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Contents

Chapter 1
Introduction

Abbreviations

AIDS	Acquired immune deficiency syndrome
AR	Agglutination reaction
BA	Blood agar
BHI	Brain heart infusion agar
BSL	Biosafety level, 1 to 4
BSC	Biosafety cabinet, I to III
CCHF	Crimean-Congo haemorrhagic fever
CDC	Centers for Disease Control and Prevention, USA
CEE	Central European encephalitis
CFT	Complement-fixation test
CNS	Central nervous system
CSF	Cerebrospinal fluid
CT	Computerized tomography
CTF	Colorado tick fever
ECDC	European Centre for Disease Prevention and Control, Stockholm
EEE	Eastern equine encephal(omyel)itis
ELISA	Immunoenzymatic serological test
EMC	Encephalomyocarditis
FMDV	Foot-and-mouth-disease virus
FAO	Food and Agriculture Organization of the United Nations
HCPS	Hantavirus cardio-pulmonary syndrome
HIT	Haemagglutination-inhibition test
HIV	Human immunodeficiency virus
HFRS	Haemorrhagic fever with renal syndrome
HPAI	Highly pathogenic avian influenza
HPS	Hantavirus pulmonary syndrome
HUS	Haemolytic-uremic syndrome
IF, IFA	Immunofluorescence microscopy, immunofluorescence assay
JE	Japanese encephalitis
KFD	Kyasanur forest disease
LB	Lyme borreliosis

LCM	Lymphocytic choriomeningitis
LD	Lethal dose
LI	Louping ill
MID	Minimum infectious dose
MLD	Minimum lethal dose
MLST	Multilocus sequence typing
MRSA	Methicillin-resistant *Staphylococcus aureus*
MVE	Murray Valley encephalitis
NFD	Natural focus of disease/infection (singular or plural)
OHF	Omsk haemorrhagic fever
OIE	World Organization for Animal Health
ONN	O'nyong nyong
PCR	Polymerase chain reaction
PFGE	Pulse-field gel electrophoresis
RDPA	Reaction of diffuse precipitation in agar (gel), immunodiffusion test
RES	Reticuloendothelial system
RFLP	Restriction fragment length polymorphism
RLB	Reverse line blot (molecular detection technique)
RIHA	Reaction of indirect (passive) haemagglutination
RMSF	Rocky Mountain spotted fever
RSSE	Russian spring-summer encephalitis
RT-PCR	Reverse transcription polymerase chain reaction
RVF	Rift Valley fever
SARS	Severe acute respiratory syndrome
SFG(R)	Spotted fever group (rickettsiae)
SFN; SFS	Sandfly fever Naples; Sandfly fever Sicily
SGA	Sabouraud glucose (dextrose) agar
s.l.	*sensu lato*
SLE	St. Louis encephalitis
SSH	Snowshoe hare virus
s.s.	*sensu stricto*
TBE	Tick-borne encephalitis
TC	Tissue (in fact, cell) culture
TOT	Transovarial transmission
TST	Transstadial transmission
vCJD	New variant of Creutzfeld-Jakob disease
VEE	Venezuelan equine encephal(omyel)itis
VNT	Virus-neutralisation test
VSV	Vesicular stomatitis virus
WB	Western blotting
WEE	Western equine encephal(omyel)itis
WHO	World Health Organization
WNV	West Nile virus (WNF, West Nile fever)
YF	Yellow fever

This book originated while lecturing a graduate course in microbiology called first "Zoonoses", and later more specifically "Microbial Zoonoses and Sapronoses" at the Faculty of Science, Masaryk University in Brno, in the years 1992–2009. It presents an up-to-date survey of the problems of microbial zoonoses and sapronoses, and can be used not only by microbiologists but also zoologists or students of veterinary and human medicine including Ph.D. students.

Preparing a modern review of this turbulent discipline has been difficult. In the last two decades or so, we have encountered a number of new emerging infectious diseases (e.g. SARS, Ebola, Nipah, hantavirus pulmonary syndrome) or diseases newly recognized (Lyme borreliosis, ehrlichiosis, anaplasmosis), re-emerging (West Nile fever in Europe), geographically expanding (West Nile encephalitis in the Americas), starting to occur at altitudes higher than before (TBE and LB in Europe), with an increasing incidence (campylobacterosis, or salmonellosis after 1988), those changing the range of hosts and vectors or caused by agents modifying their characteristics (virulence, antibiotic resistance) and clinical symptoms they produce in the host. A number of these emerging diseases has been due to the ability of some pathogens to cross the "species barrier" of their hosts, as observed with, e.g., vCJD, avian and swine influenza, SARS or AIDS. It has been estimated that from a total of about 177 (re)emerging diseases, zoonoses present as much as 75% (Taylor et al. 2001, Woolhouse and Gowtage-Sequeria 2005). A number of zoonoses and insect-borne diseases (malaria, dengue, filariosis, trypanosomiases, leishmaniasis) jeopardise the lives of millions of people every year.

Another problem in writing this book has been frequent and profound changes in nomenclature and taxonomy of many zoonotic and sapronotic agents (e.g., *Ehrlichia, Anaplasma,* other rickettsiae, *Pneumocystis, Rhinosporidium*, microsporidia). In addition, the number of known zoonotic and sapronotic aetiological agents of human diseases is high and growing steadily (more than 815 today: Woolhouse and Gowtage-Sequeria 2005), and it has been necessary to take in consideration only the important ones while neglecting those that are regarded as "minor" at present. Intentionally, more emphasis is given in this book to the ecological aspects of zoonoses and sapronoses (haematophagous vectors of the diseases and their bionomics; vertebrate hosts of zoonoses; habitats of the agents and their geographical distribution; natural focality of the diseases) than to clinical and therapeutic details.

Chapter 2
Types of Human Disease by Source of the Infectious Agent

In general, the source of infection for human beings is another human, or an animal, or the environment (extra-animal substrate). In line with this we can distinguish human infectious diseases as anthroponoses, zoonoses and sapronoses, respectively. The names have been derived from the *Greek* "νόσος" (nosos) = disease; "ανθρόπος" (anthropos) = man; "ζωός" (zoos) = living (animal); "σαπρός" (sapros) = decayed.

Type of human disease	The source of infection (habitat of the agent)	Man-to-man transmission
Anthroponosis	Human	Common
Zoonosis	Animal	Uncommon or rare
Sapronosis	Abiotic substrate	Very rare

Anthroponoses are diseases transmissible only from man to man. Typical microbial anthroponoses are typhoid fever (typhus abdominalis, caused by *Salmonella typhi*), paratyphoid fever, shigellosis (bacillary dysentery, the agents are *Shigella* spp.), whooping cough (the agent is *Bordetella pertussis*), diphtheria (*Corynebacterium diphtheriae*), streptococcal diseases (tonsillitis, scarlet fever, erysipelas), syphilis (*Treponema pallidum*), yaws (*Treponema pertenue*), gonorrhoea (*Neisseria gonorrhoeae*), *Haemophilus* infections (including Brazilian purpuric fever), chancroid (ulcus molle, *Haemophilus ducreyi*), tuberculosis caused by *Mycobacterium tuberculosis*, leprosy, trachoma (inclusion conjunctivitis) and lymphogranuloma venereum (*Chlamydia trachomatis*), chlamydial pneumonia and cardiovascular disease, mycoplasmal pneumonia, peptic ulcer disease, pneumococcal pneumonia, invasive group A streptococcal infections, meningococcal disease, common cold, epidemic influenza (except for that caused by certain types of zoonotic orthomyxoviruses – avian, swine or equine), poliomyelitis, some types of viral hepatitis (A, B, C), epidemic viral gastroenteritis, rubella, measles (morbilli, rubeola), mumps (infectious parotitis), epidemic haemorrhagic conjunctivitis, infectious mononucleosis, cytomegalovirus infection, smallpox (variola), herpes simplex, chickenpox (herpes zoster, caused by varicella-zoster virus), AIDS, ringworm caused by *Trichophyton rubrum, Epidermophyton floccosum* and some other

species of dermatophytes, candidosis, *Pneumocystis* pneumonia (caused by human genotypes of *P. jirovecii*), some microsporidial infections, cryptosporidiosis (human genotypes), giardiasis (human genotype), trichomoniasis, amoebiasis (amoebic dysenteria, *Entamoeba histolytica*), and several other human diseases.

Zoonoses are diseases transmissible from animal to man. The term was invented by Rudolf Virchow during his study of trichinellosis in 1855. In general, zoonoses are not transmissible by contact from the patient to other people, although there are notable exceptions with haemorrhagic fevers Lassa, Machupo, Ebola, Marburg, CCHF, or SARS, plague etc. The term used earlier for diseases transmissible from animals to man was "anthropo-zoonoses". By analogy, a term "zoo-anthroponoses" was used for diseases transmissible the other way, from man to animals; however the number of the latter is limited (e.g., influenza, tuberculosis). Regrettably, many epidemiologists started to use these both terms in a reverse order (zooanthroponoses as diseases transmissible from animals to man), or promiscuously. The WHO therefore suggested using "zoonoses" as the official term, and that the two previous terms should no longer be used. According to an expert commission of WHO/FAO the definition of zoonoses reads as follows: "*Zoonoses are diseases and infections which are naturally transmitted between vertebrate animals and man*" (WHO Tech Rep Ser 169, 1959). This definition was confirmed also by the third and fourth report of this Commission (WHO Tech Rep Ser 378, 1967; WHO Tech Rep Ser 682, 1982).

The number of known zoonoses is growing steadily, at present exceeding 250, about 80 of which are common. From the zoonoses discovered in recent decades we can mention for instance Lyme borreliosis, anaplasmosis, HFRS and other haemorrhagic fevers Lassa, Marburg and Ebola, hantavirus pulmonary syndrome, SARS or Nipah fever. However (and luckily), only a limited number of zoonoses can cause extensive outbreaks – e.g., salmonellosis, Q fever, yellow fever, Japanese encephalitis, West Nile fever, chikungunya, RVF and American equine encephalomyelitides. Other zoonoses attract public (and media) attention due to their high lethality, sometimes associated with a high contagiosity for attending medical personnel (haemorrhagic fevers).

The transmission of zoonotic agents from animal to man can be realized either directly, or indirectly, *via* a vector (usually a haematophagous, i.e. blood-feeding member of the phyllum *Arthropoda*); in the latter case we can speak about obligate or facultative vector transmission (so-called "meta-zoonosis" according to WHO 1967).

Zoonoses can further be divided according to the habitat or ecosystem where their agents circulate as: (i) synanthropic, with an urban (anthroponotic, domestic) cycle where the source of human infection are most often domestic animal or synanthropic vertebrates bound to human dwellings; or (ii) exoanthropic, with a sylvatic (feral) cycle, and their reservoir is in the countryside outside human dwellings – in so-called natural foci. The first group of zoonoses forms, e.g., vesicular stomatitis, brucellosis, bovine tuberculosis, glanders, listeriosis, erysipeloid or ringworm caused by *Trichophyton verrucosum* and *Microsporum canis*. These diseases are transmissible usually percutaneously, aerogenically, alimentarily or per conjunctiva, and often present typical occupational diseases in farmers, butchers and veterinary

doctors. The second group form classic diseases with natural focality in the sense of J. N. Pavlovsky, e.g. tick-borne encephalitis and other arboviroses, tularaemia, plague or scrub typhus, where man acquires the infectious agent after entering a natural focus, commonly by the attack of an haematophagous vector. However, there does not exist a clear distinction between these two groups of zoonoses, and a number of zoonoses exhibit both urban and sylvatic patterns of circulation – yellow fever, American trypanosomiasis, plague.

Sapronoses are diseases transmissible to man from an abiotic substrate in the environment – soil, water, decaying plants, animal excrement, carrion and other substrata. The most important feature is that the sapronotic agent replicates actively in these abiotic substrata – it is not the mere persistence of the microbe in the environment, nor secondary contamination of environmental objects with the agent from animal sources. The source of infection is therefore not an animal or man. Sapronotic agents are capable of reproduction both in abiotic environment (saprophytic phase) and in the organisms of homoiothermous vertebrates including man (parasitic phase). They show a "dual life": saprophytic and parasitic (pathogenic). In particular, many human mycoses belong to sapronoses, especially visceral ones, like coccidioidomycosis, histoplasmosis, blastomycosis, emmonsiosis, cryptococcosis, and also some bacterial (legionellosis) and protozoan (primary amoebic meningoencephalitis, naegleriosis) diseases, but no viral, rickettsial or chlamydial diseases (because obligate intracellular parasites are unable to reproduce extracelullarly).

The term "sapronosis" and its definition were introduced into epidemiology by Russian microbiologist V. I. Terskikh (1958) in the article entitled "On diseases of humans and animals caused by microbes capable of reproduction in external milieu . . .", and for further use it was accepted by Somov and Litvin (1988), Krauss et al. (1997), Hubálek (2003) and others. The definition of sapronoses reads after Krauss et al. (1997): *Krankheiten*, *deren Erreger keine Wirbeltiere als Reservoir erfordern*, *weil sie in Wasser*, *Boden*, *auf Pflanzen usw. vorkommen und von dort aus auch Vertebraten infizieren können*.

The difference between zoonoses and sapronoses is sometimes fuzzy to vague, and a disease can be called, depending on circumstances, either zoonosis or sapronosis (e.g., listeriosis, pseudotuberculosis, anthrax).

A characteristic feature of a majority of zoonoses and sapronoses is that man is a dead-end host in the epidemic process (inter-human transmission is absent), although his or her disease can often be serious, even fatal. The pathogenic agent is evolutionarily not adapted to its accidental new host. From an ecological point of view, all pathogenic microorganisms are parasites of their hosts (animals or plants). The evolution of parasitism in today's pathogens proceeded along the trajectory saprophyte (commensal) → facultative (occasional) parasite → obligate parasite. An obligate parasite as a rule does not kill its host on which it is evolutionarily adapted (the untimely death of the host would lead to a bad fate for it), while unusual, incidental but susceptible hosts (man in our case) where parasitic co-evolution has not occurred can be severely harmed or killed.

Classification of infectious diseases into anthroponoses, zoonoses and sapronoses is a combination of an anthropocentric view (i.e. treating only diseases

of humans, while not those of animals) and ecological view (studying the habitats in which the infectious agent lives: Hubálek 2002).

We can distinguish six classes of zoonoses and sapronoses according to systematic arrangement of their aetiological agents:

I   Viroses
II   Bacterial diseases
III   Mycoses
IV   Protozoan diseases
V   Helminthoses (<u>invasions</u>: cestodes, trematodes, nematodes)
VI   Diseases caused by arthropods (<u>infestations</u>)

However, we will concentrate on microbial zoonoses and sapronoses in this monograph, leaving aside diseases caused by multicellular organisms (helminths and arthropods, i.e. invasions and infestations). The agents of the classes IV–VI belong to the field of parasitology, and cause the so-called parasitic zoonoses and sapronoses.

Chapter 3
A History of Zoonoses and Sapronoses and Research into Them

The history of these diseases and of their study is given in a brief chronological review of the most important events (important epidemics) and milestones of their study (relevant microbiological discoveries). In some historical data it is difficult to differentiate between the year of discovery and the year of its publication.

Eighteenth century BC, Babylonian codex Eshuna: "mad dogs" (most probably rabies).

Fourth century BC, Talmud: notes on mad dogs in Israel.

1320 BC, Bible: a description of a plague epidemic among the Philistines (enlarged lymphatic nodes, overpopulation of "mice" at the same time).

556 BC, China: a description of rabies.

435 BC, HIPPOKRATES: "*Epidemion*" (the causes of diseases are in environment).

429–426 BC, THUCYDIDES: the "plague of Athens" killed about one-quarter (75,000–100,000) of the citizens of Athens during the siege by the Spartan army (the "Peloponnesian wars", 431–404 BC) while not affecting besieger, and for 3 additional years thereafter. When the Athenian navy was dispatched later against Sparta, it was also heavily affected by the disease: one-quarter of 4,000 soldiers died, including the commander Pericles and his two sons. The symptoms described by Thucydides involve high fever, facial erythema, pustular rash to ulcers on the skin (sometimes gangrenes), bleeding from gums, tongue and throat, conjunctivitis, cough, sneezing, runny nose, diarrhoea, severe vomiting, dehydration, sleeping distress; and some of those who survived lost their toes and fingers, vision or memory. According to the symptoms and some epidemiological features, as the most probable cause of this epidemic could be regarded epidemic louse-borne typhus, while plague is improbable; additional alternative hypotheses have included ergotism or smallpox; some authors also considered (but as much less probable causes) abdominal typhus, malaria, dengue, WN fever, Ebola haemorrhagic fever, CCHF, gastrointestinal anthrax or brucellosis. (In our opinion, a combination of epidemic typhus with ergotism is a feasible hypothesis). In any event, this outbreak contributed significantly to the decline of Athens.

224 BC, China: the first major epidemic of plague reported.

First century, SUSRUTA (a Brahmin priest in India) and COLUMELLA (an educated Roman farmer): the spread of fevers is caused by "biting flies".

100, RUFUS from Ephesus: a description of bubonic plague in Libya, Egypt and Syria (here was plague known since third century BC).

541–546: 1st plague pandemic ("Justinian", Byzantine Caesar) started in Egypt, continued in Palestine, Syria, Constantinople, and engulfed the whole known world including Europe (Italy, Spain, France, Germany, Denmark, England), central Asia and China (an estimated 100 million persons succumbed out of about 142 million contracting the disease).

1321, Florencia: "*Statuti sanitari*": rule of the city how to behave when there occurs an epidemic.

1346–1352, 2nd plague pandemic ("The Black Death") in Europe – it started already in about 1330 in central Asia, where almost entire populations of Tatars and Saracens had succumbed. During the siege of the Genoan fortress of Caffa (today's Theodosia) in the Crimea, Tatars catapulted the cadavers of their soldiers that had succumbed to plague within (the first "biological warfare"); Genoan merchants escaped the fortress but spread the plague to Constantinople and Messina. The ensuing pandemic engulfed the whole Italy, Dalmatia, France, England and Norway in 1348; then Germany and Moravia in 1349; and Poland, Russia (for instance in Smolensk died all citizens except for five persons) in 1350–1351. In Europe, one-quarter of inhabitants succumbed (about 25 million), and an estimated 25 million died in Asia [the pandemic probably considerably contributed to the fall of the Mongolian empire] and Africa earlier; 1361, 1371 and 1380–1382 saw follow-up outbreaks in Europe.

1348, Venezia: "*Magistrato della Sanità*": probably first hygienic office for control of plague and other diseases.

Fifteenth century, a new epidemic of plague in Germany, France and Russia.

1490, Granada: during the siege of the town kept by Maurs, a total of 17,000 Spanish soldiers succumbed to epidemic louse-borne typhus.

1493–1495, Haiti – Hispañola: first description of yellow fever (dengue?).

1528, Naples: 14,000 French besiegers succumbed to epidemic (louse-borne) typhus.

1542, Hungary: 30,000 people died from epidemic typhus.

1545, Mexico: an epidemic of haemorrhagic fever "cocolitztli" (aetiology has remained unexplained).

1546, FRACASTORO: "*De contagione et contagiosis morbis et eorum curatione*" – first theory of infectious diseases caused by germs ("*seminaria morbi*"); he

described three modes of infection (*contagiosis morbis*) – *per contactum; per fomites* (indirectly – via clothes, bedding, things); *ad distans* (via air).

1554, AGRICOLA: a treatise on plague ("*De peste libri III*").

1575–1577, an epidemic of plague in Italy (Milan and Venezia 70,000 victims, etc.).

1585, Milan: a pact between Milan and Swiss cities for control of plague (commerce etc.).

1606–1620, big outbreaks of plague in Germany, France, Switzerland, Italy.

1647–1648, an extensive epidemic of yellow fever in the Caribbean – Little Antilles (Barbados → St. Cristof → Guadeloupe), Yucatan and Cuba (e.g., in Havana one-third of citizens died); the disease was imported from West Africa during the slave trade (viraemic slaves and infected *Aedes aegypti* mosquitoes on ships).

1648–1649, Prague: a major epidemic of plague in the city besieged by Swedish soldiers at the end of the Thirty-Year's War.

1653–1654, plague in southern, western and northern (Sweden) Europe with a great number of victims (e.g., London 60,000; Genova 50,000; Amsterdam 50,000).

1675–1684, another extensive epidemic of plague in Europe (central) spreading from Poland to Moravia, Bohemia (13,000 victims in Prague alone), Austria, Germany etc.: schools and churches were closed and public religious services forbidden; *magistri sanitatis* (directors of health) and plague regulations (including obligatory notification of sick and dead persons) were installed in many towns; preventive measures were fixed for physicians, priests−confessors, and friars attending patients and dying persons; quarantine was imposed on foci of infection.

1709, plague in Poland, Hungary and Russia.

1713–1715, plague in Vienna (Austria), spread to Prague (Bohemia) by an infected tailor, and later to Moravia (Olomouc); the last plague epidemic in central Europe.

1720, plague in Marseille.

1737, plague in Mesina and environs (46,000 victims).

1741, an epidemic of yellow fever in Portugal and Spain (e.g., 10,000 victims in Cadiz).

1759, the first veterinary school in the world founded in Lyon.

1762, big outbreak of YF in Cuba.

1778, great YF epidemic in Senegal.

1779, extensive epidemic of sandfly (pappataci) fever among French soldiers in the Mediterranean (Italy) during the Napoleonic wars.

1779, big outbreak of dengue fever in Indonesia (on the island of Java).

1788, ANDRIEVSKI: human and animal anthrax are identical (autoinoculation).

1791, The Royal Veterinary College founded in London.

1793, Philadelphia (USA): yellow fever killed 10% citizens of the town.

1802–1803, YF epidemics in Portugal and Spain (80,000 victims), and on Haiti (29,000 French soldiers).

1804, ZINKE: experimental transmission of rabies by saliva from infected animals to healthy ones.

1817, beginning of pandemic cholera: India → China, Japan, Indonesia, Russia → Baltic, England, and Ireland, then → North America, Mexico.

1820, ERNST: dermatomycosis of a man, the source was a diseased cattle.

1839–1841, SCHÖNLEIN and GRUBY: the cause of human and animal favus is a fungus.

1849–1855, POLLENDER and REYER: microscopic detection of the anthrax agent (rods) in the blood of diseased sheep and humans.

1850, FRESENIUS: a description of avian aspergillosis and cultivation of the agent (*Aspergillus fumigatus*).

1851, Paris: 1st international conference on preventive measures against cholera, plague and YF.

1853, New Orleans, USA: YF (29,000 cases, 8,000 succumbed to the disease).

1854, BEAUPERTHUY: hypothesis on the transmission of YF by mosquitoes.

1855, SNOW: a monograph "*On the mode of communication of cholera*" (John Snow found that water from a certain pump on Broad Street in London was the source of infection with cholera that caused death of more than 500 people in August 1854; he laid the foundations of descriptive epidemiology).

1859, LAMBL: description of a protozoan *Cercomonas intestinalis* (now *Giardia lamblia*) as a cause of diarrhoea.

1863–1865, DAVAINE: experimental transmission of anthrax.

1866, GRAWITZ and REMAK: cultivation of the favus agent (*Trichophyton*) and demonstration of its pathogenity by autoinoculation (Remak) – cf. 1839.

1867, LISTER: introduction of aseptical and antiseptical techniques during chirurgical operations.

1870, Brazil: a big outbreak of YF.

1867–1873, OBERMAIER: description of the agent of endemic recurrent typhus (spirochete *Borrelia recurrentis*) in the blood of patients [he died 1873 after autoinoculation of a blood sample taken from a patient with cholera].

1873, LÖSCH: unraveled amoebic diarrhoea (*Entamoeba histolytica*).

1874, MÜNCH: confirmed the finding of Obermaier; a theory on transmission of spirochetes by lice, fleas and other insects.

1876, KOCH: cultivation of the anthrax agent (*Bacillus anthracis*) and detection of its ability to form spores: foundings of scientific research of infectious diseases (so-called Koch's postulates for verification of the disease agent).

1877, MANSON: mosquitoes are biological vectors of filariae *Wuchereria bancrofti* on Taiwan (first evidence on participation of mosquitoes in the transmission of diseases).

1878, plague in the Astrakhan region of Russia (Lower Volga), with 416 victims (the last outbreak of plague in Europe).

1878, an extensive epidemic of YF in the USA (132 towns were hit; 75,000 cases – 16,000 persons died).

1880, LAVERAN: discovery of the aetiological agent of malaria (*Plasmodium*) in the blood of patients [Nobel prize 1907].

1880, RITTER described psittacosis in 7 patients in Switzerland, acquired from exotic birds.

1880–1881, PASTEUR and TOUSSAINT: vaccine against anthrax (an attenuated culture of *B. anthracis*) successfully demonstrated in a public experiment on sheep (25 vaccinated, 25 controls) in Pouilly-le-Fort.

1881, FINLAY: hypothesis on transmission of YF by the mosquito *Aedes aegypti* in Cuba (cf. also 1854).

1881, KOCH: introduction of solid nutrient media (with gelatine and agar) for isolation and cultivation of pure cultures of microbes.

1882, PASTEUR: serum against rabies tested on animals.

1882–1883, LÖFFLER and SCHÜTZ: discovery of the agents of erysipeloid and glanders, and vaccination of swines against erysipeloid.

1884, GRAM: differential staining of bacteria for microscopy.

1884, NICOLAIER: microscopical evidence of the agent of tetanus.

1884, LICHTHEIM: pathogenicity of the fungus *Absidia corymbifera* for humans.

1885, PASTEUR: antiserum to rabies tested on man.

1885, CARRIÓN: fatal autoinfection with bartonellosis (connection between *verruga peruana* and *febris Oroya*).

1886, BRUCE: isolation of the agent (*Brucella melitensis*) from victims with "Maltese fever" and its experimental transmission to monkeys.

1886, an epidemic of yellow fever in the USA (20,000 victims).

1888, GÄRTNER: *Salmonella enteritidis* is a common agent of the human and cattle disease (58 human patients after eating meat from a diseased cattle = first description of food-borne human salmonellosis).

1888, Paris: Pasteur Institute founded.

1888, BABES: an intraerythrocytic protozoon is the agent of cattle piroplasmosis (described as *Haematococcus bovis*, renamed by Starkovici *Babesia bovis* in 1893).

1889, KITASATO: cultivation of tetanus bacterium (*Clostridium tetani*).

1890, EPPINGER: isolation of *Nocardia asteroides.*

1891, Berlin: Institute for Infectious Diseases (today called Robert Koch Institute) founded.

1892, LÖFFLER: isolation of *Salmonella typhimurium.*

1892, BEHRING and KITASATO prepared tetanus antiserum.

1893, SMITH and KILBORNE observed transmission of Texas cattle fever (piroplasmosis, caused by *Babesia bigemina*) by ticks *Boophilus annulatus* (first detection of transmission of a pathogen by ixodid ticks, including demonstration of both TST and TOT).

1893, MORANGE: transmission of psittacosis from parrots to humans.

1894–1930 (...1955), 3rd pandemic of plague started in Hongkong after dispersal from continental China; rats and their fleas spread the disease on ships to many harbours in the world (Japan, India, Europe, Africa, Americas and Australia): 30 million persons were affected, 12 millions of them died.

1894, YERSIN and KITASATO: isolation of the plague agent (*Yersinia pestis*) during the epidemic in Hongkong.

1895, BUSSE and SANFELICE discovered (and cultivated) the agent of human cryptococcosis.

1895, BRUCE: *Trypanosoma brucei* is transmitted by tsetse fly *Glossina morsitans.*

1897, OGATA explained the role of rats and the flea *Xenopsylla cheopsis* in the epidemics of plague.

1897, BANG: isolation of the agent of livestock brucellosis (*Brucella abortus*).

1897, FLÜGGE: evidence of transmission of epidemic typhus by the body louse.

1897, van ERMENGEM proved the aetiology of botulism (toxin of *Clostridium botulinum*).

1898, Liverpool: first School of Tropical Medicine in the world founded (Ross, Dutton).

1899, London School of Hygiene and Tropical Medicine founded (P. Manson).

1898, ROSS in birds and GRASSI in man explained epidemiology of malaria (its transmission by *Anopheles* mosquitoes) [1902 Nobel prize to Ross].

1898, SCHENCK discovered the agent of sporotrichosis (*Sporothrix schenckii*).

1898, GILCHRIST and STOKES: fungal aetiology of blastomycosis.

1900, Hamburg: Institute for Maritime and Tropical Diseases (today called Bernhard Nocht Institute for Tropical Medicine) founded.

1900, OPHÜLS and MOFFITT isolated the agent of coccidioidomycosis.

1898–1900, an extensive epidemic of YF in Cuba.

1900–1901, REED, CARROLL, LAZEAR and AGRAMONTE ("Yellow Fever Commission"): first evidence of the transmission of the YF agent by arthropods (*Aedes aegypti* – experimental mosquitoes were supplied by C. Finlay – cf. 1881) to a susceptible man; during the experiments J. Lazear infected himself unintentionally and died (first fatal laboratory infection with a virus); beginnings of arbovirology.

1901, GORGAS: eradication of the YF vector *Ae. aegypti* in Havana (larvicide control by oil; a similar action he headed in Panama during the building of the Canal, 1905).

1901, RICKETTS: isolation of the agent of blastomycosis (with autoinoculation).

1901–1902, DUTTON and BRUCE: human sleeping sickness is caused by *Trypanosoma gambiense* transmitted by the tsetse fly *Glossina palpalis.*

1902, AUJESZKY: propagation of pseudorabies virus (aetiology of *morbus Aujeszky*).

1903, NEGRI observed elementary bodies of rabies virus in the CNS of rabid animals.

1903, GRAHAM demonstrated transmission of dengue fever by mosquitoes in Lebanon.

1904–1905, DUTTON and TODD (Kenya), KOCH (east Africa), ROSS and MILNE (Uganda) elucidated the aetiology of African recurrent fever (spirochete *Borrelia duttoni*) and proved experimentally its vector (*Ornithodoros moubata*, including TOT) and susceptibility of monkeys to this disease (Dutton and Todd infected themselves at autopsy of the monkeys, and Dutton died due to the infection); this disease had already been known to Livingstone (as "human tick disease") in 1857.

1904, GAFFKY: isolation of *Clostridium botulinum.*

1905, ZAMMIT isolated *Brucella melitensis* from goat milk.

1906, RICKETTS discovered the agent of Rocky Mountain spotted fever and described its transmission (including TOT) by *Dermacentor* spp. ticks.

1906, BANCROFT found that the mosquito *Aedes aegypti* is the only vector of dengue.

1906, DARLING described histoplasmosis as protozoan disease (an error, cf. 1934).

1907, CHAGAS found that the "kissing bug" *Triatoma infestans* can transmit *Trypanosoma cruzi.*

1908, NICOLLE and MANCEAUX discovered *Toxoplasma gondii.*

1908, AFZELIUS described specific skin lesions called *erythema* (*chronicum*) *migrans* in some patients after attack of the tick *Ixodes ricinus* on them in Sweden (Lyme borreliosis – cf. 1982 and 1983).

1909, KLEINE described development of *Trypanosoma brucei gambiense* in the fly *Glossina palpalis.*

1909, DOERR, FRANZ and TAUSSIG carried out experimental transmission of the pappataci fever to volunteers by phlebotomines.

1909–1912, NICOLLE, SERGENT and FOLEY proved experimentally the transmission of epidemic typhus by body louse and discovered also so-called eclipse phase of pathogen in the vector [Nobel prize 1928].

1911, FRANCO et al. discovered in Columbia an alternative, forest cycle of yellow fever ("jungle YF") – confirmed in full in 1932 (SOPER), with monkeys and mosquitoes other than *Ae. aegypti* participating in the jungle cycle.

1911–1912, McCOY and CHAPIN isolated the agent of tularaemia from an ill ground squirrel (*Citellus beecheyi*) in the area of Tulare (California).

1911–1912, ZABOLOTNY et al. demonstrated plague in exoanthropic rodents (marmots, susliks) in Russia.

1912, SPLENDORE isolated dimorphic pathogenic fungus *Paracoccidioides brasiliensis.*

1913, PROWAZEK and ROCHA da LIMA: demonstration of the agent of epidemic typhus (*Rickettsia prowazeki*) in the body louse [S. Prowazek got laboratory infection and died of typhus in 1915].

1915, INADA and UHLENHUTH isolated *Leptospira icterohaemorrhagiae.*

1915, LANE and MEDLAR demonstrated the agent of chromoblastomycosis (*Phialophora verrucosa*).

1918, CLELAND and CAMPBELL isolated (by intracerebral inoculation of rhesus monkey) the virus of Murray Valley encephalitis from the CNS of three dead persons during an epidemic in Australia (the very first isolation of an arbovirus).

1918–1920, the pandemic of "Spanish flu" caused death of at least 21 million people (the aetiological agent originated with great probability from an avian influenza virus).

1920, STOKES passaged the yellow fever agent.

1920, FRANCIS found that the agent of tularaemia in ground squirrels, hares and rabbits is transmissible to human.

1921, BOYD and CRUTCHFIELD isolated the agent of maduromycosis (*Monosporium apiospermum*).

1923, SPENCER and PARKER found evidence that the vector of RMSF is the tick *Dermacentor andersoni.*

1924, SPENCER prepared a phenolized vaccine against RMSF.

1924, PARKER, SPENCER and FRANCIS: *D. andersoni* tick is also vector of tularaemia.

1925, RAMON and DESCOMBEY prepared anatoxin (vaccine) against tetanus *Clostridium tetani* (vaccine).

1926, MURRAY isolated *Listeria monocytogenes.*

1926, de KRUIF published a very successful book "Microbe Hunters".

1926, SILLER, HALL and HITCHENS demonstrated transmission of dengue to volunteers by *Aedes aegypti* mosquitoes.

1927, STOKES, BAUER and HUDSON verified that the agent of yellow fever is a filterable virus; the use of rhesus monkey for arbovirus isolation.

1927, RAMON applied vaccination against tetanus (toxoid).

1927–1928, a big outbreak of dengue fever in Athens, Greece.

1928, EVANS discovered that *Brucella abortus* causes undulating fever in humans.

1928, FLEMING observed antibacterial effect of the fungus *Penicillium notatum* on staphylococci (penicillin – cf. 1940).

1928, EPSTEIN and TARASOV demonstrated *Leptospira grippotyphosa* as the agent of "harvest fever" in Europe.

1929–1930, BEDSON et al. identified the agent of psittacosis (as a "virus") during a winter pandemic in USA and Europe, caused by importation of green Amazon parrots from Argentina.

1930, POOL, BROWNLEE and WILSON isolated louping ill virus from the CNS of sheep in Scotland and demonstrated the aetiology of LI by inoculating it to healthy sheep.

1930, THEILER first used white mouse (inoculated intracerebrally) for isolation of arboviruses.

1930, NAGAYO, KAWAMURA et al. demonstrated the transmission of tsutsuga-mushi fever by larval trombiculid mites ("chiggers").

1930–1932, DAUBNEY, HUDSON and GARNHAM isolated RVF virus from sheep.

1931, GOODPASTURE and WOODRUFF used chicken embryos for cultivation of viruses.

1931–1933, MEYER, ROSENBUSCH et al. isolated WEE virus from the brain of horses in California (first isolation of an arbovirus in USA).

1932, MacLEOD and GORDON demonstrated transmission of LI virus to sheep by the tick *Ixodes ricinus.*

1932, McCOY and BEDSON explained the aetiology of psittacosis (ornithosis).

1932, de KRUIF published another successful book about infectious diseases including zoonoses "Men Against Death".

1932–1933, MUCKENFUSS et al. isolated SLE virus from a patient.

1933, GILTNER, SHAHAN, TEN BROECK and MERRIL isolated EEE virus from CNS of dead horses during an extensive outbreak in USA (EEE virus was isolated from humans in USA later, in 1938).

1934, DeMONBREUN cultivated the agent of histoplasmosis, and found it to be a fungus [cf. 1906].

1934, LOVE and JUNGHERR isolated zoonotic simian B virus (*Herpesvirus simiae*).

1934, ARMSTRONG and LILLIE recovered LCM virus.

1934, PANOV reported on severe clinical symptoms and epidemiology of Russian spring-summer encephalitis in Siberia to the Ministry of Health of the USSR, and asked for a research expedition to be sent for detailed investigation of the disease.

1934, an epidemic of HFRS in Korea (aetiology unexplained at that time).

1935, DOMAGK reported about prontosil (he discovered the sulphonamide already in 1932) [Nobel prize 1939].

1935, vaccination of sheep against louping ill in Great Britain (the vaccine was formalinised, but contaminated with scrapie prions and caused the spread of scrapie in Britain).

1935, isolation of Japanese encephalitis virus from CNS of a deceased patient in Tokyo.

1935–1937, DERRICK described Q fever among slaughterhouse workers in Australia.

1936–1937, DAVID, DRBOHLAV, KŘIVINKA and VRLA: a big outbreak of tularaemia among rodents, hares, and then humans (>500 patients) in Lower Austria, west Slovakia and south Moravia (Czechland).

1937, THEILER released an attenuated vaccine (17D) against YF [Nobel prize 1951].

1937–1939, ZILBER, LEVKOVICH, CHUMAKOV, SMORODINTSEV, SHUBLADZE, PAVLOVSKY et al.: description of aetiology of RSSE (virus

isolation) and its epidemiology (*Ixodes persulcatus*) in Siberia; laboratory infection and untimely death of three investigators (N.V. Kagan, V.I. Pomerancev, N. Utkina) and chronic RSSE in M.P. Chumakov.

1938, BECK and WYCKOFF: isolation of VEE virus from horses in Venezuela.

1939, PAVLOVSKY formulated the paradigm on natural focality (nidality) of diseases.

1939, MÜLLER discovered insecticide effects of DDT (dichlordiphenyl-trichloethane), a compound synthetized by ZIEDLER already in 1874 [Nobel prize to MÜLLER in 1948].

1940, SMITHBURN et al. isolated WN virus from the blood of a patient in West Nile district, Uganda.

1940, FLOREY, CHAIN and HEATLEY prepared purified penicillin (cf. 1929), industrial production started in 1941 [Nobel prize to A. Fleming, E.B. Chain and H.W. Florey in 1945].

1940, SMORODINTSEV demonstrated viral aetiology of HFRS.

1941, HIRST introduced haemagglutination test and HIT in virological diagnostics (influenza, etc.).

1941, MEYER described two human cases of ornithosis acquired from a sick feral pigeon (in New York City).

1942, EMMONS and ASHBURN discovered adiasporomycosis (emmonsiosis) in North-American rodents.

1943–1944: KIMURA and HOTTA isolated dengue virus.

1943–1944, WAKSMAN, UGIE and SCHATZ discovered streptomycin [Nobel prize to S.A. Waksman 1952].

1944, WOODWARD synthetized quinine [Nobel prize 1965].

1944, SABIN et al. isolated viruses of pappataci fever (SFN, SFS) from the blood of patients and detected the vector (*Phlebotomus papatasi*).

1944, FLORIO et al. demonstrated experimental transmission of CTF virus by ixodid ticks.

1946–1947, CHUMAKOV et al. explained viral aetiology of Crimean haemorrhagic fever.

1946, Center for Disease Control (CDC) founded in Atlanta, USA (renamed as Centers for Disease Control and Prevention in 1994).

1946, HUEBNER, POMERANTZ and JELLISON discovered the agent of rickettsial pox and its vector (*Allodermanyssus* mites).

1947, CHUMAKOV et al. first isolated OHF virus (from a patient).

1947–1948, BURKHOLDER and DUGGAR proposed the wide-spectrum antibiotics chloramphenicol and tetracycline for treatment of rickettsial and other microbial diseases.

1949, The Gamaleya Institute for Epidemiology and Microbiology (of the USSR Academy of Medical Sciences) founded in Moscow.

1949, ENDERS, WELLER and ROBBINS used cell cultures (primary rhesus monkey kidney) for isolation and propagation of viruses [Nobel prize 1956].

1949–1950, KREJČÍ, GALLIA and RAMPAS isolated TBE virus from the blood and CSF of patients and from *Ixodes ricinus* ticks in Czechland.

1951, "The Rockefeller Foundation Virus Program": a total of 30 million USD were released for arbovirus investigations over the world until 1970 (>60 new viruses were isolated).

1951, BLAŠKOVIČ, BÁRDOŠ, RAŠKA et al. explained a big outbreak (>600 patients) of milk-borne TBE in Rožňava, east Slovakia.

1951–1954, outbreaks of HFRS among American soldiers during the Korean war (aetiology remained unexplained at that time; cf. 1956–1958).

1952, TAYLOR et al. isolated Sindbis virus from the mosquito *Culex univittatus* in Egypt.

1952–1953, Chikungunya virus was isolated during epidemics in Tanzania and Uganda.

1952, HAMMON and REEVES isolated the virus of California encephalitis from mosquitoes and detected first three cases of fatal encephalitis in children.

1953–1954, SMORODINTSEV and GREŠÍKOVÁ demonstrated experimentally the ability of the TBE virus to be transmitted by milk (cf. 1951).

1954, CHAMBERLAIN et al. used chicken embryos for isolation of arboviruses.

1955, plague in the Asian seaports Rangun, Bombay, Madras and some others (>12 million people died only in India).

1955, TAYLOR et al. used newborn laboratory mice as the most sensitive substrate for isolation of arboviruses.

1955–1957, WORK and TRAPIDO studied a big outbreak among monkeys in Kyasanur forest (southwest India), followed by an epidemic in humans; the agent was *Flavivirus* KFD, isolated also from ticks *Haemaphysalis spinigera*.

1957, SKRABALO and DEANOVIC described first case of human babesiosis (caused by *Babesia divergens*, Slovenia).

1958, PARODI, CASALS, BUCKLEY et al. described an epidemic of Argentine haemorrhagic fever with a high fatality rate, and isolated the agent (arenavirus Junin).

1958, BÁRDOŠ and DANIELOVÁ isolated Ťahyňa bunyavirus from *Aedes* mosquitoes in east Slovakia (the very first human pathogenic mosquito-borne virus isolated in Europe).

1958, SIMPSON isolated the agent of Congo haemorrhagic fever (CCHF virus).

1959, HADDOW et al.: a big outbreak of ONN fever in Uganda (2 million persons affected), the alphavirus isolated.

1960, MacKENZIE et al.: an epidemic of severe haemorragic fever ("el typho negro") in San Joaquin, Bolivia (cf. 1964–1965).

1960, BÁRDOŠ and SLUKA: certain summer flu-like cases of humans ("Valtice fever") in south Moravia (Czechland) are caused by Ťahyňa bunyavirus.

1961, Oropouche fever in Brazil, outbreak with 11,000 patients (but first cases revealed already in 1955).

1962–1964, a big outbreak of VEE in Venezuela and Columbia.

1964, GREŠÍKOVÁ and LIBÍKOVÁ isolated Tribeč/Lipovník virus (Kemerovo group) from ixodid ticks in Slovakia.

1964–1965, JOHNSON, MacKENZIE, KUNS and WEBB isolated the agent of Bolivian haemorrhagic fever (arenavirus Machupo) from humans and rodents.

1965, THOMPSON et al. isolated LaCrosse bunyavirus from the brain of a child killed by California encephalitis.

1967, CHUMAKOV isolated the agent of Crimean haemorrhagic fever in Russia (CCHF virus).

1967–1968, SIEGERT, MARTINI, HENNESSEN, STILLE et al.: three clusters of Marburg haemorrhagic fever cases (31 patients, 7 died) in pharmaceutical laboratories in Germany (Behringwerke AG in Marburg, and also Frankfurt) and in Serbia (Beograd) that were acquired from rhesus monkeys imported from Uganda in 1967.

1969, BUCKLEY, CASALS and DOWNS isolated Lassa arenavirus from the blood of a missionary during an epidemic in Nigeria.

1970, a big outbreak of Rocio (flavivirus) fever in Brazil.

1970, monkeypox in humans, Zaire (DR Congo). The virus was originally isolated from ill macaques by VON MAGNUS already in 1958.

1972, DOHERTY: a large outbreak of Ross River fever in Australia (but the virus was first isolated from mosquitoes in 1959, and then a number of human cases were reported up to 1970).

1972–1977, BUTZLER, SKIRROW et al.: *Campylobacter jejuni* causes epidemic bacterial gastroenteritis in humans.

1975, discovery of simian B herpesvirus, fatal for humans but benign for monkeys.

1976, NIME et al. found that *Cryptosporidium parvum* caused acute diarrhoea in humans (an epidemic with some 400,000 cases in Milwaukee, Wisconsin).

1976–1977, SHOPE, BEARE, CRAIG et al.: an epidemic of swine influenza among army recruits in Fort Dix, New Jersey (USA), the virus isolated; in a follow-up, 135 million USD were released for the US national vaccination campaign (however, Guillain-Barré syndrome developed in at least 1,500 of 40 million vaccinees).

1976–1977, McDADE, SHEPARD et al.: an outbreak of atypical pneumonia called "Legionnaires' disease" in a Philadelphia hotel (34 from 221 sick legionnaires died); isolation of the agent (*Legionella pneumophila*).

1976–1978, LEE, LEE and JOHNSON isolated the agent of HFRS in Korea and elsewhere (Hantaan bunyavirus).

1976–1979, BOWEN, JOHNSON, PATTYN, SUREAU, McCORMICK, et al.: extensive outbreaks of Ebola haemorrhagic fever in Zaire and Sudan, and recovery of the agent (a new filovirus).

1977–1978, a major epidemic of RVF in Egypt (18,000 persons with the disease, hundreds died).

1979–1980, BRUMMER-KORVENKONTIO et al. detected the agent of *nephropathia epidemica* in Finland (Puumala hantavirus).

1979–1980, an extensive epidemic of Ross River fever in Polynesia (more than 60,000 people affected).

1980, 2nd big epidemic of Oropouche fever in Amazonia.

1982, PRUSINER: infectious agents in spongiform encephalopathies are specific proteins ("prions") [Nobel prize 1997].

1982–1983, RILEY et al.: enteropathogenic *Escherichia coli* O157:H7 was the cause of epidemic haemorrhagic enterocolitis (hamburgers, USA) and of haemolytic-uraemic syndrome.

1982, BURGDORFER et al. clarified the aetiology of Lyme disease (an ixodid tick-borne spirochete), observed in Old Lyme (Connecticut, USA) since 1975, and clinically described as rheumatic arthritis in 1977.

1983, STEERE et al. isolated the agent of Lyme disease (*Borrelia burgdorferi*).

1983, MONTAGNIER, BARRÉ-SINOUSSI, GALLO et al. isolated a lymphotropic retrovirus (lentivirus HIV) from patients with AIDS, a syndrome described in 1981.

1985, MULLIS introduced PCR in microbiology.

1986–1987, MAEDA et al. described human tick-borne monocytic ehrlichiosis.

1988, GLIGIČ et al. isolated the agent of HFRS in Serbia (Dobrava hantavirus).

1989–1991, haemorrhagic fever in Venezuela (Guanarito arenavirus isolated).

1990–1992, REGNERY, WELCH et al. isolated the causative agent of cat-scratch fever (*Bartonella henselae*).

1991, ANDERSON, DAWSON et al. isolated the agent of human monocytic ehrlichiosis (*Ehrlichia chaffeensis*).

1993, TEMPEST, CHEEK, NICHOL, PETERS, KSIAZEK, CHILDS, LeDUC, ELLIOTT, JAHRLING, SCHMALJOHN et al.: an epidemic of lethal pulmonary syndrome among Navaha Indians in the "Four Corners" region (southwestern USA), and isolation of the agent (Sin Nombre hantavirus).

1994, BAKKEN, DUMLER, CHEN et al. described human tick-borne granulocytic anaplasmosis, and detection of the agent (*Anaplasma phagocytophilum*) in *Ixodes scapularis* ticks in USA.

1994–1995, MURRAY, SELVEY et al. isolated Hendra paramyxovirus from ill horses and from a man in Australia.

1996, WILL et al. reported occurrence of a new variant Creutzfeld-Jakob prion disease (vCJD) that is pathogenic for man (since 1994) and linked to the epizootic of bovine spongiform encephalopathy ("mad cows disease") that appeared in Great Britain in 1986.

1996, an outbreak of West Nile encephalitis in Romania (>500 patients).

1998, PHILBEY, KIRKLAND, ROSS et al. isolated a new Menangle paramyxovirus in Australia (pigs, humans, fruit bats).

1998–99, SIT and BING isolated a new Nipah paramyxovirus during a big outbreak (pigs, humans) in Malaysia.

1999 –2006, a very surprising epidemic of West Nile encephalitis in New York after an importation of WNV (probably from Israel), with a following spread over whole North (later also Central and South) America; closely before this event, big WN outbreaks in southern Russia and Israel.

2002, an extensive epidemic of SARS in southeast Asia, exported later to other countries (Canada etc.).

2005, founding of the "European Centre for Disease Prevention and Control" (ECDC), Stockholm.

2005–2006, a major epidemic of avian influenza (H5N1) in Asia, with a following wave-like rapid spread to Europe and Africa; the strain also infected 504 humans and caused 299 deaths (WHO, as of 12 August 2010); most cases have been reported from Indonesia (168), Vietnam (119), and Egypt (111).

2005–2007, to date the largest epidemic of chikungunya fever on islands in Indian Ocean (Réunion Island etc.), in India etc. (about 280,000 cases – 213 persons died).

2005–2010 (still ongoing), a major outbreak of Q fever in the Netherlands (at least 3,500 human cases up to 2009).

2007, the first outbreak of chikungunya fever in Europe (imported from India): northeastern Italy (Ravenna and surroundings), with about 334 suspected cases, 204 (of 281 tested) were laboratory confirmed.

2007, NIKLASSON et al.: rodent-borne Ljungan picornavirus was found to cause intrauterine foetal death and CNS malformations.

2008, a new arenavirus Lujo killed several persons (Zambia, South Africa).

2009, a pandemic of H1N1 swine influenza: as of April 1, 2010, it encompassed 213 countries and caused 17,483 human deaths.

Chapter 4
The Infection Process in Zoonoses and Sapronoses

The basic facts of infectology relevant for the study of microbial zoonoses and sapronoses are briefly summarized in this chapter. The infection process is an interaction between the pathogenic microorganism and the host's organism (macroorganism) which started by entry of the microbial agent in the host's body.

4.1 Infectious Agent

The infectious organism is the aetiological agent of a corresponding disease, and it belongs to one of the five large groups of organisms: (1) viruses; (2) bacteria (Prokaryota); (3) fungi; (4) protozoa; (5) Metazoa (multicellular parasites – helminths and arthropods). The metazoan parasites have been omitted in this review.

To be ascertained as the aetiological agent of a particular disease, the microorganism must fulfil Koch's Postulates – i.e., it should:

(1) be detected in all cases of the disease;
(2) be isolated from the patient and cultured in a laboratory;
(3) the isolated and cultured agent should reproduce the disease after inoculation to another host of the same or related species;
(4) be recovered from this inoculated, sick host;
(5) produce a specific immune reaction (form antibodies) in the inoculated host.

The important characteristics of the aetiological agent include its pathogenicity, virulence, invasivity and toxigenicity.

Pathogenicity is the ability of the agent to produce a specific pathological state in a susceptible host. Pathogenic microorganisms are characteristic by their specificity (affinity) against hosts: usually only some species of host (vertebrates) are susceptible, while other species are refractory (resistant) to the microorganism. For instance, while most serovars of *Salmonella* are pathogenic to a broad range of vertebrates, typhoid caused by *Salmonella typhi* is only known in man (i.e., anthroponosis). The rate of susceptibility varies, however, even within a certain species and is influenced by genetic, physiological and other factors. Pathogenicity can also be considerably

modified also by antigenic variability of the agent: e.g., out of a great number of salmonella serovars, only a few cause human disease. An important factor can be evolutionary changes due to mutations and recombinations of microorganisms. For instance, they are well known in the influenza virus and described as antigenic "drift" (small modifications of haemagglutinin) as well as antigenic "shift" (great changes of neuraminidase and haemagglutinin) occurring at simultaneous infection of a vertebrate host with both human and animal (e.g. swine) strains of influenza virus. A similar source of variability is known in bunyaviruses of California group that have a three-segmented genome and are able to produce reassortants (LaCrosse, Snowshoe hare and Ťahyňa viruses) at a mixed infection in, e.g., vector mosquitoes. An interesting change in outer glycoproteins (antigens) during the infection of single host is known in some bacteria (*Borrelia recurrentis, B. burgdorferi*) and protozoa (*Plasmodium, Trypanosoma*) and it is called "immune evasion".

Some microorganisms are potential pathogens that are able to cause disease only under condition of a host weakened by, for example, stress, malnutrition, cancer, immunosuppression (secondary zoonoses and sapronoses in HIV-infected patients), after transplantation or chirurgical operations. Such agents are sometimes also called as "opportune" (e.g. many mycoses caused by species of fungi in essence saprophytic).

Virulence is the pathogenicity rate of individual strains of infectious agent; it is determined mainly by invasivity and toxigenicity.

Invasivity is ability of the agent to penetrate a host's tissues and replicate there.

Toxigenicity is ability of the agent to damage the host by the production of poisons (exotoxins and endotoxins), sometimes even in the absence of replication. Bacteria *Clostridium tetani*, *C. perfringens*, *C. difficile*, *C. botulinum*, *Bacillus anthracis*, *B. cereus*, *Staphylococcus aureus*, *Streptococcus pyogenes*, *Corynebacterium diphtheriae*, *Vibrio cholerae*, enterotoxigenic strains of *Escherichia coli* or some fungi (*Aspergillus* spp.) are examples of microorganisms forming diverse and extremely effective toxins which can cause or considerably aggravate the disease of a host.

4.2 Infection Entry

An infection starts with a penetration of the agent into the host's body by one of the three great (skin, mucosae of respiratory and alimentary tracts) or two limited (conjunctiva, urogenital tract) epithelial surfaces. These epithelia (mucosal membranes) present so-called entrance gates ("ports"). However, certain infections remain limited to these entry sites, e.g. staphylococcal dermatitis, dermatophyte diseases, cutaneous leishmaniasis, salmonelloses. Other zoonotic and sapronotic agents usually penetrate through the mucosal membranes and disseminate in the host's organism – e.g., arboviruses, rhabdoviruses, rickettsiae, mycobacteria, chlamydiae, *Francisella tularensis*, *Coxiella burnetii*, *Cryptococcus neoformans*, *Histoplasma capsulatum*, *Naegleria fowleri*, leptospirae, trypanosomae, *Leishmania donovani*, *Toxoplasma gondii* etc.

Skin: entry *per cutis*, percutaneously. The skin is the usual gate of entry in zoonoses transmissible by haematophagous arthropods (insects or mites – "arthropod-borne infections") through the mechanism of intracutaneous inoculation. The obligately arthropod-borne diseases (i.e. transmitted exclusively by arthropods) involve arboviroses, Lyme borreliosis, tick-borne recurrent borrelioses, rickettsioses, trypanosomiases, leishmaniasis, babesiosis or malaria. The facultatively arthropod-borne diseases (i.e. transmitted also by modes other than *via* arthropods) include, for example, Q fever, plague, tularaemia and brucellosis. Transmission mediated by a vector can be either mechanical (i.e. through contaminated legs, mouth parts or excrements), which does not necessitate replication or development of the infectious agent in the vector, or, more typically biological, when the infectious agent replicates or undergoes a certain developmental cycle in the vector before it is capable to be transmitted to a new vertebrate host. In the latter, biological type, the agent is transmitted to the host by the vector's salivation, regurgitation, or deposition of excrement on the epidermis. The skin as infection gateway is also used by other sapronoses and non arthropod-borne zoonoses after epidermal damage or skin traumatisation by scratching, biting or similar: e.g., cowpox, monkeypox, simian B virus infection, milker's nodules, orf, vesicular stomatitis, rabies, pseudorabies, foot-and-mouth disease, tularaemia, pasteurellosis, anthrax, tetanus, erysipeloid, glanders, melioidosis, leptospirosis, rat bite fevers (sodoku and Haverhill fever), cat scratch disease, nocardiosis, dermatophilosis, dermatophytoses, sporotrichosis, chromoblastomycosis and maduromycosis.

Mucosa of the respiratory tract: entry *per inhalatione.* A number of zoonotic and sapronotic pathogens enter the respiratory tract (air passages, both higher and lower) by inhalation of contaminated dust particles (small solid particles in the air) or aerosol droplets, and the diseases are called "respiratory infections". Droplets with a diameter of <100 μm can be stable and persist in the air for a long time, and droplets with a diameter of 1–5 μm can easily penetrate even into pulmonary alveoli. Examples of respiratory zoonoses and sapronoses are arenaviroses (Lassa, Junin, Machupo, LCM), hantaviroses, some arboviroses (RVF, VSV), filoviroses (Marburg, Ebola), EMC, ornithosis, Q fever, bovine and avian tuberculosis, other mycobacterial infections, legionellosis, tularaemia (aerogenic infection of farmers during work with contaminated hay or straw, grass cutting machines, and in washing sugar-beet in sugar refineries), plague (urban cycle), brucellosis, listeriosis, glanders, anthrax, cryptococcosis, coccidioidomycosis (infectious dust of soil particles contaminated with fungal arthrospores can be spread for tens of kilometres), histoplasmosis and emmonsiosis (during soil excavations) or pneumocystosis.

Mucosa of the alimentary tract: entry *per os*, perorally. In the peroral (alimentary) penetration of the agent in the host's body (ingestion, swallowing), the most important role is played by contaminated foods ("food-borne diseases") and water or drinks ("water-borne diseases"). This is a very often an entry gate in zoonoses where the vehiculum of the agent are contaminated foods of animal origin, most commonly meat, milk or eggs: arenaviroses (LCM, haemorrhagic fevers), some arbovirus diseases (unpasteurized milk and cheese: TBE, LI, KFD, RVF), salmonellosis (eggs, meat), campylobacterosis (meat, especially poultry),

pseudotuberculosis, yersiniosis, listeriosis (cheese, meat), pasteurellosis, botulism (meat and salad patés), anthrax, tularaemia (water, musts, cider), brucellosis (milk), cholera, glanders, melioidosis, bovine tuberculosis (milk), leptospirosis (water), Q fever (milk), toxoplasmosis (meat), giardiosis and cryptosporidiosis (water), sarcosporidiosis (intestinal coccidiosis, sarcocystosis), balantidiosis (water).

Conjunctivae: entry *per conjunctivam*. Human infection through conjunctivae has only been described in some zoonoses and sapronoses, e.g., vesicular stomatitis, psittacosis, arenaviroses, Q fever, brucellosis, tularaemia, glanders, fusarioses, and amoebic keratitis.

4.3 Infection Course and Host Defence

An infection is the entry of the pathogenic organism into the host's body and multiplication of the agent in the host's tissues. The result of the infection is either an inapparent infection or a manifest disease of the host. Infection is thus a broader term than infectious disease.

