarse sin ambigüedad posible. En caso de duda, se escribirá el título completo. Para tener ejemplos de abreviaturas de títulos y del formato bibliográfico utilizado en la *Revista*, se sugiere a los autores consultar un número reciente.

Los datos aún no publicados y las comunicaciones personales se citarán en el cuerpo del texto y no en la bibliografía. Los autores habrán obtenido previamente la autorización de citar estos datos y comunicaciones personales.

En cada referencia, deben figurar los apellidos, seguidos de las iniciales de sus nombres, de todos los autores, el año de publicación, el título completo, el nombre del periódico o revista, el volumen, el número y las páginas, de acuerdo con los ejemplos siguientes. .

– Artículo de periódico o de revista:

Basualdo L.S., Gonzalez A.L. & Zemborain N. (1982). – Estudio de la producción de anticuerpos aftosos en bovinos con carencia de proteínas. *Rev. sci. tech. Off. int. Epiz.*, **1** (2), 875-892.

– Artículo en prensa:

I. Capua (2007). – Vacunación de aves de corral contra la influenza aviar de notificación obligatoria. In Vacunación animal. Parte 1: desarrollo, producción y utilización de vacunas. *Rev. sci. tech. Off. int. Epiz.* (en prensa).

– Capítulo de libro o informe de conferencia (debe incluir la editorial, el lugar de publicación, así como el lugar donde se celebró la conferencia y sus fechas):

Castilla D. & Diaz Arredondo G.H. (1992). – Evaluación del poder inmunógeno de varias cepas de Bacteroides nodosus. *In* Actas del IV Simposio sobre enfermedades ovinas (P. Laurentín & E. Ramírez, edit.), Madrid, 12-14 de febrero de 1991. Galerna, Madrid, 894-897.

– Los autores que desean citar documentos bajados de la web deben indicar la fecha en que han consultado las páginas citadas. La página principal de un sitio web, sin referencia a un documento particular, no se considerará como une referencia bibliográfica. Cuando el documento no tiene fecha explícita, se considerará como año de publicación el de la última actualización de la página web, que suele indicarse al pie de página:

Unión Europea (UE) (2004). – Revision of Directive 86/609/EEC on the protection of animals used for experimental and other scientific purposes. Página web: www.europa.eu.int/comm/environment/chemicals/lab_animals/revision_en.htm (fecha de consulta: 11 de abril de 2005).

– Las referencias a documentos que existen en forma electrónica y en versión papel incluirán todas las indicaciones bibliográficas habituales, agregando la dirección web como una información adicional para el lector:

> Scientists' Working Group on Biosafety (1998). – Manual for assessing ecological and human health effects of genetically engineered organisms. Part one: introductory text and supporting text for flowcharts. Part two: flowcharts and worksheets. The Edmonds Institute, Edmonds, WA. (página web: www.edmonds-institute.org/manual.html, fecha de consulta: 25 de abril de 2005).

6. Cuadros

Cada cuadro debe tener un título y un número romano y presentarse mecanografiado, con interlínea doble, en una página separada al final del texto. Cada columna tendrá su propio encabezamiento y los valores individuales se reemplazarán, en la medida de lo posible, por sus promedios y sus desviaciones estándar. Para los comentarios, notas y precisiones relativos a los datos numéricos, se utilizará como llamada una letra minúscula en exponente (por ejemplo: [a], [b], [c], [d]) que remitirá al texto al pie del cuadro. Las abreviaturas poco usuales deberán explicarse. Los cuadros deben ilustrar, y no repetir, la información contenida en el texto.

7. Leyendas de las figuras

Todas las figuras se adjuntarán al final del texto y cada una llevará su respectiva leyenda en una página separada. Los títulos deben ser explícitos, de manera que el lector no tenga que buscar su significado en el texto. Cada vez que sea posible, se indicarán el objeto, el lugar y la fecha, pudiendo completarse esta información con unidades de medida, fuentes y notas explicativas.

8. Figuras

Se recomienda a los autores utilizar figuras para ilustrar su artículo. Se consideran figuras los diagramas, gráficos, fotografías, dibujos y mapas. Las figuras se numerarán con números arábigos, en el orden en que son citadas en el texto.

La Redacción aceptará publicar únicamente los gráficos realizados en Excel® y Microsoft® Power Point, siempre que se acompañen de la planilla de cálculo asociada. Los diagramas, así como los dibujos y mapas deberán de presentarse idealmente en formato de dibujo trazable, como por ejemplo: .eps editable, .ai (Illustrator®), .fr (Freehand®); aunque se aceptarán documentos con las mismas características que las fotografías digitales. Las fotografías digitales deberán entregarse en formato .jpg, .tiff o .eps. Con un ancho de entre 455 y 2.055 píxeles (8,35 cm – 17,4 cm) y una resolución de no menos de 250 dpi (píxeles por pulgada). Las fotografías tradicionales, tendrán un formato máximo de 8 cm × 10 cm. También se aceptarán reproducciones fotográficas de documentos originales. Éstas, deberán llevar al dorso, escritos con lápiz, su número, el nombre del primer autor y una flecha que indique su parte superior.

Separatas

El primer autor recibirá cincuenta separatas de cortesía. Los pedidos de separatas suplementarias deberán remitirse a la Redacción cuando el artículo haya sido aceptado.

Los autores y coautores recibirán un ejemplar del número de la *Revista* en que su artículo es publicado.

Notes / Apuntes

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Directeur de la publication : B. Vallat

Directeur de la rédaction : P.-P. Pastoret