An inapparent (asymptomatic) infection, sometimes also called a subclinical, does not present any symptoms of disease. It can be detected by the presence of specific antibodies after the infection or by delayed hypersensitivity.

An infectious disease is a clinically manifest infection; it produces clinical signs (symptoms). Any infectious disease reveals a range of symptoms from mild to severe – sometimes fatal. A characteristic combination of certain clinical symptoms, occurring in some diseases, is called a syndrome.

The manifestation rate is the proportion of symptomatic cases out of all the infected susceptible individuals. In most infectious diseases the share of manifest forms is quite low, and only exceptionally approaches 100%, e.g. in some anthroponoses (measles). For instance, in tick-borne encephalitis it reaches only about 10%. Exact estimation of this parameter in a disease is of course difficult in humans in that it requires detailed serological and/or molecular (detection of DNA or RNA of the agent) examination of large groups of persons; it is much more simple in, for example, experimental animals.

Contagiosity is the proportion of infected (not only diseased) individuals out of the total number of individuals exposed to the pathogenic agent.

The incubation period is the time between the entry of the pathogen in the host's body and the first clinical symptoms of the disease. This incubation period varies in zoonoses and sapronoses considerably, the range being from several hours (salmonellosis, staphylococcal intoxication), a few days (tularaemia, plague, anthrax, yellow fever, sandfly fevers), 1–2 weeks (the most common case: e.g., leptospirosis, brucellosis, tick-borne encephalitis and a majority of other arboviroses, HFRS, Q fever, ornithosis, histoplasmosis, dermatomycoses, simian malaria, toxoplasmosis), to 1 month or longer (rabies, bovine tuberculosis, emmonsiosis, leishmaniasis, trypanosomiases, babesiosis); in some infectious diseases the incubation period can exceed 1 year (prion diseases, lepra).

The course of a disease according to its pace can be abortive (the symptoms subside rapidly), subacute or (per)acute (quite or very rapid to precipitous) or (sub)chronical. Most zoonoses are characterized by an acute course, while chronic zoonoses occur less often (e.g., brucellosis). Chronic diseases belong among persistent infections, which can be divided into:

(i) chronic, in which after primary infection the agent persists in the host for a long time without causing any harm to the host, is excreted, and can be easily detected (e.g., chlamydiosis, or LCM and HFRS in rodents);

(ii) latent, in which after primary infection the agent also persists in the host for a long time but is demonstrable only during relapses of the disease: e.g., Brill-Zinsser's disease (a relapsing form of epidemic louse-borne typhus caused by *Rickettsia prowazeki*) or malaria (*Plasmodium vivax*);

(iii) "slow infections" could also be regarded as persistent, e.g. the anthroponoses AIDS, subacute sclerotizing panencephalitis (measles virus), kuru, Creutzfeldt-Jakob's disease, zoonotic vCJD, or scrapie in sheep.

Mixed infection of a host by two pathogenic agents can sometimes occur – either simultaneously (co-infection) or gradually (in that case primary and secondary infection is to be differentiated). Re-infection is a repeated infection of the host with the same pathogen after convalescence, while superinfection is a repeated infection of the host with the same pathogen during the course of primary infection.

For a microbiologist it is crucially important to know the risk of laboratory infection (occupational risk) for particular pathogens and to adapt laboratory practice accordingly. Among the statistically most common zoonoses and sapronoses with reported laboratory infections are brucellosis, Q fever, tularaemia, dermatomycoses, VEE, ornithosis and coccidioidomycosis. With agents of such diseases, as well as with many other dangerous pathogens (e.g., TBE virus, arenaviruses causing haemorrhagic fevers, CCHF, filoviruses, hantaviruses, *Penicillium marneffei*), it is necessary to work in the laboratory at biosafety level BSL-3 or even at the extreme level BSL-4. Further, laboratory personnel should be vaccinated against corresponding diseases, if possible. Work at levels BSL-3 and BSL-4 is carried out in special biohazard boxes with laminar airflow through so-called HEPA filters (they capture 99.997% of particles with the size ≥ 200 nm), preventing both a contamination of the treated samples and, more importantly infection of the worker and surroundings at the same time. The same biosafety should be guaranteed for work with infected experimental animals and haematophagous arthropods. Nevertheless, work with a majority of zoonotic and sapronotic pathogens could be done at biosafety level BSL-2, while BSL-1 (the lowest grade of biosafety) is only acceptable for a limited number of potential microbial pathogens (e.g., *Bacillus cereus*, *Escherichia coli*).

A very significant factor for the "success" of an infection is the dose of the respective pathogen that penetrates into the host organism. In general, pathogenic agents as well as strains of one pathogenic species with varying virulence can be characterized in relation to different species of hosts using a number of dose types:

The infectious dose is the number of particles (cells) of the pathogen causing an infection. It can be expressed in a variety of indices, most often as **MID** (minimum infectious dose) or, more precisely, **ID_{50}** (the dose causing infection in 50% of exposed individuals). Some highly contagious diseases have very low infectious doses: e.g., for human tuberculosis the respiratory MID is 1–10 cells of *Mycobacterium tuberculosis* and for giardiosis is the MID by ingestion only 10 cysts of *Giardia lamblia*.

The pathogenic dose (the dose causing disease) is nearly always higher than the infectious dose. For instance in salmonellosis it is 10^4–10^6 cells of *Salmonella enterica* at peroral application of various serovars to man, while in human tularaemia it is as low as 10 cells of *Francisella tularensis* at intradermal inoculation.

The lethal dose is the killing dose, and can be expressed as **MLD** (minimum lethal dose) or **LD_{50}** (the dose that will kill 50% of infected individuals). Only exceptionally is the agent absolutely deadly; in mouse tularaemia the MLD at intraperitoneal or subcutaneous inoculation is no more than one living cell of *Francisella tularensis.*

The protective dose is the dose of an agent or vaccine which will protect the host against a following challenge with the fully virulent strain. It can be expressed as **MPD** (minimum protective dose) or **PD_{50}** (the dose that will protect 50% of challenged individuals).

The cytopathic dose of a pathogen (most often a virus) is estimated in cell cultures on the basis of cytopathic effect (CPE). For instance **CPD_{50}** (the same as **TCD_{50}**) is the virus dose causing CPE in 50% of cells inoculated or, more exactly, in 50% of all cell culture tubes (wells, flasks) inoculated with the same virus dilution.

All these various types of dose could be generalized as effective doses: **MED** – minimum effective dose; **ED_{50}** – the dose with a 50% effect. Various experimental procedures are used for estimation of ED_{50}: basically titration of the agents in laboratory animals and cell cultures, or direct counting of microbial particles (cultivation of serial dilutions of bacteria by plating, electron microscopy in viruses). The most common mathematical methods used for calculation of ED_{50} are those of Reed-Muench, Kärber, or moving averages.

The resistance of the host macroorganism to a pathogenic microorganism plays an important role in the infection process, as well as the ability of the microorganism to defeat it. The host resistance is vertebrate species-specific, but it varies also individually according to the state of the individual host during the infection. Mechanisms of resistance can be divided in two groups of effects:

(a) natural (non-specific) resistance/immunity formed by mechanical (skin, mucosa) and chemical (gastric juice, lysozyme) barriers, genetic and physiological (e.g. hormonal) determinants, the complex process of inflammation, phagocytosis, complement or interferon production;

(b) acquired (specific) immunity that is determined by a previous contact with the pathogenic agent, and involves humoral (production of immunoglobulin antibodies mediated through activated B-lymphocytes) and cellular (mediated via T-cells) immunity. Specific immunity can be:

> – active, acquired naturally after infection;
> – active, acquired artificially through vaccination;
> – passive, acquired naturally (during embryonal development and with the mother's milk);
> – passive, acquired artificially (by application of specific immunoglobulins).

On the other side, the pathogenic microorganism can defeat the host's defence by, e.g.:

- capsule formation (*Cryptococcus neoformans*);
- the formation of biofilms on solid substrata such as catheters, veins interior walls etc. (staphylococci, pseudomonads);
- the ability to survive intracellularly within macrophages (some bacteria are able to inhibit the connection of phagosome with lysosome, to produce catalase or hydrophobic cell walls: *Francisella tularensis*, mycobacteria etc.) or within other cell types (viruses);
- antigenic variation and immune evasion (periodic changes of surface antigen – variable surface glycoprotein VSG – during the interaction with the host's antibodies, for instance by "silent" gene or gene cassettes' expression): spirochetes, plasmodia, babesiae, trypanosomes;
- invasion of tissues and niches in the host's body inaccessible to antibodies (*Borrelia burgdorferi* s.l. in synovial fluid);
- inhibition of production of interferon (filoviruses) or complement (a number of viruses).

Many factors can be important during pathogen-host interaction, e.g.:

- dose of the infectious agent, its virulence, and entry gate (see above);
- age of the attacked host;
- state of the host's immunity (antibodies), immunosuppression;
- genetic factors controlling host immunity (cf. sickle anaemia in malaria patients, thalassemia, or ovalocytosis in southeast Asia);
- physiological state of the host (malnutrition, shortage of vitamins or other essential compounds, gravidity);
- stress factors (physical and psychological);
- parallel non-infectious diseases (atopic eczema, *diabetes mellitus*, cancer), and mixed infections.

Chapter 5
The Epidemic Process in Zoonoses and Sapronoses

This chapter could also be called eco-epidemiological basics or background information for zoonoses and sapronoses. Epidemiology is the study of the process of the origin and spread of transmissible (communicable) infectious diseases of man, and their control. The modern conception of epidemiology is broader in that it also includes non-infectious diseases (such as diabetes, heart attack, cardiovascular diseases and carcinomas) and variables that influence their distribution in the human population. In both the classical and modern conception of epidemiology, these factors include variables concerning the host (age, sex, nutrition, occupation), the agent (virulence, antigenic variability), and the environment (chemical factors, contamination, emissions, temperature, precipitation and humidity, illumination, ionising radiation, noise etc.). Epidemiological data are then analysed, and the results of these studies can then be used for the prevention and control of these human diseases. Epizootiology is characterized analogically but relates to animal diseases. In general, these two disciplines should not be differentiated; already Rudolf Virchow in the middle of nineteenth century wrote: "*Zwischen Tier- und Menschenarzneikunde ist oder sollte wissenschaftlich keine Scheidengrenze sein*". This is in line with the present approach called "One Medicine, One Health Concept" (including epidemiology). However, there are some additional characteristics specific for the "epizootiology", such as, for example, turnover of the animal population (herd), hygienic parameters of breeding, or methods of control of animal diseases including zoonoses (culling of groups or whole herds affected with dangerous diseases).

According to geographical distribution, the intensity and frequency of infectious diseases, their occurrence can be described as:

– sporadic (single cases without any obvious connection);
– familiar (cases within a family: e.g., alimentary infections with TBE);
– epidemic/epizootic (outbreak: an increased or mass occurrence of cases in a particular time and space);
– pandemic/panzootic (mass occurrence of a disease in many countries or continents);
– endemic/enzootic (a long-term or permanent occurrence of a disease or its agent in a certain locality or area, for instance in a natural focus).

Epidemics/epizootics can be explosive (with a swift onset, an exponentially increasing number of cases and then a precipitate decrease of cases) or prolonged, and can be classically (and best) described in the form of the epidemic curve (i.e., a frequency histogram with the number of cases vs. time intervals). A number of diseases (especially some viral anthroponoses) occur in epidemic cycles with intervals varying according to the aetiological agent. The inter-epidemic interval is the period in which outbreaks or new cases of a particular disease do not occur.

5.1 Characteristics of the Epidemic Process

The epidemic process is an evolutionarily stabilized mechanism consisting of three phases: (1) excretion of the agent from the host's organism (i.e., the source or donor of an infectious disease); (2) transmission of the agent; (3) its entry into a susceptible host (recipient). Contrary to the infectious process (related to an individual host level), the epidemic/epizootic process concerns the level of a population. [Analogically the disciplines of epidemiology and infectology differ from each other, even though a clear distinction between both subjects is sometimes difficult to make].

5.1.1 The Source of Infection

The source of zoonotic diseases is a vertebrate animal (donor), excreting the microbial agent during the contagious phase, sometimes being without any clinical symptoms but, more often, during the period of fully developed clinical signs. The excretion mechanisms include:

– urination (hantaviruses, leptospires),
– defecation (*Salmonella*, *Giardia*),
– regurgitation (the production of pellets, e.g. in raptors and owls: *Mycobacterium avium*),
– salivation (rabies virus),
– expectoration (coughing: e.g. Nipah or SARS viruses),
– bleeding (e.g. Lassa, Ebola or CCHF viruses),
– lactation (milk-borne zoonoses TBE, brucellosis, bovine tuberculosis),
– via pus (glanders).

In sapronoses, the source of infection is an abiotic substrate (e.g. soil or water in some visceral mycoses, atypical mycobacterioses or legionellosis), in which the agent lives as a saprophyte. However, the abiotic substrate or environment in general cannot be regarded as the source of an infectious disease when the microbe originating from an animal just contaminates and/or persists in the substrate without replication; in that case the source of infection is the particular animal.

A <u>carrier</u> of an infection is an individual that carries and excretes the infectious agent without having clinical symptoms at that time. The carrier can be:

– healthy or asymptomatic;
– in the incubation period of the disease (dog – rabies);
– in the convalescent phase of the disease;
– in the chronic state of the disease, with persisting infection (man – typhoid).

Carriership is epidemiologically a very important factor due to the asymptomatic, discreet pattern of the host. Carriership can be relatively short-term or long-term (even lifelong), and excretion of the infectious agent can be regular or irregular to erratic.

It is important to differentiate between the source and the <u>reservoir</u> of a disease. The latter is a vertebrate or invertebrate animal species (in zoonoses) or a microhabitat in the environment such as soil, rhizosphere, an organic substrate, or water (in sapronoses) in which the agent thrives also in an interepidemic period.

5.1.2 The Transmission Mode of the Infectious Disease

The transmission mode of an infectious disease is the means by which transfer of the infectious agent goes from the source (e.g., a vertebrate <u>donor</u> host) to a susceptible host (<u>recipient</u>), including all contributing factors. This transfer can be either direct or <u>indirect</u> contact, the latter mediated by contaminated excrement, food, animal products, arthropods, etc. Epidemiologists classify the transmission modes (Fig. 5.1) into four categories, as:

(a) <u>contact</u> (direct transmission: contact diseases, including perinatal infections);
(b) <u>inhalation</u> (via air, aerogenically: generally the group of respiratory, air-borne infectious diseases);
(c) <u>ingestion</u> (food-borne and water-borne alimentary, gastrointestinal diseases);
(d) <u>inoculation</u> (group of infections transmissible by haematophagous arthropods, and some nosocomial – acquired in hospital – or iatrogenic infections, i.e., acquired inadvertently during diverse medical procedures and treatments such as injections, infusions, blood transfusions or operations).

Categories (b) to (d) are also called indirect transmission, accomplished through diverse factors. Sometimes another additional group of indirect transmission is detached as so-called <u>contaminative transmission</u>, usually in infectious skin diseases; in this transmission for example underwear, bed linen, baths or medical instruments are involved.

The common mode of spread in communicable diseases in the population is <u>horizontal transmission</u> (i.e. from one individual to another), while less frequent is <u>vertical transmission</u> from female to descendants. <u>Transplacental transmission</u> is a specific means of transmission known in mammals when the pathogenic
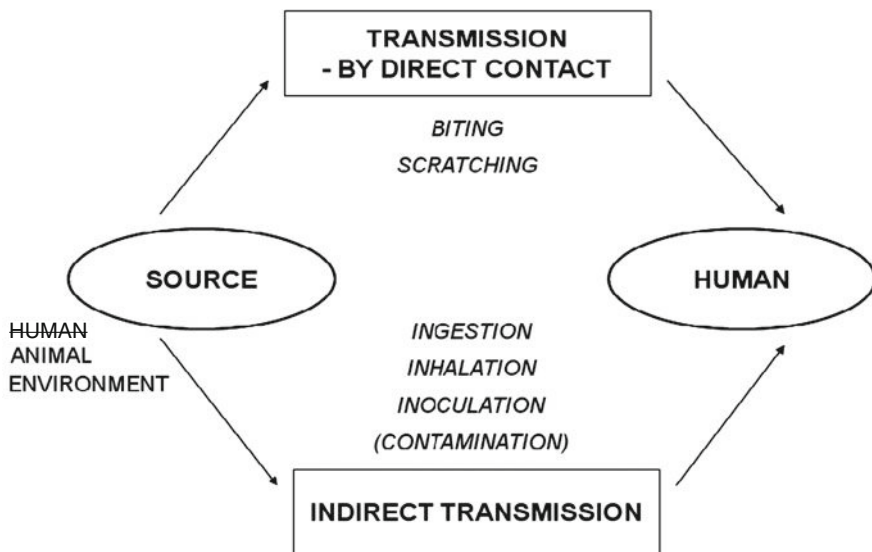
Fig. 5.1   Modes of transmission of zoonotic and sapronotic agents to man

microorganism is transmitted from the infected mother to the embryo during intrauterine development; this can cause abortion, premature birth, stillbirth, malformations, and innate diseases. These pathological effects have been observed in some zoonoses such as brucellosis, listeriosis or toxoplasmosis. The spread of the agent from mother to baby is also possible during lactation (brucellosis, Q-fever).

Tenacity (resistance of the pathogenic agent outside the host or vector organism) under different circumstances has great importance in epidemiology. Many pathogens survive for a long time in animal products such as meat, milk, eggs, skin and its adnexes (mycobacteria, listeriae, coxiellae, brucellae, salmonellae, *Clostridium botulinum*, *Bacillus anthracis*, *Trichophyton* spp., *Toxoplasma gondii*), in excreta (salmonellae, mycobacteria, leptospirae, brucellae), and also in water (salmonellae, leptospirae, atypical mycobacteria, francisellae, yersiniae) and soil (fungi, *Bacillus anthracis* spores). Sporulating microorganisms are, in general, much more resistant to refractory external conditions than vegetative forms and non-sporulating microbes.

Some very important potentially microbistatic or microbicide environmental factors, are:

– temperature (*Listeria monocytogenes* grows even at 4°C!);
– humidity (most pathogens are not very sensitive to drying, but several, e.g. *Fusobacterium necrophorum,* is very sensitive);
– light and ultraviolet radiation (*Mycobacterium bovis* is inactivated in direct light within 2–3 h, while in diffuse light much later, 30–40 h);
– pH (for instance, most arboviruses cannot tolerate pH values other than neutral while *Mycobacterium bovis* survives as long as 20 min in 10% sulphuric acid!);

– oxygen (toxic for anaerobic bacteria – clostridia);
– disinfectant compounds, antibiotics and other microbicidal substances.

Acquired resistance to antibiotics and chemotherapeutics occurs in a number of bacterial (methicillin-resistant *Staphylococus aureus,* vancomycin-resistant *Enterococcus faecium*) and protozoan (chloroquine-resistant *Plasmodium falciparum*) species.

5.1.3 Susceptible Population of the Host

Factors influencing susceptibility or resistance of a vertebrate population against an infection are, for example:

– age structure of the population;
– "herd immunity" (occurs when the proportion of immune individuals in a population or herd is sufficient to stop the spread of an infectious disease to unprotected individuals);
– physiological state of the population (malnutrition, insufficient supply of vitamins and trace elements);
– stress conditions in the population (physical, psychological);
– concurrent other diseases in the population.

5.2   External Factors in the Epidemic Process

These factors can be divided into socio-economic (acting within human society) and environmental/natural (physical, geographical, biotic) variables. The effect of both these groups of factors is especially pronounced in diseases with natural focality, and in emerging infectious diseases.

5.2.1 Socio-Economic Factors

Socio-economic (or social) factors play, of course, principal role in anthroponoses, but they are also important in zoonoses and partially in sapronoses. These factors involve:

– the density of human population in certain area;
– social and hygienic conditions (life style and level): trench fever (*Bartonella quintana*) in battlefields, refugee camps (Rwanda) or in homeless people (southern France, Seattle and Moscow);
– close contact between humans and domestic animals: "mixing" areas in southeast Asia for avian, swine and human influenza A viruses;
– a collective style of life: communal eating habits (company canteens, fast-food places: hamburgers contaminated with *E. coli* O157, or chicken meat with *Campylobacter jejuni*) and accommodation patterns;

- the degree of countryside urbanisation (expansion of towns, especially in tropical regions); formation of "slums" with good conditions for epidemic outbreaks (e.g. malaria, yellow fever, dengue, Chagas disease, plague etc.);
- "suburbanization", i.e. building of detached houses in peri-urban forested areas – e.g. in Connecticut (USA: annual incidence of Lyme borreliosis here up to 1,000 per 100,000 population, while in Central Europe only about 30–50 per 100,000); or in forested districts of Novosibirsk (presenting a very high morbidity with RSSE);
- the colonisation of new lands ("pioneers") and the following anthropogenic impact in ecosystems involving building of settlements, extensive landscaping (yellow fever during building of Panama Canal, Oroya fever during railway construction in Venezuela, RSSE at construction of the Trans-Siberian Railway), deforestation (scrub typhus, Nipah fever associated with the movement of reservoir fruit bats to farmland after the cutting down of trees, malaria in Brazil), but also reforestation (LB in eastern USA);
- the construction of water reservoirs, irrigation of farmland (schistosomiasis in Asuan, mosquito-borne diseases) and draining marshes;
- unintentional creation of new, artificial breeding places for mosquito larvae such as water-containing and for insects accessible pots, barrels, tanks, used tyres, sewage systems, etc.;
- rapid international transport and commerce: importation of new pathogenic agents, invertebrate vectors and vertebrate reservoirs (e.g. "airport malaria"; import of used tyres or decorative plants like lucky bamboo (*Dracaena*) → dispersal of Asian mosquitoes *Aedes albopictus* and *Ae. japonicus* to America, Africa and parts of Europe; yellow fever spread from Africa to America with the slave trade together with the vector mosquito *Aedes aegypti* on ships);
- the markedly increased mobility and migrability of the human population for business (each year >500 million dealers and merchants fly to foreign destinations), tourism and education, the immigration of workers from abroad;
- expanded domestic tourism connected with potential entering natural foci of infectious diseases (TBE, LB, etc.); tourism to foreign countries, foreign trade fairs and pilgrimage with potential risk of acquiring an "exotic" or other infectious zoonotic or sapronotic disease (Lassa, Ebola); "adrenaline tourism" (leptospirosis in water sports);
- leisure time activities (hunting at home and abroad, safaris, and the collection of mushrooms and forest berries), leading to an enhanced contact with vectors of diseases;
- an increased density of pets and companion animals in the human population;
- the expansion and intensification (concentration, specialisation) of agriculture (e.g. this led to enhanced incidence of leishmaniasis in the Caucasus and Nepal due to overpopulation of burrowing rodents; *Apodemus agrarius* prefers rice fields in eastern Asia → increased incidence of HFRS; new irrigated rice paddies in south Asia → Japanese encephalitis; integrated duck and swine farms in China → risk of origin of influenza pandemic strains);

- the processing and consumption of animal products and animal waste: for instance, BSE in Great Britain originated from the consumption by cows of meat-bone meal from sheep with scrapie, and led to the follow-up outbreak of a new variant of CJD in humans;
- the movements of herds and flocks of domestic ruminants to new pastures, and nomadism;
- the import and export of domestic animals, their products and foods of animal origin (eggs – salmonellosis);
- the domestication of animals (mainly in the past), deer farming in many parts of the world (at present): risk of brucellosis, bovine tuberculosis or paratuberculosis;
- the import and breeding of exotic animals in zoos, safari parks, for research or private use;
- the occupational risk of zoonotic and sapronotic diseases especially for: animal breeders and keepers, vets, butchers and slaughterhouse workers; forest workers (ixodid tick-borne diseases); casual labourers (pulmonary mycotic infections); medical laboratory workers and researchers;
- nosocomial/iatrogenic zoonotic infections (viruses Ebola, Lassa, CCHF) at blood transfusions (HIV, malaria, CTF, babesiosis etc.) and organ transplantations;
- xenotransplantation (contaminated organs of animals that are transplanted to humans), animal cell cultures used for preparation of vaccines: for instance, in pigs hepatitis E and retrovirus infections occur asymptomatically;
- drug abuse (HIV, hepatitis, anthrax);
- cosmetic operations (piercing, tattooing);
- insufficiency or absence of health prevention (including health education) and care, decline of health system infrastructure;
- social disasters and stress (wars, refugee camps, famine).

We can say with certain degree of exaggeration that a common denominator in nearly all socio-economic factors in zoonoses and sapronoses is the globalisation of: economics, the transport of goods, animals, and persons, tourism and recreation, animal and plant production, life style, landscaping and so on. An appropriate metaphor has often been used is that the world is becoming a "global village".

5.2.2 Environmental (Natural) Factors

The environmental factors that are not associated with human activity are principally diverse ecological variables that determine or affect the circulation of pathogenic agents. They involve the factors: (a) abiotic, i.e. geomorphological, geological, hydrological, pedological (soil character and structure), climatic and meteorological (actual weather conditions); (b) biotic. i.e. vegetation and fauna. This complex of environmental factors influences for example the geographical distribution of zoonotic (in connection with the distribution of reservoirs, amplifying hosts, and potential vectors) and sapronotic agents.

Among the abiotic factors, generally the most important is climate (temperature, precipitation), and then latitude, altitude and relief (geomorphology influences, e.g., mesoclimate and microclimate). For instance, a significant correlation between the El-Niňo Southern Oscillation (ENSO) activity in the Pacific Ocean and an increased incidence of certain infectious diseases (cholera, malaria, hantavirus pulmonary syndrome) in widespread regions during 1991–1993 was reported. There are substantiated fears that global warming could affect the geographical distribution of some arthropod-borne diseases as malaria, dengue, or leishmaniasis. In the analogical climatic system of North Atlantic Oscillation (NAO), which markedly affects the weather in Europe, its correlation with incidence of zoonoses and sapronoses has not yet been proven.

Abiotic environmental conditions also determine the seasonality of many infectious diseases, especially those transmitted via invertebrate vectors (e.g. TBE, LB) – this is caused by the seasonal distribution (phenology) of their vectors. For the seasonality of some vectors (mosquitoes) increased precipitation in certain periods of the year is necessary, forming at least periodic pools or wetlands favourable for development of the vectors. Environmental temperature can markedly influence the replication rate of viruses in insect vectors.

Natural disasters are also abiotic factors; they are meteorological (hurricanes or tornados), hydrological (floods) or geophysical (earthquakes, extensive landslides or tsunamis). For instance, the 1994 earthquake near Los Angeles caused the increased incidence of pulmonary mycoses (170 human cases of coccidioidomycosis and histoplasmosis) – their agents sporulate in the soil, and the spores were aerosolized during the event. Shortly after floods populations of blood-sucking insects, especially mosquitoes, usually peak, with potential subsequent outbreaks of mosquito-borne diseases (in tropical countries dengue and malaria), and also other zoonoses and sapronoses because of disruptions in water, food and power supply: e.g., colibacillosis (enteropathogenic *Escherichia coli*), salmonellosis, melioidosis (in tropics), leptospirosis, tularaemia, giardiosis and cryptosporidiosis.

Biotic factors, influencing epidemic (and epizootic) process, are in particular:

- size, density and development of populations (population dynamics) of both vertebrate hosts (reservoirs) of diseases and invertebrate vectors (dangerous "gradations");
- bionomics and behaviour (the existence of nesting colonies, common roosting sites and gathering places, synanthropism), and phenology (seasonality) of hosts and vectors;
- mobility ("home range") and migration (of birds and bats, invasions and vagrancy) of hosts and vectors;
- herd immunity in hosts populations;
- stress factors (malnutrition or overpopulation) in host populations;
- pattern and type of vegetation;
- intrinsic changes of pathogens (virulence, host and vector ranges).

A very important ecological factor in the spread of many zoonoses is the population dynamics of vertebrate hosts, and namely their overpopulation, which

can lead wild vertebrates to approach human dwellings (especially in cold seasons of the year). For instance, the overpopulated common hamster (*Cricetus cricetus*) in eastern Slovakia in 1972 (the population density was 200 ex./ha) entered farmyards and human dwellings and caused tularaemia cases in humans.

Bird migration can play a role in the spread of microbial zoonotic pathogens, in two ways: (i) migratory birds are hosts of these agents; or (ii) they transmit infected ectoparasites – vectors. According to qualified estimates, every year about half a billion birds migrate from Europe to Africa and back, and a part of them are infested with ticks. Hoogstraal and Kaiser examined 1826 birds during their northward migration in Egypt in the springs of 1960 and 1962: ixodid ticks were found on 10% individuals (about 90% of the ticks were the African subspecies *Hyalomma marginatum rufipes*). African ticks were also observed on birds in Bulgaria (*Amblyomma variegatum*, *A. hebraeum*) and in Azerbaijan (*A. lepidum*), and *Hyalomma marginatum* sporadically in Czechland and Finland. Imagoes of African ticks *A. variegatum*, *A. hebraeum* and *A. gemma* were detected on domestic animals in southern Europe (Italy, France, Bulgaria, Crimea) as a consequence of importation of their larval stages with birds. A majority of these exotic ticks cannot finish their developmental cycle under environmental conditions in Europe, but some of them could theoretically infect local vertebrate hosts and give an origin for new natural foci of infections. In addition to birds, also some bats migrate for longer distances (e.g., *Miniopterus schreibersii*). Further, even insect vectors can be imported with air currents under specific meteorological conditions (e.g. biting midges *Culicoides* infected with veterinary important arboviruses bluetongue or African horse sickness).

In some diseases (and their agents), their general association with environmental characteristics is so close and specific that it is possible to predict their distribution in new areas, using biogeographical indicators (isotherms, isohyetes, certain plant communities or collections of indicator plant and animal species). Another approach is the "remote sensing" of the Earth from satellites (e.g. Landsat); this technique was used to monitor arthropod-borne diseases in Africa (RVF, malaria and trypanosomiasis), Europe and North America (TBE and LB) and South America (malaria and Chagas disease). The most utilized data from the satellites is the "normalized difference vegetation index" (NDVI). Data received from satellites are combined with data from the geographical information system (GIS), and make it possible to model risk maps for particular infection and area (e.g., risk of human infection with LB based on the distribution of *Ixodes scapularis* in North America).

5.3 Natural Focality of Diseases

The concept of natural focality (also translated as "nidality") of diseases was first presented by E. N. Pavlovsky during a 1939 meeting of the U.S.S.R. Academy of Sciences, in a paper entitled "On the Natural Focality of Infectious and Parasitic Diseases". The idea of this doctrine is based on the fact that the aetiological agents of a number of infectious diseases circulate among free-living vertebrates and blood-sucking arthropods in nature absolutely independently of man, being an

integral component of particular ecosystems. Man can acquire the disease when entering the natural focus after contact with the host or the vector of the disease, but he/she is a "dead end" host of the enzootic cycle. The natural foci of many diseases are closely related to specific ecosystems or biomes. For instance, foci of TBE/RSSE and HFRS (bound to taiga, and mixed forests of the mild Eurasian zone), sandfly fever and Mediterranean tick typhus (macchia ecosystem), CCHF (xerothermic pastures), cutaneous leishmaniasis and tick recurrent fever (desert and semidesert ecosystems), KFD and scrub typhus (tropical rainforest). Diseases with natural focality are in fact endemic and enzootic zoonoses or sapronoses, and their study is a sort of landscape epidemiology/epizootiology.

Principal components of natural foci of infections are:

(a) the aetiological agent of the disease (the microbial pathogen);
(b) wild vertebrates – hosts of the agent (donor, recipient, or even reservoir of the agent);
(c) haematophagous arthropods (mites including ticks, insects) – vectors;
(d) the habitat and environmental factors, enabling permanent circulation of the agent.

Condition (c) need not be fulfilled in sapronoses and some zoonoses with natural focality; e.g. haemorrhagic fevers caused by Hantaan, Ebola, Marburg, Junin, Machupo and Lassa viruses, and a number of others, not transmissible by invertebrate vectors, are also classified as diseases with natural focality.

The basis of a natural focus is an ecosystem, a geobiocenosis, formed by a biocenosis (plant and animal communities) and a biotope (abiotic environmental conditions). During investigation of a natural focus, the study should involve not only the autecology of principal hosts (reservoirs) and vectors, but also their synecology, interrelationships and association rate with the habitat. Indicators of a natural focus can sometimes be characteristic features of the landscape (relief etc.) or some components of biocenoses, that could directly or indirectly reflect the presence of the agent, and make it possible to help unveil the focus in the field. Ecotones (dividing lines between two different habitats or ecosystems, e.g. forest/pasture) are very important elements in the natural foci, being contact sites for different vertebrate species from both habitats with occasional exchange of their ectoparasites and also pathogens (B. Rosický).

The natural focus of disease (NFD) is geographically defined as the territory including one or more landscape types where permanent circulation of the aetiological agent occurs without it being introduced externally. In that sense, NFD is a territorial unit, and its borders can thus be marked on a map. It has a spatial structure, being formed of a "nucleus" (a proper seat of the agent that can survive there long-term even in the epizootic period), and the surrounding "coat" appearing during increased epizootic activity of NFD. The least space in which a pathogenic agent can long-term survive in a biocenosis used to be designated as an elementary focus. In addition, the biotic structure of NFD is defined and formed by a complex of the agent, hosts, and vectors of the disease.

A very important, though often contradictory, term in epidemiology and natural focality of diseases is the <u>reservoir</u>. According to the modern conception of natural focality terminology (Kucheruk and Rosický 1983), the reservoir of an infectious disease is either an (vertebrate or invertebrate) animal species in zoonoses, or an abiotic environmental component in sapronoses (soil, organic substrate, water), where the agent survives in the interepidemic/interepizootic period.

The <u>host</u> of the agent (a vertebrate) is either <u>primary/principal</u> (secures permanent circulation of the agent), <u>secondary</u> (often involved in the epizootic process) or <u>accidental</u> (does not play a role in the epizootic process). In as far as the host becomes the source of infection of another vertebrate, it is branded as the donor, while the acceptor of the infection is the recipient. A useful term is the "<u>amplifying host</u>", i.e. that vertebrate host in whose blood the agent replicates in such a manner that it could serve as an effective donor of the agent for a haematophagous vector.

A <u>vector</u> (a haematophagous arthropod able to transmit the pathogenic agent) used to be classified analogically as <u>primary/principal</u>, <u>secondary</u> or <u>accidental</u>. In parasitological terms, the vector is an obligate ectoparasite of a temporary type, and can be associated with different species of hosts in different foci of a disease. For instance, the natural foci of TBE (and some other tick-borne infections) in central Europe can be distinguished (Rosický 1964) as "theriodic" (when the main hosts of adult *Ixodes ricinus* ticks are wild game animals), "boskematic" (when the main hosts of adult *Ixodes ricinus* ticks are grazed domestic animals like cattle, sheep, goat), "mixed theriodic-boskematic", and "mountain" (the vector might be *Ixodes trianguliceps* tick, a nidicolous ectoparasite of rodents).

The natural foci of a disease can vary in their <u>valence</u> reflecting present activity of the focus, i.e. the intensity of circulation of the agent. The foci are not stable for years, they evolve due to changes in biocenoses that affect fluctuation in valence, decline or origin of the natural foci. For instance, it is well known that a drainage in a landscape decreases activity of natural foci of tularaemia while area (re)forestation increases the valence of LB foci (northeast US states). Nowadays, the most important factor in the evolution of natural foci is not a natural succession of habitats but man with his "anthropic" activity (pasturing, deforestation, reforestation, draining, irrigation, building of water reservoirs, landscaping, etc.) resulting in "anthropisation" of the landscape. We can distinguish eubiocenoses (hardly affected by man), agrobiocenoses (farmland), and anthropobiocenoses (human habitats and urban ecosystem). A more detailed differentiation of countryside according to the land-use rate suggested Rosický and Hejný (1959) for Europe:

(1) natural landscape – practically only the tops of high mountains (no natural foci of diseases occur);
(2) landscape slightly affected by human activity – for instance mountain areas (virtually without haematophagous vectors except for the Mediterranean: natural foci of leptospirosis or, in southern Europe, some arboviroses); examples outside Europe: HPS (North America);

(3) landscape moderately affected by human activity – e.g. flood-plain forests or some less cultivated forests (natural foci of tularaemia or TBE);
(4) landscape markedly affected by man – a major proportion of countryside in Europe (foci of TBE, LB, leptospirosis, etc.);
(5) fully cultivated landscape (farmland: Q fever, brucellosis, tularaemia);
(6) landscape devastated by pasture, air pollution, mining (*Mycobacterium kansasii* infections), exploitation or dumping grounds;
(7) urbanized landscape – the urban ecosystem, consisting of: "city" – pericentre – residential areas – suburban areas. A number of zoonoses occur even in urban biocenoses, e.g. LB (city parks), psittacocis (*Chlamydophila psittaci*), toxoplasmosis (feral pigeons and cats as the source) or dermatophytosis caused by *Microsporum canis* (cat and dog). Pets or "companion animals" (cat, dog, horse, etc.) and synanthropic vertebrates (mouse, rats, feral pigeon, etc.) play a considerable role in their circulation in urban cycles.
  For examples of natural foci of zoonotic diseases, see Photos 5.1–5.49.

Epidemiologically important aspects or factors in the land use are, for example:

– global changes of biotopes;
– the formation of new biotopes (water reservoirs, pastureland, deforested or reforested areas etc. – LB);
– ecotones (balks, windbreak tree lines – emmonsiosis);
– the succession of biocenoses in anthropic biotopes (slag heaps etc. – LB);
– the synanthropisation of animals (exoanthropic vs. [eu]synanthropic animals);
– game breeding for profit (various deer spp.);
– the introduction of exotic mammals (e.g., mouflon);
– nomadism (migration of animal herds: still practiced in many tropical and subtropical countries);
– the conservation of original habitats in nature park areas – reservations (in which elementary natural foci of diseases could persist – e.g., TBE, tularaemia).

5.4 Epidemiological Examination in the Focus of an Infectious Disease

The focus of an infection is (unlike the natural focus of infection) a place where the disease spreads from. Techniques that epidemiologists use for analysis of an outbreak are descriptive and analytical.

5.4.1 Descriptive Epidemiological Methods

They aim to find answers for three principal questions: _Who? Where? When?_ (Epidemiology can be regarded, in fact, as a sort of detective story).

(1) (*Who?*) Data about patients: age; sex; ethnic group; occupation; anamnesis.
(2) (*Where?*) Description and characterization of the place of infection: geographical location of the cases (entered on a map); the character of the environment

(natural and social conditions: urban-rural; agricultural-industrial; presence of potential zoonotic and sapronotic sources).

(3) (*When?*) Time data: date, duration and dynamics of the individual disease and outbreak (an epidemic curve which can be explosive or protracted); determination of the incubation period and probable time of infection in individual cases; seasonal dynamics of the disease (graph); long-term trends of the disease; epidemic cycles.

These descriptive epidemiological methods were first, and in an exemplary way, used by John Snow in his search of the infectious source of cholera in London, 1854 (Photos 5.50 and 5.51).

Basic Statistical Indices in Epidemiology

These indices play an important role in comparative descriptive epidemiology, and most frequently involve the following coefficients.

Morbidity is the ratio of the number of sick persons to the number of all persons living in the area; usually it is given as the number of sick persons per 100,000 population per year. So-called specific morbidity is only related to a certain, well defined group (cohort) of residents characterized by for example age, sex, occupation, or related to certain period (a week, decade, month, season) or locality.

Prevalence is the number of all sick persons in a population at certain time (e.g. as for 1st July, point prevalence) or interval (interval prevalence); this index is useful especially for chronic diseases (such as tuberculosis) the incidence (annual increment) of which can be low though they are sufficiently widespread, or for those infectious diseases where it is difficult to estimate their beginning. Prevalence relates to all cases of the disease – new and old.

Incidence is the number of new cases in a certain period (e.g. a year), and can be assigned to the number of all inhabitants in certain area as with morbidity. However, incidence only concerns new cases.

Mortality is the ratio of the number of persons that died due to particular disease to the total population in the same area; it is usually given as the number of dead persons per 100,000 population per year. So-called specific mortality is only related to a certain well-defined cohort of residents.

Lethality (fatality rate) is the ratio of persons that died due to certain disease out of all persons ill from that disease (i.e. not all infected!); it is given in percent.

5.4.2 Analytical Epidemiological Methods

They aim to prove causality according to the following general algorithm:

(1) supplementary clinical, pathologic-anatomical, microbiological, serological and other examinations in the focus (Photos 5.52–5.62);
(2) definition of spatial and temporal extent of the disease;

(3) identification of the factor in common among the affected persons;
(4) formulation of a hypothesis about the source of the infectious disease.

The association of various variables and conditions with the frequency of a certain disease is statistically tested using 2×2 or 2×n contingency tables (χ^2 test, Fisher exact test), parametric or nonparametric correlation coefficients, or so-called "odd ratio" (OR). During these epidemiological analyses it is sometimes necessary to include also control group of persons in addition to the primary group of affected persons/patients ("case and control study"). Epidemiological studies can be divided into prospective (headed from cause to consequence of the disease) and retrospective (headed from consequence of the disease to case). For additional methods useful in epidemiology, see Ahrens and Pigeot (2005).

Serological surveys present a very good method for showing a contact of a population with a particular agent, and retrospective epidemiological analysis. Detection of immunoglobulines of certain classes (and especially the ratio IgM to IgG) offers in a number of diseases means for estimation of their dynamics (IgM are produced in earlier stages of infection than IgG). Serological examinations can be single, repeated or longitudinal. Comparative testing of antibody titres in paired serum samples, taken during acute and convalescent phase of the disease (at an interval of 3–4 weeks), is used as a method for detection of recent infection. Allergic skin tests are another useful and sensitive technique for revealing previous contact of population with particular disease agent.

5.4.3 The Epidemiologists' Activity

It is then based on the results of examinations, and aimed at controlling an acute epidemic situation (isolation and hospitalisation of patients, quarantine, disinfection, disinsection or deratization) or implementation of preventive measures (securing hygienic standards, vaccination, registration of carriers in some diseases). Unlike prevention, prophylaxis presents medical measures intending to achieve specific protection (immunity) of the host organism against the agent or its harmful products (toxins, allergens), and is to be applied before, or very shortly after, the exposure. Prophylaxis involves the use of chemotherapeutics (antibiotics), passive immunization (specific immunoglobulins, antitoxins) or vaccination (active immunisation with a live/attenuated, inactivated, subunit or synthetic vaccine, DNA vaccine or anatoxin). The efficacy of vaccination has been confirmed many times, for example in the control of yellow fever (Photo 5.63), or in vaccination campaign against TBE in Austria, where about 80% population has been immunised.

5.5 Epidemiological Surveillance

The term *surveillance* was first used in epidemiology in 1950 in connection with monitoring and control programs for malaria, smallpox and urban yellow fever. The

epidemiological surveillance was recommended by WHO in the years 1968–1969 as a modern approach of controlling infectious diseases. It is a complex epidemiological study of a disease as a dynamic process, including the ecology of the pathogenic agent, hosts, reservoirs and vectors of the disease, as well as the study of the environmental conditions and all mechanisms that affect spread of the disease. In short, epidemiological surveillance is monitoring of the disease and all external variables which can influence its dynamics; the data are collected, stored in a database and continuously evaluated. The final aim of epidemiological surveillance is the control (suppression) of the disease based on knowledge of those factors determining or modifying the epidemiological or epizootiological process.

According to WHO (WHO Tech. Rep. Ser. 682, 1982), "epidemiological surveillance is the process of collection, interpretation, and distribution of information on rates of occurrence of a particular disease to estimate the variation of incidence and prevalence in order to take appropriate action for the control or eradication of the disease". In short, the outline of epidemiological surveillance could be expressed as "collection → interpretation → distribution → action." Distribution here means feedback of information for medical workers in the field. The content of the surveillance is: (1) exact and rapid diagnosis of the disease (clinical, pathological and laboratory, including isolation and identification of the pathogenic agent from human, animal and vector samples – see Photos 5.53–5.62); (2) meaningful use of accessible means to control the disease. For instance in zoonoses, concentration on the animal reservoir or vector would be optimal: e.g., rat extermination, vector control, or the use of baited peroral vaccines for wild animals as foxes in the case of rabies.

The presence of a zoonosis in a certain area can be indicated by clinical observation of animals (rabies, RVF, virus encephalitides, WN disease, and hantaviroses), their post-mortem examination, meat inspection (tuberculosis, anthrax), with the use of serological surveillance (SLE in sentinel chickens or sparrows, JE in young domestic pigs, brucellosis in cattle, sheep and goats) and allergic skin testing (bovine tuberculosis), monitoring isolation tests of vectors (e.g. of mosquitoes in American equine encephalitides and other mosquito-borne diseases) and foods of animal origin. Data on overpopulation (very high population densities) of vectors and hosts (especially reservoir hosts: e.g. fox and rabies, rodents in tularaemia and hantaviruses like HFRS) are epidemiologically disturbing. The rapid international exchange of epidemiological information, based on WHO, FAO and express media such as ProMED-mail (a program of the International Society for Infectious Diseases) is always of great importance. This information exchange is a part of international "early warning" system and makes possible the "rapid response" activity of epidemiologists and other components. For this purpose, there is also International Classification of Diseases (including zoonoses and sapronoses), and a list of "notifiable (reportable) diseases" which, however, differs among countries, i.e. certain infectious diseases are to be reported only in certain countries. In addition to national epidemiological centres and national reference laboratories, there are international institutions such as WHO, CDC in USA, or ECDC in Europe, which have available a background of expert teams and reference laboratories. For instance,

many programmes concerning epidemiological surveillance of zoonoses have been established and funded by European Commission during the last decade (ENIVD, EDEN, EpiSouth, EpiNorth), others include EpiZone, GeoSentinel, CaribVET or FoodNet.

It is very important to emphasize that the collaboration of a broad and well-coordinated team of experts from different fields (epidemiology, infectology, environmental microbiology, human medicine, veterinary medicine, medical zoology and climatology) is necessary to manage a high-quality epidemiological surveillance system.

The problem of <u>bioterrorism</u> is also connected with epidemiological surveillance. Bioterrorism is defined as a wilful use of microorganisms or toxins from living organisms with the aim to cause disease or death in humans, animals or plants which are essential for mankind. The danger of modern terrorist attacks with microbes were demonstrated in the USA in 2001 with anthrax spores in mail (22 cases, including five fatalities). Because a number of zoonotic and sapronotic pathogens could serve for the production of bio-weapons we cannot avoid this issue. Out of zoonoses, plague, tularaemia, brucellosis, glanders, Q fever, viral haemorrhagic fevers (Ebola, Marburg, Lassa), and viral encephalitides (VEE, WN, SLE, JE) present a potential danger, and out of sapronoses mainly anthrax, *Clostridium botulinum* (toxin), melioidosis, cholera or coccidioidomycosis. Every new outbreak with unclear epidemiological characteristics (unusual clinical cases, indeterminate aetiology or mass outbreak) should be considered also from this perspective. Most important is an established rapid response system acting from the low level – i.e., from an adequately dense and coordinated network of human and veterinary health centres (hospitals, outpatient clinics) and diagnostic laboratories – to the rapid dissemination of information and treatment.

5.6 The Control of Zoonoses and Sapronoses

The struggle against infectious diseases proceeds at three levels: prevention . . . control . . . eradication. The basic step and integrating activity in this struggle is the strategy of epidemiological surveillance.

1. <u>Prevention</u> is the implementation of a series of measures inhibiting illness of humans (or animals) and the origin of outbreaks: conformation to hygienic rules, use of repellents (in arthropod-borne diseases), vaccination, veterinary control at borders, observance of technology rules in the meat, dairy and leather industries, and health education. Also consistent legislation is helpful: search for, and evidence of, germ-carriers, preventive examinations, and a disease reporting system.

2. <u>Control</u> is the implementation of a series of measures decreasing total incidence of a disease, and suppressing an already occurring epidemics. It consists of quarantine (isolation of infected people), medical nursing or hospitalisation in seriously ill patients, accessible medical treatment at local levels (hospitals and diagnostic laboratories), therapy or in animal-hosts reduction of numbers

(elimination/culling of infected or vagrant animals including safe collection and disposal of carrion), safe burial of human victims, safeguarding drinking water, safe removal of waste (rubbish), reduction of arthropod vector populations (by habitat changes, use of insecticides, attractants or biological methods [predators, parasites], and reduction of their fertility), environment decontamination, control of the abiotic environment (in sapronoses) and vehicles (veterinary inspection of foods of animal origin), and zoohygienic measures against the spread of infectious diseases.

Environmentally-friendly techniques of reduction of invertebrate vectors should be preferred to insecticide application. For instance in mosquitoes, the use of larvicide microbial toxin – *Bacillus thuringiensis israelensis* (effective against members of the genus *Aedes*) or *B. sphericus* (effective especially against *Culex* spp.), but also management of water level in particular areas. It is necessary to note that there is a problem with growing resistance of mosquito populations to current adulticide and some larvicide preparations (organophosphates, carbamates, pyrethroids), particularly in Africa.

By analogy, interventions against rodents (rodenticides of higher generations) and other vertebrates – hosts or reservoirs of zoonotic agents – should also be environmentally friendly. The main goal is to ensure that these biocides do not put at risk other, non-target animals (and of course humans) with either direct contact toxicity or in the form of a cummulative effect in food chains.

3. Eradication means rooting out the agent of a disease from an area. This necessitates very concentrated, i.e. expensive, procedures including consistent monitoring of the disease combined with vaccination of humans and (in zoonoses) also of animals. In this sense, only one disease has been eradicated globally: smallpox (which is, of course, an anthroponosis).

In all eradication trials the economy of expense vs. final effect plays a decisive (according to the "cost-benefit" analysis) role. This is obvious in for example African malaria. Therefore the modern control of diseases clearly prevails over the attempts of their eradication.

Chapter 6
Haematophagous Arthropods as Vectors of Diseases

The first terrestrial forms of arthropods appeared in the Palaeozoic, at the turn of the Silurian and Devonian ages some 400 million years ago. Today, arthropods form about 85% of all known animal species. They are characterized by segmentation of the body and limbs, reticulated paired appendages (antennae etc.), exoskeleton formed mainly of chitin, and bilateral symmetry. Opinions about the system of the phylum *Arthropoda* are not yet in full agreement among invertebrate taxonomists, but the basic distinction of their four classes or subphylla have recently been confirmed by sequencing their DNA:

1. Chelicerates (*Chelicerata*);
2. Crustaceans (*Crustacea*);
3. Insects (*Insecta,* or *Hexapoda*);
4. Myriapods (*Myriapoda*).

Haematophagous (i.e., blood-feeding) arthropods belong to two animal classes (involving 5 orders and 16 families): acarines (ticks and mites within *Chelicerata*) and insects (lice, bugs, diptera, and fleas).

Important characteristics of *Chelicerata* (including *Acarines*) are the absence of antennae, a head connected with the thorax in the *cephalothorax* with two pairs of appendages (chelicerae, pedipalps), and four pairs of legs (in larval acarines only three pairs). Mites realise their life cycle (metamorphosis) as Holometabola: larva → nymph (sometimes including several "instars") → imago. Some mites (Mallophaga) belong to the Hemimetabola, with a number of morphologically similar (but gradually increasing in size) larval instars until the sexually mature individual develops. In addition to mites, scorpions and spiders also belong to chelicerates.

The main features of insects are the presence of wings (but in some groups, especially in obligatory parasitic insect orders like fleas or lice, the wings are either not present or lost in maturity − hippoboscids), a respiratory tracheal system, and one pair of antennae. The insect body is divided in three main parts: head, thorax and abdomen. The wings and three pairs of legs (*Insecta* have therefore also been called *Hexapoda*) are situated on the thorax. The ontogenetic development of insects is called metamorphosis, and two types of it exist in medically important insects: usually as Holometabola (larva → pupa or puparium → imago), but some groups

like hemipteran bugs or lice are Hemimetabola (larva 1 → larva 2 → larva 3 etc. → imago; pupal stage is absent in those groups, and larval and adult stages do not differ morphologically markedly, only by the size and presence of sexual organs).

Systematic arrangement of haematophagous arthropods

Arthropods (*ARTHROPODA*)	[phylum]
Chelicerates (*Chelicerata*)	[subphylum]
1. **Mites and Ticks** (*Acarina*)	[order]
Hard ticks (*Ixodidae*)	[family]
Prostriata (*Ixodinae*)	[section (subfamily)]
Metastriata (*Rhipicephalinae*,	[sections (subfamilies)]
Amblyomminae, *Haemaphysalinae*)	
Soft ticks (*Argasidae*)	[family]
Argasinae, *Ornithodorinae*	[subfamilies]
Trombiculid mites (*Trombiculidae*)	[family]
Chicken mites (*Dermanyssidae*)	[family]
Insects (*Insecta*)	[class]
2. **Lice** (*Anoplura*)	[order]
3. **The Bugs** (*Hemiptera*)	[order]
Bedbugs (*Cimicidae*)	[family]
Triatomine bugs (*Reduviidae*)	[family]
4. **Diptera** (*Diptera*)	[order]
Mosquitoes (*Culicidae*)	[family]
Culicinae, *Anophelinae*	[subfamilies]
Sandflies (*Psychodidae*)	[family]
Phlebotominae	[subfamily]
Biting midges (*Ceratopogonidae*)	[family]
Blackflies (*Simuliidae*)	[family]
Horseflies (*Tabanidae*)	[family]
Stable-flies (*Stomoxyidae*)	[family]
Tsetse-flies (*Glossinidae*)	[family]
Deerflies (Keds) (*Hippoboscidae*)	[family]
5. **Fleas** (*Siphonaptera*)	[order]

Haematophagous arthropods are well-known as a nuisance of man and animals; but in addition, they could also transmit microbial pathogenic agents and parasitic helminths. These infections and/or infestation are collectively called "arthropod-borne diseases" or "vector-borne diseases". Among the more notorious are TBE, LB, malaria, YF, dengue, rickettsial typhus, plague, leishmaniasis, sleeping sickness or filariasis (onchocerciasis). A number of them range among the most widespread and dangerous infectious diseases. For instance 200–500 million people acquire malaria each year and up to two million people succumb to this disease annually.

6.1 Characteristics of Transmission of Infection by Arthropods

A simple scheme of the transmission can be presented as: donor (vertebrate *A*) → vector (haematophagous arthropod) → recipient (vertebrate *B*).

There exists a so-called non-specific (passive or mechanical) type of transmission of a pathogenic agent, where the agent does not multiply nor develop in the arthropod, and can be transferred onto a host contaminatively (with mouth parts, surface of limbs, or excrement) or inoculatively (mouth parts during the blood feeding, or the act of stinging). A number of non-haematophagous insect groups participate in this non-specific transmission of pathogens, e.g. cockroaches (order *Blattaria*: *Blatta orientalis*, *Blattela germanica*, *Periplaneta americana*) and flies (order *Diptera*: the families of cyclorraphan flies *Muscidae*, *Calliphoridae*, and *Sarcophagidae* can mechanically transfer for instance *Coxiella burnetii*, enteropathogenic strains of *Escherichia coli*, salmonellae, campylobacters, *Clostridium perfringens*, or cryptosporidia). Some synanthropic species of insects also participate in spreading of nosocomial (hospital) infectious diseases. They live in waste and rubbish, and feed in kitchens, pantries and storage rooms. Cockroaches prefer buildings with central heating. A number of human pathogens passage through the gut of invertebrates without a damage, remaining active: *Bacillus anthracis*, *Vibrio cholerae*, *Staphylococcus aureus*, *Salmonella typhi*, cysts of *Balantidium coli*, *Entamoeba histolytica*, *Giardia lamblia* or *Arenavirus* LCM.

In the following parts we will concentrate only on the other, more important type of transfer called specific or biological transmission of pathogens by arthropods, when the infectious agent must replicate or develop in the vector unless it could be transferred into a susceptible recipient host. The transmission can occur by inoculation via mouth parts (salivation, regurgitation) or contaminatively, by depositing on the host's skin infectious excreta (in kissing bugs or lice), or infective coxal fluid (in argasid ticks). Biological transmission can be divided in:

(a) propagative (a simple replication of the agent in the vector: rickettsiae in lice, plague bacteria in fleas and the TBE virus in ixodid ticks);
(b) cyclometamorphous (a part of the agent's life cycle takes place in the vector, but without replication: the filaria *Wuchereria bancrofti* in the *Mansonia* mosquito);
(c) cyclopropagative (a part of the agent's life cycle occurs in the vector, together with replication: malaria *Plasmodium* in the *Anopheles* mosquito).

The susceptibility of a vector to an agent is characterized experimentally by the "infection rate" that shows the proportion of the vector individuals which infect themselves during feeding on a viraemic/bacteraemic host (donor). By analogy, the effectivity of the transfer of a pathogen from the vector to another vertebrate host (recipient) is called the "transmission rate", and expressed as the proportion of infected vector individuals which infect susceptible hosts by feeding on them. The general ability of a vector species not only to infect themselves when feeding on a viraemic/bacteraemic host but also to transfer the agent to a recipient host is then called "vector competence". It has been demonstrated many times that this competence can differ between geographical variants of one vector species (e.g. the *Aedes aegypti* mosquito and EEE virus).

According to trophic specialisation (host preference) we can differentiate between vectors (e.g., mosquitoes) that are anthropophilic (feeding preferentially

or only on man), zoophilic (feeding on mammals – e.g., boophilic vectors feed on cattle), ornithophilic (feeding mainly or only on birds), and so on. An epidemiologically very important category is presented by so-called "bridge vectors" capable of alternative feeding on divergent groups of vertebrates, e.g. on both birds and humans (*Culex pipiens*), and thus making possible the inclusion of additional vertebrates into the endemic cycle or transformation of a natural cycle into an urban cycle (WNV).

An "invasive vector" is defined as an allochthonous (non-original, intruder) species which was introduced from the original area and ecosystem into a new area where it spreads and causes environmental or health damage [introduction → colonization → dispersion]. Such invasive vectors are for example the mosquitoes *Aedes albopictus* and *Ae. japonicus* that spread in America and Europe after initial introduction from Asia with import of tyres and ornamental plants (e.g., *Draceana* spp.), or ticks imported to USA with exotic vertebrate (mammalian, reptile) species.

The pathogenic agent has to overcome three main barriers in the body of its vector, to be able to effectively enter a recipient vertebrate during blood feeding: the barrier of the midgut, the haemocel with haemocytes, and that of the salivary glands – the terminal. This process is connected with a number of biotic (changes in gene expression of the agent) and abiotic (temperature) factors. The period from the sucking of an agent by a haematophagous arthropod to the ability of this vector to transmit it to a recipient host is called the "extrinsic incubation period" (EIP); the term was coined by H. R. Carter in 1898. The EIP can be affected significantly by environmental conditions, especially temperature. For instance, for yellow fever virus the mean EID in *Aedes aegypti* mosquito is 30 days at 18°C, 18 days at 21°C, 11 days at 24°C, but only 6 days at 31°C, and 5 days at 36°C. Other factors also affect the reproduction rate of the agent such as interference between different microorganisms in the vector: it is known in rickettsiae (non-pathogenic *Rickettsia peacocki* inhibits *R. rickettsii* in *Dermacentor andersoni* tick).

The dynamics of a specific infection of the vector with a certain pathogen can have quite a surprising but standard course. For instance during experimental infection of mosquitoes with a mosquito-borne virus (by feeding on a vertebrate host) the agent is detectable in mosquitoes immediately after feeding, but then it disappears for a few days (the so-called eclipse phase). Then its concentration (titre) increases exponentially and keeps the titre later at a certain stable level, usually for the whole life of the mosquitoes. In many vector species vertical transfer of the pathogenic agent from one stage of the vector to the further stage during vector metamorphosis (larva → nymph/pupa → imago), so-called transstadial transmission (TST), is also detectable: e.g. in *Ixodes ricinus* tick for TBE virus. Even more dramatic and ecologically and epidemiologically significant is transovarial transmission (TOT), when the agent is delivered by the infected female to the offspring: this mechanism was convincingly documented e.g. in *Aedes* mosquitoes for the California group orthobunyaviruses. In combination with the also documented horizontal sexual (venereal) transmission of these arboviruses from infected male to uninfected female during copulation, these three transmission mechanisms make possible long-term persistence of the disease agent in the vector population, which becomes, in fact, also the reservoir.

For infection of a vector with an agent, the host should have a certain minimal level of the agent concentration in the blood (viraemia, bacteraemia) ("threshold value") – usually in the range of some 100 infective particles/ml blood. However with arboviruses this paradigm was undermined some two decades ago when so-called "non-viraemic transmission" or "saliva-activated transmission" as a consequence of co-feeding of vectors was postulated, in that non-infected *Rhipicephalus appendiculatus* ticks became infected when they co-fed with ticks of the same species infected with Thogoto virus upon non-viraemic guinea-pigs. An analogical event has been observed with *Ixodes ricinus* ticks and TBE virus, or the spirochete *Borrelia burgdorferi.* It is to be expected that this phenomenon of co-feeding associated with co-infection might be quite widespread in tick-borne agents, and cannot be excluded also in other arthropod-borne infections (but only in those where feeding on the host lasts at least for several tens of minutes or hours).

6.2 A Survey of Haematophagous Vectors of Microbial Diseases

6.2.1 Ticks and Mites **(Acarina)**

The mites's body is composed of two main parts – the frontal (anterior) *gnathosoma* and the rear (posterior) *idiosoma*. It is interesting that the central neural ganglion is situated in the idiosoma and not in the gnathosoma. Imagoes and nymphs have four pairs of legs, while larvae have only three pairs. In some mite groups there are more nymphal stages called instars.

Ticks (*Ixodides*)

Hard ticks (*Ixodidae*)   [Ontogenesis: larva → nymph → imago]

Hard ticks (Fig. 6.1) are big mites with a dorsoventrally flattened body and a well-developed gnathosoma (the capitulum). The mouth organs are well adopted for a long-term attachment to the host's skin, and formed of toothed, harpoon-like *hypostome* spenetrating the skin of the host, further pairs of *chelicerae* and club-shaped *palpae*. The dorsal plate (*scutum*) reaches the rear part of the body in males (*conscutum*) while in females (which are usually bigger than males) it covers only the anterior part of dorsum, and the rest is formed as *alloscutum* with a softly folded integument, which can extend substantially during blood feeding. Some tick genera (*Dermacentor*, *Hyalomma* or *Rhipicephalus*) have eyes situated along the side of the shield, in other genera (*Ixodes*, *Haemaphysalis*) eyes are missing. Anal and genital (in adults) openings are situated on the ventral part of the body. The location of the anal ripple is an important morphological character differentiating the two sections: the Prostriata (*Ixodinae*: the long line runs in front of the anal opening) and the Metastriata (the semicircular ripple is at rear of the opening).

The development of an ixodid tick runs along the scheme egg → larva → nymph → imago (Fig. 6.2), and this life cycle may take several years (e.g. in *Ixodes ricinus*
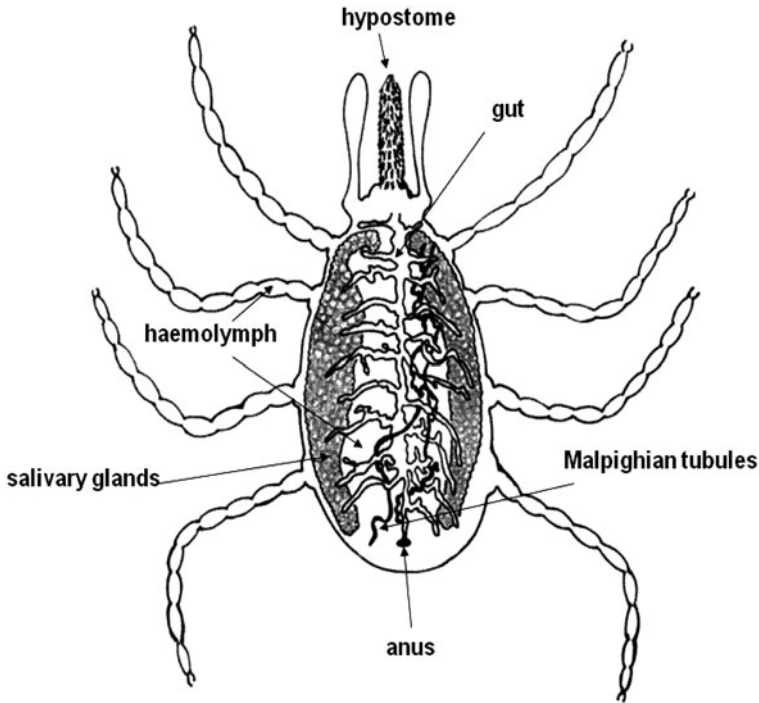
Fig. 6.1 A schematic longitudinal section through the tick *Ixodes ricinus* (drawing S. Šikutová)
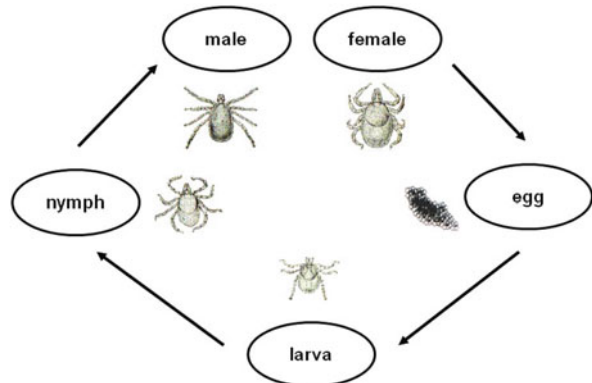
Fig. 6.2 The life cycle of the common tick *Ixodes ricinus* (drawing S. Šikutová)

usually 3 years). A female after copulation and sufficient blood feeding deposits some 500–10,000 eggs in the litter on the ground. The development of the individual life stages of the ticks proceeds on one, two, or three vertebrate hosts. The one-host cycle is characteristic for example for species of the genus *Boophilus* that live on ruminants from the stage of larva to imago permanently. The two-host cycle is common for example in the tick species *Rhipicephalus bursa* and *Hyalomma*

marginatum: blood-fed larvae remain on their host even after metamorphosis to nymphs which feed further on this host; only the blood-fed nymphs quit this host, and as adults attack another vertebrate. The three-host cycle means that each life stage of the tick feeds on other vertebrate host. Such an alternation of different vertebrate hosts, potential carriers of zoonotic agents, is of an enormous epizootiological and epidemiological importance, in that each of the hosts could harbour another pathogen.

Some tick species can cause non-infectious "tick paralysis" (a toxicosis): e.g., *Dermacentor andersoni* in North America, or *Ixodes holocyclus* in Australia. The most affected mammals are usually livestock (cattle, sheep or goats), dogs, cats, and infrequently also humans.

The habitats of ixodid ticks are strictly determined by the habitats occupied by their hosts, and especially by those host species making possible the feeding and mating of adult ticks. Rosický (1959) therefore satisfactorily classifies the natural foci of tick-borne diseases according to the habitats and the main hosts of the tick imagoes as wild (theriodic), where the principal hosts of adult ticks are wild animals (under the conditions of central Europe deer, hare, fox, badger, and hedgehog), and pastoral (boskematic), where the role of hosts of adult ticks is played by pastured domestic ruminants – cattle, sheep and goats. Mixed types of these two classes of natural foci can also occur of course. This ecological classification of tick-borne natural foci of diseases is widely acceptable in epidemiological characterization of, e.g., some zoonotic arbovirus diseases like TBE or Bhanja virus infection.

The behaviour of ixodid ticks in their attack on a host is noteworthy. They are on the watch for the victim host in vegetation (the height above the ground depends on the life stage of the tick) and use several senses for indication of a potential host. One of the most effective is the so-called Haller's organ, situated on the proximal dorsal part of the first pair of the legs, presenting in fact a pocket gas chromatographic device. (This extraordinary ability of ticks to seek for, and attack, a host is routinely exploited by arachno-entomologists during so-called flagging or dragging vegetation to collect ticks – Photo 5.62).

To prevent infestation by ticks, many sorts of repellent are used, the effective compounds usually being dimetylphthalate or dimethyltoluamide. Other important personal preventive measures against ticks include appropriate wearing and boots, and personal body inspections for ticks attached.

Ixodes ricinus (The sheep tick, also known as The common tick or The castor bean tick; Photos 6.1–6.5) is the most frequent species of *Ixodidae* in central Europe. The geographical distribution of this species covers nearly all Europe and also parts of north Africa; in eastern and north eastern Europe this species is replaced by the related species *Ixodes persulcatus* which also occurs in the Baltic countries. A typical habitat of *I. ricinus* is mixed and deciduous forests up to about 700 m a.s.l. elevation (however, it has been recorded up to 1,350 m a.s.l. in Styrian Alps and at about 1,200 m a.s.l. in the Krkonoše mountains of Czechland and in Switzerland in recent years, possibly as a consequence of global warming). During the year, the first ticks are active in March–April, and their occurrence peaks usually in May

(according to meteorological conditions), while a second, much lower, peak can sometimes occur in autumn; the last ticks can be found in October and November. Larvae feed on small mammals (most often *Apodemus* or *Myodes)*, lizards and ground-feeding birds. The nymphs attack larger mammals (squirrels, hedgehogs, hares etc.), and less often medium-sized birds. Females feed on hares, deer, fox, domestic mammals (cattle, sheep, goat, horse or dog), and pheasants. Sometimes it is possible to find larvae or nymphs even on deer and domestic ruminants. Also humans can be infested with all stages though with the larvae less often (larvae are mainly found in children); in general, feeding nymphs are observed more frequently than females in man. The whole life cycle of *I. ricinus* lasts usually 3 years (a range 2–6 years depending on climate, weather and the hosts' presence and abundance). All life stages are able to hibernate in unfed or engorged state. The tick *I. ricinus* is a vector of a number of human pathogens: TBE (TOT demonstrated) and Kemerovo group viruses (e.g. Tribeč virus), *Borrelia burgdorferi* s.l., *Anaplasma phagocytophilum* s.l., *Rickettsia helvetica*, *Francisella tularensis, Babesia microti* and *Babesia venatoria* (EU1). In addition, this tick species also carries pathogens of domestic and wild mammals: blood protozoans *Babesia divergens* (cattle), *B. canis*, *B. ovis*, *Trypanosoma theileri,* and filaria *Dipetalonema rugosicauda.*

Ixodes persulcatus (The taiga tick) occurs in European and Asian Russia, but also in the Baltic states and Finland. It is more aggressive than *I. ricinus* in attacks on humans, and vectors the RSSE virus, *Borrelia burgdorferi* s.l., *Anaplasma phagocytophilum*, and *Babesia microti*.

Ixodes scapularis (The black-legged tick or The deer tick; synonym *I. dammini*; Photos 6.6 and 6.7) of the *I. ricinus* complex is a principal vector of Powassan flavivirus (and its subtype "deer-tick virus"), *Borrelia burgdorferi*, *Babesia microti*, *Ehrlichia ewingii* and *Anaplasma phagocytophilum* in the eastern USA and Canada.

A related tick *Ixodes pacificus* (The western black-legged tick), living in western USA, transmits *B. burgdorferi, Anaplasma phagocytophilum* and *Ehrlichia chaffeensis.*

Ixodes dentatus (The eastern rabbit tick) transmits *B. burgdorferi* in North America.

Ixodes hexagonus (The hedgehog tick) occurs in Europe and parts of North Africa. Its main hosts are hedgehogs, mustelids, dogs and other canids. This species was found as a vector of *Borrelia burgdorferi* s.l. in Germany, also TST was demonstrated.

Ixodes trianguliceps is a nidicolous ectoparasite of rodents (i.e. living in their burrows − especially of voles *Microtus* spp.) in Eurasia, and transmits *Anaplasma phagocytophilum, Borrelia afzelii,* and *Babesia microti.*

Ixodes holocyclus is an Australian tick species that transmits *Rickettsia australis*, and also causes tick paralysis in cattle (intoxication). Natural hosts for this tick include koalas, bandicoots, opossums and kangaroos.

Ixodes uriae is a parasite of seabirds such as puffins (*Fratercula arctica*) and guillemots (*Uria* spp.) in northern hemisphere that transmits *Borrelia garinii*.

Haemaphysalis concinna is distributed in Eurasia. The habitats are warm but not dry deciduous and mixed forests, their clearings, and also wetlands with a higher vegetation. Imagoes are active between April and August (peak June–July), larvae from May to October, and nymphs from April till October. The whole three-host cycle lasts usually 3 years. Larvae and nymphs parasitise often birds and small mammals, while imagoes preferrentially big mammals. Humans are attacked by females and nymphs. This tick species has been found to carry TBE virus *Rickettsia sibirica*; *Francisella tularensis* has also been isolated from this species.

Haemaphysalis punctata (The red sheep tick; Photos 6.8 and 6.9) is distributed in Europe, North Africa and Asia Minor in steppe and forest-steppe habitats, and often pastures. Imagoes are active from March to June and then in October, larvae from May to August, and nymphs from April to June and then from August to October. The three-host cycle involves birds, small and big mammals and usually lasts 3 years. Humans are infrequently attacked by imagoes and nymphs. This species is a vector of TBE, Bhanja and Tribeč viruses, *Coxiella burnetii*, and blood pathogens of animals (*Babesia*, *Theileria*).

Haemaphysalis spinigera occurs in the ecosystem of tropical forest in India where it feeds on small to large vertebrates (including monkeys and humans). It is the main vector of KFD virus; in circulation of the virus in natural foci participate also other species of this genus but they do not attack humans (*H. turturis*, *H. wellingtoni*). A related species *H. intermedia,* living in forest-steppe habitats in India, is the vector of Bhanja virus.

Dermacentor marginatus (The ornate sheep tick; Photos 6.10 and 6.11) is a southern Eurasian tick of steppe and forest-steppe xerothermic (dry and warm) habitats including pastureland (biotopes similar to *H. punctata*) where the imagoes are active from March to May (peak in April) and then in September and October, while larvae and nymphs from June (July) to August. It is a three-host species: larvae and nymphs parasitise small mammals, imagoes big domestic and wild mammals. The whole life cycle usually takes only 1 year. Humans are attacked occasionally by this species which can carry TBE (RSSE), CCHF, OHF and Bhanja viruses, further *Coxiella burnetii*, *Rickettsia sibirica*, *R. slovaca*, *R. conorii*, *Francisella tularensis* and, in addition, haemoparasites of domestic animals (*Babesia*, *Nuttallia* and *Anaplasma*).

Dermacentor reticulatus (The ornate dog tick; Photos 6.12–6.14) is a (southern) Eurasian species that prefers the floodplain forest–meadow ecosystem with scrub and bushy forest edges. Seasonal activity and host range are similar to those of *D. marginatus*, imagoes appear in their habitats already in late February or early March. Infestation of humans is infrequent. The species is known as a vector of TBE and OHF viruses, *Rickettsia sibirica*, *R. conorii*, *R. slovaca, R. helvetica, Francisella tularensis* (TOT demonstrated), *Coxiella burnetii*. As in the previous species it carries the agents of animal piroplasmosis (*Babesia*, *Nuttallia*), including *Babesia canis* (documented lately in Hungary, Austria, Germany, Slovakia and Slovenia).

Dermacentor nuttalli lives in open agroecosystems in Siberia, central Asia, northern Mongolia and China. Immature stages feed on small and medium-sized mammals,

imagoes on big mammals including humans. Vector of *Rickettsia sibirica* and *Francisella tularensis*.

Dermacentor andersoni (The Rocky Mountain wood tick; Photo 6.15) occurs in North America (western USA); it parasitises ground squirrels, other rodents and domestic animals. A vector of *Rickettsia rickettsii*, Powassan and CTF viruses, *Coxiella burnetii, Ehrlichia chaffeensis,* and *Francisella tularensis*. This tick species also causes non-infectious tick paralysis in USA.

Dermacentor variabilis (The American dog tick; Photo 6.15) has a disjunct distribution in the USA, Canada and Mexico; immature stages of this three-host species parasitise rodents, and imagoes medium-sized to big mammals including man. A vector of *Rickettsia rickettsii*, *R. montana*, *R. parkeri*, *R. peacocki*, *Ehrlichia chaffeensis, Anaplasma marginale* (in cattle), *A. phagocytophilum*, *C. burnetii*, and *Francisella tularensis*. It also causes non-infectious tick paralysis in dogs and humans.

Dermacentor albipictus (The winter tick or The moose tick), a North-American one-host tick, transmits *Anaplasma phagocytophilum* and possibly *Rickettsia rickettsii*. Parasitizes mostly moose, but also other wild ruminants and pastured livestock.

Dermacentor occidentalis (The Pacific Coast tick) is a three-host tick found on many animals. Immature forms parasitise rodents. It transmits CTF virus, *Anaplasma phagocytophilum*, *C. burnetii*, *F. tularensis* and possibly *Rickettsia rickettsii*. In addition, this species causes tick paralysis in domestic animals.

Dermacentor parumapterus is a North-American vector of CTF virus and *Rickettsia rickettsii*.

Hyalomma marginatum (Photos 6.16 and 6.17) is distributed in xerothermic habitats of south Eurasia and Africa; larvae (and nymphs) are occasionally introduced into central Europe on migratory birds. During the three-host cycle immature stages infest small mammals and birds, while imagoes domestic and wild big mammals. This species attacks also man intensively. A very important vector of CCHF, Bhanja, and Dhori viruses, and *Rickettsia aeschlimanni*.

Additional species of the genus *Hyalomma* transmit CCHF, Bhanja, WN, Dhori, Thogoto and Dugbe arboviruses: *H. truncatum* (in Africa – it also transmits *Rickettsia conorii* to humans), *H. anatolicum* (in central Asia and Africa), *H. dromedarii* (in southern Asia and northern Africa) and *H. impeltatum* (in Africa). Many species of the genus *Hyalomma* also carry haemoparasites of domestic animals (*Babesia*, *Nuttallia*). The Mediterranean species *H. scupense* (= *H. detritum*) can transmit *Coxiella burnetii*, the agent of Q fever.

Rhipicephalus sanguineus (The kennel tick or The brown dog tick; Photos 6.18 and 6.19) is distributed in south Europe and in Africa and the Americas but it can be occasionally introduced into central or northern Europe on dogs from the Mediterranean. Its three-host cycle involves dogs and domestic animals (hosts for imagoes), and also humans. An important vector of *Rickettsia conorii, R. rickettsii*

(in Mexico) and *Coxiella burnetii*. It also carries a number of animal (dog, horse, sheep) pathogens: *Babesia canis* (canine ehrlichiosis), *Nuttallia* and *Theileria* spp.

Rhipicephalus bursa is an important, two-host cycle tick parasitising domestic animals in south Europe northern Africa and central Asia. It is a vector of Thogoto and Bhanja viruses, and *Babesia bigemina* and *B. bovis.*

Rhipicephalus appendiculatus (The brown ear tick) occurs frequently in the African savannah. All life stages infest various mammals including cattle. The vector of *Rickettsia conorii*, *R. aeschlimanni*, Bhanja virus, and veterinary important *Theileria parva* and *Anaplasma bovis*.

Boophilus annulatus (The cattle fever tick; Photos 6.20 and 6.21) lives in Asia, southern Europe, Africa, and Mexico on cattle. It carries CCHF and Bhanja viruses, as well as two agents of severe piroplasmosis of cattle − *Babesia bigemina* ("Texas fever") and *B. bovis* ("red water") and, in addition, *Anaplasma marginale*.

Boophilus microplus (The cattle tick; Photos 6.25 and 6.26) is a pan-tropical, one-host tick species that transmits *Anaplasma marginale*, *Babesia bovis*, *B. bigemina* and *Coxiella burnetii*. It can be found on many hosts including cattle, buffalo, horses, donkeys, goats, sheep, deer, pigs, dogs and wild mammals. Under experimental conditions, this tick can transmit *Babesia equi*, the cause of equine piroplasmosis.

Boophilus decoloratus (The blue tick) is a very common African species with an one-host cycle, infesting cattle. The vector of several viruses (CCHF, Bhanja, Dugbe, Thogoto) and bacteria (*Rickettsia conorii, R. africae*). In addition, also the vector of veterinary and economically very important diseases caused by *Babesia bigemina* (the redwater agent), *Theileria annulata* (theileriosis) and *Ehrlichia (Cowdria) ruminantium* (the heartwater agent).

Amblyomma americanum (The lone star tick; Photo 6.22) is a three-host species living in USA and Mexico, the vector of bacteria *Ehrlichia chaffeensis*, *E. ewingii*, *Borrelia lonestari*, *Coxiella burnetii*, *R. rickettsii* and *Francisella tularensis*. Imagoes infest mainly deer (e.g. white-tailed deer), dogs or coyotes, while nymphs and larvae feed on sciurids, marmots, and passeriform and galliform birds. This tick species may also cause non-infectious tick paralysis.

Amblyomma cajennense (The Cayenne tick) and *A. aureolatum* transmit *C. burnetii*, *Rickettsia rickettsii* in Texas and Brazil, and *Ehrlichia chaffeensis*. These tick species are distributed from southern Texas to northern Argentina.

Amblyomma maculatum (The Gulf Coast tick) transmits *Borrelia burgdorferi, Rickettsia rickettsii* and *R. parkeri* in USA. This tick also occurs in Central and South America.

Amblyomma triste is a neotropical tick species with a variety of hosts, the main tick species that feeds on humans in Uruguay; primary candidate vector for tick-borne rickettsioses caused by *R. parkeri*.

Amblyomma hebraeum (The South African bont tick; Photos 6.23 and 6.24) occurs in South Africa, imagoes attack large ruminants, and all stages also feed on man. The vector of *Rickettsia africae,* and the veterinary important "heartwater" agent, *Ehrlichia (Cowdria) ruminantium.*

Amblyomma variegatum (The tropical bont tick; Photos 6.25 and 6.26) is a common African species with the three-host cycle, infesting cattle. It transmits CCHF, Bhanja, Dugbe and Thogoto viruses and some other human pathogens (e.g. *Rickettsia africae*). In addition, it is the vector of very important veterinary diseases caused by *Theileria annulata* (theileriosis) and *Ehrlichia ruminantium* (heartwater), and possibly also by *Dermatophilus congolensis* (dermatophilosis of cattle). This species has been exported into some Caribbean islands (Guadelupe, Antigua) with transports of West-African cattle in the eighteenth and nineteenth centuries; it has then spread to some neighbouring Caribbean islands, probably by carriage on cattle egrets (*Ardeola ibis*).

Soft ticks (*Argasidae*)   [Ontogenesis: L → N1 → N3(...N5) → I]

Argasids, or "soft ticks", are big, dorsoventrally flattened mites with a leathery, lightly verrucose and wrinkled integument, the scutum is missing (Fig. 6.3). The mouthparts including the hypostome are situated ventrally, and are not seen from above. Their life cycle includes several instars of nymphs. The soft ticks are nidicolous and nocturnal; during the day they hide in crevices in the walls and floors of human dwellings, henhouses, pigeon lofts and stables; in the country they live in mammalian burrows, avian nests or in caves. In contrast to long-term feeding (2–14 days) of hard ticks, argasids suck the blood of the host for only a short time (1–2 min, or at most several hours), painlessly. After the feeding, a macula with an erythema appears on the skin. Argasids are long-lived invertebrates (especially members of the genus *Ornithodoros* – up to 25 years), and are able to survive hungry even for several (up to 11) years. During transmission of some pathogens (e.g. borreliae), not only the saliva but also the colourless coxal fluid which is excreted from the coxal glands between the 1st and 2nd pairs of legs at blood feeding and thereafter play a significant role as vehiculum. Soft ticks of the subfamily *Ornithodorinae* (especially those of the genus *Ornithodoros*) are vectors of endemic tick-borne relapsing fevers, while members of the subfamily *Argasinae* (genus *Argas*) are vectors of pathogens of poultry. Argasid ticks may also cause a severe allergic response in humans, accompanied occasionally even with anaphylactic shock.

Argas reflexus (Photos 6.27–6.29) attains a length up to 1 cm (female). It is distributed in European pigeon lofts and henhouses. Feeds on birds, and attacks man only exceptionally, usually after leaving the nesting place of pigeons. It carries pathogens of poultry (*Borrelia gallinarum*), but no pathogens of humans.

Argas vulgaris occurs in southern Eurasia and parasitises hollow-nesting and colonial bird species (sparrow, starling, pigeon, rook), and can also feed on man. It is the vector of *Coxiella burnetii* and pathogens of poultry (*Borrelia gallinarum*).
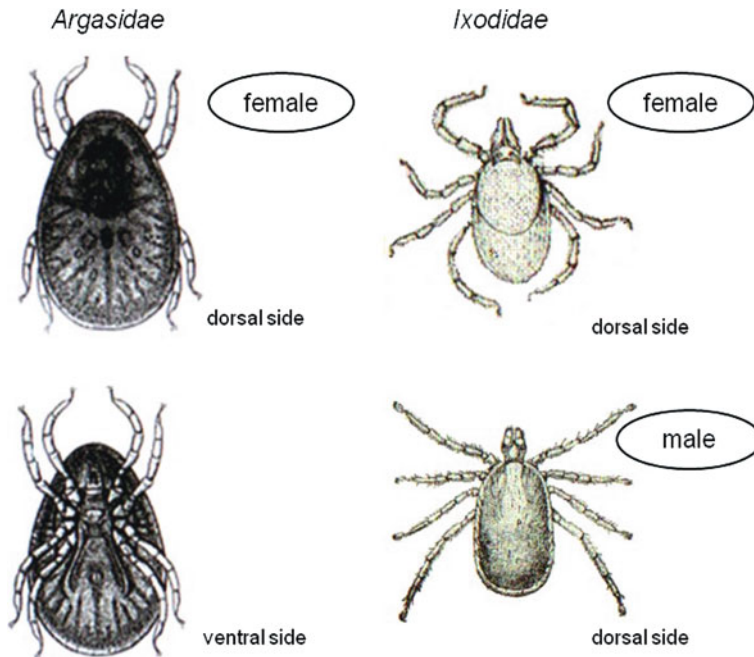
Fig. 6.3 Comparison between soft and hard ticks *Argas vulgaris* and *Ixodes ricinus* (modified according to Rosický et al. 1989 by S. Šikutová)

Argas persicus (The fowl tick) is distributed in northern Africa, southern Eurasia and Australia. It attacks poultry and pigeons, and occasionally also humans. The vector of pathogens of poultry *Borrelia gallinarum*, *B. anserina*, and *Aegyptianella pullorum.*

Argas vespertilionis, a specific ectoparasite of bats, is distributed in Eurasia and Africa. It carries *Coxiella burnetii*, and Keterah (Issyk-kul) virus.

Argas hermanni lives in pigeon lofts in Africa and Eurasia. It was demonstrated as occasional vector of WN virus.

Ornithodoros moubata s.l. (Photos 6.30 and 6.31) is distributed in Africa and its frequent habitats are the shacks of natives. The species is the main vector of *Borrelia duttonii* (the agent of African relapsing fever) and the virus of African swine fever. The nymphal stage includes five instars.

Ornithodoros erraticus s.l. lives in Spain and northern Africa. Feeding on humans is usually painfull. The vector of *Borrelia hispanica*, *B. crocidurae* and African swine fever virus.

Ornithodoros tholozani (*O. papillipes*) is distributed in central Asia and the Mediterranean in buildings, stables, etc. There are four instars of the nymphal stage.

In addition to cattle, this species also attacks carnivores, rodents and humans, as well as birds. The vector of *Borrelia persica* (TOT).

Ornithodoros tartakovskyi occurs in central Asia, including higher altitudes. The nidicolous (nest) parasite of mammals (mustelids etc.) and birds. The vector of *Borrelia latyschewi* (TOT) and *Coxiella burnetii.*

Ornithodoros hermsi, *O. turicata* and *O. rudis* occur in the Americas. The vectors of American relapsing fever caused by *Borrelia hermsii*, *B. turicatae* and *B. venezuelensis.*

Other Mites (*Acariformes*)

Trombiculid mites (*Trombiculidae*)   [Ontogenesis: L → N1...N3 →I]

Adults are small (1–2 mm) reddish, yellowish or whitish mites with a characteristic 8-shape of the body (other mites are usually oval) that is soft and looking velvety because it is covered densely by very fine feathered hairs. The life cycle (Fig. 6.4) is heteromorphous: larvae morphologically differ from nymphs and adults considerably. Only the larvae ("chiggers") attack terrestrial vertebrates (mammals and birds, in warm and humid areas also snakes and amphibians) as ectoparasites and feed on their blood. They are very small (0.1–0.2 mm), orange to red coloured, and their body is oval, not 8-shaped. The powerful mouthparts form two-segmented chelicerae and five-segmented pedipalps. Chiggers have three pairs of legs and a pair of eyes. Nymphs and adults are blind, have four pairs of legs, live in the soil and feed on small invertebrates and their eggs. Nymphochrysalis (protonymph) and imagochrysalis (pre-adult) are resting stages during which histolysis and metamorphosis to the higher stage occur.
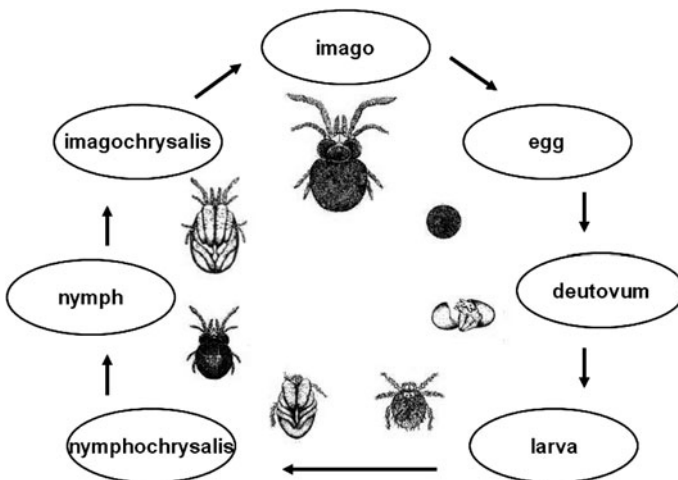
Fig. 6.4 The life cycle of trombiculid mites (*Trombiculidae*) (modified from Beaty and Marquardt 1996)

Leptotrombidium akamushi, *L. deliense*, *L. scutellare* and *L. pallidum* are the main vectors of scrub typhus ("tsutsugamushi" fever) that is caused by a rickettsia *Orientia tsutsugamushi* in south eastern Asia and Oceania. However, more trombiculid spp. participate in the endemic cycle of scrub typhus. These chiggers parasitise rodents and other wild vertebrates, and occasionally attack humans.

Several species of mites are nuisance arthropods. In central and northern Europe, *Neotrombicula autumnalis* causes trombiculosis, a seasonal skin disease (allergic urticarial reaction) accompanied with itching that occurs in the late summer and in the autumn (*erythema autumnale*). The larva feeds briefly, usually for 1–2 days, on those parts of the human body where clothing is tight on the skin. Trombiculosis could be mistaken for scabies, and its incidence might be high though underreported (underdiagnosed). Characteristic habitats for these chiggers are gardens and open places with grass, where up to several hundred of trombiculid larvae per dm^2 can occur. In North America, trombiculosis is usually caused by chiggers of *Eutrombicula alfreddugesi*.

Chicken (red) mites (*Dermanyssidae*)   [Ontogenesis: (L) → N1 → N2 → I]

Medium-sized mites (0.5–2.5 mm), parasitising mammals and birds, many species are nidicolous.

Dermanyssus gallinae, the well-known ectoparasite of fowl, has recently been shown to transmit mechanically *Salmonella enterica* serovar Enteritidis in experiment. There is also one report on isolation of *Erysipelothrix rhusiopathiae* from the chicken mites.

Liponyssoides sanguineus, an ectoparasite of synanthropic mice and rats, is the principal vector of rickettsialpox (caused by *Rickettsia akari*), and relatively often attacks also humans.

6.2.2 *Lice* (Anoplura)   [Ontogenesis: L1 → L2 → L3 → I]

Small insects (adults 2–4 mm) with a dorsoventrally flattened, prolonged and wingless body, parasitising exclusively mammals. The mouthparts are adapted to biting and sucking. Three pairs of strong legs with claws are on thorax. Lives for up to 46 days. The fertilized female lays 80–300 eggs which are glued either to hairs or fibres of clothing. Larvae hatch after 1–2 weeks (depending on temperature) and undergo three developmental stages (each lasting about a week). They have three pairs of legs, and morphologically are similar to adults except for missing reproductive organs and a rounded abdomen. Lice of all stages and both sexes are exclusively blood feeding, and they feed several times a day. Their biting hurt and itch, and consequent scratching of the skin can lead to secondary bacterial contamination of the lesions and to eczema, furuncles or pyodermia (*pediculosis*). Lice move from person to person, but also are transferable from clothes and linen. They abandon patients lying in bed with louse-borne typhus, fever and lathered in sweat, and attack healthy people – the activity which is enormously important for the epidemiology of the

Fig. 6.5 The body louse,
Pediculus humanus (modified
from Service 1996 by
S. Šikutová)
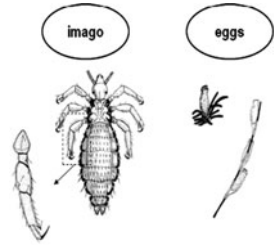
disease. Louse infestation occurs when cold weather, poor hygiene, and poverty are
pravalent. To delouse people, cloth and linen, a number of preparations can be used,
e.g. Diffusil H92 N (malathion), Diffusil H (permethrin) and shampoo Orthosan
H (pyrimiphosmethyl), newly also Diffusil H Forte (orthosan) because many louse
populations have acquired resistence to most of these biocides.

Pediculus humanus (synonym *P. corporis*, The human body louse; Photo 6.32) hides
in clothes and beds and visits the human body only during blood feeding. It is sensi-
tive to bodily hygiene. In comparison to the following species *P. capitis*, *P. humanus*
(Fig. 6.5) is larger, wider and adults differ by several morphological features on
the legs and abdomen. The body louse is the principal vector of louse-borne epi-
demic typhus (the agent, *Rickettsia prowazeki*), trench fever (*Bartonella quintana*)
and louse-borne epidemic relapsing fever (*Borrelia recurrentis*). The mechanism of
pathogen transmission to humans is by swatting of the infected louse into small
wounds in the skin or into the conjunctiva.

Pediculus capitis (The human head louse) lives in the scalp, and rarely in the beard
or eyebrows. It is more subtle than body louse, and often attacks children. Its pres-
ence is usually revealed by louse eggs glued onto hairs. The itching after bites causes
scratching and secondary inflammation or eczema mainly around ears (*pediculo-
sis*). The head louse has not been found to be a specific (biological) vector of any
infectious disease.

6.2.3 *Heteropterans* (**Heteroptera**)   [Ontogenesis: L1...L5 → I]

Bedbugs (*Cimicidae*)

These wingless insects are broadly oval, and markedly flattened dorsoventrally.
Their size is 6 mm (♂) to 9 (♀) mm, and the colour yellowish to brown (prior to
blood feeding). The mouthparts are capable of biting and sucking. The larva under-
goes five instars before it changes into an adult. Nocturnal animals, which hide in
beds, furniture fissures, wall crevices, space behind canvases, etc. during the day.
They may feed in the wild on birds or bats, but they are mainly associated with
human dwellings. Their presence in a room is often revealed by dark (red)brown
spots in such places. Bedbugs feed on blood for a short time (3–15 min), and are very

awkward human ectoparasites. Many people react to their bites with *urticaria cimicina*. A great increase in bedbugs populations has been observed since 2000, and they are usually more and more resistant to standard insecticides in many countries of the northern hemisphere. Outbreaks of healthcare-associated dermatitis caused by bedbugs in hospital nursing homes and hospices have been lately reported in many countries.

The most important, widely distributed, bedbug species is *Cimex lectularius* (Photos 6.33–6.35), while in tropical areas it is *C. hemipterus*. Fortunately the role of bedbugs as biological vectors of infectious diseases is negligible. However, they can take part in the mechanical transmission of certain pathogenic microorganisms (*Bacillus anthracis*, *Staphylococcus aureus*, *Yersinia pestis*, *Francisella*, *Trypanosoma*, *Leishmania*). *Oeciacus vicarius*, an ectoparasite of the American cliff swallow *Petrochelidon pyrrhonota* in the USA, was demonstrated to be the vector of Fort Morgan alphavirus (this virus is related to WEE virus).

Triatomine (Kissing) Bugs (*Reduviidae*)

Haematophagous reduviids belong to the subfamily *Triatominae.* In Latin America, the bugs are known under a variety of local names, including barbeiros, vinchucas, pitos and chinches. They are big (up to 35 mm), gray-black or red-black tropical bugs with a dorsoventrally flattened body, long proboscis (*rostrum*), long and thin antennae, and two pairs of membraneous wings (the forewings have a thickened base). Only adults fly – larvae are wingless, and have five instars. The total duration of the life cycle of triatomine bugs (from egg to adult) varies from 4 to 24 months, depending on the species and environmental conditions. The adults and immature forms occupy similar habitats. Eggs are laid in fissures and cracks in walls (especially mud walls) of buildings, mammalian burrows, bird nests or tree hollows. Kissing bugs are nocturnal insects, hiding in human dwellings (hiding places behind pictures, among furniture, cracks in floors, in beds or the roof), the surroundings of houses and the shelters of domestic animals (chickens, cattle, goats, cats and dogs), nests and the burrows of diverse vertebrates (mainly rodents, armadillos, bats, birds, squirrels, racoons, sloths or opossums). They feed on blood – mainly

Fig. 6.6 The adult triatomine bug *Rhodnius prolixus* (modified from Service 1996 by S. Šikutová)

mammalian. The mouthparts are for biting and sucking; their bites (often on face, therefore "kissing" bugs) are usually painless, without causing an oedema. Large populations of triatomine bugs can cause chronic anaemia through loss of blood. Kissing bugs transmit Chagas disease (the agent, *Trypanosoma cruzi*) in South and Central America, especially the species *Triatoma infestans* (Photos 6.38 and 6.39), *T. megista*, *Rhodnius prolixus* (Fig. 6.6; Photos 6.40 and 6.41) and *R. pallescens*, and *Dipetalogaster maximus* (Photos 6.36 and 6.37). Moreover, a great number of other triatomine species are involved in sylvatic, peridomestic and domestic cycles.

6.2.4 *Diptera* (Diptera)   [Ontogenesis: L1…L4 → P → I]

Insects with one pair of membranous wings (forewings) – instead of the 2nd pair there are short so-called halteres. However, in some ectoparasitic flies the wings are missing (the ked *Melophagus ovinus*) or they are lost off after finding a host (*Lipoptena cervi*). The mouthparts can lick, suck or bite. On the head they are big facetted eyes and the antennae either long (suborder *Nematocera*) or short (suborder *Brachycera*). The life cycle is holometabolic: the larva hatches from the egg (several species are viviparous – e.g. *Glossina*) and develops in four instars, before it metamorphoses into a *pupa*). The pupa is either free (mosquitoes) or forms a barrel-like puparium (flies). In haematophagous diptera, the eggs in females develop when the female feeds on blood; the period between the blood sucking and egg deposition is called the gonotrophic cycle. From a medical point of view, haematophagous and synanthropic diptera are important. Members of the second group can participate in the mechanical spread of infectious diseases, being associated with human dwellings.

Mosquitoes (*Culicidae*)

Culicine and anopheline mosquitoes possess a long *proboscis*. These biting mouthparts are composed of the lower *labium* and the upper *labrum*; paired mandibles and maxillae are transformed into biting setae and are combined with the *hypopharynx*, containing salivary duct; the trilobed salivary glands are situated in the thorax. Palpae along the sides of the proboscis are as long as the proboscis in males (and anopheline females as well), while culicine females have the palpae substantially shorter then the proboscis. For specific identification of mosquitoes one very important feature is morphology of the *hypopygium,* the male copulation organ at the rear of the abdomen.

Larvae of mosquitoes involve four instars, are without legs, live in water and respire atmospheric air with the use of two stigmates situated either on a special table (*Anophelinae*) or via a respiratory tube called *sipho* (*Culicinae*); they feed on detritus. Mosquito pupae also live in water, they do not feed, but are mobile (contrary to pupae of other groups of insects). At optimal temperature and with sufficient food supply, the development of larvae and pupae can be rapid – only about 7 and 2 days, respectively.

Only female mosquitoes feed on blood, while males feed on nectar and other plant fluids. During the feeding, the blood is imbibed into the mouth cavity, pharynx, oesophagus, and mesenteron (stomach; an appendiculate, so-called sucking stomach is situated at the rear of the oesophagus); five Malpighian glands open out into the gut behind the mesenteron (Fig. 6.7). Some species of mosquitoes are feeding specialists, they feed exclusively either on mammals (mammalophilic mosquitoes − some of those are anthropophilic or zoophilic), birds (ornithophilic) or on lower, ectothermic vertebrates (reptiles and amphibians), whereas a number of mosquito species are generalists that can use divergent hosts as a source of blood. The latter are important epidemiologically in that they can serve as so-called bridge vectors able to introduce a pathogen from one type of cycle (sylvatic, endemic, exoanthropic) to another type (urban, epidemic, synanthropic). Mosquito species are distinguished according to mating behaviour into stenogamous (mating in small tight spaces, e.g. underground) and eurygamous (mating in open spaces above ground practically everywhere). Fertilized and engorged females deposit eggs either solitarily on the water surface (*Anopheles*) or in swimming clusters (*Culex*, *Culiseta*), or on damp ground (*Aedes*). Certain culicine mosquitoes (e.g., *Cx. pipiens molestus*) are called autogenous: the females can oviposit the first egg batch without taking a blood meal (they use reserve proteins accumulated during development); all others are anautogenous – the blood meal is essential for oviposition.

Medically significant mosquitoes belong to two subfamilies (tribes): *Anophelini* and *Culicini*, which differ from each other by a number of features (Fig. 6.8): e.g., imagoes by resting position relatively to the surface (at an angle or not), the presence/pattern of dark and white scales on wing veins, palpae length and form, the shape of *scutellum* (the dorsal part of the thorax); pupae by the length and shape of breathing trumpets; larvae by their position relative to the water surface, presence/absence of a siphon, tergal plates and abdominal palmate hairs; and the eggs by the presence/absence of "floats" (air cells).
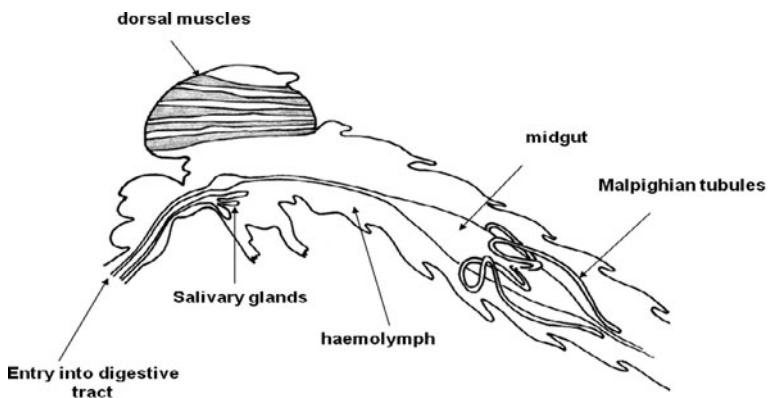
Fig. 6.7 Schematic longitudinal dorsoventral cut of a mosquito (modified from Beaty and Marquardt 1996)

Fig. 6.8 Morphological differences between two subfamilies of the family *Culicidae* (modified from Service 1996)
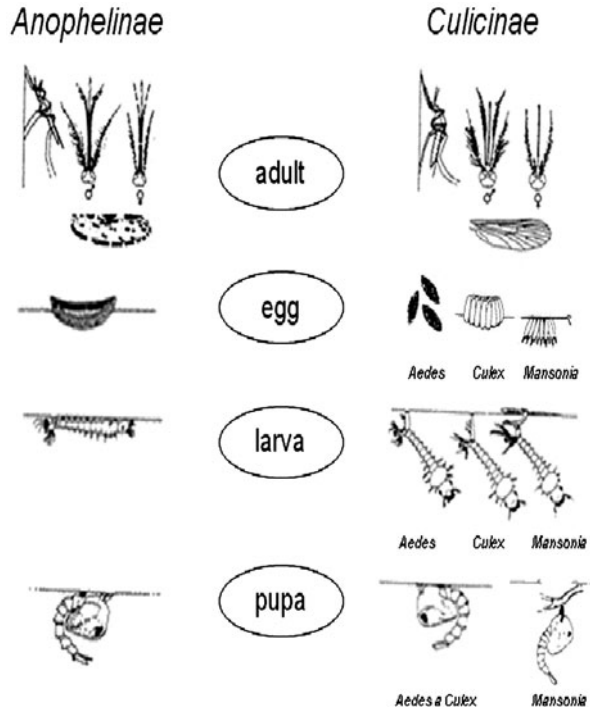
Aedes aegypti ("yellow fever mosquito"; Photo 6.42) is an anthropophilic and synanthropic species that uses every reservoir of water irrespective of its size (barrels, gutters, earthenware jars, metal drums, concrete cisterns, and even discarded pots, tins, tyres etc.). Its whole development from egg to imago lasts only 9–10 days. It has a characteristic lyre-like light pattern on the thorax, and it needs high air humidity. The original geographical region of this mosquito was tropical Africa, and the hatching habitat was tree hollows (so-called thelmes). During the slave trade since the sixteenth century, it has been dispersed through marine transport into Central, South and partially also North America, and further temporarily to Portugal and Spain, and later permanently also to tropical Asia. This species is at present distributed in the tropical and subtropical zone of the world, being the main vector of yellow fever (in the urban cycle), dengue and chikungunya fever.

Aedes albopictus (The "Asian tiger mosquito" – the name derived from agressive attacking of humans, and white colour patterns on the thorax and legs; Photos 6.43–6.45) was introduced into Texas in 1985 (in the year 2000 this species had been reported already from 30 US states), and into Brazil in 1986. In both countries the introduction mode in common was import of worn down tyres from east Asia (China, Japan and the Philippines) containing the mosquito eggs or larvae. In parallel, the mosquito reached Europe (Albania in 1979 from China; Italy and France 1989–1990 from the USA); in the twenty-first century, its occurrence has

been reported from additional European countries (Spain, Portugal, Serbia, Monte Negro, Bosnia and Herzegovina, Croatia, Slovenia, Monte Negro, Greece, Belgium, the Netherlands, Germany and Switzerland), and also from Israel (2002), and Africa (South Africa 1989, Nigeria 1991, Cameroon 1999). Another described mechanism of dispersal of this mosquito species is the trade in ornamental plants lucky bamboo (*Dracaena sanderiana*) from China and other Asian countries: the plants are transported watered, potentially with mosquito eggs or larvae. *Ae. albopictus* prefers mammals as the source of blood, and presents a significant danger as a "secondary" competent vector of dengue (sexual transmission of the virus between mosquitoes has also been described), as well as of chikungunya, Ross River fever, YF, JE, WN, RVF and California encephalitis. Already seven viruses have been detected from *Ae. albopictus* in the USA since its introduction into North America (EEE, Potosi, Tensaw, Keystone, Jamestown Canyon, LaCrosse, Cache Valley), while Chikungunya virus has been recorded in this mosquito species in Europe and southern Asia.

Aedes japonicus appeared analogically (tyre imports) in the USA (New Jersey, Connecticut) in 1998, and recently (2009) also in Switzerland. This species is a competent vector of the viruses of Japanese encephalitis and WN; the latter virus has already been isolated from this mosquito species in the USA.

Aedes vexans (Photo 6.46) is one of the most common species during mosquito overpopulations in the Eurasian floodplain forest ecosystem. The eggs laid by females on the ground remain viable for a number of years, and the hatching of larvae from them follows after the flooding of the habitat (Photos 5.15 and 5.16). This species is the principal vector of Ťahyňa *Orthobunyavirus* (California group), and its capacity for TOT for this virus has been demonstrated.

Aedes triseriatus, *Ae. dorsalis*, *Ae. melanimon* are North-American vectors of the California group orthobunyaviruses (e.g., LaCrosse virus); TOT has also been demonstrated.

Aedes vigilax is a coastal vector of the Australian viruses Ross River (TOT) and Barmah Forest.

Haemagogus is a genus of the subfamily *Culicinae*. Mosquitoes of this genus occur in Central and South America. Adults live at the canopy level in tropical rain forest, open deciduous forest or mangroves, are active during daylight, and feed mainly on monkeys. Females lay their eggs in tree-holes (endothelms), in cut bamboos, rock-holes, ground pools or artificial containers. Several species of the genus are involved in transmission of sylvatic yellow fever (*H. janthinomys*, *H. equinus*, and *H. capricornil*), Mayaro and Ilheus viruses.

Culex pipiens (Photo 6.47) is a cosmopolitan mosquito with a number of subspecies and biotypes (forms). In the northern hemisphere (Holarctic), very frequent and often sympatric biotypes are the predominantly ornithophilic *Cx. pipiens pipiens* (which is anautogenous and eurygamous, attacking occasionally also man and other mammals), and the largely mammalophilic (anthropophilic, autogenous and

stenogamous) *Cx. pipiens molestus* that often lives in the humid basements of prefabricated buildings. The two biotypes are morphologically very close, practically indistinguishable, and the hybrids *pipiens×molestus* have been described. The mosquito *Cx. pipiens* biotype *pipiens* is the vector of several arboviruses, the reservoir of which are free-living birds (e.g., *Alphavirus* Sindbis) and further of the flaviviruses WN and SLE. It took part also in the cycle of RVF during the big epidemic in Egypt in the 1970s.

Culex quinquefasciatus (synonym *Cx. fatigans*) is a tropical parallel of *Cx. pipiens*. It also involves ornithophilic and anthropophilic biotypes. In this species, Patrick Manson discovered and described the life cycle of *Wuchereria bancrofti*, the agent of lymphatic filariasis, and Ross the life cycle of avian malaria, the discovery leading later to explanation of the developmental cycle in human malaria.

Culex tritaeniorhynchus is the vector of JE in east Asia, together with mosquitoes of the complex *Cx. vishnui*.

Culex univittatus is the main vector of WN fever in northern and eastern Africa.

Culex tarsalis is an ornithophilic, zoophilic and anthropophilic mosquito playing a significant role in the transmission of *Alphavirus* WEE and *Flavivirus* SLE (TOT also demonstrated) in the USA.

Culex annulirostris is the principal vector of the Australian viruses Ross River, Barmah Forest, Murray Valley encephalitis and Kunjin (i.e., WNV).

Culiseta annulata is a big mosquito with white-striped legs, it transmits *Orthobunyavirus* Ťahyňa (TOT probable – the virus was isolated from larvae) in Europe.

Anopheles maculipennis s.l. (complex; Photo 6.48) involves several species (e.g. in central Europe *An. maculipennis* s.s., *An. messeae*, *An. atroparvus*, *An. labranchiae* – distinguishable by egg colour and the so-called "scale index"). Females are zoophilic (they attack domestic animals, also in stables and sheds) as well as anthropophilic. Vectors of malaria (some species of the complex, e.g. *An. labranchiae*, *An. maculipennis* s.s., *An. sacharovi*) and of *Orthobunyavirus* Batai.

Anopheles gambiae (Photo 6.49), *An. funestus*, *An. stephensi* and additional anopheline spp. play an important role of malaria vectors in Africa, Asia and the Americas. For instance, *An. albimanus* and *An. pseudopunctipennis* are main vectors of malaria in South and Central America and in Mexico; *An. darlingi* in Mexico and USA; *An. leucosphyrus* and *An. minimus* in Africa, India and southeast Asia. *An. hyrcanus* participates in circulation of malaria in eastern Asia, but this species also occurs in southern and central Europe. *An. gambiae* was introduced from West Africa to Brazil in 1930, where outbreaks of malaria caused by *Plasmodium falciparum* then occurred. In addition, these and some other species of *Anopheles* also participate in the transmission of several arboviruses: ONN, Bwamba, Pongola and Keterah (Issyk-kul).

Fig. 6.9 The life cycle of sandflies (modified from Beaty and Marquardt 1996 by S. Šikutová)
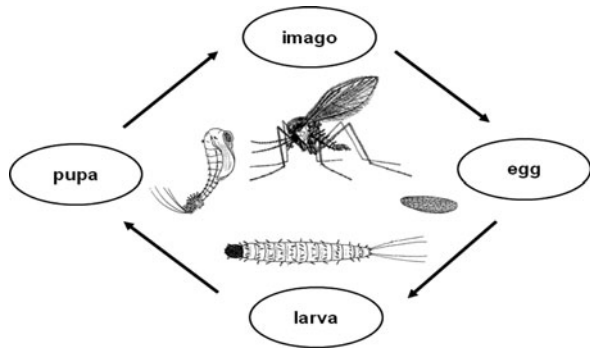
Sandflies (*Psychodidae*)

Tiny (1–3.5 mm) lightly yellowish insects with considerably dorsally bulgy thorax. In contrast to mosquitoes, their wings are hairy (legs and body as well), without scales, and the mouthparts are short – only as long as the head. The subfamily *Phlebotominae* (phlebotomine sandflies) is medically very important, involving two main genera, *Phlebotomus* (distributed in the Old World) and *Lutzomyia* (distributed in the New World), the females of which feed on the blood of terrestrial vertebrates (including reptiles). The eggs are laid in rodent burrows, tree hollows, fissures of stone walls and the floors of human dwellings, stables, folds, henhouses, and garbage (i.e., not in water as in mosquitoes).The diurnal activity of sandflies starts before sunset and finishes after sunrise while they hide during the day, though some can be active in shadowy places. Their seasonal activity begins for instance in south Europe in May and ends in October. They overwinter in the stage of the 4th larval instar (Fig. 6.9). Sandflies are widely distributed in tropics and subtropics, in Eurasia approximately up to 45°N.

Phlebotomus papatasi lives in south Europe, north Africa and central Asia. This anthropophilic species is vector of cutaneous leishmaniasis (*Leishmania major*) and sandfly (pappatasi) fever (phleboviruses SFN, SFS).

Phlebotomus perniciosus (Photos 6.50 and 6.51) and *P. perfilievi* live in the Mediterranean where they transmit visceral leishmaniasis (*Leishmania infantum*) and phlebovirus Toscana (TOT was also shown).

Phlebotomus argentipes, *P. alexandri*, *P. martini* and some other spp. transmit kala-azar (*Leishmania donovani*) in Asia and Africa.

Lutzomyia longipalpis is the main vector of visceral leishmaniasis in the New World (*Leishmania chagasi*).

Lutzomyia wellcomei is the principal vector of mucocutaneous leishmaniasis in the New World (*Leishmania braziliensis*). An additional vector of this disease is, for example, *L. carrerai*.

Lutzomyia verrucarum is a known vector of *Bartonella bacilliformis* in Peru.

Lutzomyia trapidoi transmits VSV (serotype Indiana) in USA, and *Leishmania panamensis.*

Biting Midges (*Ceratopogonidae*)

Tiny (1–2.5 mm) dipterans, coloured grayish black, with usually maculate, scaleless wings (Fig. 6.10). The biting mouthparts are short (about the size of the head), and antennae have 13–15 segments (in contrast to usually bigger blackflies). The eggs are layed on damp ground or in stagnant or slow-running water. Imagoes hide in vegetation and shadowy places during the day, and at dusk (or when overcast even during the day) the females attack mammals – especially pastured cattle (but also man and some species of birds) and briefly suck the blood. Biting midges follow the cattle returning to cowsheds but they rarely invade human dwellings. Their seasonal activity occurs from May to August. Larvae of the 4th instar hibernate. Biting midges occur in mass numbers in certain regions (Siberia, parts of European Russia, Australia, Scotland etc.).

The biting of ceratopogonids usually causes in man (but also in horses and other domestic animals) a strong allergic reaction that may persist for up to 14 days. Biting midges transfer larval stages of filariae (*Onchocerca, Mansonella*) and some bacterial (e.g., *Francisella tularensis*), protozoan (*Haemoproteus, Leucocytozoon*) and viral pathogens (bunyavirus Oropouche, and a number of veterinary very important arboviruses, especially in Australia, Africa, the USA and Japan, e.g. the viruses of bluetongue, African horse sickness, Akabane, VSV, bovine ephemeral fever or epizootic haemorrhagic fever of deer). Medically important is the ceratopogonid genus *Culicoides.*

Culicoides obsoletus, *C. pulicaris*, *C. nubeculosus* are common species in central Europe. *Culicoides imicola* is principal vector of bluetongue in Africa and southern Europe, while *C. obsoletus* is main vector of this virus in western and northern Europe. From *C. obsoletus*, two strains of *Orthobunyirus* Ťahyňa were isolated in Czechland.
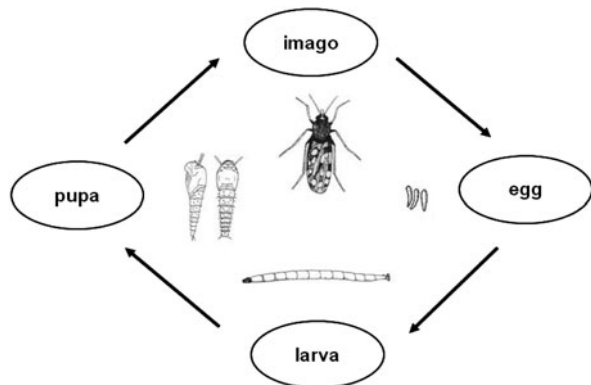
Fig. 6.10 The life cycle of the genus *Culicoides* (modified from Service 1996 by S. Šikutová)

Fig. 6.11 The life cycle of blackflies (modified from Service 1996 by S. Šikutová)
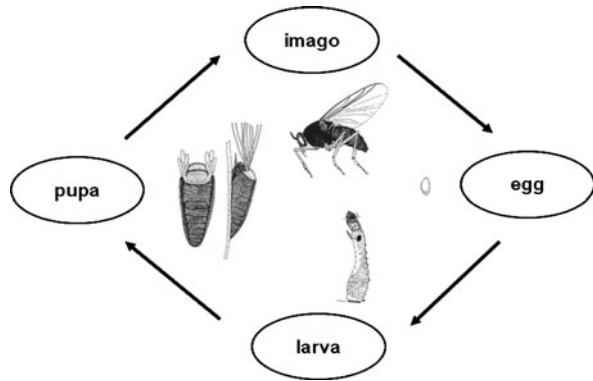
Blackflies *(Simuliidae)*

Small flies (3–6 mm; Fig. 6.11) with short legs and relatively wide round wings. The thorax is wide, strongly vaulted, and the abdomen short, pressed to the thorax. The antennae are short, composed of 9–11 segments. The eggs are laid on various objects in running water. In females after copulation, salivary glands start to produce anti-coagulation secretion, and they start to feed during the day (but largely in the morning and evening) on the blood of birds and mammals, and in some species also on man. The sucking procedure lasts 1–3 min; a strong allergic reaction can appear in humans, even of a general character (Quincke's oedema), or a tissue necrosis. There have been reported fatalities of pastured cattle associated with general intoxication after their massive infestation by blackflies (*Odagmia ornata* and some other species). Common genera in Eurasia are *Odagmia*, *Simulium*, *Eusimulium*, *Prosimulium* and *Wilhelmia*.

Blackflies are vectors (intermediate hosts) of filariae *Onchocerca volvulus* (the main vector is *Simulium damnosum*), which causes "river blindness" in man in tropical Africa, and of avian blood protozoa (*Leucocytozoon*). In addition, several arboviruses have been isolated from blackflies in North America sporadically: *Alphavirus* EEE and *Orthobunyavirus* SSH of California group).

Tabanids (*Tabanidae*)

Big flies (also called deer flies or horse flies), with a big head and short antennae (suborder *Brachycera*). The massive mouthparts are for biting and licking. The eggs are deposited on plants above the water. Imagoes are active from June to August. Females feed on the blood of big mammals, especially horses, cattle and deer. Man is attacked by tabanids of the genera *Chrysops* (e.g., *C. relictus*, *C. caecutiens, C. discalis*), *Tabanus* (*e.g., T. bromius*) and *Haematopota* (e.g. *H. pluvialis*), most often in sunny places and before storms (Photos 6.52 and 6.53).

Tabanids are mechanical carriers of *Bacillus anthracis*, *Francisella tularensis* (*Chrysops discalis* in USA and *C. relictus* in Russia), *Brucella* spp., the virus

of equine infectious anaemia, *Trypanosoma theileri*, and agents of some other infectious diseases. For instance, *Orthobunyavirus* Jamestown Canyon (California group) was isolated from deerflies of the genera *Hybomitra* and *Chrysops* in North America.

Stable-Flies (*Stomoxyidae*)

Insects resembling house-flies but with a strongly chitinised, forward projecting proboscis (as in tsetse-flies). The most common species, with a cosmopolitan distribution, is *Stomoxys calcitrans*. Both female and male feed on blood, mostly of cattle and horses, and only occasionally do they attack man. These flies often occur in stables and cowshed, while only infrequently on pastures in countryside. The eggs are deposited into cattle or equine excrement.

Stable flies can transfer *Francisella tularensis*, *Trypanosoma* spp., *Leishmania* and equine infectious anaemia virus mechanically.

Tsetse-Flies (*Glossinidae*)

These flies are conspicuous with their strongly sclerotinised, forward directed proboscis which is similar to that in stable flies but its base is bulbous. In rest, their wings are kept over the abdomen like a pair of scissors. Tsetse flies are viviparous: only one larva develops in the mother's body, is born mature and immediately penetrates into the sandy soil under bushes and trees close to animal burrows, and metamorphs in a dark, barrel-like puparium (Fig. 6.12). Tsetse-flies live in forest and savannah ecosystems of tropical Africa, usually along the river banks. Their maximum activity is around sunset, and they are also active in the morning. Both female and male feed on the blood (always for a period of about 1 min) of bigger
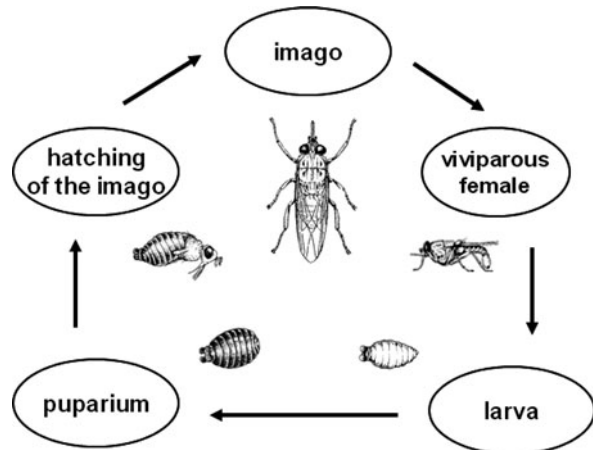
Fig. 6.12 The life cycle of tsetse-flies (modified from Beaty and Marquardt 1996 by S. Šikutová)

mammals (including man), but also on reptiles and birds. The feeding of tsetse-flies is painful for the host, often leaving a scar.

Glossina palpalis (Photos 6.54 and 6.55), *G. tachinoides, G. morsitans* (Photos 6.56 and 6.57) are important vectors of sleeping sickness in man (*Trypanosoma brucei rhodesiense*, *T. brucei gambiense*), nagana in livestock (*T. brucei*, *T. congolense*) and souma in cattle (*T. uniforme*, *T. vivax*).

Hippoboscid Flies (Keds) (*Hippoboscidae*)

Also called hippoboscids, keds, flatflies, or louseflies. Adults are usually wingless or wing discarding flies with considerably dorsoventrally flattened body. They live as ectoparasites on birds or mammals, and feed only exceptionally on man. Hippoboscids belong to the viviparous group called *Pupipara*: larval development proceeds in the mother's body, they are born fully mature, and within a very short period they metamorphose in the puparium.

Melophagus ovinus (The sheep ked), *Lipoptena cervi* (The deer ked; Photo 6.58) and *Hippobosca equina* (The forest fly) can carry some blood parasites of animals. The deer ked is the vector of *Bartonella schoenbuchensis*, a potentially zoonotic bacterium, and the sheep ked carries Candidatus *Bartonella melophagi,* also a potential zoonotic agent.

6.2.5 Fleas (**Siphonaptera**)   [Ontogenesis: L1…L4 → P → I]

Small (1–4 mm) insects with an oval body, conspicuously compressed laterally. The mouthparts are for biting and sucking, and the legs are adapted to jumping. Holometabola (four instars of larvae, pupa, imago: Fig. 6.13), adult fleas of both sexes only live from feeding on the blood of their hosts – mammals or birds. However, they are fluctuating ectoparasites, easily moving from one host to another, even that of an unrelated species: e.g. rat fleas move to man, avian fleas to mammals. After the death of its host, the fleas abandon it quickly and seek a new one. This ease of the host change is, of course, epidemiologically unusually important. The biting of fleas usually results in formation of erythematous papulae and this (non-infectious) status is called *purpura pulicosa* or *pullicosis.* Humans are attacked by several species of fleas, most often by the specific human flea *Pulex irritans* but also by the cat flea *Ctenocephalides felis*, the chicken flea *Ceratophyllus gallinae*, and less often by the canine flea *Ctenocephalides canis*, etc.

Xenopsylla cheopsis (The plague flea or The rat flea; Photos 6.59 and 6.60) is an ectoparasite of various species of rats, especially in tropics and subtropics, from where it was dispersed to the large seaports of the world. The main host is the black rat *Rattus rattus*; while in Europe another host is Norway (brown) rat *Rattus norvegicus*, and in India different rats and mice *Bandicota bengalensis*, *Golunda ellioti*, *Rattus blandfordi*, *Tatera indica*, *Mus musculus*, and even the insectivorous white-toothed shrews *Suncus caeruleus* and *S. murinus*. This flea species very often

Fig. 6.13 Life cycle of fleas
(modified from Service 1996
by S. Šikutová)
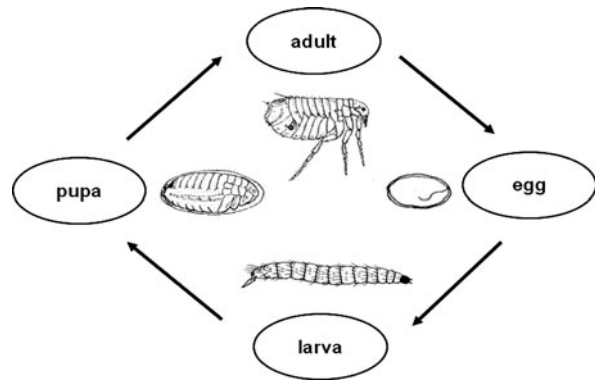
Fig. 6.14 *Yersinia pestis*
blocking proventriculum of a
flea (drawing by S. Šikutová)
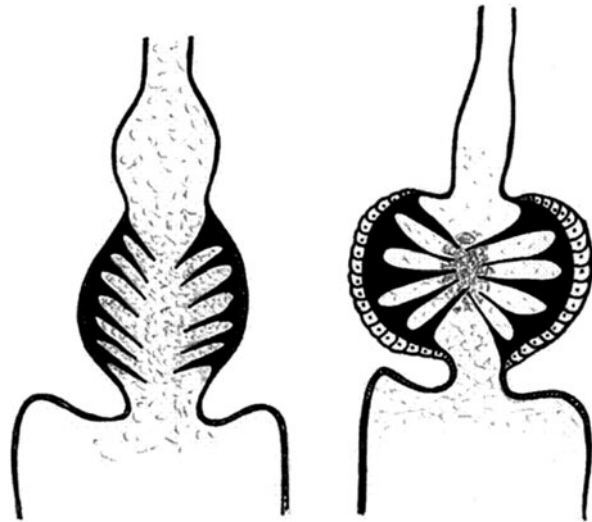
moves to man, especially when the population density of its principal host – rats –
decreases. It is a well-known, important, vector of plague (*Yersinia pestis*) and
endemic murine typhus (*Rickettsia typhi*). The mechanism of human (or another
host) infection by the flea is regurgitation: during the feeding of an already infected
flea on the blood of a non-infected host, a block of multiplying yersiniae forms in
the flea's *proventriculus* (Fig. 6.14) that inhibits further sucking; the flea throws up a
portion of blood with the block of yersiniae into the skin, thus inoculating the host.

Xenopsylla brasiliensis is an equally efficient vector of plague which prefers roofs
in rural areas in contrast to previous species.

Ctenocephalides felis (The cat flea; Photo 6.61) is the vector of cat-scratch disease (*Bartonella henselae*), and also *Rickettsia typhi* and *R. felis.* It can attack humans.

Pulex irritans (The human flea) does not transfer important human pathogens. In Africa, the DNA of *Bartonella quintana* was detected in this species.

6.3 A List of Microbial Agents Transmitted by Vectors

The following table briefly summarizes human pathogens transmitted biologically by haematophagous arthropods of individual taxa. (Sporadic or mechanical transmissions have been omitted from this list).

Arthropods	Pathogenic microorganisms			
	Arboviruses	Rickettsiae	Other bacteria	Protozoa
Family **Ixodid (hard) ticks** (***Ixodidae***)	CEE, LI, RSSE, Powassan, OHF, KFD, Kemerovo, Tribeč, Bhanja, CCHF, Dugbe, Dhori, Thogoto, CTF etc.	*Rickettsia rickettsii, R. sibirica, R. slovaca, R. helvetica, R. japonica, R. australis, R. conorii, R. africae, Ehrlichia chaffeensis, E. ewingii, Anaplasma phagocytophilum*	*Borrelia burgdorferi* s.l., *Francisella tularensis, Coxiella burnetii*	*Babesia microti, B. divergens, B. venatorum*
Family **Argasid (soft) ticks** (***Argasidae***)	Keterah (=Issyk-kul), occasionally WN		*Borrelia duttonii, B. hispanica, B. caucasica, B. hermsii* and other causative species of tick-borne relapsing fever, *Coxiella burnetii*	
Family **Trombiculid mites** (***Trombiculidae***)		*Orientia tsutsugamushi*		
Family **Other mites** (***Dermanyssidae***)		*Rickettsia akari*		
Order **Lice** (***Anoplura***)		*Rickettsia prowazeki*	*Borrelia recurrentis, Bartonella quintana*	
Family **Triatomine bugs** (***Reduviidae***)				*Trypanosoma cruzi*

(continued)

Arthropods	Pathogenic microorganisms			
	Arboviruses	Rickettsiae	Other bacteria	Protozoa
Family **Mosquitoes** (*Culicidae*)	EEE, WEE, VEE, Sindbis, Chikungunya, ONN, Ross River, Barmah Forest, YF, Mayaro, WN, SLE, YF, dengue, Murray Valley encephalitis, Rocio, Bunyamwera, Bwamba, Pongola, California group (e.g., Ťahyňa and LaCrosse), RVF, Keterah, etc.			*Plasmodium* spp.
Subfamily **Sandflies** (*Phlebotominae*)	phleboviruses of sandfly fevers (SFN, SFS, Toscana), VSV		*Bartonella bacilliformis*	*Leishmania donovani, L. infantum, L. tropica, L. major, L. braziliensis, L. mexicana*
Family **Biting midges** (*Ceratopogonidae*)	Oropouche and many other veterinary important (e.g., bluetongue, African horse fever, epizootic haemorrhagic fever, bovine ephemeral fever, Akabane, VSV etc.			
Family **Tsetse-flies** (*Glossinidae*)				*Trypanosoma brucei rhodesiense, T. brucei gambiense*
Order **Fleas** (*Siphonaptera*)		*Rickettsia typhi, R. felis*	*Yersinia pestis, Bartonella henselae*	

Chapter 7
Vertebrates as Hosts and Reservoirs of Zoonotic Microbial Agents

This chapter presents a survey of zoonotic microorganisms that have been isolated from vertebrates (*Vertebrata*), and are potentially transmissible to humans. It is intended as an aid for microbiologists, zoologists and epidemiologists, making possible better orientation among hosts (and reservoirs) of zoonoses. A great number of sources have been used in this compilation, e.g. Davis et al. (1970), Kucheruk (1979, 1989), Karabatsos (1985–1995), Hubálek (1994), etc.

The most common source of zoonoses for man are warm-blooded (endothermic, earlier called homeothermic) vertebrates, especially domestic and wild mammals, much less frequently birds, whereas only exceptionally cold-blooded (ectothermic, earlier called poikilothermic) vertebrates such as reptiles, amphibia and fishes.

A host is a vertebrate species from which a particular pathogenic agent has been isolated or detected, whereas a reservoir (host) is a vertebrate species which ensures a long-term persistence of the agent even in the inter-epizootic (inter-epidemic) period. An amplifying host is a vertebrate enabling adequate propagation (amplification) of the agent after initial infection; the pathogen then occurs in sufficient concentration and for at least several days in the blood, urine or faeces of this host. The competent host is that vertebrate species which is able not only to amplify the agent but also to transmit it to a susceptible vertebrate host or haematophagous vector (in arthropod-borne diseases). For instance in *Ebolavirus,* many primate species are amplifying and competent (but not reservoir) hosts and a source of human infection, while some species of fruit bats are the reservoir (reservoir hosts).

As long as the infected host becomes the source of infection of another vertebrate, it is considered the donor of the agent while the latter, infected vertebrate is called the recipient. The hosts are also differentiated into categories of primary (principal) host (it guarantees circulation of the agent), secondary host (it is quite often included in the epizootic process), and accidental host (does not play any role in the epizootic process). Certain vertebrates – some birds (e.g., feral urban pigeon, starling, American blackbirds) and mammalian (bat) species – can serve as so-called "lessors" (Hubálek 1994) of human-pathogenic agents (*Cryptococcus neoformans, Histoplasma capsulatum*) in that they provide for these pathogens an abiotic substrate (nest lining, droppings, guano) suitable for their propagation (an alternative term for a lessor could be a "tenant").

The following survey (an annotated list) of vertebrate hosts and pathogens shows those zoonotic microbes that were in particular host species detected by isolation, microscopy or presence of specific RNA/DNA. On the other hand agents detected only indirectly, e.g. by the presence of antibodies, have been omitted from the list. Nevertheless the isolation of a microorganism is convincing evidence of its presence in a viable state in the host ("gold standard" in microbiology) whereas its sole detection with e.g. PCR, ELISA or immunohistochemistry is not.

7.1 Mammals (Class *Mammalia*)

In general, epidemiologically the most important mammals from the zoonotic point of view are domestic and synanthropic species, as well as those hunted for their fur, i.e. the species that come often into contact with humans. Common names and the system of mammals have been adopted from Wilson and Reeder (1993), and Bisby et al. (2009), with a few exceptions.

7.1.1 Order Pouched Mammals (Marsupialia)

Family *Didelphidae*

Opossum (*Didelphis marsupialis*)

Synonym *D. virginiana*. A big American mammal (size 40–50 cm plus tail 25–50 cm – about the size of cat), living preferentially in farmland, but also found in woodland. Active usually only at night. Omnivorous: plant food prevails (fruit, vegetables, nuts); eggs, meat (mice etc.), carrion, insects. VIRUSES: *Flavivirus* YF. BACTERIA: *Rickettsia rickettsii*, *R. typhi*, *Borrelia hermsii*. PROTOZOA: *Trypanosoma cruzi, Leishmania braziliensis*.

White-eared Opossum (*Didelphis albiventris*)

A medium-sized South-American omnivorous mammal feeding on invertebrates, small vertebrates, fruit and plants. PROTOZOA: *Trypanosoma cruzi* (Brazil – competent host and reservoir).

Linnaeus's Mouse Opossum (*Marmosa murina*)

South American species, omnivorous. VIRUSES: alphaviruses EEE and VEE (Mucambo). PROTOZOA: *Leishmania braziliensis.*

Family *Phalangeridae*

Common Brushtail (*Trichosurus vulpecula*)

Occurs in forests of Australia and New Zealand (introduced into the latter). Size 30–60 cm plus tail 25–35 cm, weight 1.5–5 kg. Feeds on plants, leaves, fruit, insects and young birds. BACTERIA: *Mycobacterium bovis* (reservoir).

Family *Macropodidae*

Agile Wallaby (*Macropus agilis*)

Australian species. VIRUSES: *Alphavirus* Barmah Forest, Ross River.

7.1.2 Order Insectivores (Insect-Eaters) (Insectivora)

Family *Erinaceidae*

Four-toed Hedgehog (*Atelerix albiventris*)

African species living largely in the savannah ecosystem. Herbivorous. VIRUSES: *Alphavirus* Semliki Forest, *Bunyavirus* Bhanja, *Nairovirus* CCHF. BACTERIA: *Borrelia duttoni.* FUNGI: *Trichophyton erinacei.*

European Hedgehog, Eastern Hedgehog (*Erinaceus europaeus*, *E. concolor*)

Familiar spiny animals. They occur in western Europe (*E. europaeus*: Photo 7.1), central and eastern Europe to Central Asia (*E. concolor*: Photo 7.2). Often suburban habitats, surface nest build from leaves and other plant materials; live solitarily. In the nest (usually full of ectoparasites) they rest during day, and also hibernate. Food: invertebrates, small vertebrates and fruit. More common in lowland areas. Usually heavily infested by ectoparasites (fleas, ixodid ticks, mites, etc.). VIRUSES: *Flavivirus* TBE, *Nairovirus* CCHF. BACTERIA: *Listeria monocytogenes*, *Erysipelothrix rhusiopathiae*, *Staphylococcus aureus*, *Leptospira bataviae*, *L. bratislava*, *L. grippotyphosa*, *L. pomona*, *L. sorex-jalna*, *Borrelia burgdorferi* s.l., *Salmonella enteritidis*, *S. typhimurium*, *S. paratyphi* B, *Yersinia pseudotuberculosis*, *Francisella tularensis*, *Mycobacterium avium*. FUNGI: *Trichophyton mentagrophytes*, *T. erinacei*, *Microsporum persicolor.*

Family *Soricidae*

Common Shrew (*Sorex araneus*: Photo 7.3), **Pygmy Shrew** (*S. minutus*)
Small (*S. araneus*: 6–9 cm plus tail 4–5 cm; weight 5–14 g) or very small (*S. minutus*: 4–6 cm plus tail 3–4 cm; weight 2–6 g) widespread European insectivores, very active (because of rapid metabolism). Common in lowland and in mountains, nearly ubiquitous (a wide range of habitats). Feed on invertebrates. VIRUSES: flaviviruses TBE and LI, *Orbivirus* Tribeč, *Hantavirus* Puumala. BACTERIA: *Coxiella burnetii*, *Listeria monocytogenes*, *Erysipelothrix rhusiopathiae*, *Leptospira grippotyphosa*, *L. hebdomadis*, *L. javanica*, *L. pomona*, *L. sorex-jalna*, *Borrelia burgdorferi* s.l., *Campylobacter jejuni*, *C. coli*, *Salmonella enteritidis*, *S. typhimurium*, *Yersinia pseudotuberculosis*, *Y. enterocolitica*, *Pasteurella multocida*, *Francisella tularensis*. FUNGI: *Microsporum persicolor*, *Trichophyton mentagrophytes*, *Pneumocystis jirovecii*. PROTOZOA: *Babesia microti*, *Toxoplasma gondii.*

Water Shrew (*Neomys fodiens*: Photo 7.4), **Miller's (Mediterranean) Water Shrew** (*N. anomalus*)

Comparatively large European shrews associated with water habitats and adapted to swimming. Occasionally found far from water (especially *N. anomalus*); feed on aquatic (and other) invertebrates, small fish and frogs. VIRUSES: *Flavivirus* TBE, hantaviruses Puumala and Dobrava. BACTERIA: *Listeria monocytogenes*, *Leptospira grippotyphosa*, *L. hebdomadis*, *L. javanica*, *L. pomona*, *L. sorex-jalna*, *Borrelia burgdorferi* s.l., *Francisella tularensis*. FUNGI: *Microsporum persicolor* (*N. anomalus*), *Pneumocystis jirovecii*. PROTOZOA: *Babesia microti*, *Toxoplasma gondii*.

Greater White-toothed Shrew (*Crocidura russula*), **Lesser White-toothed Shrew** (*C. suaveolens*)

Largely west-European (*C. russula*) and east-European (*C. suaveolens*) small-sized shrews living in xerotherm habitats (scrub, gardens, vineyards, seashore) and feeding on invertebrates. VIRUSES: hantaviruses Puumala (*C. russula*) and Dobrava, *Arenavirus* LCM (*C. russula*). BACTERIA: *Listeria monocytogenes*, *Leptospira hebdomadis*, *L. pomona*, *L. sorex-jalna*, *Francisella tularensis*. PROTOZOA: *Toxoplasma gondii*.

Asian House Shrew (*Suncus murinus*)

Asian species, feeds on invertebrates. VIRUSES: *Flavivirus* KFD, *Hantavirus* Hantaan. BACTERIA: *Orientia tsutsugamushi*, *Yersinia pestis* (Madagascar).

Family *Talpidae*

Common Mole (*Talpa europaea*: Photo 7.5)

Widespread in grassland and deciduous woodland in Europe (except for Ireland and Norway). Common mole lives a subterranean life and digs extensive tunnels to catch earthworms, myriapods, insects and molluscs. VIRUSES: *Flavivirus* TBE, *Hantavirus* Puumala. BACTERIA: *Francisella tularensis*. FUNGI: *Trichophyton mentagrophytes*, *T. verrucosum*. PROTOZOA: *Babesia microti*, *Toxoplasma gondii*.

7.1.3 Order Bats (Chiroptera)

Suborder FRUIT BATS (*Megachiroptera*)

Big, flying mammals eating fruit with a head similar to canids ("flying foxes"), do not contact soil. Live in Africa, Asia and Australia. During the day they rest upside down in communal roosting sites – in trees, and some species in caves and hollows. Hunted for meat in certain areas.

Family *Pteropodidae*

Egyptian Rousette (*Rousettus aegyptiacus*: Photo 7.6)

A medium-sized fruit bat (body 13–15 cm; weight 90–170 g). Occurs in many African countries and in parts of Asia (Turkey, the Near East and Pakistan).

Roosts in caves (colonies of up to several thousand individuals), old deserted and damaged buildings, mosques etc. At least some individuals migrate seasonally between colonies distanced up to 400–500 km apart. Nocturnal, feeds on tree fruit. VIRUSES: *Alphavirus* Chikungunya, *Flavivirus* West Nile, *Marburgvirus* (competent host and reservoir).

Wahlberg's Epauletted Fruit Bat (*Epomophorus wahlbergi*; Photos 7.7 and 7.8)

This medium-sized fruit bat (body about 15 cm, weight c. 100 g) occurs mainly in tropical Africa, down to South Africa. Nocturnal, roosts in trees and feeds on fruit and nectar of flowers (baobab etc. – pollination). VIRUSES: *Ebolavirus* (experimental viraemia – potential reservoir), *Lyssavirus* Lagos bat.

Straw-coloured Fruit Bat (*Eidolon helvum*: Photo 7.9)

A common African fruit bat, lives in Egypt, Sudan, central and south Africa; feeds on tree fruit and nectar. VIRUSES: *Lyssavirus* Lagos bat (reservoir), *Henipavirus* similar (but different) to viruses Nipah and Hendra (Ghana), *Coronavirus* similar to human coronavirus 229E, *Orbivirus* Ife (Nigeria).

Black Flying Fox (*Pteropus* [*Aethalops*] *alecto*)

Australian and south-Asian species. Feeds on tree fruit and nectar. VIRUSES: *Lyssavirus* ABL ("Australian bat lyssavirus"), *Henipavirus* Menangle, *H*. Hendra.

Other south-Asian and/or Australian fruit bats (*Pteropus* spp.)

VIRUSES: henipaviruses Hendra, Nipah and Menangle: **Variable Flying Fox** *P. hypomelanus* (distributed in southern Asia and Australia), **Large Flying Fox** *P. vampyrus* (southern Asia), and **Indian Flying Fox** *P. giganteus* (southern Asia) are reservoirs of Nipah virus; **Spectacled Flying Fox** *P. conspicillatus* (Australia) is the reservoir of Menangle henipavirus.

Grey-headed Flying Fox *P. poliocephalus* (Australia) is natural reservoir of *Henipavirus* Hendra.

Madagascar Flying Fox (*Pteropus rufus*)

Lives in Madagascar and Africa. BACTERIA: *Salmonella typhimurium*.

Suborder BATS (*Microchiroptera*)

Flying nocturnal mammals. Most species eat insects. Do not contact the soil surface. A characteristic feature is gathering in summer and winter colonies (sometimes very extensive roosting places) in caves, buildings or tree hollows. They are infested with specific ectoparasites including ixodid and argasid ticks. VIRUSES: alphaviruses Sindbis and Chikungunya, flaviviruses JE, SLE, KFD, Dakar bat and Rio Bravo, *Bunyavirus* Keterah, *Thogotovirus* Dhori. BACTERIA: *Borrelia duttoni*. FUNGI: *Histoplasma capsulatum* (hosts and "lessors": the fungus grows and sporulates in the bat guano in caves). PROTOZOA: *Trypanosoma cruzi*.

Family *Rhinolophidae*

Greater Horseshoe Bat (*Rhinolophus ferrumequinum*)

Distributed in southern (partly central) Europe, North Africa and Asia. Open woodland and pastures. Feeds on flying insects, particularly beetles. Roosts singly or in large groups in caves, cellars, attics and tunnels. Movements up to 30 km. VIRUSES: *Lyssavirus* EBL1. BACTERIA: *Borrelia persica*.

Rufous Horseshoe Bat (*Rhinolophus rouxi*)

Asian species. VIRUSES: *Flavivirus* KFD, *Coronavirus* SARS (*Rhinolophus* sp.).

Family *Phyllostomidae*

Gray Short-tailed Bat (*Carollia subrufa*), **Seba's Short-tailed Bat** (*C. perspicillata*)

Central and South American species. VIRUSES: *Alphavirus* VEE.

Tent-making Bat (*Uroderma bilobatum*)

Central and South American species. VIRUSES: *Alphavirus* VEE.

Jamaican Fruit-eating Bat (*Artibeus jamaicensis*)

The Caribbean. VIRUSES: *Alphavirus* VEE.

Family *Desmodontidae*

Vampire bats (sometimes regarded as a subfamily, *Desmodontinae*) have specialised, sharp upper incissors that enable them to cut the skin of large mammals, and to feed then on their oozing blood.

Vampire Bat (*Desmodus rotundus*: Photo 7.10)

It is detrimental to livestock. A Central and South-American species, quite common. The body is 7–9 cm (no tail), weight 20–40 g; feeds exclusively on fresh blood of big mammals (livestock and occasionally man). Nocturnal, roosts in caves (colonies consist of hundreds to thousands of individuals). VIRUSES: *Alphavirus* VEE, *Lyssavirus* s.s. (also several tens of human cases described: Trinidad, Ecuador, etc.).

Hairy-legged Vampire Bat (*Diphylla ecaudata*)

Central and North America. Roosts in caves during day. VIRUSES: *Lyssavirus* s.s.

Family *Vespertilionidae*

Schreber's Yellow Bat (*Scotophilus nigrita*) and **Dwarf Dog-faced Bat** (*S. temminckii*)

African and Asian species. VIRUSES: *Alphavirus* Chikungunya, *Flavivirus* Dakar bat (*S. nigrita*), *Bunyavirus* Keterah (*S. temminckii*).

Greater Mouse-eared Bat (*Myotis myotis*: Photo 7.11), **Daubenton's Bat** (*M. daubentoni*), **Pond Bat** (*M. dasycneme*)

Large (*M. myotis*) or medium-sized European bats living in open woodland, parkland, meadows, and roosting in caves, mines, tunnels, buildings (*M. myotis*: lofts, towers). *M. dasycneme* is bound to fishpond areas, its colonies are in tree hollows and in buildings. All three spp. feed on insects, and move up to 250–350 km. VIRUSES: *Lyssavirus* EBL2. BACTERIA: *Yersinia pseudotuberculosis* (*M. myotis*).

Lesser Mouse-eared Bat (*Myotis blythi*)

Southern Europe (including parts of central Europe – Hungary and Slovakia), North Africa and Asia. Feeds on insects. Movements short, up to 15 km. VIRUSES: *Bunyavirus* Keterah.

Whiskered Bat (*Myotis mystacinus*)

Eurasian species occurring in woodland, parkland, gardens and fishpond areas. Feeds on flying insects. Movements about 40–50 km. BACTERIA: *Coxiella burnetii*, *Borrelia persica*.

Long-eared Myotis (*Myotis evotis*)

Occurs in sparsely forested areas, around buildings and occasionally in caves of western North America. VIRUSES: *Lyssavirus* s.s.

Mississippi Myotis (*Myotis austroriparius*)

Colonial species distributed in southern USA; roosts in caves, also mine tunnels, hollow trees, buildings, and migrates up to 70 km. FUNGI: *Histoplasma capsulatum* (reservoir and lessor, mainly caves in Florida).

Gray Myotis (*Myotis grisescens*)

Colonial species distributed in southern and central USA; roosts in caves. Migrate up to 70 km. FUNGI: *Histoplasma capsulatum* (reservoir and lessor, mainly caves in Tennessee).

Noctule Bat (*Nyctalus noctula*: Photo 7.12)

A large Eurasian species, living in deciduous and mixed forests, and roosting in tree hollows (e.g., woodpecker holes), feeds on large insects. Migratory at least in the northern parts of its distribution range, with the movements up to 800–1,600 km. VIRUSES: *Bunyavirus* Keterah, *Lyssavirus* EBL1.

Serotine (*Eptesicus serotinus*: Photo 7.13)

Eurasian and North-African species, common in towns and villages largely in lowlands. Hunts for insects in parkland, gardens, wooded farmland. Largely a sedentary species, but movements up to 80 km (maximum of 330 km recorded in Germany). VIRUSES: *Lyssavirus* EBL1 (reservoir), *Bunyavirus* Keterah.

Big Brown Bat (*Eptesicus fuscus*)

A large, widely distributed and common American species. Roosts singly or in small clusters, in winter common in buildings. Feeds mainly on beetles, but also

other insects. Some individuals migrate. VIRUSES: flaviviruses WN and Rio Bravo, *Lyssavirus* s.s. (often).

Meridional Serotine Bat (*Eptesicus isabellinus*)

North Africa. BACTERIA: *Coxiella burnetii* (Morocco).

Parti-coloured Bat (*Vespertilio murinus*)

Eurasian species occurring in woodland, cliffs and, in northern Europe adapted to living also in cities. Feeds on insects, migratory (up to 900 km). VIRUSES: *Lyssavirus* EBL1.

Common Pipistrelle (*Pipistrellus pipistrellus*)

The smallest Eurasian and North-African bat species (3–5 cm body and head, plus tail 2–3 cm; weight 4–9 g), almost ubiquitous and widespread, also common in cities where it roosts in buildings. In Europe, it often invades living rooms during August-September. Feeds on insects. Largely sedentary, but movements up to 770 km were recorded. VIRUSES: bunyaviruses Ťahyňa (Tadjikistan) and Keterah, *Lyssavirus* EBL1. BACTERIA: *Coxiella burnetii*, *Borrelia persica*, *Yersinia enterocolitica*. FUNGI: *Microsporum persicolor*.

Red Bat (*Lasiurus borealis*), **Hoary Bat** (*L. cinereus*), **Eastern Yellow Bat** (*L. intermedius*)

North-American species occurring in woodland and roosting in trees, occasionally in caves. Migrate south in autumn. VIRUSES: *Lyssavirus* s.s.

Schreiber's Bat (*Miniopterus schreibersii*: Photo 7.14)

The most widespread bat species: Eurasia, sub-Saharan Africa, Madagascar, New Guinea and northern Australia. A highly social species, often roosts in big numbers (hundreds to thousands) in caves, usually in karst areas. Feeds on moths, beetles and diptera. Frequent movements among roosting caves, up to 350 km recorded. VIRUSES: *Lyssavirus* Duvenhage, EBL1.

Family *Molossidae*

Mexican Freetail Bat (Guano Bat) (*Tadarida brasiliensis,* synonym *T. mexicana*)

American species roosting in large colonies (sometimes thousands of individuals – Mexico, New Mexico, Texas) in caves and buildings, migratory (up to 1,280 km). Feeds largely on moths but also other insects. VIRUSES: *Flavivirus* Rio Bravo (reservoir), *Lyssavirus* s.s. (often). FUNGI: *Histoplasma capsulatum* (lessor).

Angolan Free-tailed Bat and Little Free-tailed Bat (*Mops* [*Tadarida*] *condylurus*, *Chaerephon* [*Tadarida*] *pumila*)

African migratory species. VIRUSES: *Flavivirus* Dakar bat (*M. condylurus*, reservoir).

7.1.4 Order Apes **(Primates)**

Sometimes the species of particular monkeys are not reported in the literature, and these "group records" are listed here. VIRUSES: alphaviruses Sindbis and ONN, flaviviruses YF, dengue (rarely), filoviruses Marburg and Ebola, *Bunyavirus* Oropouche, *Herpesvirus simiae* (monkeys of the Old World – reservoir), *Orthopoxvirus simiae, Tanapoxvirus.* BACTERIA: *Erysipelothrix rhusiopathiae*, *Campylobacter jejuni*, *Salmonella enterica*, *Yersinia pseudotuberculosis*, *Corynebacterium ulcerans.* FUNGI: *Trichophyton simii, Microsporum nanum.* PROTOZOA: *Trypanosoma cruzi*, *Plasmodium knowlesi*, *P. simium*, *P. cynomolgi*, *Balantidium coli.*

Family *Galagonidae*

Senegal Galago (*Galago senegalensis*)

A large West-African prosimian, feeds mainly on insects and small vertebrates. VIRUSES: *Flavivirus* West Nile, *F*. YF.

Family *Callithricidae*

White-tufted-Ear Marmoset (*Callithrix jacchus*)

A very small South-American monkey (the size of a squirrel). Feeds largely on insects. Often kept as a household pet in South America. VIRUSES: *Alphavirus* Mayaro, *Flavivirus* YF, *Bunyavirus* Oropouche, *Lyssavirus* genotype 7 (8 lethal human cases acquired from this source were described in Brazil 1991–1998).

Family *Cebidae*

Howler monkeys (*Alouatta* spp.) **squirrel monkeys** (*Saimiri* spp.), **spider monkeys** (*Ateles* spp.), and **sakis** (*Pithecia* spp.)

South-American monkeys living in tropical rain forest ecosystem. VIRUSES: *Alphavirus* Mayaro, *Flavivirus* YF.

Family *Cercopithecidae*

King Colobus (*Colobus polykomos*) **and other colobus monkeys** (*Colobus* spp.)

African monkeys of the tropical rain forest ecosystem. VIRUSES: *Flavivirus* YF.

Patas Monkey (*Erythrocebus patas*)

African species, living in tropical rain forest ecosystem. VIRUSES: *Flavivirus* YF.

Hanuman Langur (*Semnopithecus* [*Presbytis*] *entellus*)

Indian monkey, omnivorous. VIRUSES: *Flavivirus* KFD.

Greater Spot-nosed Monkey (*Cercopithecus nictitans*)

African species. VIRUSES: *Flavivirus* YF (also in *C. mitis*), *Ebolavirus.* PROTOZOA: *Trypanosoma brucei gambiense.*

Vervet (Green) Monkey (*Chlorocebus aethiops*: Photo 7.15)

Syn.: *Cercopithecus aethiops.* Common monkey species from Senegal and Ethiopia to south Africa, feeds on insects (locusts, termites etc.) and plants. VIRUSES: *Alphavirus* Chikungunya, *Flavivirus* YF, filoviruses Marburg and Ebola.

Rhesus Monkey (*Macaca mulatta*: Photo 7.16)

South-Asian species, feeds on plants and small animals. VIRUSES: *Alphavirus* Chikungunya, *Flavivirus* KFD, *Herpesvirus simiae* (reservoir). MICROSPORIDIA: *Enterocytozoon bieneusi.*

Sooty Mangabey (*Cercocebus atys*)

African species feeding on insects and plants. VIRUSES: *Flavivirus* YF, SIV ("simian immunodeficiency syndrome virus", ancestor of HIV-2).

Olive Baboon (*Papio anubis*: Photo 7.17)

East-African species living mostly on the ground and feeding on tubers and small animals. VIRUSES: *Alphavirus* Chikungunya, *Flavivirus* YF. FUNGI: *Trichophyton simii.*

Family *Pongidae*

Chimpanzee (*Pan troglodytes*)

Ape living in African tropical forests, feeds on plants and animals, e.g. monkeys that are occasionally killed and eaten by chimps. VIRUSES: *Filovirus* Ebola, SIV ("simian immunodeficiency syndrome virus", ancestor of HIV-1, in the subspecies *P. t. troglodytes*).

7.1.5 Order Carnivores (Flesh-Eaters) (Carnivora)

Family *Canidae*

Gray Fox (*Urocyon cinereoargenteus*)

North-American species living in semi-open country (open woodland, chaparral). Omnivorous: small mammals, birds, eggs, invertebrates, fruit, acorns. VIRUSES: *Flavivirus* SLE, *Lyssavirus* s.s.

Red Fox (*Vulpes vulpes*: Photo 7.18)

Distribution widely Eurasian, but possibly conspecific with the New World Red Fox (*V. fulva*). Habitat is woodland and open land (farmland), nearly ubiquitous, occurs also in urban habitats. Feeds on small mammals (largely rodents), rabbits, hares, occasionally birds (pheasants, grouse and poultry) and invertebrates (larger insects), and sometimes vertebrate carrion and fruit. Home range about 2–3 km, but longer trails in snowy winters. Young foxes can disperse up to 200 km from their birth dens. VIRUSES: *Flavivirus* TBE, *Lyssavirus* s.s. (reservoir), *Herpesvirus suis* 1. BACTERIA: *Ehrlichia* spp., *Listeria monocytogenes*, *Staphylococcus aureus*, *Borrelia burgdorferi*, *B. afzelii*, *B. garinii*, *Leptospira grippotyphosa*, *Salmonella typhimurium*, *S. infantis*, *S. derby*, *Yersinia pseudotuberculosis*, *Brucella suis* biovar *2, B. microti* (lymphadenitis), *Mycobacterium bovis*,

Dermatophilus congolensis. FUNGI: *Trichophyton mentagrophytes*. PROTOZOA: *Leishmania infantum*, *Toxoplasma gondii*.

Corsac Fox (*Vulpes corsac*)

Central Asian species of steppe, semidesert and desert habitats. Feeds on rodents, young birds, reptiles and insects. VIRUSES: *Lyssavirus* s.s. PROTOZOA: *Toxoplasma gondii*.

Arctic Fox (*Alopex lagopus*)

Northernmost parts of North America and Eurasia, tundra and boreal forest biome. Scavenger, following the Polar Bear; also hunts for lemmings, hares, birds and eats eggs and berries. Makes very long journeys. Often bred in captivity (kept for fur). VIRUSES: *Lyssavirus* s.s. (reservoir). BACTERIA: *Listeria monocytogenes*, *Bacillus anthracis*, *Leptospira* spp. (acquired from rodents), *Brucella abortus, Francisella tularensis.*

Raccoon Dog (*Nyctereutes procyonoides*: Photo 7.19)

Eurasian species (originally from east Asia) about the same size as the Red Fox, with a short bushy tail. Occurring in variable habitats. Feeds on small animals, fruit and other plant components. VIRUSES: *Lyssavirus* s.s. (very susceptible), *Coronavirus* SARS. BACTERIA: *Yersinia pseudotuberculosis*, *Francisella tularensis*.

Wolf (*Canis lupus*)

Islet-like distribution in Eurasia and North America. Prefers forest habitats and tundra biome, and hunts (in packs) deer, wild boar, rabbits, hares, occasionally birds, sheep, goats, and feeds even on frogs, fruit and insects. Hunting range is up to 100 km. VIRUSES: *Lyssavirus* s.s. (reservoir). BACTERIA: *Borrelia burgdorferi*, *Leptospira grippotyphosa*, *Francisella tularensis*, *Brucella abortus*. FUNGI: *Trichophyton mentagrophytes*.

Dog (*Canis familiaris*)

VIRUSES: *Flavivirus* TBE, *Lyssavirus* s.s., *Herpesvirus suis* 1. BACTERIA: *Rickettsia conorii* (reservoir), *R. rickettsii*, *Ehrlichia ewingii*, *Leptospira interrogans*, *Borrelia duttoni*, *B. burgdorferi* s.l., *Erysipelothrix rhusiopathiae*, *Staphylococcus aureus*, *S. epidermidis*, *S. intermedius*, *Campylobacter jejuni*, *Helicobacter bizzozeronii*, *Yersinia enterocolitica*, *Y. pseudotuberculosis*, *Pasteurella multocida*, *Capnocytophaga canimorsus*, *Burkholderia mallei*, *Mycobacterium bovis*. FUNGI: *Microsporum canis*, *Blastomyces dermatitidis*, *Pneumocystis jirovecii*. PROTOZOA: *Trypanosoma cruzi*, *T. brucei rhodesiense*, *T. brucei gambiense*, *Leishmania tropica*, *L. major*, *L. donovani*, *L. infantum* (reservoir), *Giardia lamblia*, *Cryptosporidium parvum*. MICROSPORIDIA: *Encephalitozoon cuniculi*, *Enterocytozoon bieneusi*.

Dingo (*Canis dingo*)

Australian wild dog living in dry bushland and semidesert. Hunts different mammals, birds, reptiles and insects, also feeds on carrion. BACTERIA: *Rickettsia australis*.

Jackal (*Canis aureus*)

Steppe, semi-open and wetland ecosystems of south-eastern Europe, Asia and north Africa, often around towns and villages. Feeds mostly on carrion, also on some small animals including insects, and fruit. VIRUSES: *Lyssavirus* s.s. (reservoir in many parts of south Asia, north Africa, Ethiopia). PROTOZOA: *Leishmania infantum* (reservoir).

Coyote (*Canis latrans*)

North-American species, the size of a medium dog, living in open woodland, bushland or prairies – very adaptable. Omnivorous, feeding on rodents, rabbits, often on carrion (scavenger), but also vegetables. Occasionally can kill sheep or calves. Extensive home range (usually up to 15 km, occasionally 160 km recorded). VIRUSES: *Lyssavirus* s.s. BACTERIA: *Mycobacterium bovis*.

Family *Ursidae*

Brown Bear (*Ursus arctos*)

Fragmentary distribution in forest ecosystem of mountainous areas in Europe and North America (Alaska). A huge mammal, with a usual weight of 150–250 kg, but sometimes up to 450 kg. Omnivorous: eating berries, insects, honey, smaller vertebrates and carrion. VIRUSES: *Lyssavirus* s.s. BACTERIA: *Campylobacter jejuni*. FUNGI: *Trichophyton mentagrophytes*.

Black Bear (*Ursus americanus*)

Forest, swamps and mountainous habitats in North America. Feeds on berries, nuts, tubers, insects, honey, small mammals, eggs, carrion, and garbage. Long movements (up to about 25 km). VIRUSES: *Lyssavirus* s.s.

Family *Procyonidae*

Raccoon (*Procyon lotor*: Photo 7.20)

North-American, medium-sized (about that of a small dog) species living near wooded areas, closely to streams and lakes, rock cliffs, but also in urban areas. Rests in tree hollows, ground burrows or rock crevices. Activity is largely nocturnal. Omnivorous: feeds on fruit, nuts, grain, rodents, frogs, carrion of larger mammals, eggs, insects, crayfish and other invertebrates. Home range 1–3 km, but young animals disperse up to 50 km from the birth place (one record of 260 km). Raccoon was introduced into Europe for its fur and now is spreading in Germany, the Baltic states, and NW Russia. VIRUSES: *Lyssavirus* s.s. (reservoir). BACTERIA: *Listeria monocytogenes*, *Leptospira autumnalis*, *L. grippotyphosa*, *L. hebdomadis*, *L. pomona*, *L. icterohaemorrhagiae*, *L. australis*, *Borrelia burgdorferi* s.s., *Yersinia pseudotubeculosis*, *Pasteurella multocida*, *Francisella tularensis*. PROTOZOA: *Trypanosoma cruzi*.

Family *Mustelidae*

Weasel (*Mustela nivalis*: Photo 7.21)

This is the smallest Eurasian carnivore (11–25 cm, tail 15–85 cm; weight about 100 g, maximum 200 g); also occurs in north-western Africa and North America. Lives usually in open country in varied habitats, and normally nests in rodent burrows. Hunts small mammals, largely rodents – even in their burrows. BACTERIA: *Staphylococcus aureus*, *Leptospira grippotyphosa*, *Yersinia pseudotuberculosis*, *Francisella tularensis*. PROTOZOA: *Toxoplasma gondii*.

Stoat (Ermine) (*Mustela erminea*: Photo 7.22)

Eurasian and North-American species, nearly ubiquitous but usually in open country. It nests in burrow or in rocks. In their diet prevail small mammals (up to the size of small rabbit). BACTERIA: *Staphylococcus aureus*, *Leptospira grippotyphosa*, *Yersinia pseudotuberculosis*, *Francisella tularensis*. PROTOZOA: *Toxoplasma gondii*.

Polecat (*Mustela putorius*: Photo 7.23), **Steppe Polecat** (*M. eversmanni*: Photo 7.24)

European and NW African (*M. putorius*) and Eurasian (*M. eversmanni*) species of medium-sized mustelids, closely related to the domestic ferret. They occur mostly in woodland (*M. putorius*) or in grassland and steppe habitats (*M. eversmanni*). They rest above ground (*M. putorius*) or in burrows (*M. eversmanni*), hunting small vertebrates (rodents and birds), and occasionally feeding on insects. VIRUSES: *Lyssavirus* s.s., *Herpesvirus suis* 1. BACTERIA: *Staphylococcus aureus*, *Leptospira grippotyphosa*, *Francisella tularensis*., *Yersinia pestis* (*M. eversmanni*, during epizootics in Central Asia and the Caucasus). PROTOZOA: *Toxoplasma gondii*.

American Mink (*Mustela vison*), **European Mink** (*Mustela lutreola*)

Medium-sized mustelids living along streams, lakes and in wetlands (excellent swimmers). Mainly nocturnal and solitary species. They feed on small vertebrates (mammals, birds and their eggs, frogs and fish). Hunted or bred for valuable fur. The American Mink was introduced to Eurasia, where it has occasionally escaped from captivity (domesticated mink) and lives in the wild now. The population of European Mink has been reduced markedly and the species is endangered, while feral American Mink is expanding in Europe and is considered a pest. BACTERIA: *Bacillus anthracis*, *Erysipelothrix rhusiopathiae*, *Pasteurella multocida*, *Yersinia pseudotuberculosis*.

Black-footed Ferret (*Mustela nigripes*)

A large "ornamental weasel" with light brown body and dark face mask. It occurs in prairies of midwestern US states, often close to prairie dog towns, feeding on prairie dogs and other small mammals and birds. BACTERIA: *Yersinia pestis*.

European Pine Marten (*Martes martes*: Photo 7.26), **Beech (Stone) Marten** (*Martes foina*: Photo 7.25)

Medium-sized Eurasian mustelids living in woodland, forests, and the Beech Marten also in dry and more open scrub, rocky habitats and close to human habitation. They hunt small mammals and birds (*M. foina* also hunts chickens). VIRUSES: *Lyssavirus* s.s., *Herpesvirus suis* 1. BACTERIA: *Erysipelothrix rhusiopathiae*, *Listeria monocytogenes*, *Salmonella enteritidis*. PROTOZOA: *Toxoplasma gondii*.

Wolverine (*Gulo gulo*)

North Asian and North American medium-sized to large mammal (about 85 cm plus bushy tail 30 cm; 15–30 kg). Habitat: mountains near the timberline, and tundra biome. Solitary and omnivorous (often including carrion), with a considerable home range. BACTERIA: *Brucella abortus.*

Badger (*Meles meles*: Photo 7.27)

The largest mustelid species (70–90 cm plus tail about 12–20 cm; weight c. 10–20 kg, sometimes up to 30 kg). Lives in Eurasian woodlands where it builds quite extensive burrows. Nocturnal and omnivorous (small mammals, carrion of large mammals, bird eggs, earthworms and other invertebrates, seeds, fruit). VIRUSES: *Lyssavirus* s.s., *Herpesvirus suis* 1. BACTERIA: *Bacillus anthracis*, *Leptospira grippotyphosa*, *Salmonella enteritidis*, *Mycobacterium bovis* (reservoir: England). FUNGI: *Trichophyton mentagrophytes*. PROTOZOA: *Babesia microti*.

Chinese Ferret-Badger (*Melogale moschata*)

Southeastern Asian species, omnivorous. VIRUSES: *Coronavirus* SARS, *Lyssavirus* s.s.

Striped Skunk (*Mephitis mephitis*)

A medium-sized (about the same as a cat) North-American species of semi-open country (mixed wood, bushland and prairie). Largely nocturnal. Dens in ground burrows or tree hollows. Omnivorous: rodents, eggs, invertebrates, carrion and fruit. VIRUSES: *Lyssavirus* s.s. (reservoir), *Coltivirus* CTF. BACTERIA: *Listeria monocytogenes*, *Leptospira autumnalis*, *L. grippotyphosa*, *L. hebdomadis*, *L. pomona*, *L. icterohaemorrhagiae*, *L. australis*, *Yersinia pseudotuberculosis*, *Pasteurella multocida*, *Francisella tularensis.*

Otter (*Lutra lutra*)

This Eurasian and NW African species lives along streams, fishponds, lakes and wetlands as largely an aquatic animal, but can travel several kilometres overland to reach another river. Feeds mainly on fish, also frogs, crayfish and other aquatic invertebrates, and vertebrate carrion. VIRUSES: *Lyssavirus* s.s. BACTERIA: *Erysipelothrix rhusiopathiae*, *Yersinia pseudotuberculosis.*

Family *Herpestidae*

Egyptian Mongoose (*Herpestes ichneumon*: Photo 7.28)

Predominantly African species (also lives in Spain and Portugal) in scrub and woods. Mainly nocturnal. VIRUSES: *Lyssavirus* s.s. (often, also human cases).

Indian Mongoose (*Herpestes javanicus* [*auropunctatus*])

South Asian species (also introduced into the Caribbean in 1870). VIRUSES: *Lyssavirus* s.s. (often, also human cases).

Yellow Mongoose (*Cynictis penicillata*)

South African social species. VIRUSES: *Lyssavirus* s.s. (often).

Family *Viverridae*

Genet (*Genetta genetta*)

African, south Asian and south-west European (Spain, France) nocturnal species living in moist, dark woods. VIRUSES: *Lyssavirus* s.s.

Masked Palm Civet (*Paguma larvata*)

South-Asian, medium sized (50–75 cm plus tail 50–60 cm; weight 3.5–5 kg) species. Lives mostly on trees, and feeds on small vertebrates, insects and fruit. It rests usually in tree hollows. VIRUSES: *Coronavirus* SARS (competent host).

Family *Hyaenidae*

Striped Hyena (*Hyaena hyaena*)

African and south-Asian species (size 90–120 cm) living in the savannah ecosystem and around human habitation. Feeds on carrion, refuse and small vertebrates. VIRUSES: *Lyssavirus* s.s. (also human cases). BACTERIA: *Bacillus anthracis*. PROTOZOA: *Trypanosoma brucei rhodesiense.*

Family *Felidae*

Lynx (*Lynx lynx*)

Fragmented distribution in Eurasia and North America in forest habitats (and scrub). Hunts birds and mammals (including cat, fox, up to the size of roe deer). VIRUSES: *Lyssavirus* s.s. PROTOZOA: *Toxoplasma gondii.*

Bobcat (*Lynx rufus*)

North American lynx living in chaparral, swamp and forest habitats. Feeds on small mammals and birds. Home range usually 3 km, but can move up to 50 km. VIRUSES: *Lyssavirus* s.s. PROTOZOA: *Toxoplasma gondii.*

Domestic Cat (*Felis catus*), **Wild Cat** (*Felis silvestris*: Photo 7.29)

Wild cat lives in fragmented woodland areas in Europe, and feeds on small mammals, birds and other small animals. VIRUSES: *Lyssavirus* s.s. (also human cases), *Henipavirus* Nipah, *Herpesvirus suis* 1. BACTERIA: *Rickettsia typhi*,

R. felis, *Bartonella henselae* (reservoir), *Chlamydophila felis*, *Staphylococcus aureus*, *Campylobacter jejuni*, *Helicobacter felis*, *Leptospira interrogans, Yersinia pestis*, *Y. pseudotuberculosis* (reservoir), *Y. enterocolitica*, *Francisella tularensis*, *Pasteurella multocida*, *Streptobacillus moniliformis*, *Burkholderia mallei*, *Mycobacterium bovis*. FUNGI: *Microsporum canis*, *Sporothrix schenckii*. PROTOZOA: *Trypanosoma cruzi*, *Giardia lamblia*, *Toxoplasma gondii* (reservoir, final host), *Cryptosporidium felis*. MICROSPORIDIA: *Enterocytozoon bieneusi*.

Mountain Lion (Cougar) (*Felis concolor*)

A large, tawny to grayish cat, living in rugged mountains, forests and swamps of western North America and in Central America. Feeds on deer, hares, rodents, and domestic animals. BACTERIA: *Yersinia pestis*.

Lion (*Panthera leo*)

African steppe habitats (savannah). BACTERIA: *Bacillus anthracis*. PROTOZOA: *Trypanosoma brucei rhodesiense*.

*7.1.6 Order Sloths and Armadillos (*Xenarthra*)*

Family *Bradypodidae*

Pale-throated (Three-toed) Sloth (*Bradypus tridactylus*: Photo 7.30)

Large mammal (50–60 cm; weight about 4 kg) living in the South-American tropical forest ecosystem. Specialized feeding on leaves and fruit of the tree *Cecropia lyratifolia*. VIRUSES: *Bunyavirus* Oropouche. BACTERIA: *Borrelia hermsii*.

Family *Dasypodidae*

Armadillo (*Dasypus novemcinctus*)

Medium-sized (40 cm plus tail 40 cm, weight 4–8 kg) American (including southern US states) mammal species occurring in bushland, woods and on cliffs. Largely insectivorous, less frequently feeds on fruit and avian eggs. The body is "armoured" – covered with a protective horny substance. Den is situated in deep burrows. BACTERIA: *Borrelia hermsii*. FUNGI: *Paracoccidioides brasiliensis*. PROTOZOA: *Trypanosoma cruzi*.

7.1.7 Order Elephants (**Proboscidea**)

Family *Elephantidae*

African (Savannah) Elephant (*Loxodonta africana*: Photo 7.31)

The largest terrestrial mammal (up to 7.5 m long, weight up to 6,000 kg). Distributed in sub-Saharan Africa in savannah with bushes and in woodland. Herbivorous (leaves, grasses, fruit). PROTOZOA: *Trypanosoma brucei rhodesiense*.

7.1.8 Order **Hyracoidea**

Family *Procaviidae*

Rock Hyrax (*Procavia capensis*: Photo 7.32)

Medium-sized mammal (30–60 cm long; weight up to 4 kg), lives on rocks in southern Africa and Angola. Herbivorous and social (up to 100 individuals in a colony) species. PROTOZOA: *Leishmania tropica*.

7.1.9 Order Rodents **(Rodentia)**

Rodents are, together with domestic mammals (namely ruminants and carnivores), epidemiologically the most important group of vertebrates as a source of human zoonotic infections (Davis et al. 1970, Kucheruk 1979, 1989, Blood et al. 2007, Hubálek and Halouzka 1996, etc.).

Family *Sciuridae*

Woodchuck (*Marmota monax*)

The "groundhog" is an approximately 50 cm long marmot living in North-American open woodland and rocky ravines. It hibernates, and builds extensive ground burrows. Feeds on plants. VIRUSES: *Flavivirus* Powassan, *Orthobunyavirus* SSH. BACTERIA: *Rickettsia rickettsii*, *Leptospira* spp., *Listeria monocytogenes*, *Erysipelothrix rhusiopathiae*, *Yersinia pestis* (reservoir), *Y. pseudotuberculosis*, *Pasteurella multocida*.

Tarbagan Marmot (*Marmota sibirica*), **Himalayan Marmot** (*M. himalayana*), **Long-tailed Marmot** (*M. caudata*), **Bobak Marmot** (*M. bobak*)

Big stocky herbivorous rodents living in steppe and grassland often at higher elevations (up to alpine) in eastern Europe (*M. bobak*) and central Asia. They form colonies and build extensive burrow systems where they also hibernate (Photo 5.41). Hunted for their fur. BACTERIA: *Rickettsia sibirica* (*M. sibirica*)*, Listeria monocytogenes*, *Erysipelothrix rhusiopathiae*, *Bacillus anthracis* (*M. caudata*)*, Leptospira* spp.*, Yersinia pestis* (all 4 spp. are a significant reservoir in Asia, and the source of human infection with plague in Mongolia and China), *Y. pseudotuberculosis*, *Pasteurella multocida* (an epizootic in Mongolia)*, Francisella tularensis*. PROTOZOA: *Toxoplasma gondii*.

Blacktail Prairie Dog (*Cynomys ludovicianus*: Photo 7.33)

Comparatively large (25–40 cm long plus tail 7–12 cm; weight about 1 kg) rodent, living in steppe habitat (dry upland prairies) of central and southern areas of USA, and forming extensive colonies ("towns") with deep burrows. Related to marmots in its bionomics. Eats plants (grasses), and occasionally insects (e.g. locusts). VIRUSES: *Orthopoxvirus simiae* (lesions) – via import of African rodents to USA in 2003. BACTERIA: *Yersinia pestis* (reservoir), *Francisella tularensis*

(interestingly, several infected captive prairie dogs from Texas imported *Francisella t. tularensis*, i.e. the highly virulent type A, as pets into the Czech Republic in 2002, but fortunately the spread of infection was controlled).

California Ground Squirrel (*Spermophilus* [=*Citellus*] *beecheyi*)

About 25 cm long rodent (with a bushy tail about 20 cm long) living in western North-American pastures, cornfields and rocky ridges. Builds long burrows, and eats plants, seeds, acorns, fruit, mushrooms, insects, and even small birds and eggs. BACTERIA: *Yersinia pestis, Francisella tularensis.*

Golden-mantled Grand Squirrel (*Spermophilus lateralis*), **Richardson Ground Squirrel** (*S. richardsoni*: Photo 7.34), **Columbian Ground Squirrel** (*S. columbianus*), and **other American ground squirrels**

North-American species of grassland steppe ecosystem, herbivorous. VIRUSES: *Alphavirus* WEE, *Flavivirus* Powassan, *Orthobunyavirus* LaCrosse, SSH and other viruses of California group (*S. lateralis*), *Coltivirus* CTF (*S. lateralis*, *S. columbianus* – reservoir hosts). BACTERIA: *Rickettsia rickettsii*, *Yersinia pestis* (competent hosts and reservoirs, as well as many other ground squirrel spp. in North America, e.g. *S. columbianus*, *S. beldingi*, *S. armatus*, *S. washingtoni*, *S. townsendi*, *S. brunneus*, and *S. variegatus*), *Francisella tularensis* (*S. richardsoni*, *S. townsendii*, *S. columbianus*, *S. armatus*), *Pasteurella multocida.*

European Ground Squirrel (Souslik) (*Spermophilus citellus*: Photo 7.35)

Xerotherm habitats (steppes, meadows, pastures, scrub) in Eurasia. Lives in colonies in simple burrows where it also hibernates. Herbivorous (seeds of grasses, grain, green herbage), occasionally feeds on insects. BACTERIA: *Coxiella burnetii*, *Listeria monocytogenes*, *Leptospira pomona*, *Yersinia enterocolitica*, *Y. pestis*, *Pasteurella multocida*, *Francisella tularensis*. PROTOZOA: *Leishmania infanti.*

Long-tailed Ground Squirrel (*Spermophilus undulatus*), **Little G. S.** (*S. pygmaeus*), **Caucasian Mountain G. S.** (*S. musicus*), **Daurian G. S.** (*S. dauricus*)

East-European and Asian species of steppe habitats, herbivorous. VIRUSES: orthobunyaviruses of California group (*S. undulatus*). BACTERIA: *Rickettsia sibirica* (*S. undulatus*), *Coxiella burnetii*, *Listeria monocytogenes* (*S. undulatus*, *S. dauricus*), *Erysipelothrix rhusiopathiae*, *Bacillus anthracis* (*S. undulatus*), *Leptospira grippotyphosa*, *L. icterohaemorrhagiae* (*S. undulatus*), *Yersinia pestis* (reservoir: *S. pygmaeus* around Caspian Sea; *S. musicus,* the Caucasus; *S. undulatus,* Mongolia; *S. dauricus*, eastern Asia), *Y. pseudotuberculosis* (*S. pygmaeus*), *Y. enterocolitica* (*S. undulatus*), *Francisella tularensis*, *Brucella abortus* (*S. pygmaeus, S. undulatus*), *Pasteurella multocida.* PROTOZOA: *Toxoplasma gondii* (*S. pygmaeus*).

Yellow Ground Squirrel (*Spermophilus fulvus*), **Russet G. S.** (*S. major*), **Red-cheeked G. S.** (*S. erythrogenys*), **Tien Shan G. S.** (*S. relictus*)

East- and Central-Asian species of steppe habitats, herbivorous. BACTERIA: *Rickettsia sibirica* (*S. erythrogenys*), *Coxiella burnetii* (*S. relictus*), *Bacillus anthracis* (*S. fulvus*)*, Yersinia pestis* (*S. fulvus, S. major*), *Y. pseudotuberculosis* (*S. fulvus*), *Francisella tularensis* (*S. major*, *S. erythrogenys*). PROTOZOA: *Toxoplasma gondii* (*S. fulvus*, *S. erythrogenys*).

Whitetail Antelope Squirrel (*Ammospermophilus leucurus*)

A smaller (*c*. 15 cm long) semidesert rodent similar to both squirrel and ground squirrel, living in southwestern parts of North America. Feeds on seeds, insects and even meat. BACTERIA: *Yersinia pestis* (New Mexico).

Red (Spruce) Squirrel (*Tamiasciurus hudsonicus*: Photo 7.36)

Coniferous and mixed forests of North America, also swamps. Feeds on seeds, nuts, eggs, and fungi (the latter sometime stored). Nests in tree hollows or outside in tree branches. VIRUSES: *Alphavirus* WEE, *Flavivirus* Powassan, *Bunyavirus* LaCrosse and other viruses of the California group, *Coltivirus* CTF. BACTERIA: *Leptospira grippotyphosa*.

Long-clawed Ground Squirrel (*Spermophilopsis leptodactylus*)

Lives in deserts of central Asia. Feeds on grasses and subterrestrial parts of plants. Often contacts with *Rhombomys opimus*. BACTERIA: *Rickettsia sibirica*, *Coxiella burnetii*, *Borrelia persica*, *Yersinia pestis.* PROTOZOA: *Leishmania major*, *Toxoplasma gondii.*

Striped Ground Squirrel (*Xerus erythropus*)

A big (body size 30 cm plus long tail up to 28 cm; weight 350–650 g) squirrel, living in bushland and woodland of sub-Saharan Africa, builds underground burrows. VIRUSES: *Bunyavirus* Bhanja.

Congo Rope Squirrel, Thomas's Rope Squirrel, Red-legged Sun Squirrel (*Funisciurus congicus*, *F. anerythrus*, *Heliosciurus rufobrachium*)

African tropical forest herbivorous inhabitants. Occasionally feed on invertebrates. VIRUSES: *Orthopoxvirus simiae* (reservoir).

American chipmunks (*Tamias striatus*, *T. minimus* – Photo 7.37, *T. ochrogenys*, *Tamias* spp.)

North-American species building ground burrows, herbivorous and insectivorous (occasionally meat, eggs). *T. striatus* occurs in deciduous forests while the species of the genus *Eutamias* prefer coniferous forests and woods. VIRUSES: bunyaviruses of California group (LaCrosse etc.), *Coltivirus* CTF (*T. minimus*). BACTERIA: *Rickettsia rickettsii*, *Anaplasma phagocytophilum* (competent host), *Yersinia pestis* (USA – during epizootics), *Francisella tularensis*, *Pasteurella multocida.* PROTOZOA: *Toxoplasma gondii*.

Siberian Chipmunk (*Tamias sibiricus*: Photo 7.38)

A small (about 15 cm, tail of the same length) North-Asian ground squirrel species (also called "burunduk"), building burrows. Occurs in coniferous and mixed forests. Herbivorous (seeds of trees and herbs), occasionally insectivorous. VIRUSES: *Flavivirus* TBE. BACTERIA: *Rickettsia sibirica*, *Listeria monocytogenes*, *Erysipelothrix rhusiopathiae*, *Yersinia pseudotuberculosis*, *Francisella tularensis.* PROTOZOA: *Toxoplasma gondii.*

(European) Red Squirrel (*Sciurus vulgaris*: Photo 7.39)

Widespread in Eurasian forests, woods and parks. Nests on tree branches or in tree hollows. Feeds on seeds (coniferous cones, nuts and fruit), the bast of trees, mushrooms, insects, bird eggs and nestlings. This mammal is usually heavily infested with ectoparasites (fleas etc.). VIRUSES: *Flavivirus* TBE, *Orthobunyavirus* Ťahyňa, *Hantavirus* Puumala, *Lyssavirus* s.s. BACTERIA: *Listeria monocytogenes*, *Erysipelothrix rhusiopathiae*, *Leptospira hebdomadis*, *Borrelia burgdorferi* s.l. (competent host), *Pasteurella multocida*, *Francisella tularensis*. FUNGI: *Trichophyton mentagrophytes* (skin lesions). PROTOZOA: *Toxoplasma gondii.*

Western Gray Squirrel (*Sciurus griseus*)

Distributed in the westernmost part of North America, in semi-open oak and pine-oak habitats. Feeds largely on acorns and seeds of conifers. VIRUSES: *Lyssavirus* s.s. (1 case, California). BACTERIA: *Borrelia burgdorferi* s.s. (competent host with a persistent infection up to 14 months).

Eastern Gray Squirrel (*Sciurus carolinensis*: Photo 7.40)

Widespread in hardwood forests in the whole eastern part of North America. Feeds mainly on nuts and other seeds, also fruit, mushrooms, phloem under bark of trees. Nests in tree holes or builds a leaf nests in branches high above ground. VIRUSES: *Alphavirus* WEE, *Flavivirus* Powassan, *Orthobunyavirus* LaCrosse and other viruses of California group, *Coltivirus* CTF. BACTERIA: *Leptospira grippotyphosa, Clostridium tetani.*

Eastern Fox Squirrel (*Sciurus niger*)

Open broad-leaved and pine woods in the whole eastern part of North America. Spends much time on the ground. Feeds mainly on nuts and other seeds, also bird eggs, mushrooms, phloem under bark of trees. Nests in tree holes or builds a twig and leaf nest in branches high above ground. VIRUSES: *Orthobunyavirus* LaCrosse and other viruses of the California group, *Coltivirus* CTF. BACTERIA: *Leptospira grippotyphosa*, *Francisella tularensis, Borrelia burgdorferi* s.s.

Southern Flying Squirrel (*Glaucomys* [*Pteromys*] *volans*)

North-Eurasian species of flying nocturnal squirrel. Lives in mixed or deciduous forests, gregarious in winter. Feeds on seeds, nuts, insects, and avian eggs. Nests in tree holes or on tree branches. BACTERIA: *Rickettsia prowazekii*, *Yersinia pseudotuberculosis.*

Family *Castoridae*

European Beaver (*Castor fiber*), **Canadian Beaver** (*C. canadensis*: Photo 7.41)

A very big European and North-American rodent (*c.* 70 cm long, plus tail 25 cm, weight up to 30 kg). Bound to water ecosystem (streams, lakes and swamps with trees on banks). Feeds on aquatic vegetation, tree bark and small twigs. Builds a lodge in water or burrows into banks along streams. BACTERIA: *Salmonella typhimurium*, *Francisella tularensis* (*C. canadensis* – epizootics, but not in *C. fiber*). PROTOZOA: *Toxoplasma gondii*, *Giardia lamblia.*

Family *Heteromyidae*

Trinidad Spring Pocket Mouse (*Heteromys anomalus*)

South-American and Caribbean species. Feeds on seeds, other plant parts, and also arthropods. VIRUSES: *Alphavirus* VEE (Mucambo). PROTOZOA: *Leishmania mexicana.*

Great Basin Pocket Mouse (*Perognathus parvus*)

North-American species (size about 7 cm plus tail 8–10 cm), living solitarily in sagebrush, chaparral and pine stands. Herbivorous (mainly seeds). BACTERIA: *Francisella tularensis.*

Family *Pedetidae*

Springhare (*Pedetes capensis*)

Central- and South-African species with long legs living in deserts and semideserts, herbivorous. Hunted for the skin and meat. BACTERIA: *Yersinia pestis* (human cases at contact).

Family *Dipodidae*

Great Jerboa (*Jaculus jaculus*: Photo 7.42)

Small, jumping rodent living in deserts and semideserts in Egypt and Asia. Feeds on roots, seeds, leaves of succulent plants. BACTERIA: *Coxiella burnetii*, *Yersinia pestis*, *Y. pseudotuberculosis, Francisella tularensis.*

Severtzov's Jerboa, Five-toed Jerboa, Mongolian Five-toed Jerboa (*Allactaga severtzovi, A. elater, A. saltator* [=*sibirica*])

Small, jumping Asian rodents living in deserts and semideserts. Feed on roots, seeds, leaves of succulent plants. BACTERIA: *Erysipelothrix rhusiopathiae* (*A. saltator*), *Salmonella enteritidis* (*A. saltator*), *Borrelia caucasica* (*A. elater*), *Yersinia pestis* (*A. elater*, *A. saltator*), *Y. pseudotuberculosis.* PROTOZOA: *Leishmania major* (*A. severtzovi*).

Northern Three-toed Jerboa (*Dipus sagitta*)

Small, jumping Asian rodent living in deserts and semideserts. Herbivorous. BACTERIA: *Erysipelothrix rhusiopathiae*, *Salmonella typhimurium*, *Yersinia pestis.*

Small Jerboa (*Pygerethmus platyurus*) **and other jerboas** (*Alactagulus acontion, Scirtopoda telum, Paradipus ctenodactylus, Eremodipus lichtensteini*)

Small and jumping Asian rodents living in deserts and semideserts. Herbivorous.

BACTERIA: *Yersinia pestis, Y. pseudotuberculosis* (*P. platyurus*).

Northern Birch Mouse (*Sicista betulina*)

Northern Eurasian species widely, but sporadically distributed. In central Europe a glacial relict at higher mountain levels in humid coniferous forests. Nest on the ground or in tree hollows close to the ground, hibernates underground. Omnivorous (seeds, berries, insects). VIRUSES: *Flavivirus* TBE. BACTERIA: *Erysipelothrix rhusiopathiae*, *Leptospira hebdomadis*, *Francisella tularensis*.

Family *Cricetidae*

Common Hamster (*Cricetus cricetus*: Photo 7.43)

European species living in dry habitats of steppe character and in farmland in colonies and building deep burrows with stored food, where it also hibernates. Largely herbivorous, feeding on all agricultural crops, grain, seeds, roots, and occasionally on invertebrates. Irregular mass overpopulation events (e.g., east Slovakia 1971/1972). VIRUSES: *Alphavirus* Sindbis, *Flavivirus* OHF, *Lyssavirus* s.s. BACTERIA: *Rickettsia sibirica*, *Coxiella burnetii*, *Listeria monocytogenes*, *Erysipelothrix rhusiopathiae*, *Leptospira grippotyphosa*, *L. pomona*, *L. sejroe*, *L. icterohaemorrhagiae*, *Yersinia pestis* (one isolation in Kazakhstan)*, Francisella tularensis* (an important host in steppe habitats; 160 infected persons during the 1971/1972 east-Slovakian overpopulation of the hamster; a number of human infections at hunting hamster for the skin in eastern Europe). FUNGI: *Microsporum persicolor*.

Golden Hamster (*Mesocricetus auratus*: Photo 7.44)

Origin in the Near East. Feeds on grasses and grain. Often in captivity as pet or laboratory animal. VIRUSES: *Arenavirus* LCM (reservoir).

Ciscaucasian Hamster (*Mesocricetus raddei*)

Occurs in Ciscaucasian steppes, hibernates. Feeds on grasses, seeds, and roots. BACTERIA: *Francisella tularensis* (very susceptible, outbreaks).

Brandt's Hamster (*Mesocricetus brandti*)

Asia Minor, Transcaucasia. BACTERIA: *Francisella tularensis, Erysipelothrix rhusiopathiae*.

Greater Long-tailed (Rat-like) Hamster (*Cricetulus* [*Tscherskia*] *triton*), **Striped Dwarf Hamster** (*Cricetulus barabensis*: Photo 7.45), **Gray Hamster** (*C. migratorius*)

Small, mainly East-Asian (but *C. migratorius* also lives in the Balcans and Asia Minor) hamsters living mainly in grass steppe and brush habitats. They feed on seeds, and build burrow systems, where they store seeds for winter, and hibernate. BACTERIA: *Rickettsia sibirica*, *Orientia tsutsugamushi* (*C. triton*), *Coxiella*

burnetii (*C. migratorius*), *Listeria monocytogenes, Erysipelothrix rhusiopathiae* (*C. barabensis*), *Leptospira* spp., *Borrelia persica* (*C. migratorius*)*, Yersinia pestis* (*C. barabensis*: China, *C. migratorius*: the Urals, Transcaucasia), *Y. pseudotuberculosis* (*C. triton*), *Pasteurella multocida* (*C. triton, C. barabensis*), *Francisella tularensis* (*C. migratorius*).

Dzhungarian Hamster (*Phodopus sungorus*)

Distributed in steppes of Central Asia. Feeds on seeds, less on insects. Also in captivity as pet. BACTERIA: *Rickettsia sibirica, Erysipelothrix rhusiopathiae, Yersinia pestis, Francisella tularensis.*

White-footed Mouse (*Peromyscus leucopus*: Photo 7.46)

One of the most common rodent species in North America (in eastern and central parts). Body size about 10 cm (plus the tail *c.* 10 cm). It lives in forest ecosystem, but also in bushland and open land, and in the human environment (gardens etc.). In winter it invades human dwellings. It feeds mainly on fruit, seeds and nuts, but also on insects. It nests in diverse places (old avian or squirrel nests, buildings, etc.). VIRUSES: *Alphavirus* EEE, VEE. BACTERIA: *Anaplasma phagocytophilum, Borrelia burgdorferi* (reservoir). FUNGI: *Microsporum persicolor*. PROTOZOA: *Babesia microti.*

Deer Mouse (*Peromyscus maniculatus*)

Widely distributed over the whole of North America in moist, cool, coniferous and other forests, but also grassland. It nests in ground burrows or in trees and even buildings. It feeds on fruit, seeds, nuts, acorns, and occasionally insects. VIRUSES: *Alphavirus* VEE, *Flavivirus* Powassan, *Hantavirus* Sin Nombre (reservoir), *Coltivirus* CTF. BACTERIA: *Yersinia pestis* (California). FUNGI: *Microsporum persicolor*. PROTOZOA: *Cryptosporidium parvum, Babesia microti.*

Piñon Mouse (*Peromyscus truei*)

It lives in western and central North-American rocky habitats with scattered pines and junipers. Feeds on seeds and nuts. VIRUSES: *Hantavirus* Sin Nombre.

Long-tailed Pygmy Rice Rat (*Oligoryzomys longicaudatus*)

South-American species, called *raton colilarge* (in Spanish). VIRUSES: *Hantavirus* Andes (reservoir), *Arenavirus* Whitewater Arroyo.

Fulvous Pygmy Rice Rat (*Oligoryzomys fulvescens*)

Central-American species. VIRUSES: *Hantavirus* Choclo (reservoir).

Yellow Pygmy Rice Rat (*Oligoryzomys flavescens*)

South-American species (Argentina). VIRUSES: *Hantavirus* Lechiguana (reservoir).

Azara's grass mouse (*Akodon azarae*)

South-American species (Argentina). VIRUSES: *Hantavirus* Lechiguana (reservoir).

Eastern Woodrat, Bushytail Woodrat, Dusky-footed Woodrat, White-throated Woodrat, Southern Plains Woodrat (*Neotoma floridana*, *N. cinerea, N. fuscipes, N. albigula, N. micropus*, respectively)

Big North-American mouse-like species (about 20–22 cm, plus tail 15–20 cm). *N. floridana* lives in diverse habitats: rocky cliffs, swamps, gardens, or in semidesert. Builds nest houses from various material, and feeds on seeds, fruit and vegetables. *N. cinerea* lives in high mountains of western North America on cliffs and pine stands where it usually does not build nest houses and feeds on green vegetation. VIRUSES: *Coltivirus* CTF (*N. cinerea*), *Arenavirus* Whitewater Arroyo (*N. albigula*). BACTERIA: *Anaplasma phagocytophilum* (*N. fuscipes*). PROTOZOA: *Trypanosoma cruzi* (*N. micropus*).

Hispid Cotton Rat (*Sigmodon hispidus*)

A small rat (13–20 cm, tail 11–20 cm), lives in southern USA and in Mexico on humid meadows, nests on surface or in burrow, and feeds on green vegetation and eggs of birds. VIRUSES: *Hantavirus* Black Creek Canal (reservoir). PROTOZOA: *Trypanosoma cruzi*.

Rice rat (*Oryzomys palustris*) **and related rats** (*O. laticeps*, *O. capito*)

American semi-aquatic and nocturnal species, *O. palustris* occurs in USA (southwestern), 12–13 cm plus tail 11–18 cm. They live in humid habitats, sedges or marshes, and feed on green vegetation and seeds. They nest under vegetation litter close to the upper level of the water. VIRUSES: alphaviruses EEE a VEE (Mucambo), *Hantavirus* Bayou (*O. palustris,* reservoir).

Short-tailed Cane Mouse (*Zygodontomys brevicauda*)

This nocturnal, terrestrial species is abundant in grassland, clearings, marshy areas, second growth, and agricultural areas of South America. Its diet includes seeds, fruit, and green plant material. It makes short burrows in banks or under tree roots, leading to nests made of grasses and plants. VIRUSES: *Hantavirus* Calabazo, *Arenavirus* Guanarito.

Family *Microtidae*

Norway Lemming (*Lemmus lemmus*), **Black-footed Lemming** (*L. sibiricus*), **Brown Lemming** (*L. trimucronatus*)

Scandinavian, north-Siberian and Canadian species, respectively, of higher mountain levels (tundra and sub-alpine meadows). Nests either in burrows or aboveground. They feed on vegetation. Overpopulation every 3–4 years, followed by emigration southwards. VIRUSES: *Hantavirus* Topografov (*L. sibiricus* reservoir). BACTERIA: *Listeria monocytogenes* (in *L. trimucronatus*), *Leptospira grippotyphosa* (*L. sibiricus*), *Francisella tularensis* (outbreaks in *L. sibiricus, L. lemmus* – waterborne cases of human tularaemia have been described from this source).

Bering's (Tundra) Collared Lemming (*Dicrostonyx rubricatus*), **Greenland Collared Lemming** (*D. groenlandicus*), **Collared (Arctic) Lemming** (*D. torquatus*)

North-Palaearctic species – *D. rubricatus* and *D. groenlandicus* are Nearctic. VIRUSES: *Bunyavirus* SSH (*D. rubricatus*). BACTERIA: *Listeria monocytogens* (disease: *D. groenlandicus*), *Francisella tularensis* (disease: *D. torquatus*, *D. groenlandicus*).

Steppe Lemming (*Lagurus lagurus*)

Central-Asian and east-European species living in steppe habitats. Body size about 10 cm (tail absent), maximum weight 40 g. Herbivorous. BACTERIA: *Rickettsia sibirica, Coxiella burnetii, Yersinia pestis, Francisella tularensis* (epizootics in south Russia).

Sagebrush Vole (*Lagurus curtatus*)

Eastern states of USA. Size about 11 cm, tail only 2–3 cm + weight 20–40 g. Habitat is arid scattered sagebrush. Feeds on green vegetation. BACTERIA: *Francisella tularensis.*

Indian Gerbil (*Tatera indica*)

South-Asian species of steppe habitats. BACTERIA: *Orientia tsutsugamushi, Yersinia pestis.* FUNGI: *Trichophyton simii.*

Great Gerbil (*Rhombomys opimus*: Photo 7.47)

Asian species of big mouse with bushy tail, associated with desert, semidesert and steppe habitats. It usually builds systems of burrows (Photo 5.44), and feeds on roots, grasses and invertebrates. BACTERIA: *Coxiella burnetii, Yersinia pestis* (principal host in Central Asia, reservoir), *Borrelia duttonii, B. persica, Pasteurella multocida, Borrelia latyshevi, Listeria monocytogenes, Erysipelothrix rhusiopathiae, Bacillus anthracis.* PROTOZOA: *Leishmania tropica* and *L. major* (reservoir), *Toxoplasma gondii.*

Fat Sand Rat (*Psammomys obesus*: Photo 7.48)

African species of big mouse with bushy tail, living in desert and semidesert habitats. Feeds on roots, grasses and invertebrates. BACTERIA: *Coxiella burnetii, Yersinia pestis* (reservoir), *Borrelia duttonii, B. persica, Listeria monocytogenes, Erysipelothrix rhusiopathiae.* PROTOZOA: *Leishmania tropica* and *L. major* (reservoir).

Libyan Jird, Persian J., Shaw's J. (*Meriones libycus, M. persicus, M. shawi*)

African and Asian species of big mice with bushy tail, associated with desert, semidesert and steppe habitats. They usually build systems of burrows, and feed on roots, grasses and invertebrates. VIRUSES: *Arenavirus* LCM (*M. shawi*). BACTERIA: *Coxiella burnetii, Yersinia pestis* (reservoir), *Borrelia duttonii,*

B. persica, Francisella tularensis (*M. libycus*)*, Listeria monocytogenes, Erysipelothrix rhusiopathiae, Bacillus anthracis* (*M. libycus*). PROTOZOA: *Leishmania major* (reservoir).

Mid-day Jird, Tamarisk J., Vinogradov's J., Mongolian J. (*Meriones meridianus, M. tamariscinus, M. vinogradovi, M. unguiculatus*)

Asian species of mice with bushy tail, associated with desert, semidesert and steppe habitats. They feed on roots, grasses and invertebrates. BACTERIA: *Orientia tsutsugamushi* (*M.t.*)*, Coxiella burnetii* (*M. meridianus*)*, Yersinia pestis* (reservoir – all spp.)*, Leptospira* spp. (*M. tamariscinus*)*, Borrelia persica* (*M. meridianus*)*, Francisella tularensis* (*M. meridianus, M. tamariscinus*)*, Listeria monocytogenes* (*M. meridianus*)*, Erysipelothrix rhusiopathiae* (*M. meridianus, M. vinogradovi, M. unguiculatus*). PROTOZOA: *Leishmania major* (*M. meridianus, M. tamariscinus*).

Silver Mountain Vole, Flat-headed Vole (*Alticola argentatus*, *A. strelzowi*)

Central-Asian (Kazakhstan and Mongolia) and North-Asian species of high rocky mountains. Herbivorous; they store hay between stones. BACTERIA: *Yersinia pestis* (Mongolia)*, Y. pseudotuberculosis* (*A. strelzowi*).

Bank Vole (*Myodes* [*Clethrionomys*] *glareolus*: Photo 7.49)

A widespread Eurasian species living in deciduous and mixed forests, woods, coppices and parks. Builds a system of shallow runs and burrows in the soil. Mainly herbivorous (seeds, nuts, fruit, roots and bast), but occasionally feeds on invertebrates (mainly insects). Active also in winter. Cyclic overpopulations every 3–5 years. VIRUSES: flaviviruses TBE and LI, *Hantavirus* Puumala (principal reservoir), orbiviruses Tribeč and Kemerovo, *Arenavirus* LCM, *Parechovirus* Ljungan*, Orthopoxvirus bovis.* BACTERIA: *Coxiella burnetii, Anaplasma phagocytophilum* s.l., *Listeria monocytogenes*, *Erysipelothrix rhusiopathiae*, *Borrelia afzelii*, *B. garinii*, *Leptospira australis*, *L. grippotyphosa*, *L. hebdomadis*, *L. jalna*, *L. pomona*, *Campylobacter jejuni*, *C. coli*, *Salmonella typhimurium*, *S. enteritidis*, *Yersinia pseudotuberculosis*, *Y. enterocolitica*, *Pasteurella multocida*, *Francisella tularensis*, *Mycobacterium microti*. FUNGI: *Microsporum persicolor* (skin lesions), *Trichophyton mentagrophytes* (skin lesions), *Pneumocystis jirovecii*. PROTOZOA: *Babesia microti* (splenomegaly), *Toxoplasma gondii*, *Cryptosporidium parvum*. MICROSPORIDIA: *Encephalitozoon cuniculi*.

Red-backed (Ruddy) Vole (*Myodes* [*Clethrionomys*] *rutilus*), **Gray-sided Vole** (*M. rufocanus*)

Asian species living in forest habitats, birch woodland, tundra biome; *M.rut.* occurs also in Alaska and northernmost Europe. They feed on seeds, berries and other plant components, and nest in burrows. VIRUSES: *Flavivirus* TBE (RSSE), *Hantavirus* Puumala. BACTERIA: *Rickettsia sibirica* (*M. rufocanus*), *Orientia tsutsugamushi* (*M. rufocanus* – possible reservoir in the Primorye region, Siberian Russia)*, Coxiella burnetii*, *Listeria monocytogenes*, *Erysipelothrix rhusiopathiae*, *Leptospira*

grippotyphosa, *L. javanica*, *Salmonella typhimurium* (*M. rutilus*)*, Yersinia pseudo-tuberculosis* (*M. rufocanus*), *Francisella tularensis*. FUNGI: *Microsporum persi-color*. PROTOZOA: *Toxoplasma gondii*.

Common Vole (*Microtus arvalis*: Photo 7.50)

An abundant Eurasian species; cultivated steppe is the typical habitat (mead-ows and arable fields). Lives in colonies, builds extensive burrows and runs, active also in winter. Herbivorous (mainly green parts of plants, in winter also roots, etc.). Overpopulation usually every 3–4 years (up to 1,500 ind./ha), and the stressed animals are often affected with fatal infectious and non-infectious diseases and, at the same time, serve as an easy source of food for predators like foxes, raptors and owls. VIRUSES: *Flavivirus* TBE, hantaviruses Tula (reser-voir) and rarely Puumala, *Arenavirus* LCM, *Parechovirus* Ljungan. BACTERIA: *Rickettsia slovaca*, *Coxiella burnetii*, *Borrelia afzelii, Listeria monocytogenes*, *Erysipelothrix rhusiopathiae*, *Bacillus anthracis, Borrelia burgdorferi* s.l. (com-petent host), *Leptospira grippotyphosa* (reservoir), *L. bataviae*, *L. hebdomadis*, *L. pomona*, *Campylobacter jejuni*, *C. coli*, *Salmonella enteritidis*, *S. paraty-phi* B, *Yersinia pseudotuberculosis*, *Y. enterocolitica*, *Y. pestis* (mountainous Transcaucasia), *Brucella suis* biotype 2, *B. microti* (systemic disease), *Pasteurella multocida* (epizootics), *Francisella tularensis* (often human aerogenic infections during tularaemia epizootics of voles in agroecosystems), *Mycobacterium microti*. FUNGI: *Microsporum persicolor* (skin lesions), *Trichophyton mentagrophytes*, *T. erinacei, Pneumocystis jirovecii*. PROTOZOA: *Babesia microti* (splenomegaly), *Giardia lamblia*, *Toxoplasma gondii*, *Cryptosporidium parvum, Encephalitozoon cuniculi*.

Field (Short-tailed) Vole (*Microtus agrestis*: Photo 7.51)

Eurasian, very widespread and abundant species. Lives in grassland (usually humid), moorland, also arable fields in colonies, builds extensive runs and bur-rows, and is active also in winter. Feeds on grasses and other vegetation (mainly green parts, but in winter also roots, bast etc.). Populations are cyclic, and dur-ing the overpopulation peak the voles serve as rich source of food for foxes and birds of prey. VIRUSES: *Flavivirus* TBE, *Hantavirus* Tula (and rarely Puumala), *Orthopoxvirus bovis*. BACTERIA: *Listeria monocytogenes*, *Borrelia burgdorferi* s.l. (competent host), *Leptospira grippotyphosa* (reservoir), *Yersinia enterocolitica*, *Francisella tularensis*, *Mycobacterium microti*. FUNGI: *Microsporum persicolor*, *Trichophyton mentagrophytes*, *T. erinacei, Pneumocystis jirovecii*. PROTOZOA: *Babesia microti* (splenomegaly), *Toxoplasma gondii*, *Cryptosporidium parvum*.

Root (Tundra) Vole (*Microtus oeconomus*)

Distributed in north-eastern Europe, northern Asia and Alaska. There are also some places in central Europe where smaller populations thrive as a glacial relict (Neusiedler See in Austria and Hungary, and south Slovakia). The normal habitat is humid tundra, or wetlands in central Europe. Feeds on green vegetation, roots, and nests usually under plant litter or in shallow burrows. VIRUSES: *Flavivirus*

OHF. BACTERIA: *Rickettsia sibirica*, *Coxiella burnetii*, *Listeria monocytogenes*, *Erysipelothrix rhusiopathiae*, *Leptospira grippotyphosa* (reservoir), *L. javanica*, *L. pomona*, *L. hebdomadis*, *Francisella tularensis.* FUNGI: *Microsporum persicolor.*

Maximovicz's Vole (*Microtus maximowiczii*)

Occurs in open taiga ecosystem of the Russian Far East. Herbivorous. VIRUSES: *Flavivirus* TBE (RSSE), *Hantavirus* sp. BACTERIA: *Rickettsia sibirica*, *Orientia tsutsugamushi, Listeria monocytogenes*, *Erysipelothrix rhusiopathiae*, *Leptospira grippotyphosa* (reservoir)*, L. hebdomadis*, *Yersinia pseudotuberculosis, Francisella tularensis.*

Middendorf's Vole, North Siberian V., Sakhalin V. (*Microtus middendorffi, M. hyperboreus, M. sachalinensis*)

These herbivorous vole spp. occur largely in tundra of North Siberia or in the Russian Far East. BACTERIA: *Orientia tsutsugamushi (M. sachalinensis), Erysipelothrix rhusiopathiae (M. hyperboreus)*, *Francisella tularensis (M. middendorffi).*

Social Vole (*Microtus socialis*)

Steppe habitats in southeastern Europe, Caucasus, Israel, and Kazakhstan. BACTERIA: *Coxiella burnetii, Leptospira hebdomadis, Erysipelothrix rhusiopathiae*, *Yersinia pestis, Francisella tularensis.*

Narrow-headed Vole (*Microtus gregalis*)

Asian (and Israeli) herbivorous species of steppe habitats. VIRUSES: *Flavivirus* TBE (RSSE). BACTERIA: *Rickettsia sibirica, Leptospira hebdomadis, L. grippotyphosa, L. pomona, Listeria monocytogenes*, *Erysipelothrix rhusiopathiae*, *Yersinia pestis, Francisella tularensis.*

Major's Pine Vole (*Microtus majori*)

Occurs in meadow habitats and woodland at high altitudes in Asia Minor and Caucasus. BACTERIA: *Leptospira pomona, L. grippotyphosa, Erysipelothrix rhusiopathiae*, *Francisella tularensis.*

Meadow Vole (*Microtus pennsylvanicus*)

North-American vole living in lowland moist habitats or in high grassland near streams or lakes, much less often in forests. It can swim, feeds on grasses, seeds, grain and bark. Population fluctuates considerably, with spikes every 3–4 years. BACTERIA: *Francisella tularensis.*

California Vole (*Microtus californicus*)

It only occurs in the westernmost part of North America in marshes (even with brackish water) and on wet meadows, but also on grassy hills. Feeds on green vegetation (grasses, sedges). VIRUSES: *Alphavirus* WEE.

Mountain Vole (*Microtus montanus*)

Distributed in western North America in mountain areas. Feeds on grasses and other green vegetation. VIRUSES: *Parechovirus* Ljungan.

Common Pine Vole (*Microtus* [*Pitymys*] *subterraneus*)

Eurasian less abundant species occurring usually in humid forests and woods (alder stands), meadows, or banks of brooks. Herbivorous (grasses, seeds and roots). VIRUSES: *Hantavirus* Tula, *Orbivirus* Tribeč. BACTERIA: *Leptospira grippotyphosa*, *L. jalna*, *Campylobacter jejuni*, *C. coli*, *Yersinia pseudotuberculosis*, *Francisella tularensis*. FUNGI: *Pneumocystis jirovecii*. PROTOZOA: *Babesia microti*, *Toxoplasma gondii*, *Giardia lamblia.*

Water (Ground) Vole (*Arvicola terrestris*: Photo 7.52)

A large vole (the size of a rat: up to 20 cm long, plus tail about 10 cm; weight up to 200 g), occurring in Eurasian aquatic habitats; some populations occur in humid sites far from water (gardens and orchards). Mainly subterranean pattern of life. Nests underground. Feeds on root vegetables, roots of fruit trees (pest), occasionally fish, carrion. Active also in winter. VIRUSES: *Flavivirus* OHF (reservoir) and TBE, *Hantavirus* sp. BACTERIA: *Rickettsia sibirica*, *Coxiella burnetii*, *Listeria monocytogenes* (disease – reservoir), *Erysipelothrix rhusiopathiae* (reservoir), *Bacillus anthracis*, *Leptospira bataviae*, *L. grippotyphosa* (probable reservoir), *L. icterohaemorrhagiae*, *L. hebdomadis*, *Salmonella enteritidis*, *S. paratyphi* B, *S. typhimurium*, *Yersinia pseudotuberculosis*, *Y. enterocolitica*, *Y. pestis, Pasteurella multocida*, *Brucella abortus, Francisella tularensis* (reservoir in floodplain forest ecosystem; disease, also many human cases). FUNGI: *Trichophyton mentagrophytes*. PROTOZOA: *Babesia microti*, *Toxoplasma gondii.*

Muskrat (*Ondatra zibethicus*: Photo 7.53)

Medium-sized (25–40 cm, tail 20–25 cm, the weight of adults 1–2.5 kg) water rodent occurring in North America and, since nineteenth century, has been introduced into Eurasia (first bred in captivity for the fur) where it has spread widely. Burrows in banks of streams, and in reed stands of fishponds, marshes and lakes it builds big houses (up to 1 m high). Feeds on aquatic vegetation, occasionally on water invertebrates (snails, mussels, etc.) or frogs and rarely fish. Seasonally it moves overland. In some countries (e.g. Russia) it is hunted for its fur. VIRUSES: *Flavivirus* OHF (epizootics), *Hantavirus* Puumala, *Lyssavirus* s.s. BACTERIA: *Rickettsia sibirica, Coxiella burnetii, Chlamydophila abortus* (an epizootic), *Listeria monocytogenes*, *Erysipelothrix rhusiopathiae*, *Fusobacterium necrophorum*, *Staphylococcus aureus*, *Leptospira bataviae*, *L. grippotyphosa*, *L. hebdomadis*, *L. icterohaemorrhagiae*, *L. pomona*, *L. tarassovi*, *Salmonella enteritidis*, *S. typhimurium*, *Yersinia pseudotuberculosis*, *Y. enterocolitica*, *Pasteurella multocida*, *Francisella tularensis* (human infections during hunting muskrats for skins), *Actinomyces bovis* (fatal disease of muskrats). FUNGI: *Trichophyton mentagrophytes* (lesions), *Microsporum cookei*. PROTOZOA: *Toxoplasma gondii*, *Cryptosporidium parvum*, *Giardia lamblia.*

Northern Mole Vole (*Ellobius talpinus*)

A species specialized for subterranean life in steppe, semidesert, desert and mountain habitats from Ukraine and Central Asia to west China. Feeds mainly on roots of herbs, but also on insects and worms. BACTERIA: *Rickettsia sibirica, Coxiella burnetii, Yersinia pestis, Francisella tularensis.*

Transcaucasian Mole Vole (*Ellobius lutescens*)

A species specialized for subterranean life in mountainous meadow and steppe habitats in Asia Minor, Caucasus and Central Asia. Feeds mainly on roots of herbs. BACTERIA: *Yersinia pestis* (Iran, Kurdistan).

Family *Muridae*

Giant Rat (*Cricetomys emini*), **Gambian Rat** (*C. gambianus*)

Big omnivorous rats (up to 40 cm plus bicoloured tail 35 cm in *C. gambianus*) of African tropical forests. VIRUSES: *Nairovirus* Dugbe (*C. gambianus*), *Orthopoxvirus simiae* (lesions).

Drylands Vesper Mouse (*Calomys musculinus*), **Large Vesper Mouse** (*C. callosus*)

South-American species of agrocenoses. VIRUSES: arenaviruses Junin (*C. musculinus*, reservoir) and Machupo (*C. callosus*, reservoir).

Striped Field Mouse (*Apodemus agrarius*: Photo 7.54)

Occurs mainly in northern Eurasia in more humid habitats with dense vegetation but in winter it approaches human settlements and isolated buildings. Feeds on seeds, but animal component (insects) makes up about one-third of the diet. VIRUSES: *Flavivirus* TBE, hantaviruses Hantaan (reservoir), Dobrava (reservoir in SE. Europe), Saaremaa and Puumala, *Arenavirus* LCM. BACTERIA: *Rickettsia sibirica*, *R. slovaca*, *Orientia tsutsugamushi*, *Coxiella burnetii*, *Listeria monocytogenes*, *Erysipelothrix rhusiopathiae*, *Bacillus anthracis*, *Leptospira grippotyphosa*, *L. australis*, *L. bataviae*, *L. hebdomadis*, *L. pomona* (*mozdok* – reservoir), *L. sejroe*, *L. javanica*, *L. icterohaemorrhagiae*, *Borrelia burgdorferi* s.l. (competent host), *Salmonella typhimurium*, *Yersinia pseudotuberculosis*, *Y. pestis*, *Brucella abortus*, *Francisella tularensis*. FUNGI: *Trichophyton mentagrophytes*. PROTOZOA: *Babesia microti*, *Toxoplasma gondii*.

Korean Field Mouse (*Apodemus peninsulae*)

Distributed in eastern-Asian brush and woodlands. In winter season attracted to human habitations. Feeds on seeds and vegetation. VIRUSES: *Flavivirus* TBE, *Hantavirus* Hantaan. BACTERIA: *Orientia tsutsugamushi, Leptospira autumnalis* (reservoir), *Yersinia pseudotuberculosis*, *Francisella tularensis*, *Erysipelothrix rhusiopathiae*. PROTOZOA: *Toxoplasma gondii*.

Yellow-necked Mouse (*Apodemus flavicollis*: Photo 7.55)

A common European species, living in woodland (less in open country habitats). Feeds on seeds (acorns etc.) and invertebrates (insects etc.). VIRUSES: *Flavivirus* TBE, hantaviruses Dobrava (reservoir), Saaremaa, Hantaan and Puumala, *Arenavirus* LCM, *Orthopoxvirus bovis*. BACTERIA: *Rickettsia slovaca, Orientia tsutsugamushi, Anaplasma phagocytophilum* s.l., *Listeria monocytogenes, Borrelia burgdorferi* s.l., *B. afzelii, Leptospira australis, L. bataviae, L. bratislava, L. grippotyphosa, L. hebdomadis, L. jalna* (reservoir), *L. pomona, L. saxkoebing* (reservoir), *L. sejroe, Campylobacter jejuni, C. coli, Salmonella* spp., *Yersinia enterocolitica, Francisella tularensis*. FUNGI: *Trichophyton mentagrophytes, Pneumocystis jirovecii*. PROTOZOA: *Babesia microti, Toxoplasma gondii*.

Wood Mouse (*Apodemus sylvaticus*)

A very common Eurasian mouse species, living usually in small woods and wooded habitats of open country (coppices, windbreak tree lines, brush, etc.). Feeds on seeds (acorns, etc.) and invertebrates (up to a quarter of the diet – insects, etc.). VIRUSES: flaviviruses TBE and LI, hantaviruses Hantaan and Puumala, *Arenavirus* LCM. BACTERIA: *Rickettsia sibirica, Coxiella burnetii, Orientia tsutsugamushi, Listeria monocytogenes, Erysipelothrix rhusiopathiae, Bacillus anthracis, Borrelia burgdorferi* s.l., *B. afzelii, B. caucasica, Leptospira australis, L. bratislava, L. grippotyphosa, L. hebdomadis, L. jalna, L. pomona, L. saxkoebing, L. sejroe, L. icterohaemorrhagiae, Campylobacter jejuni, C. coli, Salmonella typhimurium, S. enteritidis, Yersinia enterocolitica, Y. pseudotuberculosis, Francisella tularensis, Brucella abortus, Mycobacterium microti*. FUNGI: *Trichophyton mentagrophytes, T. erinacei, T. verrucosum, Microsporum persicolor, Pneumocystis jirovecii*. PROTOZOA: *Babesia microti, Toxoplasma gondii*.

Pygmy Field Mouse (*Apodemus uralensis* [syn. *A. microps*])

Associated with dry steppe-like habitats. Feeds on grain, seeds, grasses. BACTERIA: *Leptospira bataviae, L. pomona, L. sejroe, L. grippotyphosa*.

House Mouse Western (*Mus domesticus*) and **Eastern** (*Mus musculus*: Photo 7.57)

Synanthropic species with a cosmopolitan distribution. However, *M. domesticus* is distributed in western (partly also central) Europe and the Americas, while *M. musculus* in central and eastern Europe and in Asia. The dividing line between these two similar species goes across Europe from southern Sweden through Denmark, eastern Germany, western Czechland to Italy. Both species are associated with human habitation but in the summer some populations also live in fields. They feed on plant and animal remnants, seeds (grain – often considered a pest), small roots and insects. Epidemiologically very important rodent species. Many zoonotic and sapronotic agents have also been recorded in laboratory mice, which are bred forms of wild house mouse. VIRUSES: hantaviruses Puumala, Leakey, Seoul, Sin Nombre, arenaviruses LCM (reservoir) and Junin, *Cardiovirus* EMC, murine *Orthopoxvirus, Orthopoxvirus bovis*. BACTERIA:

Rickettsia akari (reservoir), *R. typhi*, *R. sibirica*, *Orientia tsutsugamushi*, *Coxiella burnetii*, *Listeria monocytogenes*, *Erysipelothrix rhusiopathiae*, *Bacillus anthracis*. *Borrelia persica*, *Leptospira sejroe* (reservoir), *L. grippotyphosa*, *L. icterohaemorrhagiae*, *L. pomona*, *L. javanica*, *L. hebdomadis*, *Salmonella typhimurium*, *S. enteritidis*, *S. paratyphi* B, *Yersinia pestis* (Ural and Volga rivers during epizootics in 1937–1938, 1946, and 1958)*, Y. pseudotuberculosis*, *Y. enterocolitica*, *Y. pestis, Pasteurella multocida*, *Francisella tularensis*, *Brucella abortus*, *Streptobacillus moniliformis*. FUNGI: *Trichophyton mentagrophytes*, *T. quinckeanum*, *T. verrucosum*, *T. erinacei*, *Microsporum canis*, *Histoplasma capsulatum*, *Pneumocystis jirovecii*. PROTOZOA: *Babesia microti*, *Trypanosoma cruzi*, *Leishmania major*, other *Leishmania* spp., *Toxoplasma gondii*, *Cryptosporidium parvum*.

Harvest Mouse (*Micromys minutus*)

A small (5–8 cm plus tail 4–7 cm; weight 5–12 g) Eurasian species living close to water (reed beds, meadows, ditches) and building typical spherical grassy nests woven in vegetation 40–80 cm above the ground while in winter it uses ground nests. Feeds on seeds and insects (30%). The population density is usually low, and the epidemiological role therefore decreased. VIRUSES: *Flavivirus* TBE, hantaviruses Hantaan and Puumala, *Arenavirus* LCM. BACTERIA: *Orientia tsutsugamushi*, *Erysipelothrix rhusiopathiae*, *Leptospira bataviae* (reservoir), *L. pomona*, *Yersinia enterocolitica*, *Y. pestis* (China), *Francisella tularensis*. PROTOZOA: *Babesia microti*.

Nile Grass Rat (*Arvicanthis niloticus*: Photo 7.58)

Synanthropic omnivorous African species, widely distributed. VIRUSES: *Flavivirus* West Nile. BACTERIA: *Yersinia pestis*. PROTOZOA: *Leishmania tropica*, *L. major*.

Black Rat (*Rattus rattus*: Photo 7.59)

A synanthropic species with cosmopolitan distribution but rare in colder areas. The Black Rat has a tail longer than its body plus head and also longer ears (when bent they reach to the eye) than the Brown Rat. Contrary to the Brown Rat, the Black Rat prefers drier and warmer sites in buildings (attics), granaries, storehouses etc. (often in seaports). Omnivorous (crops, foodstuffs, refuse, fruit, etc.). Epidemiologically a very important rodent. VIRUSES: *Flavivirus* KFD, *Hantavirus* Seoul, *Cardiovirus* EMC, *Arenavirus* LCM, *Orthopoxvirus bovis*. BACTERIA: *Rickettsia akari* (reservoir), *R. typhi*, *R. conorii*, *Orientia tsutsugamushi*, *Coxiella burnetii*, *Listeria monocytogenes*, *Erysipelothrix rhusiopathiae, Leptospira grippotyphosa*, *L. icterohaemorrhagiae*, *L. pomona*, *L. javanica*, *Borrelia burgdorferi* s.l., *B. duttonii, Spirillum minus* (reservoir), *Streptobacillus moniliformis* (reservoir), *Burkholderia mallei*, enteropathogenic *Escherichia coli, Salmonella typhimurium*, *Yersinia pestis*, *Y. pseudotuberculosis* (reservoir), *Francisella tularensis*, *Brucella abortus*, *Mycobacterium bovis*, *M. avium*. FUNGI: *Microsporum canis*, *Trichophyton mentagrophytes*. PROTOZOA: *Leishmania infantum*, *Trypanosoma cruzi*, *Toxoplasma gondii*.

Brown Rat (*Rattus norvegicus*: Photo 7.60)

A synanthropic species with a nearly cosmopolitan distribution. The Brown Rat has a shorter tail and ears than the Black Rat. The Brown Rat often lives in the cellars of urban buildings and farmyards near water (sewers etc.), and is extremely adaptable. Omnivorous – the animal component of the food (e.g., offal) is substantial, and it feeds commonly on rubbish, fodder for domestic animals (sometimes directly in the manger). It is found regularly in farm buildings and in slaughterhouses. Epidemiologically a very important rodent, especially at an enhanced population density when control by rat extermination is necessary. Many zoonotic agents have also been reported in the laboratory rat (which is, in fact, a bred albino form of the Brown Rat). VIRUSES: *Hantavirus* Seoul (reservoir), *Lyssavirus* s.s., *Cardiovirus* EMC, *Herpesvirus suis* 1 (pseudorabies). BACTERIA: *Rickettsia akari* (reservoir), *R. typhi*, *R. sibirica, Orientia tsutsugamushi, Coxiella burnetii, Bartonella quintana, Listeria monocytogenes, Erysipelothrix rhusiopathiae* (reservoir), *Bacillus anthracis, Leptospira icterohaemorrhagiae* (reservoir), *L. pomona, L. copenhageni, Helicobacter heilmannii* (gastritis), *Borrelia burgdorferi* s.l., *Spirillum minus* (reservoir), *Streptobacillus moniliformis* (reservoir), *Burkholderia mallei*, enteropathogenic *Escherichia coli, Salmonella enteritidis, S. typhimurium, S. dublin, S. paratyphi* B, *Yersinia pestis* (in epizootics), *Y. enterocolitica, Y. pseudotuberculosis* (reservoir), *Y. pestis, Pasteurella multocida, Francisella tularensis, Brucella abortus, B. melitensis, B. suis* (biotype 2), *Mycobacterium bovis, M. avium, M. paratuberculosis*. FUNGI: *Microsporum canis, Trichophyton mentagrophytes, T. erinacei, T. simii, Pneumocystis jirovecii*. PROTOZOA: *Trypanosoma cruzi, Toxoplasma gondii, Giardia lamblia, Balantidium coli.*

Turkestan Rat (*Rattus turkestanicus*)

Deciduous forests of mountainous Central and southern Asia and China, also synanthropic species. BACTERIA: *Rickettsia typhi, R. sibirica, Orientia tsutsugamushi, Coxiella burnetii.*

Polynesian Rat (*Rattus exulans*)

Indonesian, Australian and Oceanic species, not strictly synanthropic. Omnivorous. BACTERIA: *Leptospira australis, Yersinia pestis.* PROTOZOA: *Toxoplasma gondii.*

Natal Multimammate Rat (*Mastomys natalensis*: Photo 7.56)

A widely distributed rat of sub-Saharan Africa. It occurs in savannah, in agroecosystems and in human habitation including houses. Sometimes serves as food for local humans. VIRUSES: *Arenavirus* Lassa (reservoir). BACTERIA: *Yersinia pestis.* PROTOZOA: *Leishmania* spp.

Great Bandicoot Rat (*Bandicota indica*), **Lesser Bandicoot Rat** (*B. bengalensis*), **Short-tailed Bandicoot Rat** (*Nesokia indica*)

Common, omnivorous species of Indian rats. VIRUSES: *Hantavirus* Thailand (*B. indica* reservoir). BACTERIA: *Orientia tsutsugamushi, Leptospira* spp.,

Yersinia pestis, Francisella tularensis (*N. indica*). FUNGI: *Trichophyton simii*. PROTOZOA: *Leishmania major* (*N. indica*).

Family *Rhizomyidae*

Large Bamboo Rat (*Rhizomys sumatrensis*), **Lesser Bamboo Rat** (*Cannomys badius*)

R. sumatrensis occurs in south-east Asia, feeds on stems and leaves of bamboo; *C. badius* is an Indian species. FUNGI: *Penicillium marneffei* (reservoir).

Family *Ctenodactyliidae*

Common Gundi (*Ctenodactylus gundi*)

A guinea pig-like stocky rodent (body size 16–20 cm, tail very short), occurs in desert rocky habitats of north Africa (Maghreb), feeds on plants. PROTOZOA: *Toxoplasma gondii* (very first observed and described from this host in 1908 – but the gundis were from captivity).

Family *Gliridae*

Edible Dormouse (*Glis glis*: Photo 7.61)

Eurasian species living in warm deciduous forests, scrub, gardens, orchards. Feeding on fruit and seeds. Hibernating. VIRUSES: *Cardiovirus* EMC (disease). BACTERIA: *Borrelia burgdorferi* s.l. (competent host).

Forest Dormouse (*Dryomys nitedula*)

Eurasian species living in woods with thick undergrowth, often in hills and mountains. Omnivorous, but the animal component forms about 80% of the diet (insects, snails, avian eggs and nestlings, small mammals). Hibernating. VIRUSES: *Flavivirus* TBE. BACTERIA: *Leptospira pomona*, *Francisella tularensis*.

Garden Dormouse (*Eliomys quercinus*: Photo 7.62)

European, largely a woodland species but also occurs in orchards, gardens and scrub. Feeds on invertebrates, nestling birds (a good tree climber), small mammals, and in autumn on fruit (berries), nuts, and seeds. Hibernates in common nests. VIRUSES: *Flavivirus* TBE. BACTERIA: *Borrelia burgdorferi* s.s., *B. spielmanii*. FUNGI: *Microsporum persicolor*.

Hazel Dormouse (*Muscardinus avellanarius*)

A small, nocturnal European dormouse, occurring in deciduous and coniferous woodland and coppices from lowland to mountains. Omnivorous, feeds on fruit, seeds, nuts and invertebrates. Hibernating. BACTERIA: *Erysipelothrix rhusiopathiae*. PROTOZOA: *Babesia microti*.

Family *Hystricidae*

Porcupine (*Hystrix cristata*)

African herbivorous mammal with long spiny quills, digging burrows and occurring in bushland, farmland and arid rocky areas. BACTERIA: *Borrelia duttonii*, *B. persica* (reservoir).

Indian Crested Porcupine (*Hystrix indica* [=*H. leucura*])

Central and southern Asia, herbivorous. BACTERIA: *Borrelia persica* (reservoir). PROTOZOA: *Leishmania infantum.*

Family *Erethizontidae*

(Canadian) Porcupine (*Erethizon dorsatum*)

A big (about 50 cm long plus tail 20 cm; weight 5–12 kg) North-American species resembling African porcupine but it lives mostly on trees (or in bushland) and feeds on bark, twigs and buds. VIRUSES: *Coltivirus* CTF. BACTERIA: *Rickettsia rickettsii, Coxiella burnetii, Francisella tularensis.*

Family *Chinchillidae*

Chinchilla (*Chinchilla laniger*)

Living in rocky habitats in high mountains (the Andes) in Chile and Bolivia, feeds on roots, rootstocks, tubers and green plants. Bred for its excellent fur. BACTERIA: *Listeria monocytogenes.*

Family *Caviidae*

Guinea Pig (*Cavia aperea porcellus*)

Originally a wild South-American herbivorous species living in open lowland. As domesticated animal, it serves as pet and an important laboratory animal. In South America also used for human food. BACTERIA: *Chlamydophila caviae*, *Leptospira pomona*, *L. grippotyphosa*, *Leptospira* spp.*, Yersinia pestis*, *Y. pseudotuberculosis*. FUNGI: *Trichophyton mentagrophytes*. PROTOZOA: *Trypanosoma cruzi* (reservoir).

Cavy (*Microcavia australis*)

Mountainous areas of Argentina, also close to human habitation. Hunted for food. BACTERIA: *Yersinia pestis* (human cases after contact).

Family *Echimyidae*

Cayenne Spiny Rat (*Proechimys cayennensis*)

Central- and South-American medium-sized rat-like rodent species in wooded habitats and scrub. VIRUSES: alphaviruses VEE (Mucambo) and EEE, bunyavirus group C, *Arenavirus* Machupo. PROTOZOA: *Leishmania mexicana* (competent host).

Spiny Rat (*Proechimys semispinosus*)

A common rat-like rodent of Central and South America. BACTERIA: *Leptospira* spp. (incl. *L. icterohaemorrhagiae, L. pomona*), *Salmonella* spp.

Punaré (*Trichomys apereoides*)

A caviomorph rodent living in South America (Brazil). PROTOZOA: *Trypanosoma cruzi* (reservoir), *Leishmania braziliensis* (competent host, possibly reservoir).

Family *Hydrochaeridae*

Capybara (*Hydrochaeris hydrochaeris*)

A huge South-American rodent (the largest rodent: 100–130 cm long; weight about 50 kg). Forest and grassland areas close to water. Feeds on aquatic plants. BACTERIA: *Rickettsia rickettsii*.

Family *Myocastoridae*

Coypu (Nutria) (*Myocastor coypus*: Photo 7.63)

A large South-American (Argentina) rodent (about 60 cm long plus tail 30–40 cm, weight 7–9 kg), living in marshes, ponds and lakes. Herbivorous (aquatic plants). Kept for its fur and meat in Eurasia; occasionally some individuals escape from captivity. VIRUSES: *Lyssavirus* s.s. BACTERIA: *Leptospira hebdomadis*, *L. australis*, *L. icterohaemorrhagiae*, *L. autumnalis*, *L. australis, L. bataviae*, *Listeria monocytogenes*, *Salmonella typhimurium, Yersinia enterocolitica*, *Y. pseudotuberculosis*, *Francisella tularensis* (susceptible). FUNGI: *Trichophyton mentagrophytes* (skin lesions). PROTOZOA: *Toxoplasma gondii*.

7.1.10 Order Lagomorphs (*Rabbits, Hares, and Pikas; Lagomorpha*)

Family *Ochotonidae*

Daurian Pika (*Ochotona dauurica*), **Pallas's (Mongolian) Pika** (*O. pallasi* [= *O. pricei*])

Small central Asian and east Asian mammals, quite abundant in grassland or rocky steppe habitats in foothills and at higher mountain elevations. Exclusively herbivorous (grasses), they store large quantities of hay for winter periods. They are very often massively infested by fleas of many species (mainly *Ctenophyllus* spp., but also *Citellophilus tesquorum*, a known vector of plague). BACTERIA: *Rickettsia sibirica, Erysipelothrix rhusiopathiae, Yersinia pestis* (Mongolia, Altai Mts.: reservoir – mainly *O. pallasi*, epizootics), *Y. pseudotuberculosis, Francisella tularensis*.

Afghan Pika (*Ochotona rufescens*), **Alpine Pika** (*O. alpina*)

Small Asian species of mountain or foothill (*O. rufescens*) steppes, rocks (*O. alpina*); exclusively herbivorous and storing hay. BACTERIA: *Coxiella burnetii, Erysipelothrix rhusiopathiae* (*O. alpina*).

Family *Leporidae*

Rabbit (*Oryctolagus cuniculus*: Photo 7.65)

Wild and domestic rabbit. The wild rabbit lives in scrub and grassland on lighter (sandy) soil where it builds a system of burrows (colonies). This originally European

species was introduced to Australia where it is a pest. Obligately herbivorous (grass, herbage; bark and twigs in winter), with an extraordinary reproduction rate. Free living populations may nearly reach the density of rodent populations, but are periodically and drastically reduced by epizootics of myxomatosis. VIRUSES: *Coltivirus* Eyach, *Herpevirus suis* 1, *Orthopoxvirus bovis*. BACTERIA: *Rickettsia conorii*, *Orientia tsutsugamushi*, *Coxiella burnetii, Staphylococcus aureus*, *Listeria monocytogenes*, *Yersinia pseudotuberculosis*, *Pasteurella multocida*, *Francisella tularensis*, *Fusobacterium necrophorum*, *Mycobacterium paratuberculosis*. PROTOZOA: *Trypanosoma cruzi*, *Toxoplasma gondii*, *Giardia lamblia*. MICROSPORIDIA: *Encephalitozoon cuniculi*, *Enterocytozoon bieneusi*.

Eastern Cottontail (*Sylvilagus floridanus*)

A small rabbit distributed widely in central, eastern and southern North America in brushland, small woods with open areas, coppices, farmland, edges of marshes. It was introduced into Europe (France, Italy, Switzerland). Herbivorous (grasses, herbs, in winter also feeds on bark and twigs). VIRUSES: *Coltivirus* Eyach. BACTERIA: *Coxiella burnetii*, *Yersinia pseudotuberculosis*, *Francisella tularensis*.

Brown (European) Hare (*Lepus europaeus*: Photo 7.64)

Well-known European mammal, most often occurring in farmland (agrocoenoses in lowlands and warmer hilly land) or open woodland, but also appears in suburban woody areas and gardens. Herbivorous: grasses, herbs, leaves; bark and twigs in winter. It does not build burrows like the rabbit but rests in shallow depressions in the ground. VIRUSES: *Flavivirus* TBE, *Flavivirus* WN (Volga Delta), *Orthobunyavirus* Ťahyňa, *Nairovirus* CCHF (southern Russia), *Herpesvirus suis* 1. BACTERIA: *Rickettsia slovaca*, *Coxiella burnetii*, *Chlamydophila abortus*, *Listeria monocytogenes*, *Erysipelothrix rhusiopathiae*, *Bacillus anthracis*, *Staphylococcus aureus*, *S. intermedius*, *Streptococcus* spp., *Bacteroides fragilis* (necrobacillosis), *Leptospira grippotyphosa*, *Borrelia burgdorferi* s.l., *Campylobacter jejuni*, *C. coli*, *Salmonella typhimurium*, *S. enteritidis*, *Yersinia pseudotuberculosis* (disease), *Y. enterocolitica*, *Pasteurella multocida* (disease), *P. haemolytica*, *Brucella suis* var. *leporis* (biotype 2 – chronic disease; carriership; reservoir), *Francisella tularensis*, *Mycobacterium bovis*. FUNGI: *Trichophyton mentagrophytes*, *Pneumocystis jirovecii*. PROTOZOA: *Babesia microti*, *Toxoplasma gondii*. MICROSPORIDIA: *Encephalitozoon intestinalis*, *E. hellem*.

Mountain (Blue) Hare (*Lepus timidus*)

Northern parts of Eurasia and North America. Habitat is tundra, heathland, and farmland. Sociable, forming herds of up to 50 individuals. Feeds on grasses, heather and twigs of willow, birch, etc. VIRUSES: flaviviruses LI and TBE. *Orthobunyavirus* SSH. BACTERIA: *Listeria monocytogenes*, *Erysipelothrix rhusiopathiae, Salmonela typhimurium, Yersinia pseudotuberculosis*, *Francisella tularensis*. PROTOZOA: *Toxoplasma gondii*.

Snowshoe Hare (*Lepus americanus*)

This species is widely distributed in northern parts of North America, occurring in forests, coppices, swamp areas; it is herbivorous and largely nocturnal. VIRUSES: *Orthobunyavirus* SSH. BACTERIA: *Rickettsia rickettsii*, *Listeria monocytogenes*, *Yersinia pseudotuberculosis*, *Francisella tularensis*.

Blacktail Jackrabbit, Whitetail Jackrabbit (*Lepus californicus*, *L. townsendii*)

North-American hare species living in open areas (grassland, prairies, semidesert); herbivorous. VIRUSES: *Orthobunyavirus* California encephalitis (*L. californicus*), *Coltivirus* CTF (*L. californicus*). BACTERIA: *Rickettsia rickettsii*, *Coxiella burnetii* (*L. californicus*), *Listeria monnocytogenes* (*L. californicus*), *Yersinia pseudotuberculosis* (*L. californicus*), *Francisella tularensis*, *Brucella suis* (*L. californicus*).

Tolai Hare (*Lepus tolai*)

Central-Asian species. BACTERIA: *Rickettsia sibirica*, *Listeria monocytogenes*. *Burkholderia pseudomallei*, *Salmonela typhimurium*, *Yersinia pseudotuberculosis*, *Pasteurella multocida*, *Brucella suis* var. *leporis*, *Francisella tularensis*. PROTOZOA: *Toxoplasma gondii*.

7.1.11 Order Odd-Toed Ungulates (Perissodactyla)

Family *Equidae*

Horse (*Equus caballus*) and other equids (**donkey, mule**)

VIRUSES: alphaviruses EEE, WEE and VEE, flaviviruses JE and WN, *Nairovirus* CCHF, *Vesiculovirus* VSV, *Lyssavirus* s.s., orthomyxoviruses Dhori and influenza A, *Paramyxovirus* Hendra, *Aphthovirus* FMDV. BACTERIA: *Streptococcus equi* ssp. *zooepidemicus*, *Bacillus anthracis*, *Clostridium tetani*, *C. botulinum*, *Salmonella enterica*, *Burkholderia mallei*, *B. pseudomallei*, *Fusobacterium necrophorum*, *Corynebacterium pseudotuberculosis*, *C. ulcerans*, *Arcanobacterium pyogenes*, *Rhodococcus equi*, *Dermatophilus congolensis*. FUNGI: *Trichophyton equinum*.

Family *Rhinocerotidae*

Black Rhinoceros (*Diceros bicornis*: Photo 7.66)

Huge African (mainly eastern and southern Africa) herbivorous mammal (body length about 3.5 m; weight 1,400 kg). PROTOZOA: *Trypanosoma brucei rhodesiense*.

7.1.12 Order Even-Toed Ungulates (Artiodactyla)

Family *Suidae*

Domestic Pig and Wild Boar (*Sus scrofa*: Photo 7.67)

Eurasian and North-African wild species, also introduced into North America, common in deciduous humid forests (oak and beech) and visits neighbouring fields.

Extensive home range. Can swim well (also in great rivers). Omnivorous: acorns, beechmast, roots, field vegetables, maize, rodents, bird eggs and nestlings, molluscs, insects and other invertebrates, carrion of large mammals etc. The food is collected from the ground or dug from the soil. A very dangerous infectious disease of wild boars and domestic pigs is swine pest (not transmissible to man); for pigs it is highly contagious and lethal. VIRUSES: *Flavivirus* JE (amplifying host), *Vesiculovirus* VSV, *Orthomyxovirus* influenza A, *Henipavirus* Nipah, *Cardiovirus* EMC, *Aphthovirus* FMDV, *Hepevirus* hepatitis E, *Herpesvirus suis* 1 (reservoir), *Parapoxvirus bovis* 2. BACTERIA: *Orientia tsutsugamushi*, *Chlamydophila abortus*, *Leptospira pomona* (reservoir), *L. australis, L. tarassovi, Listeria monocytogenes*, *Erysipelothrix rhusiopathiae* (reservoir), *Streptococcus suis* (reservoir), *Bacillus anthracis*, *Clostridium difficile*, *C. botulinum*, *Campylobacter jejuni*, *C. coli*, *Helicobacter bizzozeronii*, *Brachyspira pilosicola*, *Salmonella enteritidis* and other serovars, enteropathogenic *Escherichia coli*, *Yersinia enterocolitica*, *Y. pseudotuberculosis*, *Brucella suis* (biotypes 1–3), *Pasteurella multocida*, *Francisella tularensis*, *Burkholderia pseudomallei*, *Fusobacterium necrophorum*, *Rhodococcus equi*, *Mycobacterium bovis*, *M. avium*. FUNGI: *Trichophyton mentagrophytes*, *Microsporum nanum*. PROTOZOA: *Trypanosoma cruzi*, *T. brucei rhodesiense*, *T. brucei gambiense*, *Toxoplasma gondii*, *Sarcocystis suihominis*, *Giardia lamblia*, *Balantidium coli*. MICROSPORIDIA: *Enterocytozoon bieneusi*. OTHER EUKARYOTA: *Blastocystis*.

Warthog (*Phacochoerus africanus*: Photo 7.68)

African species of savannah, omnivorous. Male weights up to 150 kg. BACTERIA: *Mycobacterium bovis*. PROTOZOA: *Trypanosoma brucei rhodesiense*.

Family *Hippopotamidae*

Hippopotamus (*Hippopotamus amphibius*)

A huge (up to 3,200 kg) African hydrophilic species, herbivorous. PROTOZOA: *Trypanosoma brucei rhodesiense.* BACTERIA: *Bacillus anthracis.*

Family *Camelidae*

Dromedary, Bactrian Camel (*Camelus dromedarius*, *C. bactrianus*)

Origin of *C. bactrianus* is in Central Asia, in *C. dromedarius* unknown. Today distributed in arid areas in Africa and Asia (central, southern, the Near East). Herbivorous. VIRUSES: *Flavivirus* WN, *Phlebovirus* RVF, orthomyxoviruses Thogoto and Dhori, *Orthopoxvirus bovis*. BACTERIA: *Mycobacterium bovis.*

Family *Cervidae*

Elk (Moose) (*Alces alces*)

The greatest deer-like ruminant (weight up to 800 kg), distributed in northern Eurasia and America. Lives in semi-open humid to marshy forests. Feeds on herbage, grasses, shoots, leaves, twigs and bark of trees, brushes and aquatic plants. Migrates or travels for long distances. BACTERIA: *Erysipelothrix rhusiopathiae*,

Bacillus anthracis (brucellosis, with abortus), *Staphylococcus aureus*, *Brucella abortus* (causing problems in the Greater Yelowstone Ecosystem, Wyoming), *B. suis* (biotype 4), *Mycobacterium bovis*.

White-tailed Deer (*Odocoileus virginianus*)

North-American species (but introduced into Finland), occurring in humid forests and open bush in their environment. Feeds on twigs, acorns, mushrooms, grasses and other plants. In Europe kept in deer farms. VIRUSES: bunyaviruses Jamestown Canyon and SSH, *Vesiculovirus* VSV, *Herpesvirus suis* 1. BACTERIA: *Anaplasma phagocytophilum* (competent host), *E. chaffeensis*, *Erysipelothrix rhusiopathiae*, *Listeria monocytogenes*, *Bacillus anthracis*, *Fusobacterium necrophorum*, *Leptospira pomona*, *Pasteurella multocida*, *Yersinia pseudotuberculosis*, *Y. enterocolitica*, *Brucella abortus*, *Mycobacterium bovis*, *M. paratuberculosis*, *Dermatophilus congolensis*. FUNGI: *Trichophyton mentagrophytes*.

Reindeer (*Rangifer tarandus*), **Caribou** (*R. tarandus caribou*: Photo 7.69)

North of Eurasia and America, occurring in woodland and tundra biome. Feeds on grasses, herbage, twigs of trees and shrubs. Reindeer live in great herds and migrate twice a year, up to hundreds of kilometres, whereas the Caribou migrates short distances (usually up and down mountains). BACTERIA: *Brucella abortus, B. suis* (biotype 4, also in *R. t. caribou*).

Fallow Deer (*Cervus* [*Dama*] *dama*)

The original distribution area of this deer species is the Mediterranean and SW Asia, but since Middle Ages it has been introduced into central Europe – game reserves situated in deciduous forest and parkland. Feeds on leaves, herbage, shoots, grasses, acorns and chestnuts. VIRUSES: *Lyssavirus* s.s. BACTERIA: *Anaplasma phagocytophilum* s.l., *Bacillus anthracis*, *Pasteurella multocida*, *Yersinia pseudotuberculosis*, *Brucella abortus*, *Mycobacterium bovis*, *M. paratuberculosis*.

Red Deer (*Cervus elaphus*: Photo 7.70)

A large (weight 70–250 kg) Eurasian, North-American and North-African deer, occurring in lowland to mountain forests. Feeds on leaves, twigs, shoots, grasses, herbage, acorns, beechmast, mushrooms etc. VIRUSES: *Flavivirus* LI, *Lyssavirus* s.s., *Vesiculovirus* VSV, *Herpesvirus suis* 1. BACTERIA: *Anaplasma phagocytophilum* s.l., *Listeria monocytogenes*, *Bacillus anthracis*, *Staphylococcus aureus*, *Leptospira grippotyphosa*, *L. pomona*, enteropathogenic *Escherichia coli*, *Yersinia pseudotuberculosis*, *Pasteurella multocida*, *Brucella abortus*, *Mycobacterium bovis*, *M. a. paratuberculosis*. FUNGI: *Trichophyton mentagrophytes*. PROTOZOA: *Toxoplasma gondii*, *Cryptosporidium parvum*.

Sika Deer (*Cervus nippon*)

Eastern Asiatic deer species of woodland and parkland, in the twentieth century introduced into Europe where it thrives in a number of game parks. Herbivorous (grasses, leaves, shoots). VIRUSES: *Hepevirus* hepatitis E. BACTERIA: *Ehrlichia chaffeensis*, *Anaplasma phagocytophilum* s.l., *Yersinia pseudotuberculosis*.

Roe Deer (*Capreolus capreolus*: Photo 7.71)

Eurasian species living in forests and woods of all types, lately also in agrocoenoses. Feeds on grasses, herbage, leaves, shoots, bark, mushrooms and fruit. VIRUSES: *Lyssavirus* s.s., *Vesiculovirus* VSV, *Herpesvirus suis* 1. BACTERIA: *Anaplasma phagocytophilum* s.l., *Listeria monocytogenes*, *Erysipelothrix rhusiopathiae*, *Bacillus anthracis*, *Clostridium tetani*, *Staphylococcus aureus*, *Salmonella enteritidis*, *S. typhimurium*, *Yersinia pseudotuberculosis*, *Y. enterocolitica*, *Brucella abortus*, *B. melitensis*, *Pasteurella multocida*, *Mycobacterium bovis*, *M. avium*, *M. paratuberculosis*. PROTOZOA: *Toxoplasma gondii.*

Family *Bovidae*

Red Lechwe (*Kobus leche*)

A gazelle living in wetlands, largely in southern Africa. Herbivorous (aquatic plants). BACTERIA: *Mycobacterium bovis.*

Greater Kudu (*Tragelaphus strepsiceros*), **Gray (Bush) Duikker** (*Sylvicapra grimmia*)

Mammals of African savannah ecosystem. BACTERIA: *Mycobacterium bovis* (*T. strepsiceros*). PROTOZOA: *Trypanosoma brucei rhodesiense.*

Chamois (*Rupicapra rupicapra*)

Isolated populations occur in the alpine zone of some mountains in Europe, Asia Minor and the Caucasus. Feeds on grasses, herbage, and bark. Can move for longer trails. VIRUSES: *Parapoxvirus ovis*. BACTERIA: *Brucella melitensis* (biotype 3), *B. abortus*, *Mycobacterium bovis*, *Dermatophilus congolensis.*

Goat (*Capra hircus*)

VIRUSES: *Flavivirus* TBE (transmission to man by milk), *Phlebovirus* RVF, *Nairovirus* CCHF, *Bunyavirus* Bhanja, *Orbivirus* Tribeč, *Lyssavirus* s.s., *Parapoxvirus ovis*. BACTERIA: *Coxiella burnetii*, *Chlamydophila abortus*, *Salmonella enterica*, enteropathogenic *Escherichia coli*, *Brucella melitensis* (reservoir), *Fusobacterium necrophorum*, *Listeria monocytogenes*, *Mycobacterium paratuberculosis*. PROTOZOA: *Trypanosoma brucei rhodesiense*, *Leishmania tropica*, *Toxoplasma gondii.*

Sheep (*Ovis aries*)

Domestic (and feral) sheep has a cosmopolitan distribution. It is a grazing herbivore. VIRUSES: flaviviruses LI and TBE (transmission to man by milk products), *Phlebovirus* RVF, *Nairovirus* CCHF, *Bunyavirus* Bhanja, *Lyssavirus* s.s., *Parapoxvirus ovis*, *P. bovis* 2. BACTERIA: *Coxiella burnetii* (competent host, reservoir), *Ehrlichia chaffeensis*, *Chlamydophila abortus* (enzootics of abortion), *Leptospira interrogans*, *Listeria monocytogenes*, *Erysipelothrix rhusiopathiae*, *Campylobacter jejuni*, *C. foetus*, *Salmonella enterica*, enteropathogenic

Escherichia coli, *Bacillus anthracis*, *Brucella melitensis* (reservoir), *Francisella tularensis*, *Pasteurella multocida*, *Burkholderia pseudomallei*, *Fusobacterium necrophorum*, *Corynebacterium pseudotuberculosis*, *C. ulcerans*, *Arcanobacterium pyogenes*, *Mycobacterium paratuberculosis*, *Dermatophilus congolensis*. FUNGI: *Trichophyton verrucosum*. PROTOZOA: *Trypanosoma brucei rhodesiense*, *T. brucei gambiense*, *Toxoplasma gondii*, *Giardia lamblia, Cryptosporidium parvum.*

Mouflon (*Ovis musimon*)

The original area of this European species is Corsica and Sardinia (mountain grasslands), but the mouflon has been introduced into other European countries where its herds live in many game reserves and on deer farms. Herbivorous. BACTERIA: *Mycobacterium paratuberculosis*. PROTOZOA: *Toxoplasma gondii.*

African (Cape) Buffalo (*Syncerus caffer*)

Huge (up to 170 cm high; male weights up to 900 kg) herbivorous mammal living in sub-Saharan African savannah. BACTERIA: *Mycobacterium bovis*. PROTOZOA: *Trypanosoma brucei rhodesiense.*

Cattle (*Bos taurus*)

Big herbivorous domesticated ruminants with a cosmopolitan distribution. PRIONS: prion vCJD. VIRUSES: *Flavivirus* TBE (transmission to man by milk), *Phlebovirus* RVF, *Nairovirus* CCHF, *Nairovirus* Dugbe, *Bunyavirus* Bhanja, *Vesiculovirus* VSV, orthomyxoviruses Thogoto and Dhori, *Lyssavirus* s.s., *Aphthovirus* FMDV, *Orthopoxvirus bovis*, *Herpesvirus suis* 1, *Parapoxvirus bovis* 1 and 2. BACTERIA: *Rickettsia sibirica*, *Coxiella burnetii*, *Chlamydophila abortus*, *Leptospira tarassovi*, *L. hardjoe*, *L. interrogans*, *Listeria monocytogenes*, *Bacillus anthracis*, *Clostridium difficile*, *C. botulinum*, *Staphylococcus aureus*, *Streptococcus zooepidemicus*, *Campylobacter jejuni*, *C. foetus*, enteropathogenic *Escherichia coli* (reservoir), *Salmonella enterica*, *Yersinia pseudotuberculosis*, *Brucella abortus*, *Pasteurella multocida*, *Burkholderia pseudomallei*, *Fusobacterium necrophorum*, *Corynebacterium pseudotuberculosis*, *C. ulcerans*, *Arcanobacterium pyogenes*, *Mycobacterium bovis*, *M. avium*, *M. paratuberculosis*, *Dermatophilus congolensis*. FUNGI: *Trichophyton verrucosum* (reservoir). PROTOZOA: *Trypanosoma brucei rhodesiense*, *T. brucei gambiense*, *Toxoplasma gondii*, *Sarcocystis bovihominis*, *Cryptosporidium parvum*, *Babesia divergens*. MICROSPORIDIA: *Enterocytozoon bieneusi.*

American Bison (*Bison bison*: Photo 7.72), European Bison (*Bison bonasus*: Photo 7.73)

The American Bison lives on the open plains of North America, while European bison in forests in Poland, Belarus, Russia, the Caucasus, and Romania. Both species are gregarious, long living (up to 30 years) and herbivorous (mostly grazing on grasses, but also feeding on the leaves of trees, twigs and other vegetation). BACTERIA: *Fusobacterium necrophorum* (Poland – lesions in male bisons), *Brucella abortus* (brucellosis – abortions) and *Mycobacterium bovis*

(bovine tuberculosis) – the latter two causing big epizootiological problems in North America, e.g. in the Greater Yelowstone Ecosystem (Wyoming), *Bacillus anthracis.*

7.2 Birds (*Aves*)

Domestic and free-living birds may be involved in the circulation of zoonotic and sapronotic microorganisms in nature generally as:

(1) biological amplifying hosts of zoonotic microorganisms (the pathogen multiplies in/on the avian body) with an acute, chronic or latent infection, and in some cases as carriers shedding the agent for a prolonged period – such bird species may be characterized as a reservoir of infection when they ensure a long-term reproduction or survival of the agent, especially in the inter-epizootic periods;

(2) "lessors" (Hubálek 1994) or "tenants" of some sapronotic microorganisms, by providing a substrate (droppings) suitable for reproduction of an agent. Such microbes are, e.g., fungi *Cryptococcus neoformans* or *Histoplasma capsulatum*. These fungi utilize uric acid, the main component of avian excreta. and other low-molecular nitrogen compounds in the bird droppings for their growth. Most often the lessors are bird species congregating in communal roosting sites where they produce considerable amounts of droppings.

In addition, some birds can host and disseminate ectoparasite vectors (ixodid ticks or fleas) infected with zoonotic agents such as tick-borne viruses (TBE, CCHF, Kemerovo viruses etc.) and bacteria (*Borrelia burgdorferi* s.l., *Anaplasma phagocytophilum*).

Nevertheless, domestic and free-living birds have a substantially lower significance than mammals as a primary (direct) source of human infection. Therefore they are not given here as particular species, but mostly in blocks of avian groups and only some species are listed in more details.

Colonial ardeids: egrets and herons (family *Ardeidae*; Photo 7.74)

VIRUSES: Flaviviruses JE (competent hosts, reservoir) and MVE. BACTERIA: *Chlamydophila psittaci.*

Storks (*Ciconiidae*)

VIRUSES: *Flavivirus* West Nile (White Stork *Ciconia ciconia* is a competent host: Photo 7.75).

Domestic and wild waterfowl (ducks, geese, swans; Photo 7.76**)**

VIRUSES: *Flavivirus* WN, *Orthomyxovirus* avian influenzua A (H5N1). BACTERIA: *Chlamydophila psittaci, Clostridium botulinum*, *Listeria monocytogenes*, *Erysipelothrix rhusiopathiae*, *Salmonella enterica* serovar Enteritidis (reservoir), *Listeria monocytogenes, Mycobacterium avium.* PROTOZOA:

Cryptosporidium meleagridis. OTHER EUKARYOTA: *Blastocystis hominis, Encephalitozoon intestinalis*, *E. hellem.*

Domestic chicken and turkey

VIRUSES: *Orthomyxovirus* avian influenzua A (H5N1, H7N1), *Paramyxovirus* NDV, *Hepevirus* E. BACTERIA: *Chlamydophila psittaci, Campylobacter jejuni* (reservoir), *Salmonella enterica* serovar Enteritidis (reservoir), enteropathogenic *Escherichia coli, Pasteurella multocida, Clostridium difficile*, *C. botulinum*, *Listeria monocytogenes*, *Erysipelothrix rhusiopathiae*, *Mycobacterium avium.* FUNGI: *Microsporum gallinae, Trichophyton simii.* PROTOZOA: *Cryptosporidium meleagridis.* OTHER EUKARYOTA: *Blastocystis hominis, Enterocytozoon bieneusi.*

Pheasant (*Phasianus colchicus*)

VIRUSES: alphaviruses EEE and WEE. BACTERIA: *Mycobacterium avium.*

Red grouse (*Lagopus lagopus scoticus*)

VIRUSES: *Flavivirus* LI.

Gulls (*Larus* spp.; Photos 7.78 and 7.79)

BACTERIA: *Chlamydophila psittaci, Campylobacter jejuni, Salmonella enterica* serovars Typhimurium and less often Enteritidis, enteropathogenic (and multiresistant) *Escherichia coli, Campylobacter laridis, Listeria monocytogenes*, *Mycobacterium avium.*

Puffin and Guillemots (*Fratercula arctica, Uria lomvia, U. algae*; Photos 7.77, 5.37).

BACTERIA: *Borrelia garinii.*

Feral pigeon (*Columba livia* f. *domestica*: Photo 7.80)

Especially feral pigeons (*Columba livia* f. *domestica*) can present a risk as hosts or lessors of several human pathogenic microorganisms. VIRUSES: *Flavivirus* SLE. BACTERIA: *Chlamydophila psittaci* (source of human ornithosis), *Coxiella burnetii* (at least 5 human cases have been described)*, Salmonella enterica* serovar Typhimurium, *Mycobacterium avium.* FUNGI: *Cryptococcus neoformans* (urban pigeons as a lessor, worldwide). MICROSPORIDIA: *Enterocytozoon bieneusi.*

Wood Pigeon (*Columba palumbus*)

BACTERIA: *Mycobacterium avium* (Great Britain).

Guacharo (oilbird, *Steatornis caripensis*)

Communal breeding and roosting sites in caves, Central America. FUNGI: *Histoplasma capsulatum* (lessor).

Thrushes (family *Turdidae*)

VIRUSES: *Alphavirus* Sindbis. BACTERIA: *Borrelia garinii, Mycobacterium xenopi* (European blackbird).

Red-winged Blackbird (*Agelaius phoeniceus*), **Common Grackle** (*Quiscalus quiscula*), **Brown-headed Cowbird** (*Molothrus ater*)

Common North-American birds with communal roosting sites in woods and tree groves, often in urban parks. FUNGI: *Histoplasma capsulatum* (lessors).

Starling (*Sturnus vulgaris*)

Communal roosting sites in trees or in reeds, often in urban parks.

VIRUSES: *Orbivirus* Tribeč. FUNGI: *Histoplasma capsulatum* (lessor – probably only in North America).

House Sparrow (*Passer domesticus*: Photo 7.82)

VIRUSES: Alphaviruses EEE, WEE. *Flavivirus* SLE. BACTERIA: *Salmonella enterica* serovar Typhimurium, *Mycobacterium avium, M. xenopi.*

House Finch (*Carpodacus mexicanus*)

VIRUSES: Alphaviruses EEE and WEE, *Flavivirus* WN.

Corvids (family *Corvidae*)

VIRUSES: *Flavivirus* WN (American crow – Photo 7.81, blue jay – competent hosts). BACTERIA: *Listeria monocytogenes*, *Mycobacterium avium* (rook).

Other birds

VIRUSES: alphaviruses Sindbis (various passerines), EEE and WEE (mainly passerines), Semliki Forest, Mayaro, Ross River and Barmah Forest, flaviviruses JE (bitterns, passerines), WN (sporadically passerines, turtle dove etc.), SLE, MVE (cormorants), Bagaza, Rocio (passerines), Usutu, TBE (forest birds) and KFD, *Orthobunyavirus* Oropouche, orbiviruses Kemerovo (redstart) and Tribeč (chaffinch), *Paramyxovirus* NDV (cormorants in Canada).

BACTERIA: *Chlamydophila psittaci* (reservoir: wild birds of many species and orders), *Staphylococcus aureus*, *Borrelia garinii* (ground-foraging forest birds and sea birds), *Campylobacter jejuni, C. coli*, *C. laridis*, *Salmonella enterica* different serovars, *Yersinia pseudotuberculosis*, *Y. enterocolitica*, *Pasteurella multocida.*

FUNGI: *Cryptococcus neoformans* (some pet birds are "lessors").

PROTOZOA: *Giardia lamblia*, *Toxoplasma gondii.*

MICROSPORIDIA: *Encephalitozoon hellem* (parrots, water birds), *E. intestinalis* (water birds).

7.3 Reptiles (*Reptilia*)

Ectothermic (poikilothermic) vertebrates. Their role as hosts of zoonotic agents is relatively very low.

VIRUSES: alphaviruses WEE (snakes of three genera), VEE, and Mayaro (varan *Ameiva ameiva*, iguana *Tropidurus torquatus*), *Flavivirus* OHF (lizards) and WN (alligators, *Natrix natrix*).

BACTERIA: *Salmonella enterica* – some serovars (snakes), *Borrelia hermsii* and related species (agama), *B. lusitaniae* (lizards), *Listeria monocytogenes*, *Salmonella enterica* (some serovars pathogenic for humans), *Yersinia enterocolitica, Mycobacterium haemophilum.*

FUNGI: *Basidiobolus* (crocodiles).

PROTOZOA: *Trypanosoma brucei rhodesiense*.

7.4 Amphibians (*Amphibia*)

Ectothermic (poikilothermic) vertebrates. Their role as hosts of zoonotic agents is very low.

VIRUSES: alphaviruses WEE (*Rana pipiens*) and Sindbis (*Rana ridibunda*), *Flavivirus* OHF (frogs) and WNV (*Rana ridibunda*).

BACTERIA: *Yersinia enterocolitica.*

FUNGI: *Basidiobolus ranarum.*

PROTISTA: *Rhinosporidium seeberi.*

7.5 Fishes (*Pisces*)

Their role as hosts of zoonotic agents is relatively very low.

BACTERIA: *Neorickettsia sennetsu*, *Yersinia enterocolitica*, enterotoxigenic *Escherichia coli, Salmonella* spp., *Vibrio cholerae, V. parahaemolyticus*, *V. vulnificus*, *V. metschnikovii*, *V. alginolyticus*, *V. harveyi*, *V. furnissii*, *V. mimicus*, *Grimontia hollisae*, *Photobacterium damselae*, *Listeria monocytogenes*, *Erysipelothrix rhusiopathiae, Streptococcus iniae, Clostridium botulinum* types E and F, *C. perfringens*, *Mycobacterium marinum*, *M. haemophilum, M. abscessus, M. ulcerans.*

PROTISTA: *Rhinosporidium seeberi.*

Chapter 8
Systematic Survey of Zoonotic and Sapronotic Microbial Agents

Viruses, bacteria, fungi, protozoa, and metazoa (parasitic worms and arthropods) are among the aetiological agents of zoonoses and sapronoses. Our survey only concerns the agents of microbial diseases (i.e., not the metazoan invasions and infestations), and is arranged according to systematic position. In addition, a new zoonotic disease caused by prions – variant of Creutzfeld-Jakob disease – is included.

Sapronotic agents are marked with an asterisk *, and those agents the source of which can be simultaneously both animals and extraanimal substrates (i.e., the agents of zoonoses plus sapronoses), are marked with an asterisk in parentheses (*). Occasionally also those anthroponotic agents are mentioned, biological vectors of which are haematophagous arthropods −those are labelled with **, while (**) marks the anthroponotic agents which can occasionally also cause a zoonosis under certain circumstance (e.g., avian influenza A virus).

In the paragraph named "Bio-containment", the grades of Biosafety Level (BSL) practices and containment are given, which are related to laboratory work with the particular agent (Anonymous 2007). The scores mean, in short:

BSL-1 – the agent does not usually cause any illness, and standard microbiological practices are required (work on open bench);

BSL-2 – the agent can cause an illness and care is required to control aerosols and contamination (class I or II biosafety cabinets [BSC] are required for aerosol producing procedures; limited access);

BSL-3 – the agent causes a serious illness and it can spread aerogenically; class II BSC required for all manipulations with infectious material; restricted access, air lock facility, controlled uni-directional air flow, exhaust air discharged away from building (with certain viruses via HEPA filtration);

BSL-4 – the agent causes serious, life-threatening disease and it can spread aerogenically, there is no known effective therapy nor vaccination against it; the work with such agent necessitates rigorous containment of all manipulations, change of clothing and shower; class II BSC is adequate only when all laboratory personnel are immunized or insusceptible to the agent, otherwise

class III BSC or hermetically closed positive pressure suits are required; the facility must be equivalent to a separate building with HEPA filtration of all exhaust air, and provided with a double-door autoclave.

For dangerous agents of the bio-containment grade BSL-3, the BSL-2 practices and facilities can be generally used for the less risky nonpropagative laboratory procedures such as serology with inactivated antigens or for staining of fixed (inactivated) impression smears, while BSL-3 practices and facilities have to be used for propagative techniques such as inoculation (and harvesting) of cell cultures, embryonated eggs or experimental animals, the necropsy of infected animals and manipulations with infected tissues. A more detailed information on BSL and risk groups of microbes is given below (CDC 2007).

Classification of infectious microorganisms by risk group

Risk group classification	NIH guidelines for research involving recombinant DNA molecules 2002	World Health Organization laboratory biosafety manual 3rd edition 2004
Risk group 1	Agents that are not associated with disease in healthy adult humans	(No or low individual and community risk) A microorganism that is unlikely to cause human or animal disease
Risk group 2	Agents that are associated with human disease which is rarely serious and for which preventive or therapeutic interventions are *often* available	(Moderate individual risk; low community risk). A pathogen that can cause human or animal disease but is unlikely to be a serious hazard to laboratory workers, the community, livestock or the environment. Laboratory exposures may cause serious infection, but effective treatment and preventive measures are available and the risk of spread of infection is limited
Risk group 3	Agents that are associated with serious or lethal human disease for which preventive or therapeutic interventions *may be* available (high individual risk but low community risk)	(High individual risk; low community risk). A pathogen that usually causes serious human or animal disease but does not ordinarily spread from one infected individual to another. Effective treatment and preventive measures are available
Risk group 4	Agents that are likely to cause serious or lethal human disease for which preventive or therapeutic interventions are *not usually* available (high individual risk and high community risk)	A pathogen that usually causes serious human or animal disease and that can be readily transmitted from one individual to another, directly or indirectly. Effective treatment and preventive measures are not usually available

Biosafety Level 1 is suitable for work involving well-characterized agents not known to consistently cause disease in immunocompetent adult humans, and present minimal potential hazard to laboratory personnel and the environment. BSL-1 laboratories are not necessarily separated from the general traffic patterns in the building. Work is typically conducted on open bench tops using standard microbiological practices. Special containment equipment or facility design is not required, but may be used as determined by appropriate risk assessment. Laboratory personnel must have specific training in the procedures conducted in the laboratory and must be supervised by a scientist with training in microbiology or a related science.

Biosafety Level 2 is suitable for work involving agents that pose moderate hazards to personnel and the environment. It differs from BSL-1 in that (1) laboratory personnel have specific training in handling pathogenic agents and are supervised by scientists competent in handling infectious agents and associated procedures; (2) access to the laboratory is restricted when work is being conducted; and (3) all procedures in which infectious aerosols or splashes may be created are conducted in BSCs or other physical containment equipment.

Biosafety Level 3 is applicable to clinical, diagnostic, teaching, research, or production facilities where work is performed with indigenous or exotic agents that may cause serious or potentially lethal disease through inhalation route exposure. Laboratory personnel must receive specific training in handling pathogenic and potentially lethal agents, and must be supervised by scientists competent in handling infectious agents and associated procedures. All procedures involving the manipulation of infectious materials must be conducted within BSCs (preferably Class II or Class III), or other physical containment devices, other physical containment devices, or by personnel wearing appropriate personal protective equipment. The laboratory must be separated from areas that are open to unrestricted traffic flow within the building.

Biosafety Level 4 is required for work with dangerous and exotic agents that pose a high individual risk of life-threatening disease, aerosol transmission, or related agent with unknown risk of transmission. Agents with a close or identical antigenic relationship to agents requiring BSL-4 containment must be handled at this level until sufficient data are obtained either to confirm continued work at this level, or re-designate the level. Laboratory staff must have specific and thorough training in handling extremely hazardous infectious agents. Laboratory staff must understand the primary and secondary containment functions of standard and special practices, containment equipment, and laboratory design characteristics. All laboratory staff and supervisors must be competent in handling agents and procedures requiring BSL-4 containment. Access to the laboratory is controlled by the laboratory supervisor in accordance with institutional policies. There are two models for BSL-4 laboratories:

(1) A *Cabinet Laboratory* where all handling of agents must be performed in a Class III BSC.

(2) A *Suit Laboratory* where personnel must wear a positive pressure protective suit.

BSL-4 Cabinet and Suit Laboratories have special engineering and design features to prevent microorganisms from being disseminated into the environment. The BSL-4 cabinet laboratory consists of either a separate building or a clearly demarcated and isolated zone within a building. For BSL-4 containment agents, the BSL-3 laboratory procedures can be sometimes used when all personnel is effectively vaccinated against the disease and stringent laboratory protocols are applied (example: TBE virus).

8.1 Prions

Since 1986, an explosive epizootic of bovine spongiform encephalopathy (BSE) has occurred in the UK: about 166,000 cattle were affected up to 1997, with peak incidence between 1992 and 1993. According to the main symptom – loss of neuromuscular coordination – the syndrome has informally been called "mad cow disease". It originated from the use of ovine meat-bone meal added to the fodder of cattle; some of the source sheep cadavers had been infected with scrapie, another spongiform encephalopathy, known and described in Great Britain since 1732. Scrapie was widely dispersed in Britain by the mass use of a vaccine against louping ill (1938 and later) unintentionally contaminated with scrapie. In cattle a disease similar to scrapie was never observed before 1986; in the 1980s, scrapie obviously overcame the species barrier and adapted to cattle (a "host-jumping event").

The infectious agent in BSE and in similar "slow" or "unconventional" diseases, called generally transmissive spongiform encephalopathies (TSE), with an incubation period usually over 3 years in diverse mammals (mink, cat, deer) including man (Creutzfeld-Jakob disease, or kuru) are so-called prions (Stanley B. Prusiner 1982: "**pro**teinaceous **in**fectious particles", a modified acronym), causing vacuolisation of the brain tissue that looks like a sea sponge or an Emmental cheese. It is interesting that the prion PrP^c is a physiological normal protein in the CNS, which however can change its conformation (spatial configuration) from the molecule with a prevailing α-helix structure to the pathogenic β-folded flat structure PrP^{Sc} (the index Sc means scrapie). Following this structural rearrangement, the molecule becomes hydrophobic and resistant to disintegration. An accumulation of these PrP^{Sc} prions in the CNS leads to the subsequent destruction of neurons. The pathological isoform of the prion posseses several other quite unusual characteristics: it is non-antigenic (no humoral antibodies are produced against the prion), resistant against proteases (this is why it accumulates in tissue not being metabolised), high temperature (it does not inactivate at 121°C for 60 min nor at 240°C for 1 min) and common disinfection liquids (peracetic acid, phenol, alcohols, formalin, ultraviolet and microvave irradiation), while undiluted sodium hypochlorite, 4% NaOH, or autoclaving at 134°C for 60 min are effective.

8.1.1 Prion vCJD

Creutzfeld-Jakob disease (CJD) was described in 1920–1921. It is a rare, but absolutely fatal disease with a mean incidence of about 0.4 cases per 10 million population, usually hereditary but there is also a transmissible form e.g. during corneal transplantation or on application of hypophyseal growth hormone. A new variant of human CJD (abbreviated as "vCJD") has been observed in Britain since 1994, differing from classic CJD by a substantially shorter incubation period (months rather than years), a much higher incidence rate (the original inter-annual increase of the incidence was 20–30%) and in also affecting young people. This vCJD was detected disproportionately more often in families of farmers in areas where BSE was endemic and after contact with diseased cattle. There is a justified hypothesis that vCJD is, in fact, BSE transmitted to humans, it means a zoonosis. This hypothesis has been promoted by results of several experiments with BSE on animals (monkeys and mice). The CNS, bone marrow, neural ganglions, ileum, retina, and so-called mechanically-recovered meat (remnants of meat detached from backbone and ribs, used for preparation of hamburgers, sausages and minced meat in tins for humans and pet animals) are regarded as risky bovine tissues for consumption. Until August 2010, "only" 212 people had died of vCJD (against the originally expected many hundreds or thousands of victims), out of which 43 patients were from outside Great Britain (France 25 cases, Ireland 6, the Netherlands 2, Spain 1, Portugal 1, Italy 2, Canada 1, USA 3, Japan 1, and Saudi Arabia 1). The epidemic was efficiently controlled by drastic veterinary measures: culling, and the ban on feeding meat-bone meal to cattle.

> Bio-containment: BSL-2.
> Diagnosis of transmissive spongiform encephalopathies is carried out using electroencephalography, CT, histopathology, immunohistochemistry, molecular biology (PCR), ELISA and WB.

8.2   Viruses

Most zoonotic viruses have an RNA genome, although some zoonoses are also caused by DNA herpetic and pox viruses. Numerically most common viral agents of zoonoses are arboviruses. The acronym arbovirus (for "**ar**thropod-**bo**rne **virus**", coined by W. M. Hammon in 1958) was suggested by W. C. Reeves, and recommended for general use by the WHO in 1960. Arboviruses do not constitute a taxonomic unit, but an ecological group with a common mode of transmission to vertebrates. Their existence in nature is conditioned by replication in blood-feeding (haematophagous) arthropods and by an interaction between these arthropods and vertebrates. It is important that transmission from the vector arthropod to the recipient vertebrate is biological, not mechanical. The ability of replication in two phylogenetically and physiologically diverse organisms – poikilothermic arthropods

and homeothermic vertebrates – and, at the same time, at largely varying ambient temperatures, is a remarkable characteristic and one that is in other viruses unusual.

For the transmission of arboviruses by competent arthropod vectors the following conditions must be fulfilled:

(1) the vector is infected by feeding on a viraemic vertebrate host or during co-feeding with infected vectors,
(2) the infectious dose must pass to the vector's gut lumen,
(3) the virions are adsorbed on the membrane of epithelial gut cells, penetrate into them by endocytosis or fusion of the virion envelope with the cell membrane, and replicate intracellularly,
(4) infectious virions disseminate from epithelial gut cells in the haemocel of the vector,
(5) they further migrate into salivary glands,
(6) the virions are transmitted in saliva by feeding of the vector on a susceptible host.

Nearly 500 arboviruses are registered (*International Catalogue of Arboviruses*) at present. They belong to 9 families: *Bunyaviridae* (52% of all arboviruses), *Reoviridae* (17%), *Flaviviridae* (12%), *Rhabdoviridae* (10%), *Togaviridae* (6%), *Orthomyxoviridae*, *Poxviridae*, *Asfarviridae* and *Nodaviridae* (the latter four all less than 1%). However, only about 100 arboviruses have been reported as pathogenic to humans.

The diagnosis of arbovirus and other virus infections is largely based on serological laboratory analysis, optimally by using paired blood serum samples taken at an interval of 2–3 weeks. Recent infection is said to be demonstrated when the patient seroconverts between the two intervals (i.e., the 1st sample is without antibodies, while the 2nd sample contains antibodies to a particular virus) or when there is an at least fourfold increase of antibody titre against the virus, as revealed in different serological tests, e.g. ELISA, HIT, CFT, VNT, IFA etc. (Lennette, Schmidt et al. 1974). If there is only one (convalescent) serum sample of the patient available, the differentiation of recent infection from a past one can be done by testing for IgM and IgG immunoglobulins – in recent infections IgM antibodies prevail over IgG. A very significant, but difficult and time-consuming technique is the isolation of the virus agent from the blood, CSF or bioptic samples of the patient using inoculation of suckling mice, cell cultures or chicken embryos. In the last decade, isolation techniques have started to be intensively replaced by molecular techniques detecting specific nucleic acids of particular viral agents: traditional or real-time PCR and RT-PCR, nested (RT-)PCR, RLB, sequencing, and others. These methods are excellent tools, although they also have some specific limitations (e.g., they do not show whether the nucleic acid fragments originate from a viable virus). Various immunohistochemistry techniques are also very convenient for the detection of viruses in the vertebrate tissues, and usually they are highly specific.

Specific therapy for viral diseases usually does not exist, and therefore symptomatic treatment, rest in bed, a sufficient supply of fluids, infusions and antipyretics

are recommended; in certain cases antiserum (a specific immunoglobulin) may help, when applied immediately after infection. Only a few virus diseases can be treated with antivirals such as nucleotide analogues, e.g. ribavirin in RNA virus infections, or acyclovir in some DNA viruses. The optimum specific measure against virus infection is vaccination. Unfortunately, very few vaccines are available against zoonotic virus diseases at present: YF, JE, TBE, WEE/EEE/VEE, RVF, and lyssa.

8.2.1 Family Togaviridae

Alphaviruses EEE, WEE, VEE (American Equine Encephalomyelitides)

Virions of the genus *Alphavirus* are spherical (60–70 nm), enveloped, contain one molecule of ss(+)RNA sized 10–12 kbp, and 2–3 surface glycoproteins E1, E2 (E3). Alphaviruses occurring in New and Old World differentiated phylogenetically some 2–3 thousands of years. The first virus of this group (WEE) was isolated from a dead horse in 1933.

> Source of infection (natural host range): birds (EEE, WEE: house sparrow, house finch, and others; less in VEE), horse, rodents (VEE and EEE: *Peromyscus*, *Oryzomys*, *Proechimys*; WEE: *Spermophilus*, *Sciurus*, *Microtus*), leporids (WEE: *Lepus californicus*), marsupials (VEE, EEE), bats (VEE), snakes and frogs (VEE, WEE). Migratory birds can transport EEE virus.
>
> Animal disease: encephalomyelitis in horses (EEE, WEE, VEE), some other mammals, and in pheasants (EEE).
>
> Transmission mode (Figs. 8.1, 8.2): culicine mosquitoes, less other arthropods. Main cycles in nature: sylvatic (endemic) and urban (epidemic, synanthropic): **WEE** – *Culex tarsalis* and *Ae. melanimon* (but also *Cx. pipiens, Cx. quinquefasciatus, Cx. restuans, Ae. dorsalis, Ae. nigromaculis, Culiseta inornata, An. freeborni* as secondary or occasional vectors) + birds as vertebrate hosts (house sparrows in the urban epidemic cycle); *Ae. melanimon* acts as a bridge vector of WEE virus → horse and man; reptiles may also host the virus (virus overwintering in them is possible). In **EEE**, the main vector is *Culiseta melanura* (also *Cx. salinarius, Ae. mitchellae*, *An. crucians*, *Cx. restuans* as secondary vectors) + birds as vertebrate hosts; *Ae. sollicitans*, *Ae. vexans*, and *Coquillettidia perturbans* act as bridge vectors of EEE → man and horse. **VEE** (including the enzootic subtypes Mucambo, Tonate, Everglades, and Pixuna) is vectored by *Ae. taeniorhynchus* (primary vector), *Psorophora columbiae, P. discolor*, *Culex* spp*., An. crucians*, *Coquillettidia perturbans, Mansonia titillans* (*Coquillettidia* and *Aedes* spp. in epidemic cycle) + rodents. Congenital infections have also been described (foetal abnormalities, abortion).
>
> Human disease: <u>EEE, WEE, VEE</u> – fever (also chills, headache, body aches, lethargy, nausea, vomiting, prostration, inflammation of throat, cervical

Fig. 8.1 Natural cycle of WEE and EEE viruses (*Cx. tarsalis* is the principal vector of WEE, while *Cs. melanura* in EEE) (drawing by Ivo Rudolf)
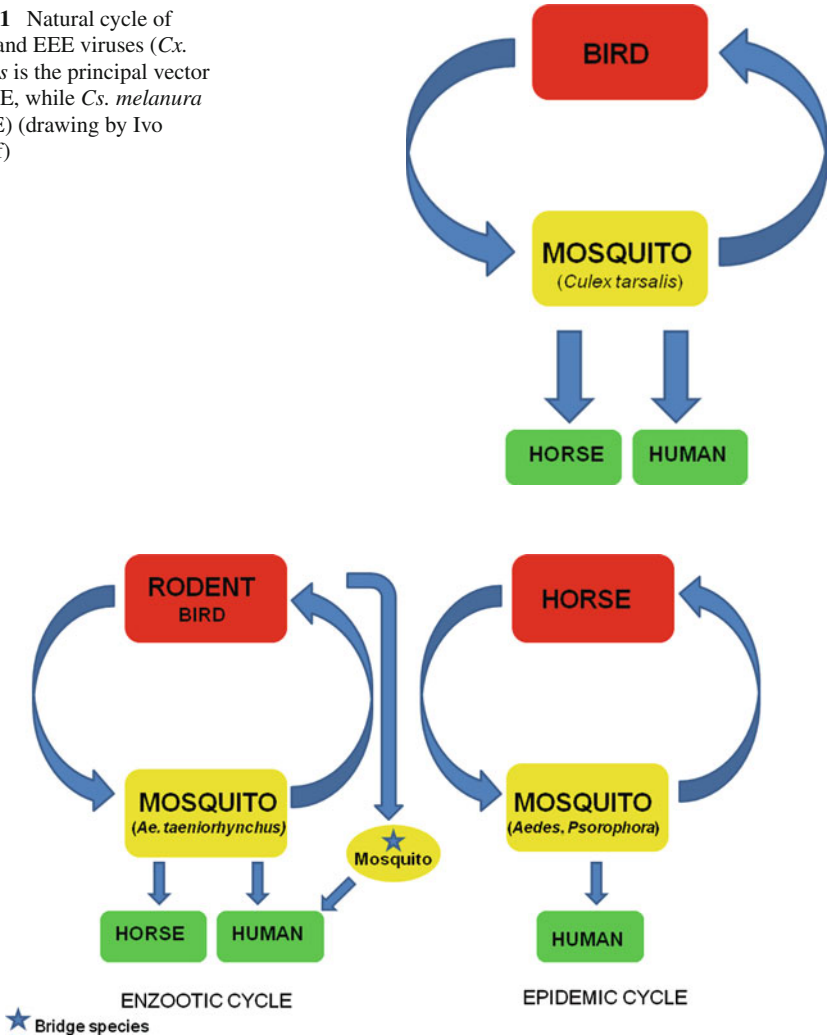
Fig. 8.2 The cycles of VEE virus (drawing by Ivo Rudolf)

lymphadenitis, dizziness, encephalitis). The highest fatality rate has been observed in EEE (35–75%, against about 20% in WEE), and persistent sequelae (paralysis, pareses) are comparatively frequent, especially in children – some of them may remain mentally deficient. A total of 221 humans cases of EEE were confirmed in the USA in 1964–2005. South-American strains of EEE virus are obviously less virulent, only 2 fatal cases have been reported. WEE epidemics: 1941 USA (3,000 human cases); 1964–1997, USA a total 639 cases. A major outbreak of VEE occurred in Venezuela and Columbia in 1962–1964 when about 30,000 persons were ill

and 300 of them died, a smaller one in Mexico and Texas in 1971 (84 cases), and a huge one in 1995 in Columbia and Venezuela with 75,000–100,000 cases.

Bio-containment: BSL-2 (WEE, EEE), BSL-3 (VEE: a high risk of laboratory infections).

Prevention: there are mono-, bi- and tri-valent inactivated vaccines (horse, human), their use is however limited (usually applicable for laboratory personnel).

Geographical distribution: North, Central and South America. However, only the less virulent subtypes of VEE occur in North America (Tonate, Everglades). Rio Negro and Pixuna viruses are other subtypes of VEE, occurring in Argentina and Brazil, respectively. Brand new data indicate that EEE virus strains that circulate in Central and South America might represent a distinct species compared to North American strains, needing a possible reclassification of EEE (J. Virol. 84: 1014, 2010).

Alphavirus Chikungunya (CHIK)

Antigenically related with the next alphavirus; three genotypes of CHIK virus are differentiated: West African, and South/Central/East African, and Asian. In Svahili language, "chikungunya" means "something what causes people becoming bended".

Source of infection (natural host range): mammals (wild primates, bats), man.

Animal disease: asymptomatic course.

Transmission mode (Fig. 8.3): culicine mosquitoes (*Aedes aegypti, Ae. albopictus*, *Mansonia* spp.). The natural cycles are: (a) sylvatic (gray vervet monkeys, baboons + canopy mosquitoes *Ae. africanus*, *Ae. furcifer*, *Ae. taylori*); (b) urban (synanthropic: man + *Ae. aegypti*, *Ae. albopictus*, *Culex pipiens fatigans*).

Human disease: chikungunya fever with a sudden onset and chills, strong headaches, severe arthralgia, myalgia, backache, nausea, vomiting, erythema on face and trunk, up to maculopapular rash, lymphadenitis, photophobia,
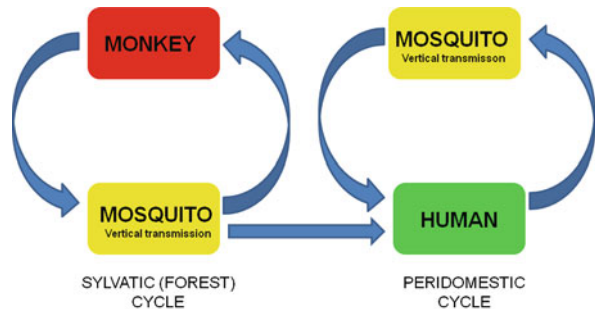
Fig. 8.3 The cycles of chikungunya virus (drawing by Ivo Rudolf)

sometimes (e.g. with Asian strains) haemorrhages; fatality rate is very low, but arthralgia may develop into severe arthritis and persist for several months (even years). Epidemics of CHIK: 1952–1953, first recognized outbreak in eastern Africa; 1955, another big epidemic in Africa; 1964–1965, major outbreaks in India (at least half a million of cases) and Thailand; 2001–2003, a number of smaller outbreaks in Indonesia; 2004, an epidemic in Kenya; 2005–2006, a huge outbreak (about 280,000 cases including 213 fatalities) on islands of Indian Ocean which started on the Comoros and Réunion Island where 255,000 persons out of 775,000 inhabitants were infected (about 130 cases have been still reported in 2010); many additional cases were reported on the Seychelles archipelago (9,000), Mayotte (5,800), Mauritius (6,000), Madagascar, and continued in India (Andhra Pradesh, Karnataka, Maharashtra – with a total of about 1.4 million of cases), later it also struck Sri Lanka, Malaysia, Thailand, Indonesia (even in 2009–2010 >12,000 people ill in province Lampung, SE. Sumatra), Madagascar and continental Africa and, moreover, tens of human cases (tourists etc.) were imported in Europe and North America from Asia; the vector in this epidemics was *Ae. albopictus*, and in India *Ae. aegypti*. In August and September 2007 broke out the first European epidemic (about 330 cases, 204 were laboratory-confirmed) of chikungunya fever – in northern Italy (in the town Ravena and environs); the index case was a resident returning from India (he picked up the infection in Kerala, and arrived on 21 June); the local vector in Italy was *Ae. albopictus,* a mosquito species introduced into the area several years ago.

Bio-containment: BSL-3.

Geographical distribution: tropical Africa (Uganda, Kenya, Tanzania, Mozambique, Zimbabwe, Malawi, Senegal, DR Congo), southern and SE. Asia (Photo 5.14).

Alphavirus O'nyong nyong (ONN)

Antigenically closely related to CHIKV. **Igbo-Ora** virus, isolated from febrile patients in Nigeria, is now considered a variant of ONN virus. In Svahili language, "o nyong nyong" means a "grinder or crusher/breaker of joints".

Source of infection (natural host range): mammals (wild primates, bats), man.

Animal disease: asymptomatic course.

Transmission mode: *Anophelinae* mosquitoes (*Anopheles funestus*, *An. gambiae*). The natural transmission of the virus also includes sylvatic and urban cycle as in chikungunya.

Human disease: o'nyong nyong fever, clinically indistinguishable from CHIK. Epidemics of ONN: 1959–1961, 2 million people infected in eastern Africa (Uganda, Kenya, Tanzania, Zambia); 1996–1997, a return of ONN after 35 years in Uganda, with hundreds of ill persons (60–80% of population infected); the epidemic later spread into neighbouring Tanzania; 2004, a smaller outbreak in Kenya.

Bio-containment: BSL-2.

Geographical distribution: tropical Africa (Uganda, Kenya, Tanzania, Zambia, Mozambique, Malawi, Senegal, DR Congo).

Alphavirus Semliki Forest

Synonym or a subtype: Zingilamo virus.

Source of infection (natural host range): rodents, insectivores (*Atelerix albiventris*), wild birds.

Animal disease: asymptomatic course.

Transmission mode: mosquitoes *Aedes* spp., *Culex pipiens*.

Human disease: febrile illness with severe persistent headache, myalgia, arthralgia, occasional encephalitis; weakness in convalescence. Numerous human cases have been observed.

Bio-containment: BSL-3.

Geographical distribution: tropical Africa.

Alphavirus Mayaro

Recent molecular analyses have recognized two lineages: genotypes D and L.

Source of infection (natural host range): mammals (largely monkeys – *Alouatta*, *Colobus*, *Pithecia*, *Saimiri*, *Callithrix*, and rodents), birds, reptiles (*Ameiva*, *Tropidurus*).

Animal disease: asymptomatic course.

Transmission mode: *Culicinae* mosquitoes (*Haemagogus* etc.). Natural (sylvatic) cycle: between mosquitoes and monkeys.

Human disease: Mayaro fever – dengue-like illness with sudden high fever, headache, severe arthralgia (lasting for several weeks and affecting principally ankles, wrists, and toes, but occasionally also major joints), arthritis, oedema, myalgia and rash; retroorbital pain, vomiting; fatal cases have not been reported. On average, 20–50% of Indians in the Amazon river basin have antibodies to the virus. In 1955, the first epidemic was observed in Brazil and Bolivia, and since that time other three big and a number of smaller outbreaks have been reported – the last one in Belém, northern Brazil, with 105 patients, and a recent outbreak (2010) in Venezuela affected 77 people.

Bio-containment: BSL-3.

Geographical distribution: South America (Trinidad, Surinam, French Guyana, Columbia, Panama, Brazil, Peru, Bolivia, Venezuela).

Alphavirus Sindbis (Synonyms: Ockelbo, Pogosta, Karelian Fever Virus; Babanki: An African Subtype)

This virus is related to the American equine encephalitis viruses, it belongs to the WEE antigenic group.

Source of infection (natural host range): birds (e.g. *Turdidae*), less often mammals (e.g. rodents, marmoset *Callithrix*) or amphibians.

Animal disease: inapparent course (sometimes symptoms in pigeon, chicken and rodents).

Transmission mode: largely mosquitoes (*Culex* spp. – e.g. *Cx. univittatus*, *Cx. pipiens*, *Cx. torrentium*, *Culiseta* spp.). Principal cycle in nature: between ornithophilic mosquitoes and birds (for instance, 27% of resident tetraonids *Tetrao urogallus*, *T. tetrix*, *Bonasa bonasia*, *Lagopus lagopus* in Scandinavia were found with antibodies in 2003).

Human disease: <u>Sindbis fever</u> (syn. <u>Karelian/Ockelbo/Pogosta fever</u> in Fennoscandia) with headache and arthralgia or arthritis, rash on thorax and extremities ("fever-arthritis-rash" triade); no fatal cases have been reported, but convalescence is long (the joints may be painful for months or even years; arthralgia, arthritis and rheumatic symptoms are observed in nearly 25% of patients for up to 3 years after the acute disease). Interestingly, the epidemics of Pogosta disease have repeated at least since 1974 (first noted epidemic) nearly regularly at the interval of 7 years, in a coincidence with the population cycles of tetraonid birds. In summary, extensive epidemics were recorded in 1974, 1981 (thousands of cases in Scandinavia and Karelia), 1988, and 1995. A total of 2,183 laboratory-confirmed cases were reported during 1981–1996. The last large epidemic in Finland took place in 2002 with almost 600 reported cases, while in 2009 only 105 laboratory-confirmed cases were reported. Seasonal peaks of the disease in Scandinavia are in the months August–September. Relatively frequent are Sindbis fever cases in African countries.

Bio-containment: BSL-2.

Geographical distribution: nearly worldwide (except for Americas).

Alphavirus Ross River

The RR virus was first isolated from mosquitoes in Australia in 1963.

Source of infection (natural host range): mammals (kangaroo *Macropus agilis*, brushtail possum *Trichosurus vulpecula*; rats; horse), birds (*Grallina cyanoleuca, Microeca fascinans*).

Animal disease: inapparent course.

Transmission mode: mosquitoes *Aedes vigilax* (coastal areas, TOT), *Ae. tremulus* (TOT), *Culex annulirostris* (inland), but also *Verrallina funerea* (competent vector*), Ae. camptorhynchus, Ae. normanensis, Ae. polynesiensis, Ae. alterans, Ae. bancroftianus, Ae. daliensis, Ae. flavifrons, Ae. funereus, Cx. quinquefasciatus, Cx. australicus, Cx. sitiens, Coquillettidia linealis, Anopheles amictus, An. annulipes,* and other species.

Human disease: <u>epidemic polyarthritis</u>, with fever, headache, malaise, arthralgia (the joint pain involves fingers, toes, ankles, knees and elbows), myalgia,

rash, apathy, lymphadenitis, affection of kidneys (haematuria, glomeru-lonephritis), splenomegalia; fatalities have not been reported; persistent or recurrent arthralgia and arthritis (polyarthritis) and lethargy can occur for up to 6–12 months. Ross River fever is the most frequent arboviro-sis in Australia, with a mean incidence of about 4,800 patients annually (for instance 47,500 cases were reported in the period 1991–2000). Five big epidemics of RR occurred: in 1927/1928; during the 2nd WW; 1956; 1979/1980 an explosive outbreak, with >65,000 persons affected on the Polynesian islands and archipelagos of Fiji, Samoa, New Caledonia, Cook and Tonga, and 2003/2004 (re-emergence on Fiji). Diagnostics and treat-ment of patients with RR fever costs Australia annually 2.7–5.6 million dollars. Risk factors for outbreaks include among others heavy rain-falls and higher maximum tides, causing increased mosquito population densities.

Bio-containment: BSL-2.

Geographical distribution: Australia, New Guinea, Polynesia (Solomon Islands, New Caledonia, Fiji, American Samoa, Tonga, Cook Islands).

Alphavirus Barmah Forest

The virus was first isolated from mosquitoes in the Barmah Forest in Victoria, Australia, 1974.

Source of infection (natural host range): mammals (e.g. kangaroo *Macropus agilis*), birds.

Animal disease: inapparent course.

Transmission mode: mosquitoes *Aedes vigilax* (competent vector), *Culex annulirostris*, *Ae. funereus, Ae. camptorhynchus, Ae. normanensis, Ae. ban-croftianus*, *Ae. eidsvoldensis*, *Cx. globocoxitus, Verrallina funerea* (compe-tent vector)*, Anopheles amictus, An. annulipes,* and other species.

Human disease: epidemic polyarthritis, with symptoms similar to those caused by Ross River virus – fever, headache, arthralgia, myalgia, rash (the rash more marked than in RR), apathy, lymphadenitis, affection of kidneys (haematuria, glomerulonephritis), splenomegalia; fatalities have not been reported; persistent arthralgia and polyarthritis occur for shorter time than in RR (a few months at maximum). First big epidemic of Barmah Forest arthritis was reported in 1993/1994 (but before, in 1992, also a number of cases occurred), and a total of 1,084 cases were reported between 1992 and 1999 (at the same time, the number of Ross River fever patients attained 5,863); a major epidemic occurred in 2005/2006, with 1,895 reported cases.

Bio-containment: BSL-2.

Geographical distribution: Australia.

8.2.2 Family **Flaviviridae**

8.2.2.1 Mosquito-Borne Flaviviruses

(**)*Flavivirus* **Yellow Fever (YF)**

Virions of the genus *Flavivirus* [Lat. *flavus,* yellow] are spherical (40–50 nm), enveloped, they contain one molecule of ss(+)RNA sized 10–12 kbp, and surface proteins E and M. This genus involves 75 viruses of 9 antigenic groups, most of them being transmissible by arthropods (mosquitoes and ticks). The strains of YF virus can be divided in Ethiopian and Neotropical (Theiler and Downs 1973), and according to sequencing analyses of genes for protein E in 3 genotypes: IA – West-African, IB – South-American, II – East-African. More detailed classifications differentiate even 7 genotypes.

> Source of infection (natural host range): monkeys (species of the genera *Colobus*, *Cercopithecus*, *Erythrocebus*, *Cercocebus*, baboons, probably also galagoes in Africa, while *Alouatta*, *Ateles*, *Saimiri*, *Pithecia*, and *Callithrix* in South America), other mammals of tropical forests, opossum (*Didelphis marsupialis*); man (the main amplifying host of the virus in the urban cycle).
> Animal disease: inapparent course (however, some species of New World monkeys die after experimental inoculation).
> Transmission mode (Fig. 8.4): mosquitoes – *Aedes aegypti* (first demonstrated by the Walter Reed commission in 1902; TOT in *Aedes* spp. mosquitoes was demonstrated later) transmission to humans in the urban cycle, while "canopy" mosquito species *Haemagogus* spp. (in Brazil e.g. *H. janthinomys*) and *Aedes* spp. (in Africa e.g. *Ae. africanus*, *Ae. simpsoni*) in the sylvatic (jungle) cycle between wild primates of the jungle ecosystem. In the sylvatic cycle, man acquires infection accidentally during occupational (e.g., hunters) or recreational (tourism) activities. In Africa, an intermediate (savannah) cycle involves transmission of YF from tree hole-breeding *Aedes* spp. (e.g., *Ae. opok*, *Ae. luteocephalus*) to humans living or working in jungle border areas. For typical habitats of sylvatic YF, see Photos 5.1 and 5.2.
> Human disease: yellow fever – high fever with chills, headache, backache, vomiting (in severe cases black vomit containing blood), haemorrhages, necrotic hepatitis (YF virus reveals a marked hepatotropism) with icterus, nephritis, albuminuria, sometimes also encephalitis; fatality rate is 5–40%. YF was introduced in the Caribbean from western Africa during the slave trade (introduction of viraemic persons and infected *Ae. aegypti* mosquitoes on slave ships): the first extensive epidemic occurred here in 1647–1648 and involved Little Antilles (Barbados, St. Cristof, Guadeloupe), Yucatan and Cuba (in Havana one-third of citizens died); epidemics occurred frequently in Central America also in the eighteenth and nineteenth centuries. In 1741 and 1802–1803, big outbreaks of YF were reported in Portugal and Spain (80,000 victims – also an import on ships, with a consequent spread from seaports to inland); in 1793, 15% citizens of Philadelphia died on YF; in 1853, 8,000 persons from 29,000 infected died in New Orleans; 1900, Havana:
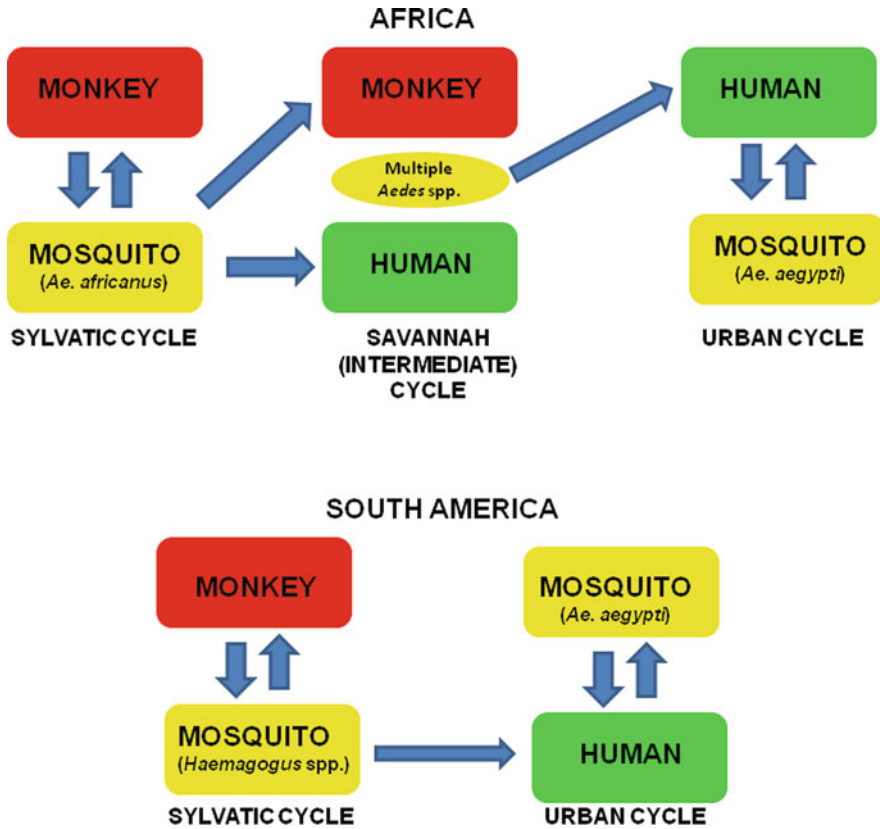
Fig. 8.4 Different cycles of Yellow fever virus (drawing by Ivo Rudolf)

Walter Reed Commission examined YF in Havana and evaluated the transmission mode of this disease; 1901, eradication of the *Ae. aegypti* vector of YF in Havana (Gorgas), later also in the Panama Canal area; since 1940 periodic epidemics have occurred in eastern Africa: 1940, Sudan 15,000 cases; 1951, Ethiopia 100,000 cases (30,000 people died); 1959, Sudan 1,800 cases; 1960–1962, Ethiopia 300,000 cases; 1965, Senegal 20,000 cases; 1969, Ethiopia 2,200 cases, and also a major epidemic in West Africa (Nigeria, Mali, Burkina Faso, Togo, Ghana); 1978 Gambia 8,400 cases (1,600 fatal); 1987, Nigeria 1,450 cases (565 fatal); 2000, Guinea 688 cases (225 fatal); 2005, Sudan 600 cases. 1998–2002, Brazil a total of 251 cases (96 fatal). According to the WHO data of 2000, mean annual incidence of YF in Africa is about 180,000 cases (of them about 27,000 fatal), elsewhere *c.* 20,000 cases (3,000 fatal).

Bio-containment: BSL-3.

Prevention: attenuated vaccine (attenuated clonal strain 17D prepared by Max Theiler) is recommended for travel in endemic tropical countries.

Geographical distribution: tropical Africa and America (in the urban cycle mainly seaport areas).

(**)*Flavivirus* **Dengue 1–4**

Four virus species (sometimes regarded as serotypes), each involves several geno-
types. The name of dengue is a Spanish homonym derived from the Svahili "ki
denga pepo", meaning "sudden convulsive attacks (cramplike seizure) caused by
an evil spirit". First recognized (identified) in the 1950s during epidemics in the
Philippines and Thailand.

> Source of infection (natural host range): man (the main amplifying host of the
> virus), exceptionally monkey and other primates. Interestingly, RNAs repre-
> senting the four DENV serotypes were detected in the livers and/or sera of
> wild mammals (rodents, marsupials, bats) caught in neotropical forests.
> Animal disease: inapparent course, sometimes pathogenic for monkeys.
> Transmission mode: mosquitoes *Aedes aegypti*, *Ae. albopictus* (TOT and sex-
> ual transmission in the mosquitoes), *Ae. niveus* in Malaysia, *Ae. scutellaris*
> in Polynesia, and other species; blood transfusion. Sylvatic cycle occurs in
> southeastern Asia – monkeys (*Macaca* and *Presbytis* spp.) + *Ae. niveus*; West
> Africa (Senegal, Nigeria – DEN-2) – monkeys *Erythrocebus patas* + *Ae. fur-
> cifer*, less often other species of *Aedes* (*Ae.africanus*, *Ae. taylori* etc.). For a
> habitat, see Photo 5.6.
> Human disease: dengue, usually biphasic fever with an intense headache, myal-
> gia, arthralgia, acute pain of eyes (retroorbital pressure), stiff neck, rash,
> nausea, insomnia; convalescence long (fatigue and depression for several
> weeks). Much more severe form of the disease is the dengue haemorrhagic
> fever (DHF), sometimes combined with syndrome of haemorrhagic shock
> (petechial bleeding into the skin and visceral organs) and fatality rate
> 5–20%. DHF manifests most frequently when infection with DEN-2 virus
> follows about 20 years after primoinfection with DEN-1 virus (demon-
> strated in Cuba). Infection with one dengue serotype results in a long-term
> immunity against reinfection with the same type, there is, however, no
> cross-protection among other serotypes (i.e., a person could experience four
> independent infection). Dengue is an important tropical pandemic, com-
> parable to malaria. Epidemics: 1897–1902 Australia; 1927/1928 Greece
> (650,000 persons from 704,000 citizens in Athens and Piraeus became
> ill); 1931 Tchaiwan; 1943–1944 Pacific islands and New Guinea – a fre-
> quent disease of American soldiers (a major health problem during war
> operations); 1954 the Philippines; 1980–1987 South and Central America
> recorded 40,000−390,000 cases annually (the Cuba alone reported >500,000
> cases of DEN-1 in 1977, >400,000 cases of DEN-2 (DHF 10,000, 158
> patients died on DHF shock) in 1981); and 5,208 cases of DEN-2 (DHF
> 205 cases, 12 patients succumbed) in 1997. Brazil reported 56,000 patients
> in 1994, 530,000 in 1998, and 290,000 in 2002 (all four dengue serotypes).
> In Argentina, dengue re-emerged in 1997, after the last epidemic in 1917.
> Dengue epidemics are less frequent in Africa, but the activity has increased
> also here: 1977 Seychelles; 1982 Kenya (DEN-2); 1985 Mozambique (DEN-
> 3); 1982 and 1993 Somalia (DEN-2); 1994 Saudi Arabia (DEN-2). Epidemic

occurrence of dengue is also known from India, Singapore, Malaysia and elsewhere in tropical countries of Asia and Oceania. 1998 south Vietnam 120,000 cases (including DHF), 342 of them fatal. According to WHO, dengue occurs in >100 tropical and subtropical countries at present, about 40% of the world population is at risk, and approximately 50 million people are infected annually, 300–400 thousands have DHF and 22,000 die. Recent expansion of DEN-3 was reported in West Africa (Cape Verde Islands, 2009, more than 17,000 cases). According to WHO reports, about 160 per 100,000 tourists visiting endemic areas acquire dengue infection and export it worldwide annually.

Bio-containment: BSL-2.

Vaccine: not available at present.

Geographical distribution: the whole tropical and subtropical belt – largely southeastern Asian region (original area), Africa, and Central and South America, less often Mexico and southern Texas (2004), and since the 1990s also northeastern Australia.

Flavivirus **Japanese Encephalitis**

JE virus forms the antigenic group JE together with WN, SLE, MVE and some other viruses.

Source of infection (natural host range): birds (reservoir: colonial ardeids), pig (amplifier host), horse, bats.

Animal disease: abortions in swines, horse encephalitis (China).

Transmission mode (Fig. 8.5): mosquitoes of the genus *Culex*: *Cx. tritaeniorhynchus* (Japan, China – a common species in rice fields), *Cx. pipiens* group (*Cx. pallens*, *Cx. quinquefasciatus*,), *Cx. gelidus* (a zoophilic species – Malaysia), *Cx. vishnui*, *Cx. pseudovishnui*, *Cx. japonicus*. Many of them are species breeding in rice fields.
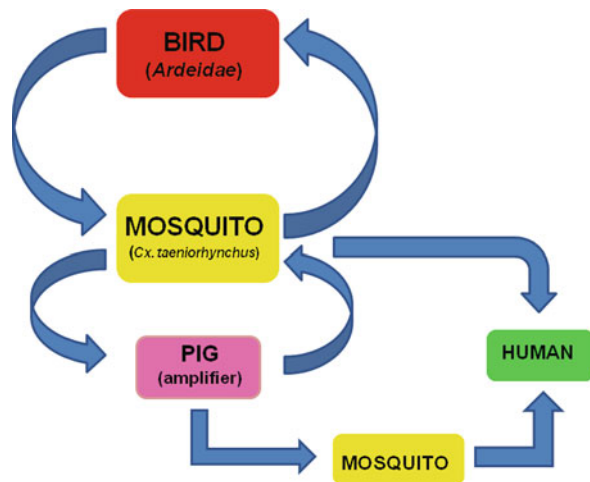
Fig. 8.5 The cycles of Japanese encephalitis virus (drawing by Ivo Rudolf)

Human disease: Japanese encephalitis with fever, headaches, myalgia, decreased consciousness, convulsions, pareses, aggravated breathing; fatality rate 20–40%; common are severe irreversible sequelae in 30–40% of patients (and even higher proportion among children). The first recorded extensive JE epidemic occurred in 1924, with 6,125 cases including 3,197 deaths. The present incidence in Asia is thousands of patients yearly: in the 1990s it was on average 45,000 (about 10,000 fatal), while in the last years 20–50 thousands annually, out of them 6,000–10,000 fatal (for instance, China reported a total of 38,000 cases in 1990, about 15,000 in 1995, 8,100 in 1999, and 5,104 cases with 214 deaths in 2005) (most of the deaths occurred in infants); India – Uttar Pradesh 4,544 cases (1,413 fatal) in 1990, and 5,737 cases (1,344 fatal) in 2005.

Bio-containment: BSL-3.

Prevention: vaccine (inactivated Japanese strain Nakayama, or attenuated Chinese SA14-14-2).

Geographical distribution: east and southern Asia (Japan, Korea, China, India, Pakistan, Bangladesh, Nepal, Sri Lanka, Cambodia, Laos, Vietnam, Thailand, Indonesia, New Guinea). In northern Australia (Queensland) JE virus appeared in 2005.

Flavivirus **West Nile** (Subtypes: Kunjin, Rabensburg)

Three genomic lineages of WNV are known at present (1–3), considered are two additional lineages (Indian and Caucasian), although the Caucasian (Russian) strain isolated from ixodid ticks seems to be closer to Koutango virus than to WNV. The Australian Kunjin is a member of the lineage 1b, most virulent are strains of the lineage 1a.

Source of infection (natural host range): birds (waterbirds, corvids); occasionally probably rodents (*Arvicanthis*), equids, ruminants (camel), bats (*Rousettus leschenaulti*, *Eptesicus fuscus*), amphibians (*Rana ridibunda*) and reptiles (*Natrix natrix,* alligator). Migratory birds and corvids contribute to the spread of WNV to distant areas. Rabbits and squirrels have been shown experimentally to develop viraemia titres capable of infecting biting mosquitoes.

Animal disease: encephalomyelitis of equids with a high fatality rate, 25–30% (France, Italy, Morocco, USA etc.). Virulent strains of WNV (e.g. NY-99) cause systemic disease in free-living birds (corvids – Photo 7.81 – and some other passerines, birds of prey etc.) and domestic birds (goose) with a mass dying, and also alligators in Florida were severely affected during the US epizootic.

Transmission mode (Fig. 8.6): largely by ornithophilic mosquitoes (*Culex univittatus*, *Cx. pipiens*, *Cx. modestus*, *Cx. quinquefasciatus*, *Cx. salinarius*, *Cx. restuans*, etc.), exceptionally ixodid (*Hyalomma*) and argasid (*Argas*) ticks. WNV cycles: (1) sylvatic (exoanthropic, natural foci) involving wild, mainly wetland and water birds as amplifying hosts of the virus and ornithophilic

Fig. 8.6 The cycles of West Nile virus (drawing by Ivo Rudolf)
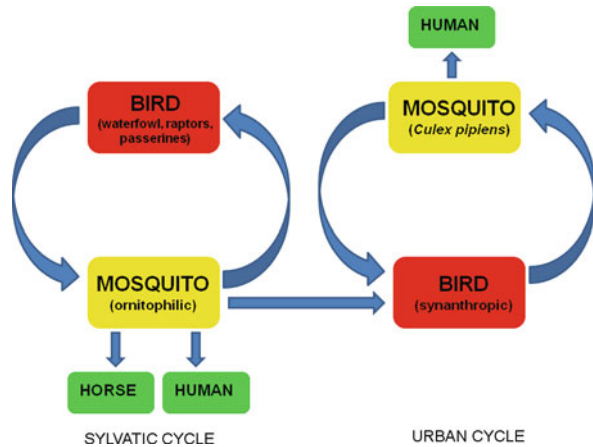
mosquitoes as vectors; (2) urban (synanthropic), with synanthropic and domestic birds as hosts and mosquito species feeding on both birds and mammals as vectors. Palaearctic natural foci of WNV infections are bound to wetland ecosystems, e.g. big river deltas (Volga, Danube, Rhône in Europe, or Djudj in west Africa). WNV transmission is also possible by blood transfusion, organ transplantation, or aerosol (laboratory infections). Contact and alimentary transmission between birds (raptors, corvids) has also been documented.

Human disease: <u>West Nile fever</u> (West Nile encephalitis, West Nile disease) with a sudden onset of fever, pharyngitis, headaches, myalgia, arthralgia, fatigue, nausea, sometimes conjunctivitis, lymphadenitis, quite often maculopapulose rash on the trunk and extremities with erythema in face, encephalitis (in about 10% of patients, i.e. roughly 1% of all infected persons); fatality rate is 5–10% (most frequently succumb persons older than 60 years). Convalescence used to be long in adults, but longterm sequellae are rare. Epidemics: 1950–1957 hundreds of cases in Israel, especially among immigrants; 1974 a big outbreak in South Africa (about 3,000 sick persons); 1994 Algeria, human cases; 1996 >800 patients (50 died) in Romania – mainly in Bucharest and environs (in the consequent years 1997–2006 a total of only 90 cases with 6 fatalities); 1997 Tunisia, tens of human cases; 1997 south Moravia (Czechland) 5 cases; 1999 Volgograd, Astrakhan and Krasnodar regions 826 ill persons (40 died), and 2000–2006 here 316 additional patients; 1999–2000 Israel >430 cases (37 patients died); 1999 first introduction of WNV (Israeli strain) into USA – New York (62 ill persons, 7 of them died) while in subsequent years followed thousands of WNF cases in the USA (and several cases in Canada), and hundreds of patients succumbed (at the same time, thousands of horses and birds died as well); the virus spread rapidly across the continent – in 2002 it reached western coasts of North America being detected then in virtually all US states

Table 8.1 Spread of West Nile virus in the USA and its establishment in the country, 1999–2009 – numbers of affected and reported individuals (CDC data)

	1999	2000	2001	2002	2003	2004	2005	2006	2007	2008	2009
US States	4	12	27	44	46	47	48	46	43	40	35
Humans	62	21	66	4,156	9,862	2,539	3,000	4,269	3,630	1,356	722
Died	*7*	*2*	*9*	*284*	*264*	*100*	*119*	*177*	*124*	*44*	*25*
Horses	25	63	738	9,157	4,146	1,341	1,072	1,121	507	224	298
Birds	295	4,323	7,333	14,122	11,350	7,074	5,204	4,106	2,182	3,026	759

(Table 8.1). WNV has also occurred in Mexico, Central America including the Caribbean, and in 2006 as far southwards as in Argentina. 2003 France, 2 human cases. 2004 several patients in western Siberia. In 2008, several human neuroinvasive WN cases were reported from Italy (3), Hungary (12), and Romania (2). In 2009, 11 human cases of WNV encephalitis were recorded in Italy (Emilia-Romagna), and recently a WNF outbreak has occurred in northern Greece where 164 cases including 14 deaths have been reported as of September 6, 2010.

Bio-containment: BSL-3.

Prevention: There is a WNV vaccine for horses, but not yet a vaccine for humans. However, human vaccine against JE cross-protects against WNV.

Geographical distribution: nowadays worldwide – mainly Africa, the Mediterranean region, south Asia, Australia and Indonesia; less often southern and central Europe, and since beginning of the twenty-first century also the Americas. For habitats, see Photos 5.3–5.5, 5.42.

Flavivirus St. Louis Encephalitis

Source of infection (natural host range): wild synanthropic (peridomestic) birds such as house sparrow, pigeon etc., bats (*Tadarida brasiliensis*), fox.

Animal disease: mostly an inapparent course.

Transmission mode (Fig. 8.7): mosquitoes of the genus *Culex*: *Cx. quinquefasciatus* in the urban cycle, *Cx. tarsalis* (TOT), *Cx. nigripalpus*, and *Cx. pipiens* in the rural cycle; SLEV was also isolated from *Psorophora ferox*.

Human disease: St. Louis encephalitis with a fatality rate up to 20% (higher mortality occurs especially in persons older than 55 years), but more commonly only fever or meningitis. In USA, tens of SLE cases occur annually. Epidemics: 1932–1933 USA 1,350 cases (266 fatalities, and isolation of SLEV from a patient); 1962 Florida; 1975 USA 1,815 cases; 1990–1991 Florida and SE. Texas 226 cases (11 fatal). A total of 480 cases including 8 deaths were reported in Cordoba, Argentina in 2005.

Bio-containment: BSL-3.

Geographical distribution: North America, sporadically Central (Panama, Trinidad, Jamaica) and South (Brazil, Argentina) America.

Fig. 8.7 The cycles of St. Louis encephalitis virus (drawing by Ivo Rudolf)
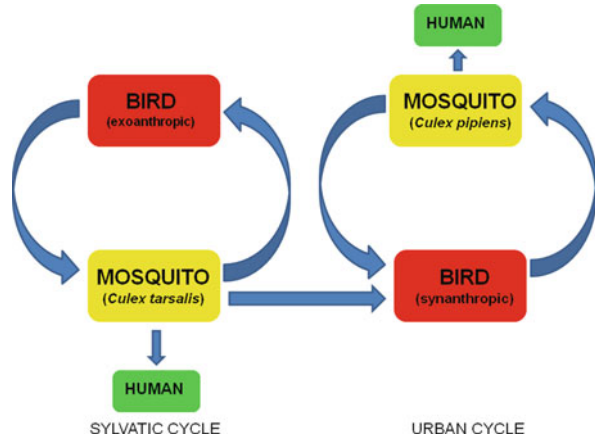
Flavivirus Murray Valley Encephalitis

Source of infection (natural host range): birds (especially waterbirds); (equids).

Animal disease: usually inapparent course, sometimes encephalitis.

Transmission mode: mosquitoes (largely *Culex annulirostris*). In natural foci the virus circulates between wetland birds (herons, cormorants) and ornithophilic mosquitoes.

Human disease: Australian encephalitis (MVE), with fever, headaches, nausea and vomiting, fatality rate 20–60% and sequelae (paralysis of extremities, mental damage). Big epidemics were reported in 1917–1925 (>180 cases, 68% fatality rate), 1950–1951, 1956, 1971, and 1974. Outbreaks usually occur after heavy rains followed by extreme drought.

Bio-containment: BSL-3.

Geographical distribution: northern Australia, New Guinea.

Flavivirus Bagaza

Serologically it cross-reacts (in CFT) with distantly related WNV and JEV, being more closely related to Ntaya flavivirus. Interestingly, Bagaza virus might be genomically identical with Israel turkey meningoencephalitis virus.

Source of infection (natural host range): birds.

Animal disease: unknown in Bagaza virus (but Israel turkey meningoencephalitis is a serious veterinary disease).

Transmission mode: *Culex* spp. mosquitoes, e.g. *Cx. neavei* and *Cx. poicilipes* in Africa, and *Cx. tritaeniorhynchus* in India. In Senegal, the virus was also isolated repeatedly from *Mimomyia* mosquitoes (*M. hispida, M. splendens, M. lacustris*).

Human disease: fever and encephalitis; the disease has not yet been well characterised. Eight patients with encephalitis revealed neutralizing antibodies against Bagaza virus during an epidemic in the Kerala state (India) in 1996.

Bio-containment: BSL-2.

Geographical distribution: Africa (Central African Republic, Cameroon, Senegal, Mauretania), India.

Flavivirus Rocio

Source of infection (natural host range): passerine birds.

Animal disease: inapparent course.

Transmission mode: by mosquitoes *Psorophora* spp.

Human disease: Rocio encephalitis with neurological and psychotic sequellae; fatality rate about 15 (5–30)%. First epidemic recorded in Brazilian state Sao Paolo in 1975 (462 patients reported, 61 of them died; the total number of cases was however estimated at about 900).

Bio-containment: BSL-3.

Geographical distribution: South America (Brazil).

Flavivirus Zika

The closest relative is Spondweni virus (see below). Zika virus was first isolated from a febrile monkey caged on a tree platform in the Zika forest in Uganda, 1947.

Source of infection (natural host range): monkeys (*Cercopithecus aethiops, Erythrocebus patas*).

Animal disease: inapparent course.

Other mosquito-borne flaviviruses associated with human disease

Virus	Geographical distribution	Vector	Vertebrate hosts	Human cases	Disease	BSL
Banzi	South Africa	*Culex rubinotus, Cx. neavei*	rodents, possibly also birds	2	Febrile illness	2
Bussuquara	Brazil, Colombia, Panama	*Culex* spp.	monkeys; birds?	1	Febrile illness	2
Edge Hill	Australia	*Aedes vigilax, Aedes* spp.	wallabies	few	Febrile illness	2
Ilheus	Brazil	*Aedes* and *Haemagogus* spp.	primates	8	Fever, CNS signs	2
Kokobera	Australia	*Cx. annulirostris*	birds?	3	Fever, arthritis	2
Sepik	Papua New Guinea	*Mansonia septempunctata*	sheep	1	Fever, headache	3
Spondweni	Tropical Africa	*Aedes circumluteolus*	primates?	7	Febrile illness	3
Usutu	Africa, Europe	*Culex* spp.	birds	2	Fever, rash	2
Wesselsbron	Africa, Madagascar	*Aedes* spp.	rodents, ruminants	9	Biphasic fever, CNS signs	3

Transmission mode: *Aedes* mosquitoes (*Ae. furcifer, Ae. africanus, Ae. apicoargenteus, Ae. aegypti, Ae. luteocephalus, Ae. dalzieli*).

Human disease: self-limited disease manifested by fever, maculopapular rash, arthralgia and conjunctivitis; other symptoms include malaise, back pain, diarrhoea and abdominal pain. In 2007, an extensive outbreak of Zika fever occurred on the Yap Island in Micronesia (first detection of the disease outside Africa and Asia), with at least 100 cases (49 were confirmed in laboratory, 59 were rated as probable). Prior to this epidemic, only about 14 human cases were reported in several African countries, India and Indonesia.

Bio-containment: BSL-2.

Geographical distribution: Africa (Uganda, Nigeria, Senegal, Tanzania, Central African Republic, Sierra Leone, Gabon, Egypt), Asia (India, Malaysia, Thailand, Vietnam, Indonesia), Oceania (the Philippines, Micronesia).

8.2.2.2 Tick-Borne Flaviviruses

Flavivirus of Tick-Borne Encephalitis (CEE, LI, RSSE)

There are four subtypes of TBEV: louping ill (LI); Central European encephalitis (CEE, e.g. topotype strains Hypr or Neudoerfl); Ural-Siberian, causing Russian spring-summer encephalitis (RSSE, e.g. strain Vasilchenko); and Far-Eastern (FEE, e.g. strain Sofyin). Dendrograms (Fig. 8.8) show antigenic and genomic similarity among viruses of the TBE complex: CEE virus is closer to LI virus than to RSSE virus, and thus LI should not be regarded as a separate virus, (because RSSE and CEE are considered as subtypes of one (TBE) virus). At the same time, Spanish sheep encephalitis virus is closely related to CEE virus.

Source of infection (natural host range): small forest mammals, especially rodents (*Apodemus*, *Myodes*, less often *Microtus* – CEE, RSSE, but probably not with LI virus), insectivores (hedgehog: CEE), goat (CEE) and sheep (CEE, LI), exceptionally cattle (CEE), certain forest birds (CEE, RSSE), red grouse *Lagopus lagopus scoticus* (LI) and mountain hare *Lepus timidus* (LI).

Animal disease: occasionally encephalitis in certain hosts (lamb, kid, dog, red grouse). LI virus causes disease in sheep and red grouse.

Transmission mode (Fig. 8.9): by ixodid ticks (reservoir – TOT), especially *Ixodes ricinus* (CEE, LI), *I. persulcatus* (RSSE); alimentary (consumption of raw, thermally untreated milk and dairy products from goats and sheep: 1951 Rožňava, Slovakia: about 500 patients), aerogenic (laboratory infections).

Circulation of TBE virus in a natural focus shows Fig. 8.9. About 0.5–3% of *Ixodes* ticks are usually infected in natural foci of TBE. For different natural foci of TBE (and LI), see Photos 5.7–5.13.

Human disease: tick-borne encephalitis usually with typical biphasic course: the 1st phase starts with fever and flu-like symptoms (severe headache, myalgia, arthralgia), sometimes conjunctivitis; after a short interval of usually 4–7 days of an apparent recovery, the 2nd phase starts with affection of the CNS (meningoencephalitis) accompanied with photophobia, dizziness, pareses of
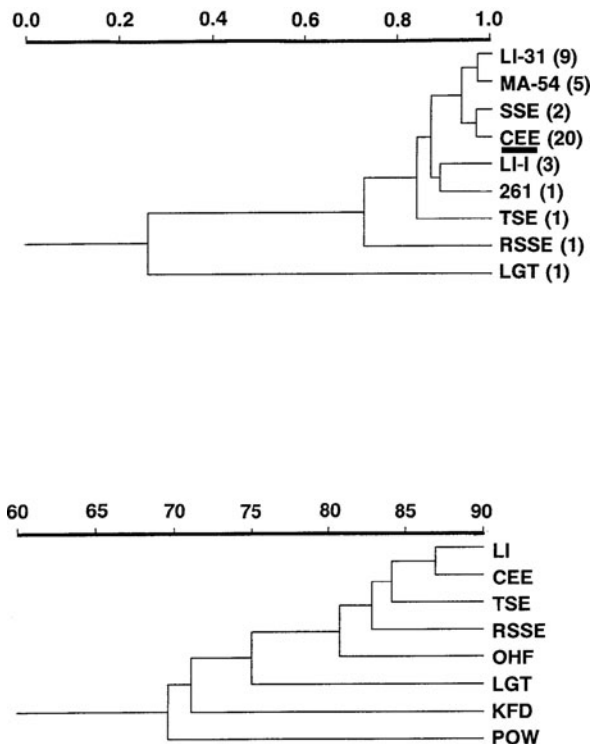
Fig. 8.8 Antigenic similarity among TBE complex viruses and strains based on IFA (the upper dendrogram: Acta Virol 39, 251–256, 1995). Louping ill strains are LI-31 (plus 8 related strains), MA-54 (plus 4 related strains; LI-I (plus two related strains), 261; SSE, Spanish sheep encephalitis; CEE, Hypr (plus 19 related strains); TSE; Turkish sheep encephalitis; LGT, Langat virus) The lower dendrogram: nucleotide sequence homology of the E gene among TBE complex viruses and strains (modified from Virus Res. 30: 129–144, 1993; and J. Gen. Virol. 75: 227–232, 1994) (drawing by Zdenek Hubalek)

cranial nerves and extremities, ataxia; fatality rate ranges from 1% (CEE, LI) to 20–30% (RSSE, FE); convalescence is long-term, and neurological sequellae (residua) sometimes including pareses are quite common. Subtypes of TBE viruses were isolated in Eurasia first in 1930 (LI), 1937 (RSSE) and 1948 (CEE). In some European countries, TBE morbidity is quite frequent: for instance, on average 368 (140–744) cases of TBE was reported in Czechland (population about 10 million) annually in the two-decade period 1970–1999; In the year 2006 the TBE incidence in Czechland peaked at 1,026 patients. In 1999, an unusual epidemic of TBE (caused by a variant of FE subtype) occurred in the Novosibirsk area of Russia: out of 447 cases, 8 patients died with haemorrhagic syndrome. 1993–2000 were described first TBE human cases in Japan (caused by FE subtype).

Bio-containment: for CEE, RSSE and FE subtypes BSL-4 (when the personnel is vaccinated, BSL-3 is enough). For other TBE virus subtypes: BSL-3.
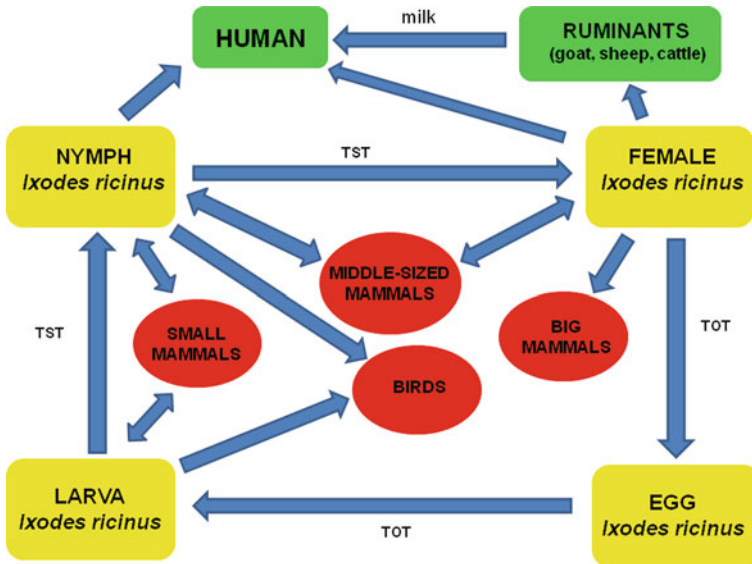
Fig. 8.9 Natural cycle of tick-borne encephalitis virus (drawing by Ivo Rudolf)

Diagnosis: serology (ELISA, HIT, CFT, VNT), detection of IgM in early phase or seroconversion in paired serum samples; less common is the isolation of the virus from the blood or CSF in cell cultures (e.g., PS pig embryo kidney cells) or in mice, detection of the virus' RNA by using RT-PCR.

Prevention: inactivated vaccine ("FSME-Immun" Immuno/Baxter; "Encepur" Behring); specific immunoglobulins (but effective only when applied immediately, i.e. within 1–2 days after infection, otherwise it could be even detrimental); repellents against ticks.

Geographical distribution: Eurasia (LI: UK and Norway, CEE: western and central Europe; RSSE: European and Asian Russia, Baltic countries, Finland; FEE: Far East Russia, northern China, Japan).

Flavivirus **Powassan**

This virus (with its subtype "**Deer tick virus**") is distantly related to TBE virus. The complete nucleotide sequence of the genome was determined (a total of 10,839 nucleotides).

Source of infection (natural host range): small and medium-sized mainly forest mammals, especially rodents (*Marmota monax, Peromyscus leucopus, Tamiasciurus hudsonicus*), skunk, fox.

Animal disease: inapparent course. Experimental inoculation of adult laboratory mice and *Macaca mulatta* monkeys caused their encephalitis and death.

Transmission mode: ixodid ticks (*Ixodes cookei, I. marxi, Dermacentor andersoni*). Virus is secreted in milk of experimentally infected goats.

Human disease: Powassan encephalitis with fever, headache, prostration, meningitis and encephalitis; pleocytosis in CSF; spastic pareses, rarely paralyses and death (fatality rate about 10%); neurological sequelae often persist. An infrequent disease in North America. For instance, 36 cases (without fatalities) were reported in the USA between 1958 (first human case) and 2005, but since the late 1990s, the incidence of human disease seems to be increasing.

Bio-containment: BSL-3.

Diagnosis: serology (ELISA, HIT, CFT, VNT).

Prevention: TBE vaccine could be cross-protective against Powassan virus infection.

Geographical distribution: North America (northern USA, Canada), Far East (Russia).

Flavivirus **Omsk Haemorrhagic Fever (OHF)**

The virus belongs to the so-called TBE complex. It was first isolated in 1947.

Source of infection (natural host range): rodents (muskrat *Ondatra zibethica* – imported to Siberia from Canada in 1928; *Arvicola terrestris, Microtus gregalis*), frogs and lizards.

Animal disease: occasional epizootics – mass dying of muskrats.

Transmission mode: ticks *Dermacentor reticulatus*, *Ixodes apronophorus*; alimentary (consumption of raw milk of goats and sheep, or drinking contaminated water); direct contact – e.g. in muskrat hunters.

Human disease: Omsk haemorrhagic fever – high fever, headaches, myalgia, nasal bleeding, pharyngitis, encephalitis (occasionally), haemorrhages; fatality rate 1–3%; long convalescence. During 1988–1997, a total of 165 cases were reported from Siberia.

Bio-containment: BSL-4.

Diagnosis: serology (ELISA, VNT); virus isolation, RT-PCR.

Prevention: TBE vaccine might partially cross-protect.

Geographical distribution: steppe ecosystem in southern and western Siberia – regions Omsk, Novosibirsk, Kurgan and Tyumen.

Flavivirus **Kyasanur Forest Disease (KFD)**

This virus also belongs to the so-called TBE complex. Very closely related to KFD virus (in fact, its subtype) is the virus **Alkhurma** (its overall genomic homology with KFDV is 89%) and **Nanjianyin** virus (China).

Source of infection (natural host range): monkeys, rat *Rattus blanfordi*, striped forest squirrel *Funambulus tristriatus*, bats and insectivores (*Suncus murinus*) in KFD; and probably sheep and goat with Alkhurma virus in Saudi Arabia.

Animal disease: occasional epizootics – mass dying of primates. For
instance, high mortality due to KFD was observed in the black-faced lan-
gur (*Semnopithecus entellus*) and the red-faced bonnet monkey (*Macaca
radiata*).

Transmission mode: ticks *Haemaphysalis spinigera* in KFD (reservoir – TOT
demonstrated) and probably *Ornithodoros savignyi* in Alkhurma virus;
alimentary (consumption of raw milk of goats and sheep, or drinking
contaminated water).

Human disease: Kyasanur Forest disease – fever, headaches, pain in extrem-
ities, erythema on face, pharyngitis, encephalitis (in about 20% of cases),
hepatitis, haemorrhages (nasal and gastrointestinal bleeding); fatality rate
8–15%; long convalescence. Big outbreaks of KFD in Indian state Mysore
in 1957, and Karnataka (213 cases with 14 fatalities) in 1986; the KFD foci
activated in India in the 1990s; an average of 400–500 human cases have
been reported annually over the last decades. In Saudi Arabia, 45 cases of
Alkhurma haemorrhagic fever occurred in 2001−2003, the fatality rate was
25%; additional 4 cases were reported in Makkah province in 2009. There is
an occupational risk with Alkhurma virus (e.g., slaughtering of sheep).

Bio-containment: BSL-4.

Diagnosis: serology (ELISA, VNT); virus isolation, RT-PCR.

Prevention: TBE vaccine might partially cross-protect.

Geographical distribution: forests in India and West China – province
Yunnan (KFD – Nanjianyin virus), and semidesert habitats in Saudi Arabia
(Alkhurma).

8.2.2.3 Bat-Borne Flaviviruses

Flavivirus Dakar Bat

Source of infection (natural host range): bats *Tadarida condylura, Scotophilus
nigrita*.

Animal disease: inapparent course.

Transmission mode: contact or aerogenic (?)

Human disease: febrile illness – two cases have been reported.

Bio-containment: BSL-2.

Geographical distribution: tropical Africa.

Flavivirus Rio Bravo

Source of infection (natural host range): the bats *Tadarida brasiliensis,
Eptesicus fuscus.*

Animal disease: inapparent course.

Transmission mode: contact or aerogenic (?)

Human disease: febrile illness with respiratory involvement and lym-
phadenopathy – several cases have been reported.

Bio-containment: BSL-2.

Geographical distribution: Mexico and USA (Texas, New Mexico, California).

8.2.3 Family **Bunyaviridae**

Virions are spherical (80–120 nm), enveloped, containing segmented molecule of a ss(–)RNA (the three segments are labeled L, M and S) sized 11–19 kbp (genus *Orthobunyavirus* – 12 kbp), with two surface glycoproteins G1 and G2, nucleoprotein N and transcriptase protein L. During a mixed simultaneous infection of cells in a vector or vertebrate host with different but related bunyviruses, reassortant (hybrid) virions can arise through incorporation of RNA segments from different viruses.

Orthobunyaviruses of California Group: LaCrosse (LAC), California Encephalitis (CE), Snowshoe Hare (SSH), Ťahyňa (TAH), Inkoo (INK), Jamestown Canyon (JC)

Another, a more distant member of this group is **Guaroa** virus. Ťahyňa virus (synonym: Lumbo) is the first mosquito-borne virus isolated in Europe (by V. Bárdoš and V. Danielová in 1958).

> Source of infection (natural host range): leporids, rodents (*Tamias*, *Spermophilus*, *Sciurus*; *Dicrostonyx*), hedgehog, white-tailed deer (JC).
> Animal disease: inapparent course.
> Transmission mode (Fig. 8.10): mosquitoes of the genera *Aedes* and *Culiseta* (reservoir). LAC: *Ae. triseriatus* (TOT). CE: *Ae. dorsalis* (TOT, also sexual transmission in mosquitoes). TAH: *Ae. vexans* (TOT), *Ae. caspius*, *Ae. cinereus*, *Ae. cantans* and others; SSH, INK: *Ae. communis*, *Ae. punctor*, *Ae. hexodontus*.
> Human disease: flu-like syndrome (e.g., Valtice fever: TAHV) lasting about a week, with fever, headache, myalgia, fatigue, lethargy, pharyngitis,
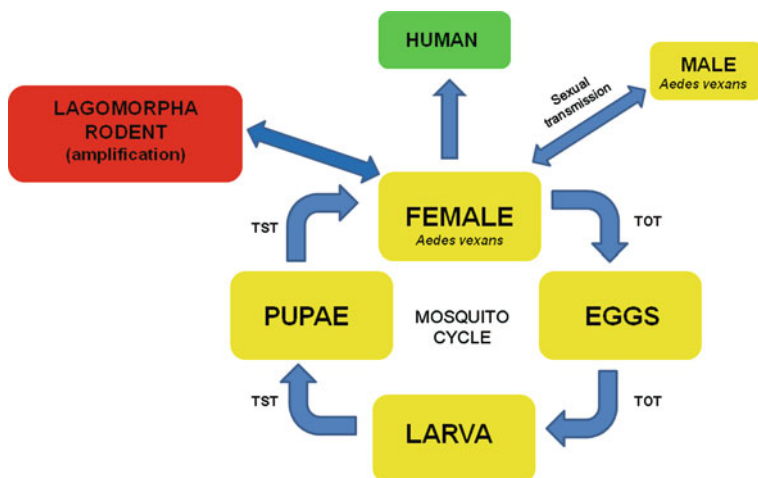
Fig. 8.10 Circulation of Ťahyňa virus in a natural focus of Valtice fever (drawing by Ivo Rudolf)

conjunctivitis, nausea, gastrointestinal difficulties, sometimes also mening-
itis (TAHV) or encephalitis (California encephalitis: CEV, LACV), more
common in children (however, JC virus is more pathogenic for adults); fatal-
ities are rare and occur in paediatric patients (LACV). The disease occurs
usually in the late summer and early autumn when the vector mosquito den-
sity increases markedly. In the USA, the California group viruses are the
most frequent cause of arboviral encephalitides (except for WN encephalitis
since the year 2000). In the years 1963–1985, a total of 1,700 human cases
of California encephalitis were reported from 23 states. During 2003–2007,
a total of 355 patients (83% occurred in children <16 year old) were reported
with probable and confirmed LACV infection in USA: 77% of them exhib-
ited encephalitis or meningoencephalitis, 17% meningitis, and the rest were
uncomplicated febrile cases; deaths occurred in 1.9% of confirmed cases,
and in 8.6% of patients with encephalitis. In central and eastern Europe,
many tens of cases of Valtice fever have been described since the 1970s,
and a majority of people of the higher age groups living in, or close to, nat-
ural foci have antibodies to TAHV. However, many symptomatic cases of
Valtice fever remain undiagnosed and characterized only as a (summer) *sta-
tus febrilis* because there is no routine laboratory diagnosis of this disease
in the region at present. Guaroa virus has been reported to cause 5 cases of
febrile illness with headache, myalgia and prostration in Brazil.

Bio-containment: BSL-2.

Diagnosis: serology (ELISA IgM, HIT, VNT, IFA), exceptionally PCR or (tech-
nically more difficult) isolation attempts from the blood samples on Vero
cells or on suckling mice.

Geographical distribution: Eurasia (TAH, INK) including China (TAH), Africa
(TAH), North America (CE, LAC, SSH, JC), South America (Guaroa virus).
Natural foci of TAHV infection in central Europe are characteristically situ-
ated in the floodplain-forest ecosystem with a mosaic of meadows and close
arable fields (Photos 5.15 and 5.16).

Orthobunyaviruses Batai (Synonyms: Čalovo, Olyka, Chittoor), Ilesha, Bwamba and Tensaw

Source of infection (natural host range): livestock, domestic pig, primates.

Animal disease: usually inapparent course, some observed in goat and sheep.

Transmission mode: zoophilic mosquitoes of the genus *Anopheles* (*An.
maculipennis* s.l., *An. barbirostris, An. gambiae* – Ilesha, Bwamba, *An.
gambiae* – Bwamba, *An. crucians* – Tensaw virus), less *Aedes* and *Culex*
spp. (*Cx. gelidus*).

Human disease: flu-like symptoms with fever, lethargy and lack of appetite.
Incidence of this disease is low. The disease with Ilesha (7 cases) and
Bwamba (9 cases) viruses included fever with headache, myalgia and rash –
of the observed Ilesha cases, one was fatal. One encephalitic case caused by
Tensaw virus was reported.

Bio-containment: BSL-2.

Diagnosis: serology (ELISA IgM, HIT, VNT, IFA).

Geographical distribution: Europe (Batai: Czechland, Slovakia, Austria, Hungary, Croatia, Serbia, Moldova, Ukraine, Russia, Finland, Sweden and Norway), Asia (Batai: Malaysia, India, China), central Africa (Ilesha, Bwamba), and southern USA (Tensaw: Florida, Alabama, Georgia).

Orthobunyavirus **Bunyamwera**

Antigenically related to Batai virus.

Source of infection (natural host range): monkeys, possibly rodents.

Animal disease: asymptomatic course.

Transmission mode: mosquitoes, mainly *Aedes* spp.

Human disease: febrile illness with rash – eight cases have been described.

Bio-containment: BSL-2.

Geographical distribution: tropical Africa.

Orthobunyavirus **Germiston**

Source of infection (natural host range): rodents.

Animal disease: asymptomatic course.

Transmission mode: *Culex rubinotus*.

Human disease: febrile illness with rash (3 cases), headache, backache, weakness and mental confusion.

Bio-containment: BSL-3.

Geographical distribution: tropical central, eastern and southern Africa.

Orthobunyavirus **Oropouche (Antigenic Group Simbu)**

Source of infection (natural host range): mammals (sloths, marsupials, monkeys *Callithrix* spp., man), birds.

Animal disease: inapparent course.

Transmission mode: biting midges (*Culicoides paraensis* – in urban cycle) and culicine mosquitoes (*Aedes serratus, Culex quinquefasciatus*). *C. paraensis* is the major biological vector of the virus to humans during urban epidemics of the disease. However, the vector(s) of the virus in its "silent" sylvatic cycle remain unknown.

Human disease: Oropouche fever – with chills, headache, myalgia, arthralgia, diarrhoea, nausea, vomiting, anorexia, conjunctivitis, rash, photophobia, dizziness and meningitis or meningoencephalitis, leucopenia, infrequently haemorrhagic phenomena; no mortality nor sequellae have been reported. The virus was first isolated from the blood of febrile forest workers in Trinidad, 1954. A big outbreak (11,000 cases) occurred in Brazil in 1961; further Brazilian epidemics were reported in the years 1967/1968 and 1979/1980 (200,000 cases), and a reemergence occurred in 2003/2004 and

2006/2008 with several tens of cases. In 2010, 282 cases of Oropouche fever have been reported in Peru (as of August). A total of about 500,000 cases of Oropouche fever have been estimated to occur in 27 epidemics in Brazil, Peru and Panama since 1960; this is the second (after dengue) most frequent arbovirosis in South America.

Bio-containment: BSL-3.

Geographical distribution: South America – largely Amazonia (Brazil, Peru), Ecuador, Trinidad, and Central America (Panama).

Orthobunyaviruses Guama and Catu (Antigenic Group Guama)

Source of infection (natural host range): rodents (*Oryzomys capito*, *Proechimys guyannensis* and other spp.), monkeys, possibly also birds.

Animal disease: asymptomatic course.

Transmission mode: mosquitoes, mainly *Aedes* and *Culex* spp.

Human disease: febrile illness with headache, myalgia and arthralgia. Both viruses have caused 7–8 human cases each.

Bio-containment: BSL-2.

Geographical distribution: Brazil, Trinidad, French Guiana, Surinam, Panama.

Bunyaviruses Apeu, Caraparu, Marituba, Murutucu, Nepuyo, Oriboca, Ossa and Restan (Antigenic Group C)

Tropical mosquito-borne viruses.

Source of infection (natural host range): rodents (*Caluromys philander*, *Proechimys guyannensis, P. spinosus, Oryzomys capito, O. laticeps, Didelphis marsupialis, Nectomys squamipes*), monkeys (*Cebus apella*), and also fruit bats (Nepuyo virus: *Artibeus jamaicensis, A. lituratus*).

Animal disease: inapparent course.

Transmission mode: mosquitoes of the genera *Aedes* and *Culex (Ae. arborealis, Cx. aikenii, Cx. portesi, Cx. vomerifer*).

Human disease: up to 15 cases of a febrile illness have been reported for each of these viruses.

Bio-containment: BSL-2.

Geographical distribution: South America and Central America (tropical forests): Brazil, Trinidad, French Guiana, Surinam, Guatemala, Honduras, Panama, southern Mexico.

Bunyavirus Keterah (Synonym: Issyk-Kul)

This virus has not yet been assigned to a genus.

Source of infection (natural host range): bats; (birds?).

Animal disease: asymptomatic in green monkeys, but damage to visceral organs.

Transmission mode: soft ticks (*Argasidae*), biting midges (*Culicoides schult-zei*), and mosquitoes.
Human disease: a fever. An outbreak in Tadjikistan in 1982.
Bio-containment: BSL-3.
Geographical distribution: central Asia, Malaysia.

Bunyavirus Bhanja (Synonym or Subtype: Palma)

This virus has not yet been assigned to a genus.

Source of infection (natural host range): sheep, goat, cattle; hedgehog.
Animal disease: CNS affection in young ruminants (kid, lamb, calf).
Transmission mode: metastriate ticks (*Haemaphysalis intermedia*, *H. punctata*, *Dermacentor marginatus, Hyalomma marginatum*).
Human disease: fever, headache, conjunctivitis, sometimes meningoencephalitis. About 10 human cases have been described, one of them serious (quadriparesis).
Bio-containment: BSL-3.
Geographical distribution: Asia, Africa, southern Europe, eastern Slovakia. Examples of natural foci in Photos 5.18–5.21.

Nairovirus Dugbe

The virus is related to Ganjam virus (Nairobi sheep disease group).

Source of infection (natural host range): cattle (zebu), *Cricetomys gambianus*.
Animal disease: not observed.
Transmission mode: *Amblyomma variegatum* and other metastriate ticks (*Boophilus decoloratus* and other *Boophilus* spp., *Rhipicephalus muhsamae* and other *Rhipicephalus* spp., *Hyalomma marginatum, H. truncatum*).
Human disease: fever, aseptic meningitis, thrombocytopenia. Two human cases have been reported.
Bio-containment: BSL-3.
Geographical distribution: tropical Africa – Nigeria, Senegal, Ivory Coast, Cameroon, Guinea, Central African Republic, Uganda.

Nairovirus Crimean-Congo Haemorrhagic Fever

It was recognized in 1969 that Congo virus, isolated in Africa, is identical to the agent of Crimean haemorrhagic fever, known in south Russia and southeast Europe, and Harry Hoogstraal thus proposed the combined name of the virus and disease – CCHF.

Source of infection (natural host range): leporids, hedgehog, other small mammals, cattle, horse, goat, sheep.
Animal disease: inapparent course.
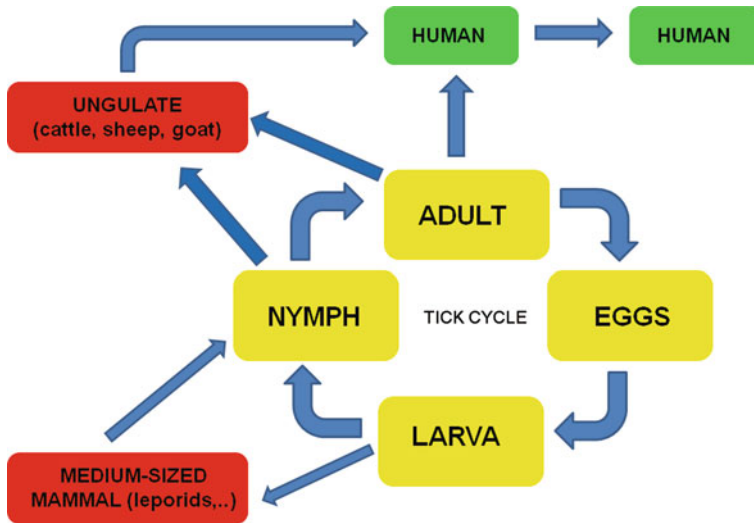
Fig. 8.11 Natural cycle of CCHF virus (drawing by Ivo Rudolf)

Transmission mode (Fig. 8.11): mostly bites by metastriate vector ticks – *Hyalomma marginatum*, *H. anatolicum, Dermacentor marginatus*, *Rhipicephalus bursa* and other spp. (reservoir – TOT; also venereal transmission in some species), frequently also by contact (nursing and care for patients, removal of feeding vector ticks, slaughtering of infected animals or those with attached ticks, sheep shearing). Occupational disease: cattle breeders, butchers, livestock industry, health professionals (nosocomial spread), specialized infectious laboratory workers (aerosol).

Human disease: Crimean-Congo haemorrhagic fever with severe headache, neckache, back pains, myalgia, dizziness, stiffness, petechial rash on the trunk, conjunctivitis, abdominal pain, vomiting, diarrhoea, photophobia, lymphadenopathy, hepatomegaly, hepatitis, psychotic signs (depression, sleepiness, lassitude), bleeding from mucous membranes (nose, gums, gastrointestinal tract) and kidneys, sometimes bleeding into brain, liver failure, pulmonary failure, haemorrhagic shock. Increased levels of transaminases, leucopenia, thrombocytopenia and coagulopathy. Long convalescence. Fatality rate 5–30% (in nosocomial infections up to 50%). Epidemics: e.g. in Bulgaria 487 notified cases in 1954/1955; Kosovo 119 cases (13% fatal) during 1995–2001; 1997–2003 Bulgaria 138 cases (29 died); a total 1,568 cases were notified in Bulgaria from 1953 to 2008, with a mean fatality rate of 17%. Since 1999 (but especially in 2006–2007), a reactivation of natural foci and re-emergence of CCHF occurred in southern Russia (regions Stavropol, Astrakhan, Rostov, Volgograd, Kalmykia, Dagestan) – a total of >1,300 patients were diagnosed with CCHF until 2007, the fatality rate has been 3–5%. Albania reported 8 cases in 2001 and additional cases (an

outbreak) occurred in Kosovo at the same time. A continuous epidemic process started in Turkey in 2002, and until 2009 a total of 4,430 cases were reported from 680 settlements mainly in the Tokat and Sivas provinces (but as many as 2,615 cases were notified solely in the last 2 years 2008 and 2009), with a mean overall fatality rate of 5%; in addition, 16% of healthy population have antibodies to CCHF virus in Turkey at present (most often farmers and village residents). This exceptional epidemiological upsurge of CCHF in Turkey (largely in the Asian part of the country) has been associated ecologically with fragmentation and use of agricultural land and the formation, by this way, of optimal habitats for *Hyalomma marginatum* vector ticks. Some epidemics outside Europe: 2000–2004 Iran 248 cases; 2003 Mauretania 38 cases (11 fatal). In 2009, cases of CCHF were also reported from southern Russia, Georgia, Kazakhstan, Tajikistan, Iran, and Pakistan. Several US soldiers acquired CCHF during field operations in Afghanistan.

Bio-containment: BSL-4.

Diagnosis: RT-PCR, detection of antibodies or antigen (ELISA, IFA), isolation of the virus (extreme risk).

Treatment: in acute phase (if diagnosed very early) ribavirin, or hyperimmune serum (globulin).

Prevention: a vaccine of Bulgarian provenience (inactivated, suckling mouse brains; not commercial, a small scale production).

Geographical distribution: Africa, Arabian peninsula, Minor and central Asia, Iran, Pakistan, China (Xinjiang province), Albania, Kosovo, Bulgaria (Photo 5.17), Turkey, Greece, south Ukraine, south European Russia.

Phlebovirus Rift Valley Fever (Synonym Zinga)

Source of infection (natural host range): ruminants (sheep, goat, cattle, camel) – viraemia up to 10^{10} PFU/ml (!), rodents.

Animal disease: sheep and goats, less often cattle suffer from necrotic hepatitis, abortions, teratogenic, often fatal (up to 90% of lambs die, but also adult sheep – *c.* 50%). Big economic losses: e.g. in South Africa died about 100,000 sheep, and 500,000 ewes aborted in 1950. Susceptible (but the infection is inapparent) are equines, pig, dog, cat, guinea pig, rabbit.

Transmission mode (Fig. 8.12): mosquitoes *Aedes* spp. (TOT) (*Ae. caballus*, *Ae. lineatopennis, Ae. vexans*), *Culex theileri*, *Cx. pipiens* (Egypt), *Cx. quinquefasciatus*, *Cx. poicilipes* (Mauretania), *Ae. caspius*, less often (during epidemics) biting midges *Culicoides*, sandflies (strain Zinga of RVF virus) and other biting insects, and also ticks – e.g., *Amblyomma variegatum* on cattle in slaughterhouses; transmission also by direct or indirect contact with infected livestock, e.g. handling of sick animals and their tissue during slaughtering, disposal of carcasses, assisting with animal births (due to the high virus content in the blood and amniotic fluid of infected animals), aerogenic (e.g. laboratory infections), alimentary (consumption of raw milk, meat), iatrogenic (blood transfusion, injections).
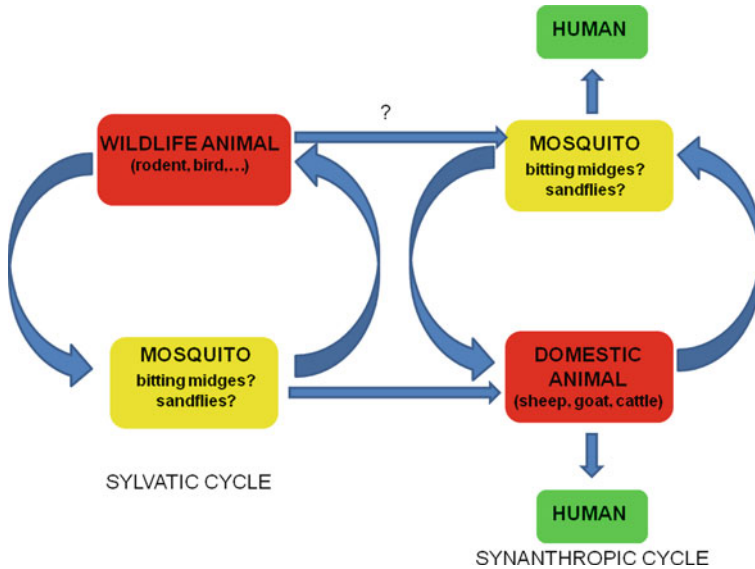
Fig. 8.12 The cycles of RVF virus (drawing by Ivo Rudolf)

Human disease: Rift Valley fever, usually biphasic course, severe headaches, arthralgia, photophobia, vision defects (up to blindness), retinitis, necrotic hepatitis, thromboses, sometimes haemorrhages (bleeding from nose) and encephalitis, occasionally lethal (fatality rate about 3%). Occupational risk in herders, farmers, abbatoir workers, butchers, veterinarians. Epidemics of RVF have occurred in Kenya since beginning twentieth century, however the agent was identified only in 1930–1931; an outbreak in 1950–1951, with about 100,000 sheep and cattle, and 20,000 human cases; 1977–1978 Egypt 18,000 cases (600 fatal), but as much as about 200,000 persons were demonstrated serologically to be infected; 1980, South Africa about 20,000 cases; 1987–1988 Mauretania (220 patients died); 1993 and 1997–1998 Egypt, Somalia, Tanzania, Kenya and Mauretania – 90,000 cases (770 fatal); 2000–2001 for the first time in Arabian peninsula – Yemen 1,087 cases (121 died) and Saudi Arabia 884 diseased persons (124 died); 2003 Mauretania min. 25 cases (4 fatal). More recent outbreaks: Tanzania and Kenya 2007, Mayotte 2007–2008, Somalia 2010, an extensive outbreak affected livestock and humans in South Africa in 2010 (42 human laboratory-confirmed cases including 2 deaths).

Bio-containment: BSL-3.

Treatment: ribavirin helps when applied early.

Prevention and risk avoidance: vaccine (inactivated). Movement of cattle, goat and sheep herds is hazardous in epidemic situation. Cooking animal products before eating them is highly recommended. As a part of surveillance, reporting of abortion in cattle, sheep and goats and mortality in young animals is important.

Geographical distribution: Africa (Kenya, South Africa, Sudan, Egypt, Zimbabwe, Mozambique Zambia, Senegal (Photos 5.24 and 5.25), Nigeria, Burkina Faso, Mauretania), Arabian peninsula.

(**) *Phlebovirus* of Sandfly Fever: Sandfly Fever Naples (SFN), Sandfly Fever Sicilian (SFS), Toscana (TOS)

Source of infection (natural host range): man.
Transmission mode: sandflies (*Phlebotomus* spp. – reservoir, TOT).
Human disease: sandfly fever, pappataci fever 2–4 days long, with lethargy, headaches and arthralgia, photophobia, retrobulbar pain, and heavy sweat; not lethal. Toscana virus causes also aseptic meningitis, while only sporadically encephalitis. Sandfly fevers escape attention and are most probably underdiagnosed.
Bio-containment: BSL-2.
Geographical distribution: the Mediterranean (Italy, Spain, southern France, Croatia – Dalmatia, Greece, Cyprus, Egypt; Photos 5.22 and 5.23), Portugal (TOS), Black Sea region (Crimea, Bulgaria, Turkey), central and south Asia. Common infections in holidaymakers or other visitors of the Mediterranean from central and northern Europe.

(**) Phleboviruses Chagres and Punta Toro

Source of infection (natural host range): man, possibly also rodents.
Transmission mode: sandflies (*Lutzomyia* spp. – reservoir).
Human disease: fever with chills, headache, myalgia, retroorbital pain, vomiting – four cases have been reported with Chagres, and two other with Punta Toro virus.
Bio-containment: BSL-2.
Geographical distribution: Panama.

Old World Hantaviruses: Hantaan, Dobrava, Saaremaa, Puumala, Seoul

The name of the prototype virus is derived from the river Hantaan in Korea. Saaremaa virus is closely related to Dobrava (syn. Belgrade) virus. The number of all known hantaviruses approaches 45 at present, but a number of them (e.g. **Tula** virus, common in Eurasia) are practically nonpathogenic for man.

Source of infection (natural host range): murine and microtine rodents – *Apodemus agrarius* (Hantaan, Saaremaa), *A. flavicollis* (Dobrava), *A. ponticus* (Dobrava), *A. peninsulae* (Amur), *Myodes glareolus* (Puumala), *Microtus arvalis* (Tula), *M. fortis* (Khabarovsk), *Rattus rattus, R. norvegicus* and *Mus musculus* (Seoul), *Lemmus sibiricus* (Topografov), etc. The rodents are not only the competent hosts of hantaviruses, but at the same time also the reservoir, due to their persistent latent infection and long-term excretion of hantaviruses by saliva, urine and faeces.

Survey of human pathogenic Old World hantaviruses

Virus	Main rodent reservoir	Disease	Disease distribution
Hantaan	*Apodemus agrarius*	HFRS	Asia
Saaremaa	*Apodemus agrarius*	HFRS	East Europe
Dobrava	*Apodemus flavicolis*	HFRS	The Balkans
Amur, Far East	*Apodemus peninsulae*	HFRS	Far eastern Russia
Seoul	*Rattus norvegicus, R. rattus*	HFRS (mild)	Mainly Asia
Puumala	*Myodes glareolus*	HFRS (mild)	Europe

Animal disease: inapparent course.

Transmission mode (Fig. 8.13): aerogenic (dried particles of rodents' infectious excreta), contact (rodent bite), and alimentary – from rodents. Hantaviruses are thus not arboviruses, but "rodent-borne" viruses.

Human disease: haemorrhagic fever with renal syndrome (HFRS: Hantaan virus – Korean haemorrhagic fever, Dobrava virus) with fever, headache, backaches, nausea, abdominal pain, vomiting, oedema and petechiae in mouth cavity and on face, later haemorrhages in gastrointestinal and urogenital tract, proteinuria, haematuria, renal insufficiency, kidney failure; fatality rate about 5% (higher one in Asia); a milder form, *nephropathia epidemica* (NE, Puumala, Saaremaa), often with myopia but rare fatalities, occurs
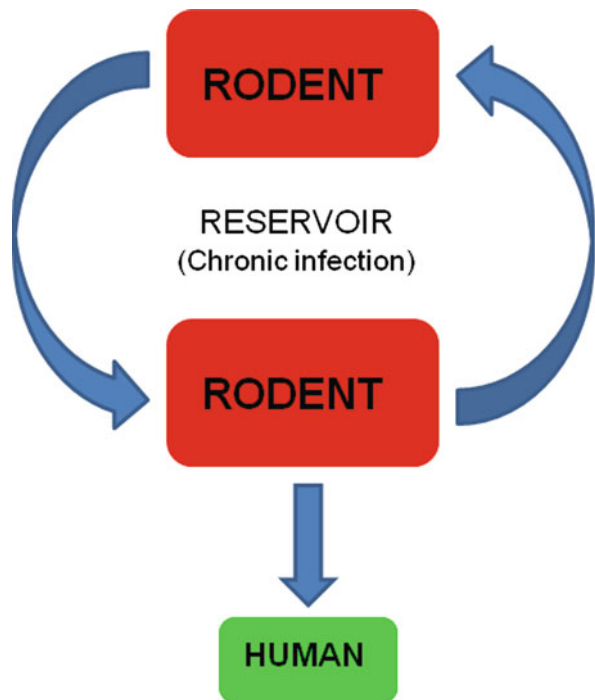
Fig. 8.13 Natural cycle of hantaviruses (drawing by Ivo Rudolf)

in Scandinavia, Belgium, the Netherlands, France, Germany, central and eastern Europe. HFRS was first reported in Korea in 1934 (Korean haemorrhagic fever); during the Korean War in 1951–1954, about 2,500 US soldiers acquired HFRS, and 120 of them died; Korea 1955–1957: 9,000 cases registered; on average, about 100,000 cases of HFRS are reported annually in China (some of them caused by Seoul virus); in the 1950s, several fatal cases of HFRS were also reported in east Slovakia and Serbia. Annual number of hantavirosis cases (mainly NE) in Europe including European Russia approaches 100,000 by expert estimations. In Sweden occur usually 200–600 NE cases annually, but in 2007 it was >1000 cases. An unusual epidemic of Puumala infection appeared in the urban park in Cologne (Germany) where 89 patients were reported in 2005. As many as 736 persons were infected with Puumala virus in Germany from January to August 2010.

Bio-containment: BSL-3.

Diagnosis: serology (ELISA, IFA), RT-PCR, immunohistochemistry (biopsy).

Treatment: ribavirin helps when applied early.

Geographical distribution: Eurasia. (Photos 5.26 and 5.27)

New World Hantaviruses: Sin Nombre, Andes, Choclo, Lechiguanas, etc.

Source of infection (natural host range): neotomine and sigmodontine rodents – *Peromyscus maniculatus* and *P. truei* (Sin Nombre), *P. leucopus* (New York or Shelter Island virus), *Oligoryzomys longicaudatus* (Andes), *O. fulvescens* (Choclo), *O. flavescens* (Lechiguanas), *Akodon azarae* (Lechiguanas), *Sigmodon hispidus* (Black Creek Canal), *Oryzomys palustris* (Bayou) etc. Also these American species of rodents are not only the competent hosts of hantaviruses, but at the same time also the reservoir, due to their persistent infection combined with long-term excretion of the virus by saliva, urine and faeces.

Animal disease: inapparent course.

Transmission mode: aerogenic (dried particles of rodents' infectious excreta – urine, faeces, saliva), contact (rodent bite), and alimentary – food contaminated by infected rodents.

Epidemiological cycle is analogical to that of the Old World hantaviruses. The main risk factor for acquiring HPS or HCPS is being a "male farmer seeing or killing rats in the fields or dwellings".

Human disease: hantavirus pulmonary syndrome (HPS) – high fever, chills, headaches, myalgia, arthralgia, diarrhoea, nausea, sweat, thrombocytopenia, severe hypotension, cough, pneumonia (marked oedema of the lungs) – intensive care including assisted ventilation is necessary, carditis (when also carditis is associated with the syndrome, the disease is called hantavirus cardio-pulmonary syndrome, HCPS); high fatality rate, 45–70%. Sin Nombre: 186 cases in USA, 1993–1998. Andes virus has caused several epidemics with a high lethality; e.g., a total of 628 cases have been reported in Chile, with a mean fatality rate of 30–35%.

Survey of human pathogenic New World hantaviruses

Virus	Main rodent reservoir	Disease	Disease distribution
Laguna Negra	*Calomys laucha, C. callosus*	HCPS	Paraguay, Bolivia, Argentina
Lechiguanas	*Oligoryzomys flavescens*	HCPS	Central Argentina
Andes	*Oligoryzomys longicaudatus*	HPS	Southwest Argentina
Bayou	*Oryzomys palustris*	HPS	Southeast USA
New York-1	*Peromyscus leucopus*	HPS	Eastern USA
Sin Nombre	*Peromyscus maniculatus*	HPS	USA and West Canada
Black Creek Canal	*Sigmodon hispidus*	HPS	Southern Florida
Muleshoe	*Sigmodon hispidus*	HPS	Texas
Araraquara	*Bolomys lasiurus*	HPS	Southeastern Brazil
Castelo dos Sonhos	Unknown	HPS	Central Brazil
Bermejo	*Oligoryzomys chacoensis*	HPS	Argentina, Bolivia
Oran	*Oligoryzomys longicaudatus*	HPS	Argentina, Bolivia
Anajatuba	*Oligoryzomys fornesi*	HCPS	Northern Brazil
Rio Mearim	*Holochilus sciureus*	HCPS	Brazilian Amazon
Rio Mamore	*Oligoryzomys microtis*	HPS	Bolivia, Peru, French Guiana
Central Plata	*Oligoryzomys flavescens*	HPS	Southern Uruguay
Choclo	*Oligoryzomys fulvescens*	HPS	Panama
Calabazo	*Zygodontomys brevicauda*	HPS	South America
Monongahela	*Peromyscus maniculatus*	HPS	North America

Bio-containment: BSL-3.

Diagnosis: serology (ELISA, IFA), RT-PCR, immunohistochemistry (biopsy).

Treatment: ribavirin helps when applied early.

Geographical distribution: North, Central and South America – Sin Nombre virus in USA; Andes virus in Argentina, Uruguay and Chile, Choclo virus in Panama.

8.2.4 *Family* Reoviridae

Virions spherical (60–100 nm), they contain 10–12 segments of dsRNA with a total size of 19–21 kbp; reoviruses do not have lipoprotein envelope, and thus are insensitive to diethyl ether contrary to other arboviruses. They also do not produce haemagglutinin (HIT cannot be used in diagnostics). Due to the highly segmented genome, they are genomically (and phylogenetically) markedly variable.

Coltivirus Colorado Tick Fever

Virions (60–80 nm) contain 12 segments of dsRNA with a total size of 21 kbp.

Source of infection (natural host range): rodents (reservoir hosts: mainly *Spermophilus lateralis*, *Tamias minimus*, *Erethizon dorsatum*, *Neotoma cinerea*, *Peromyscus maniculatus*).

Animal disease: inapparent course (but teratogenic in mice).

Transmission mode: ixodid ticks (*Dermacentor andersoni, D. occidentalis, D. parumapertus, D. albipictus*); iatrogenic – blood transfusion (the virus causes in humans persistent viraemia up to 120 days being localized in erythrocytes).

Human disease: <u>Colorado tick fever</u> (CTF) – usually biphasic, with headache, myalgia and arthralgia, conjunctivitis, photophobia, sometimes orchitis and affection of the CNS (mainly in children), temporary rash occurs less often (5–10% of patients) than in RMSF, occasionally myopericarditis, pneumonia, hepatitis; leucopenia. Rare complications with this disease have included aseptic meningitis, encephalitis, and haemorrhagic fever. Laboratory findings include leucopenia, thrombocytopenia, and mildly elevated liver enzyme levels. Mortality is low, but the convalescence long (fatigue, lethargy).

Bio-containment: BSL-2.

Diagnosis: serology (IgM ELISA, IFA, VNT; not HIT); the virus is detectable since the 4th day of disease (and for a long time) in erythrocytes (by isolation in suckling mice, IF, PCR) – this is a specific pattern of CTF.

Geographical distribution: North America (natural foci in the Rocky Mts. – USA, Canada, most often at altitudes of 1,200–3,000 m above sea level).

Coltivirus Eyach

Closely related to CTF virus.

Source of infection (natural host range): rodents and leporids.

Animal disease: inapparent course.

Transmission mode: ticks *Ixodes ricinus* and *I. ventalloi*.

Human disease: probably neuroinfections (not yet reliably demonstrated).

Bio-containment: BSL-2.

Diagnosis: serology (IgM ELISA, IFA, VNT; but not HIT); virus isolation.

Geographical distribution: Germany (1972), France; antibodies in several neurological patients in Czechland but the virus was not isolated. There is a hypothesis that this virus, a descendant of CTF agent, could have been imported from North America with U.S. Army dogs and their *Dermacentor* ticks to a military base situated in Germany after the 2nd WW, and evolved into Eyach virus under selective pressure of European ecosystems. Another hypothesis suggests that CTF virus could have been introduced in Europe with imported *Sylvilagus floridanus* cottontail rabbits, and evolved to Eyach virus.

Orbiviruses Kemerovo and Tribeč

Contrary to coltiviruses, orbiviruses of the Kemerovo group have only 10 segments of dsRNA with a total size of 19 kbp. Synonyms of Tribeč: Lipovník, Koliba, Cvilín, Brezová (subtype), Mircha, Kharagysh.

Source of infection (natural host range): birds (European starling, chaffinch), rodents, goat.
Animal disease: inapparent course.
Transmission mode: ticks *Ixodes ricinus* and *I. persulcatus*.
Human disease: fever, sometimes meningitis.
Bio-containment: BSL-2.
Diagnosis: serology (CFT, VNT; but not HIT because these viruses do not form haemagglutinin).
Geographical distribution: Eurasia, exceptionally northern Africa.

Orbivirus Orungo

Source of infection (natural host range): probably primates including man.
Animal disease: not observed.
Transmission mode: *Anopheles funestus, An. gambiae,* and other mosquitoes.
Human disease: febrile illness with headache, conjunctivitis, myalgia, vomiting and rash – 60 cases have been reported.
Bio-containment: BSL-2.
Geographical distribution: central Africa and Senegal.

Seadornavirus Banna

This reovirus, a prototype species of the genus *Seadornavirus*, with a dsRNA genome consisting of 12 segments, is considered an emerging mosquito-borne virus in eastern and southeastern Asia. It was first isolated in China (Yunnan province) in 1987. Two groups (A, B) and subgroups (A1, A2) have been disclosed occurring in different areas.

Source of infection (natural host range): pig, cattle.
Animal disease: CNS affection in pigs.
Transmission mode: mosquitoes *Culex tritaeniorhynchus, Cx. pipiens, Cx. pseudovishnui, Cx. modestus, Cx. annulus, Aedes vagus, Ae. albopictus, Ae. vexans, Ae. dorsalis,* and *Anopheles sinensis.*
Human disease: encephalitis, with symptoms sometimes similar to Japanese encephalitis – febrile illness with myalgia and the CNS affection. The disease could be misdiagnosed as JE. Public health impact of this virus is probably underestimated.
Bio-containment: BSL-3.
Geographical distribution: China, Vietnam, Indonesia (from tropics to temperate ecosystems).

Orthoreovirus Pulau (Synonym Melaka)

The virus was first isolated from fruit bats in Tioman Island, Malaysia in 1999. Melaka virus, isolated in 2007 from a patient, and Pulau virus are genomically identical.

Source of infection (natural host range): fruit bats.

Animal disease: not observed.

Transmission mode: aerogenically.

Human disease: high fever, sore throat and acute respiratory illness, with headache, myalgia, malaise, anorexia, and severe prostration – 3 cases were reported in Melaka, Malaysia, in 2007. Antibodies against the virus were found in about 13% inhabitants of the Tioman Island.

Bio-containment: BSL-2.

Geographical distribution: Malaysia.

Rotavirus (Serotypes A and C)

Virions of this genus of the family *Reoviridae* are spherical (80–100 nm), not enveloped, with 11 segments of dsRNA sized 19 kbp.

Source of infection (natural host range): cattle, pig (some genotypes of the serotype C of swine rotaviruses are obviously zoonotic; also zoonotic are some strains of serotype A which causes disease in small children under 5 years old), dog; man.

Animal disease: diarrhoea in calves and piglets.

Transmission mode: alimentary (this is, of course, not an arbovirus).

Human disease: gastroenteritis.

Bio-containment: BSL-2.

Geographical distribution: worldwide.

8.2.5 *Family* Rhabdoviridae

Virions are rod-like [Greek ραβδος (rhabdos) = rod or rivet] with a characteristic bullet shape, about 100–500×45–100 nm, enveloped, containing one molecule of ss(–)RNA sized 11–12 kbp.

Vesiculovirus – Vesicular Stomatitis Complex (VSV) – VSI (Indiana), VSNJ (New Jersey), Alagoas, Piry, Chandipura

Virions are 100–430×45–100 nm, the molecule of RNA has 11 kbp.

Source of infection (natural host range): equids, cattle, pig, wild mammals.

Animal disease: vesicular stomatitis of cattle and horses.

Transmission mode: percutaneous, through conjunctiva, aerogenic, and also by haematophagous diptera (mosquitoes, sandflies [*Lutzomyia trapidoi*: TOT in VSI], biting midges, tabanids, blackflies). Non-viraemic transmission of VSV by co-feeding was experimentally demonstrated among blackflies.

Human disease: vesicular stomatitis – a short-term flu-like disease with myalgia, occasionally with vesicles in the oral and nasal cavities. Chandipura virus can cause encephalitis in children in India.

Bio-containment: BSL-2.
Diagnosis: isolation on cell cultures, IF, serology (ELISA, IFA, VNT).
Geographical distribution: America, Africa (Senegal), India.

Lyssavirus s.s.

Genotype 1 of lyssavirus, rabies virus. Virions 100–435×45–100 nm, enveloped, containing one molecule of ss(–)RNA sized 12 kbp.

Source of infection (natural host range): canids and other carnivores (fox, dog, jackal, coyote, raccoon, skunk, badger *Melogale moschata* (China)); bats (in North America mainly *Lasiurus cinereus* and *Myotis evotis* while in South America *Desmodus rotundus* and other species of vampire bats). The source of human rabies were in Europe in the 1980s: wild mammals 78.8% (of them fox 74.1%, badger and other mustelids 4.0%, roe deer 3.9%), cattle 9.4%, dog 7.4% (the dog most often in Turkey, Greece, and Spain; in Czechland only 3.2%), cat 4.3%, man 0.1% (23 cases: the first patient mostly infected by a dog, 8 times through transplantation of cornea). In Africa, Asia and South America the dog prevails as the source of human infection ("dog rabies": the proportion is 75% and more), followed by domestic ruminants and man, while in North America, similarly as in Europe, wild mammals (76.5%). According to the source of human infection we can distinguish:

(1) "urban (canine) rabies": main source are stray dogs; this form is dominating in Asia, Africa and South America, with mortality 11–83 human cases per 1,000 cases of rabies in animals;
(2) sylvatic (wildlife) rabies: main source are wild mammals; dominating in Europe and North America, with mortality <1 (0.3–0.5) human cases per 1,000 cases of rabies in animals.

Animal disease: lyssa, rabies – paralysis, aggressive behaviour. Rabies was also occasionally diagnosed in cattle, donkey, cat, roe deer, rabbit, and some rodents (squirrel, kangaroo rat, cotton rat, vole, hamster).
Transmission mode: percutaneous – biting of an infected mammal (virus in saliva), transplantation of cornea (8 cases), liver and kidneys (2 cases in Germany, infection of the donor originated in India).
Human disease: rabies (lyssa) – encephalomyelitis with excitation, aerophobia (patient cannot stand breath of air over face), hydrophobia, anxiety, hallucinations and bizzare aggressive behaviour, eventually followed by paralysis, coma and terminating with death at full consciousness (without treatment, the fatality rate is 100%). Acute neurologic disease is divided into furious and paralytic (dumb) forms. Incubation period is long, 10 days to 6 months. The disease was known already in the Old Babylon, Egypt, Israel, India, China, ancient Greece and Rome. According to WHO, about 50,000–60,000 human deaths from rabies are estimated to occur annually, most often in Asia: e.g.,

about 20,000 cases in India (the Buddhist and Hindu ethics forbid culling the stray dogs; also in Thailand the killing of stray dogs is prohibited).

Bio-containment: BSL-2/3.

Diagnosis: anamnesis (animal bite); observation of the captured biting animal for the period of 5–10 days by a veterinarian, or post-mortem examination of the animal (histological slides of the brain and salivary glands – detection of antigen by the technique IF, Negri bodies or intracerebral inoculation of mouse); in the human patient, IF microscopy of corneal, mucosal and bioptic samples; also isolation on mice.

Treatment: post-exposure prophylaxis (PEP) is an immunization with several doses of vaccine combined with antiserum application (antirabic equine immunoglobuline).

Prevention: vaccination of exposed persons – several effective inactivated rabies vaccines are currently available worldwide (the virus is cultivated in human diploid cells); obligatory immunization of pet dogs, reduction of foxes or their vaccination (baited oral vaccine with strain SAD, suggested by George M. Baer); the latter method has 80% effectivity in the fox populations. Oral vaccination of foxes started in Europe in Switzerland (1978), and it has then been used in France, Germany, Czechland and other countries.

Geographical distribution: *Lyssavirus* s.s. occurs worldwide with the exception of Great Britain (the UK has applied a very strict policy since 1886: a 6-month quarantine for import or visit of dogs and cats), Ireland, Iceland, Japan, Australia, New Zealand and some other islands. The highest prevalence of the virus is in South and East Asia, and Central Africa.

Bat Lyssaviruses

Virions are similar to those of *Lyssavirus* s.s.

Genotype 2: **Lagos bat** virus (isolated from African bats *Eidolon helvum, Micropterus pusillus, Epomophorus wahlbergi*, *Epomops dobsonii,* and from the carnivore *Atilax paludinosus*) – probably not pathogenic to humans.

Genotype 3: **Mokola** virus (from African rodent *Lophuromys sikapusi*, shrews *Crocidura* spp.; also domestic dogs and cats) – few fatal human cases have been reported.

Genotype 4: **Duvenhage** virus (from bats *Miniopterus schreibersii, Nycteris gambiensis, N. thebaica*) – exceptional fatal cases in humans (South Africa 2006, Kenya 2007 – a Dutch tourist).

Genotype 5: **European bat lyssavirus type 1** (EBLV-1: bats, mainly *Eptesicus serotinus,* further *E. isabellinus, Tadarida teniotis, Myotis myotis, M. nattereri, Miniopterus schreibersii, Rhinolophus ferrumequinum*); bat to cat transmission of EBLV-1 was documented in France (2003); two human fatal cases have been reported (Ukraine 1977, Russia 1985).

Genotype 6: **European bat lyssavirus type 2** (EBLV-2: bats *Myotis dasycneme, M. daubentoni*) – two human fatal cases were reported (Finland 1985,

Scotland 2002). Relatively closely inter-related are the genotypes 4 and 5, EBL1 is closer to Duvenhage than is EBL2.

Genotype 7: **Australian bat lyssavirus** (ABLV: fruit bats *Pteropus alecto, P. scapulatus, P. poliocephalus, P. conspicillatus, Saccolaimus flaviventris*) – two fatal human cases were reported in Queensland, 1996 and 1998.

Genotype 8: "**Marmoset" lyssavirus** (from the marmoset *Callithrix jacchus* kept as a pet animal in Brazil) – a total of eight fatal cases in humans in the years 1991–1998.

Some additional bat (*Microchiroptera*) lyssaviruses were isolated in Asia (**Aravan, Khujand**, **Irkut**, and **West Caucasian**) but their pathogenicity for man is unknown, no human cases have been reported.

Source of infection (natural host range): bats – EBL1 largely *Eptesicus serotinus* (in the Netherlands in 21% from 1,219 bats of this species in 2005, also in central Europe), further *Vespertilio*, *Nyctalus*, less *Miniopterus schreibersii*, *Rhinolophus ferrumequinum*; EBL2 *Myotis* spp. (*M. daubentoni*, *M. dasycneme*); ABL fruit bat *Aethalops alecto*. Numbers of bats with demonstrated EBL1 or EBL2 in Europe, 1954–1992: the Netherlands 194, Denmark 164, Germany 76, Spain 7, former Yugoslavia 7, Poland 3, Sweden 3, France 2, Switzerland, Czechland, Russia and Turkey one each.

Animal disease: in bats – sometimes paralysis, aggressive behaviour.

Transmission mode: percutaneous – bites of an infected bat (virus in saliva); aerogenic (by aerosol in caves populated by rabies-infected bats).

Human disease: rabies (lyssa) – encephalomyelitis with excitation etc. Some of the bat lyssaviruses can kill humans (see above).

Bio-containment: BSL-2/3.

Diagnosis: anamnesis (animal bite); examination of the animal (histological slides of the brain and salivary glands – detection of antigen by IF or intracerebral inoculation of mouse); in the human patient, IF microscopy of corneal, mucosal and bioptic samples; also isolation on mice.

Treatment: post-exposure prophylaxis is an immunization with several doses of vaccine combined with antiserum application (antirabic equine immunoglobuline).

Geographical distribution: mainly in Europe and Africa, e.g. EBL1 in Poland, Czechland, Ukraine, Russia, Finland, France, Germany, Denmark, the Netherlands, former Yugoslavia, Spain; EBL2 in Great Britain, Finland, the Netherlands, Germany, Switzerland. However, some human pathogenic bat lyssaviruses occur also on other continents (see above).

8.2.6 *Family* Arenaviridae

Virions spherical to pleomorphic (85–130 nm), enveloped, contain two segments of ss($\pm$) RNA sized 11 kbp. Interestingly, they also contain ribosomes (20–25 nm) that cause their "sandy" appearance under electron microscopy. Zoonotic arenaviruses

belong to two complexes: (1) Old World (LCM-Lassa) complex with *Murinae* rodents as reservoir (with the viruses LCM, Lassa, Mopeia, Mobala, Dandenong, Morogoro, Ippy, Kodoko, and Lujo), and New World (Tacaribe) complex, with *Sigmodontinae* rodents as reservoir (with the viruses Tamiami, Whitewater Arroyo, Pichinde, Amapari, Flexal, Guanarito, Junin, Latino, Oliveros, Machupo, Parana, Pirital, Sabiá, Bear Canyon, Catarina, Allpahuayo, Chapare, Cupixi, and Tacaribe).

Arenavirus Lymphocytic Choriomeningitis (LCM virus)

Source of infection (natural host range): some species of wild and pet rodents (mainly *Mus musculus* and *M. domesticus*, but also *M. spretus*, *Apodemus agrarius*, *A. flavicollis*, *A. sylvaticus*, *A. mystacinus*, *Micromys minutus*, *Microtus levis*, *Chionomys roberti*, *Myodes glareolus*, *Arvicola schermani, Mesocricetus auratus, Cavia porcellus*) are reservoir of the infection (congenital infection with the long-life carriership and occasional excretion of LCM virus with saliva, faeces and urine, also horizontal and vertical transmission among rodents).

Animal disease: LCM (only in adult mice – young rodents, when infected, form subsequently an immunotolerant state with persistent infection).

Transmission mode: contact, percutaneous (biting), aerogenic, alimentary (contaminated food), congenitally (from infected mother to her foetus); organ transplantations.

Human disease: lymphocytic choriomeningitis – flu-like aseptic meningitis or only fever (usually biphasic) with headache, rhinitis, bronchitis, photophobia; miscarriage, embryo malformations (hydrocephalus, chorioretinitis); fatality rate about 1%, convalescence long. Immunocompromised patients are highly susceptible to the infection. Occupational disease in pet rodent breeders. **Dandenong** is a new arenavirus related to LCM that was recently isolated from patients after transplantation from deceased donor, who had traveled in eastern Europe.

Bio-containment: BSL-2/3.

Diagnosis: serology (indirect IFA, ELISA, CFT, VNT).

Geographical distribution: probably worldwide.

Arenavirus Lassa

Source of infection (natural host range): rat *Mastomys natalensis* (reservoir: viraemia, virus excretion in urine; Photo 7.56), *M. erythroleucus* (in Sierra Leone), less often other rodents; man.

Animal disease: inapparent course (carriership – reservoir).

Transmission mode (Fig. 8.14): contact, alimentary (capture and consumption of *Mastomys*), aerogenic (aerosolized *Mastomys* urine); high contagiosity – potential transmission to medical personnel who can acquire the disease in the hospital or in village settings from the index patients (blood, excreta).

Human disease: Lassa haemorrhagic fever with shivers (chills), pharyngitis, lymphadenitis, headaches, tinitus, myalgia, arthralgia, abdominal pain, diarrhoea, vomiting, rash, haemorrhages (bleeding into gastrointestinal tract

Fig. 8.14 Natural cycle of Lassa virus (drawing by Ivo Rudolf)
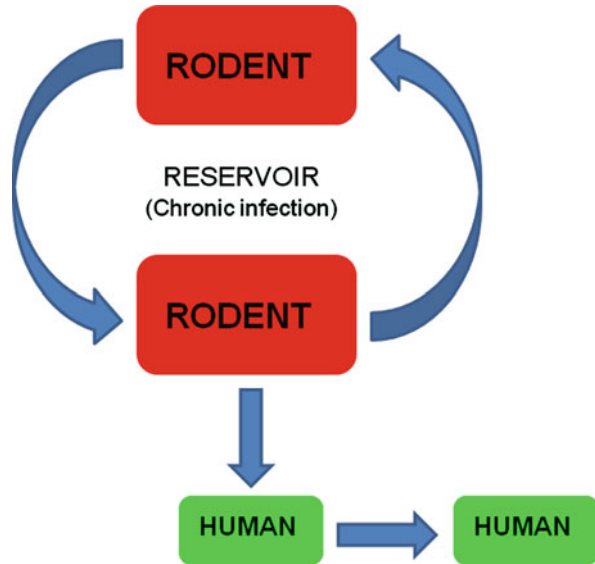
etc.), facial and neck oedema, tinnitus to deafness (common complication), hypovolaemia, abortion; fatality rate 10–50%, in severe cases up to 70%. In 1969–1975, a number of epidemics were recorded in tropical Africa (Nigeria, Liberia, Sierra Leone); hundreds of cases have been observed in northern Nigeria each year. 1996–1997 Sierra Leone: 823 cases, 53 died. In Sierra Leone, up to 40% of adult persons were found to be seropositive to Lassa virus in some areas (while the national mean seropositivity rate was 9%). It is estimated that 100,000–300,000 human infections per year occur in West Africa (with approximately 5,000 deaths). It is interesting that only 6–20% of persons infected with Lassa virus become clinically ill (according to serological survey in the latter two African countries). Imported cases of Lassa fever are occasionally reported in tourists and hunters returning from endemic regions.

Bio-containment: BSL-4.

Diagnosis: ELISA (IgM and IgG), IFA, RT-PCR, immunohistochemistry (post-mortem).

Treatment: ribavirin (helps only when applied in the first 3 or so days of disease: fatality rate decreases to 5–10%); immune serum; supportive care (e.g. fluid and electrolyte balance, oxygenation, blood pressure monitoring).

Prevention: avoiding contact with *Mastomys* spp. rodents.

Geographical distribution: western and central Africa (Nigeria, Liberia, Sierra Leone, DR Congo, Mali, Guinea, Senegal and the Central African Republic).

Arenavirus Lujo

Source of infection (natural host range): rodents.
Animal disease: unknown.

Transmission mode: contact or aerosol (highly contagious).

Human disease: haemorrhagic fever with severe headache, myalgia, vomiting, chest pain, sorethroat (pharyngitis), thrombocytopenia, granulocytosis; acute respiratory distress syndrome, and renal dysfunction. Outbreak of disease involving 5 patients, 4 of whom died, occurred in South Africa, 2008 (the first patient had been transferred from Zambia to South Africa for medical management, 3 cases involved secondary spill over of infection from the first patient, and one was a tertiary infection).

Bio-containment: BSL-4.

Diagnosis: ELISA (IgM and IgG), RT-PCR, immunohistochemistry (post mortem).

Treatment: ribavirin or immune plasma in the acute phase.

Geographical distribution: Zambia (South Africa).

Arenavirus **Junin**

Source of infection (natural host range): drylands vesper mouse *Calomys musculinus* (reservoir) – chronic infection with a persistent viraemia and excretion of the virus by urine, saliva and faeces.

Animal disease: inapparent course (but lethal in guinea pig).

Transmission mode: alimentary, contact (percutaneously – bites), aerogenic (in farmers); high risk of laboratory infections (aerosol).

Human disease: Argentine haemorrhagic fever – high fever, headaches, pain in abdomen and extremities, conjunctivitis, erythema on head and chest, petechial bleeding in the mouth, gastrointestinal and urogenital tracts, petechiae also in the skin, neurological defects (palsy, epilepsy), hypovolemic shock; fatality rate up to 30%, very long convalescence. Epidemics and cases have occurred in Argentina since 1958 (discovery date); up to now a total of at least 25,000 clinical cases.

Bio-containment: BSL-4.

Diagnosis: ELISA (IgM and IgG), RT-PCR, immunohistochemistry (post mortem).

Treatment: should start early with immune serum (plasma) and ribavirin.

Prevention: vaccine.

Geographical distribution: South America (Argentina).

Arenavirus **Machupo**

Source of infection (natural host range): rodents *Calomys* spp. (mainly *C. callosus*, reservoir) - chronic infection with persistent viraemia, excretion of the virus *via* urine, saliva and faeces.

Animal disease: inapparent course (but lethal for guinea pig).

Transmission mode: alimentary, contact (percutaneous – bites), aerogenic (in farmers); exceptionally high risk of laboratory infection (aerosol).

Human disease: Bolivian haemorrhagic fever – high fever, headaches, pain in abdomen and extremities, conjunctivitis, erythema on head and chest, petechial bleeding in the skin, mouth, gastrointestinal and urogenital tracts,

neurological defects (palsy, epilepsy), hypovolemic shock; fatality rate 10–30%; very long convalescence. Epidemics and sporadic cases have occurred in Bolivia since 1964 (discovery date), e.g. in 1994 or the latest in 2007 with 20 cases (3 fatal); in total, several thousand cases have been estimated to occur in Bolivia.

Bio-containment: BSL-4.

Diagnosis: ELISA (IgM and IgG), RT-PCR, immunohistochemistry (post mortem).

Treatment: immunne serum (immediately after infection), ribavirin in the early phase.

Prevention: vaccine (Junin, partially cross-protecting).

Geographical distribution: South America (Bolivia).

Arenavirus Guanarito

Source of infection (natural host range): short-tailed cane mouse *Zygodontomys brevicauda* (reservoir), *Sigmodon alstoni*.

Animal disease: unknown.

Transmission mode: alimentary, contact (percutaneous), aerogenic (in farmers).

Human disease: Venezuelan haemorrhagic fever with malaise, myalgia, anorexia, headache, back pain, dizziness, nausea, vomiting, prostration, petechiae, bleeding gums, later bleeding from mucous membranes, tremor, lethargy, convulsions, occasional coma and death.

Bio-containment: BSL-4.

Diagnosis: ELISA (IgM and IgG), RT-PCR, immunohistochemistry (post mortem).

Treatment: immune plasma or ribavirin in the early phase.

Geographical distribution: South America (Venezuela).

Arenaviruses Sabiá and Chapare

Closely related viruses, but distinct species.

Source of infection (natural host range): rodents (reservoir).

Animal disease: unknown.

Transmission mode: contact, alimentary, aerogenic.

Human disease: haemorrhagic fever with a course similar to the other New World arenaviruses. First case of Sabiá virus disease (fatal) reported in Sao Paolo in 1990, and several laboratory infections have been also described. Chapare virus caused a small epidemic in Bolivia (2003/2004) with at least one fatal case.

Bio-containment: BSL-4.

Diagnosis: ELISA (IgM and IgG), RT-PCR, immunohistochemistry (post mortem).

Treatment: immune plasma or ribavirin in the early phase.

Geographical distribution: South America – Brazil (Sabiá) and Bolivia (Chapare).

Arenavirus **Whitewater Arroyo**

Source of infection (natural host range): white-throated woodrat *Neotoma albigula*.
Animal disease: unknown.
Transmission mode: contact or aerosol.
Human disease: haemorrhagic fever (3 fatal human cases reported in California).
Bio-containment: BSL-2.
Diagnosis: ELISA (IgM and IgG), RT-PCR, immunohistochemistry (post mortem).
Treatment: ribavirin in the acute phase.
Geographical distribution: Southwest USA.

8.2.7 Family Filoviridae

Filoviruses are characteristic for their long filamentous virions (under electron microscopy, some of them appear ring-like, others in the shape of the figure "9", or even branching), 800–1000×80 nm or longer, up to 14 µm (!), enveloped, containing one molecule of ss(–) RNA sized 19 kbp.

Marburgvirus

Source of infection (natural host range): monkeys (*Cercopithecus aethiops* and *C. ascanius*), fruit bats (the reservoir – 2007 Gabon, Uganda, Kenya: detection of the virus RNA in 1–5% of examined *Rousettus aegyptiacus* (Photo 5.28), several virus isolations from bat tissues, and IgG antibodies detected in 2–12% bat individuals), insectivorous bats *Rhinolophus eloquens, Miniopterus inflatus* in DR Congo; man.
Animal disease: sometimes inapparent, but often fatal haemorrhagic disease in primates such as *Cercopithecus aethiops* or chimpanzee (up to 50% fatality rate) and also at experimental infection of some other monkey species of the Old World. Asymptomatic course in fruit bats.
Transmission mode (Fig. 8.15): contact (primate saliva, blood and excreta; faeces of infected fruit bats: experimentally infected bats shed the virus for up to 3 weeks), iatrogenic (injections, blood transfusion), less often aerogenic; a high contagiosity – a number of nosocomial and several laboratory infections.
Human disease: Marburg haemorrhagic fever, one of the most fatal infectious diseases, with chills, severe headaches, myalgia, pharyngitis, nausea, abdominal pain, diarrhoea, extreme fatigue, maculopapular rash most prominent on the trunk (chest, back, abdomen), skin hypersensitivity, neurological disturbances, conjunctivitis, lymphadenitis, splenomegaly, leucopenia, thrombocytopenia, massive haemorrhages – bleeding from nose, ears and eyes, bloody faeces and bloody (black) vomites, severe cachexia, multiorgan dysfunction

Fig. 8.15 The cycle of
Marburg virus (drawing by
Ivo Rudolf)
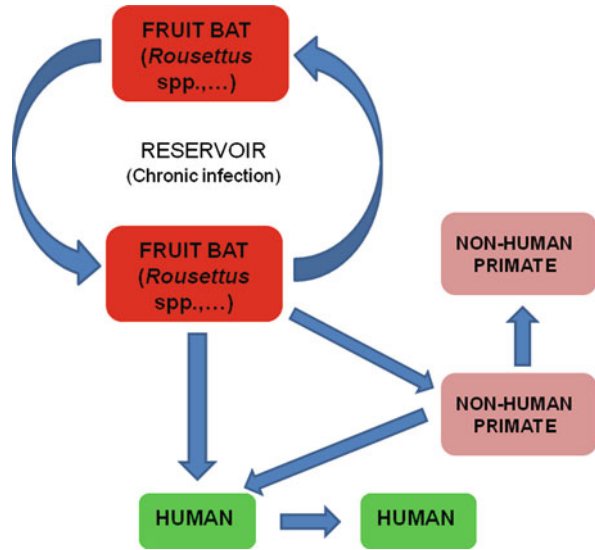
(liver etc.), hypovolemic shock and renal failure, delirium, the face looking
as a mask; fatality rate 50–90%. Long-term sequelae in many convalescents
(chronic hepatitis, transverse myelitis, uveitis, parotid gland, orchitis, impo-
tence in some men). Epidemics: 1967–1968 Germany (Marburg, Frankfurt)
and Serbia (Beograd): marmosets *Cercopithecus aethiops* imported from
Uganda – 31 laboratory personnel fell ill, 7 died; 1975 South Africa 3 cases
in Australian tourists; 1980 and 1987 Kenya (Mt. Elgon) 3 tourists visiting
the Kitum Cave died; 1999–2000 DR Congo (Durba: a gold mine) – 149
cases, 86% of patients died; 2004–2005 north Angola: 252 ill persons, 90%
succumbed; 2007 Uganda: 10 miners working in a lead mine died; 2008, a
Dutch tourist (visiting a cave with fruit bats in Uganda) died.

Bio-containment: BSL-4.

Diagnosis: isolation of the virus from the blood or other body fluids on Vero
cells or in guinea pigs (a risky procedure), electron microscopy, RT-PCR;
detection of IgM in the blood.

Treatment: therapy does not exist (ribavirin ineffective), only supportive treat-
ment.

Geographical distribution: tropical Africa (Uganda, DR Congo, Zimbabwe).

Ebolavirus

Five genotypes: Sudan (1976), Zaire (1976), Côte d'Ivoire (1994), Bundibugyo
(Uganda, 2007) and for man nonpathogenic Reston virus (1989: monkeys
Macaca fascicularis imported in the USA from the Philippines).

Source of infection (natural host range): primates including man, bats.
However, the reservoir are bats including fruit bats (experimental viraemia

lasted up to 21 days in some species – *Tadarida condylura*, *T. pumila*, *Epomophorus wahlbergi*); hunted fruit bats can also be the source of infection. Recently, *Ebolavirus* RNA was detected in visceral organs of fruit bats *Hypsignathus monstrosus, Epomops franquetti,* and *Myonycteris torquata*.

Animal disease: usually inapparent except for chimpanzee (fatality rate up to 50%), but experimental infection of monkeys often fatal. Reston virus is pathogenic to pig.

Transmission mode: contact and alimentary (saliva, blood and excretions of primates, hunter contacts with dead chimpanzees and monkeys and their meat; tree fruit contaminated with bat saliva and other excreta), iatrogenic (inoculation, transfusions), less often aerogenic; high contagiosity – nosocomial and laboratory infections occur quite frequently. Funeral rituals are also a common risk factor during the outbreaks.

Human disease: Ebola haemorrhagic fever, one of the most fatal human infectious diseases, with acute and severe headaches, pharyngitis, pains of muscles, sore back, abdominal pain, hiccups, severe diarrhoea, nausea, vomiting, extreme fatigue and weakness, rash over whole body, neurological and psychotic disturbances (disorientation etc.), conjunctivitis, leucopenia, thrombocytopenia, effective blocking of interferon production, systemic haemorrhages, bloody faeces and bloody vomites, cachexia, hypovolemic shock and renal failure, the face looking as a mask; fatality rate 50–80%; a long-term convalescence. A number of mild or asymptomatic *Ebolavirus* infections have been observed in humans – in some enzootic areas (e.g., in Gabon) a relatively high proportion of population (about 10%) has antibodies to ebolavirus Zaire without being clinically ill before. Epidemics in the years 1976, 1979 and 1995 in Sudan and Zaire caused a total of 818 cases with a very high mortality: e.g., during the 1995 outbreak in Zaire 79% and in Sudan 53%. Other epidemic in Gabon 1994–1996 had 80 cases, 2000–2001 outbreak in Uganda with 428 cases (52% fatality rate), 2002–2004 central Africa (Gabon, DR Congo) 428 cases (fatality rate 78%). During the years 1976–2000 a total of 1,524 cases of Ebola fever were recorded, and 1,010 of the patients died. A more recent outbreak was reported from DR Congo (Luebo, Kasai province) in 2007: 372 cases (186 patients died); this outbreak was linked to a masssive migration of fruit bats hunted by villagers at the bat daily communal roosts situated often on palm trees close to the village. A large proportion of the human population living in forested areas of Gabon has both humoral and cellular immunity to Zaire ebolavirus. A new genotype, Bundibugyo virus, was the cause of 116 confirmed or probable haemorrhagic cases in western Uganda, 2007–2008, with a fatality rate of 34%). The Côte d'Ivoire virus caused only one non-fatal haemorrhagic human case.

Bio-containment: BSL-4.

Diagnosis: isolation of the virus from the blood or other body fluids on Vero cells or in guinea pigs (extreme Biohazard level: BSL-4), electron microscopy, RT-PCR; detection of IgM in the blood (ELISA); immunohistochemistry (post-mortem).

Treatment: therapy does not exist, ribavirin ineffective; only supportive care.

Prevention: avoiding contact with infected humans or non-human primates and fruit bats (and their habitats like roosting sites in caves) infected with the virus.

Geographical distribution: tropical Africa (Zaire = DR Congo (Photos 5.29, 5.30, 5.52), Gabon, Sudan, Uganda, Ivory Coast, Cameroon); the Philippines (Reston virus).

8.2.8 *Family* Orthomyxoviridae

(**) *Orthomyxovirus* Influenza A

Virions are spherical to pleomorphic 80–120 nm, enveloped, with 8 segments of ss(–)RNA sized 10–15 kbp and with two surface antigens (glycoproteins): haemagglutinin (16 H subtypes) and neuraminidase (nine N subtypes). All these antigenic subtypes have been detected in birds, while in mammals only some of them: for instance in humans they have been found only H1,2,3,5,7,9 and N1,2,7. Changes and evolution of influenza A viruses: antigenic "drift" (restricted mutations in H). . .antigenic "shift" (changes in H and N associated with recombinations or reassortement of individual subtypes in vertebrates (duck, pig) leading to appearance of new subtypes).

Source of infection (natural host range): man; in zoonotic influenza pig, horse, and fowl. Free-living waterfowl, gulls and shorebirds are the main reservoir of influenza A viruses in nature. Some wild anseriforms (swans, geese, ducks) also participate in the spread of highly pathogenic avian influenza (HPAI) viruses.

Animal disease: inapparent (in a majority of waterbirds) or fever, sinusitis, bronchopneumonia, oedema of the face and neck, cyanosis – e.g., "fowl plague" in hens due to HPAI virus subtypes H5 and H7, with a fatality rate 70–100%; these subtypes may cause clinical disease also in some terrestrial and aquatic mammals.

Transmission mode: aerogenic (a droplet infection), also by conjunctivae and contact. In the zoonotic HPAI H5N1 virus is the highest risk of human infection associated with processing of fowl (killing, removal of feather, cutting into portions), and in Asian animal markets with living fowl if it is infected. Human-to-human transmission of HPAI viruses has been absent or very rare.

Human disease: influenza (an anthroponosis), often complicated with pneumonia, with the fatality rate of approximately 1%. The known anthroponotic influenza pandemics occurred in 1918/19 ('Spain flu', H1N1, which killed about 21 million persons but some sources give as many as 40 million victims); 1957/58 ('Asian flu', H2N2: >1 million victims; 1968/70 ('Hongkong flu', H3N2: at least 700,000 deaths; 1977 ('Russian flu', H1N1); and the recent pandemic of 'swine (Mexican) influenza' (H1N1) has encompassed

213 countries and caused 18,449 deaths (as of 1 August 2010). Except for the recent outbreak, all pandemic influenza A strains had in its genome inserted characteristic fragments of avian influenza viruses; they therefore emerged as reassortants of avian and mammalian influenza strains during co-infection of a susceptible mammal host (usually domestic pig as a 'mixing vessel'). However, sporadical zoonotic influenza infections of humans with equine, swine and avian strains have been reported, usually without a further spread in human population. For instance in Wisconsin (1990) several pig breeders became ill, and one pregnant women died. This so-called swine influenza caused about 50 laboratory-confirmed cases (7 patients died) worldwide up to 2008. Another zoonotic problem is avian influenza; first cases of it were described in Hongkong (1997), where 18 persons were infected from chickens with the HPAI virus type H5N1 from chickens, and 6 of the patients died (one million hens had to be put down). In 2003, one veterinarian died after being infected with HPAI virus type H7N7 in the Netherlands (30 million chickens were then killed, and during this action 89 persons get fever with severe conjunctivitis). Further cases of infection with HPAI virus H5N1 acquired directly from chickens were reported in 2003–2010: from a total of 504 laboratory-confirmed human cases in 15 countries (reported to the WHO as of August 12, 2010), the highest incidence was in Indonesia (168 cases), Vietnam (119), and Egypt (111) – 299 patients died (case fatality rate of 59%). The fatality rates of H5N1 avian influenza in humans have varied from 32% (Egypt) to 50% (Vietnam), 67% (China), 68% (Thailand), and 83% (Indonesia). During the same period, at least 200 million fowls were culled. Close contacts of humans with domestic ducks, chickens and pigs (common dwellings) in southeastern Asia present a permanent epidemiological risk into the future.

Bio-containment: BSL-2 (but HPAI H5N1: BSL-2/3).

Diagnosis: isolation and cultivation in chick embryos or on cell cultures, detection of antigen or RNA in the samples, serology (HIT).

Treatment: antivirotics amantadin, rimantadin, zanamivir (Relenza), oseltamivir (Tamiflu) might moderate the clinical course, but resistant strains of influenza A have already appeared.

Prevention: vaccine (human and fowl), culling of infected animal breeds.

Geographical distribution: worldwide; most strains of zoonotic influenza originate in Southeast Asia.

Thogotoviruses Thogoto and Dhori

Virions are spherical, 80–120 nm, enveloped, contain ss(–)RNA arranged in 6 (Thogoto) or 7 (Dhori) segments with a total size of 10 kbp, and one surface glycoprotein.

Source of infection (natural host range): cattle, camel.
Animal disease: inapparent course.

Transmission mode: metastriate ticks *Rhipicephalus* and *Amblyomma* spp.
Human disease: severe fever, the course can be complicated occasionally.
Bio-containment: BSL-3.
Geographical distribution: Africa, southern Europe (Sicily, Portugal), Asia, southern Russia.

8.2.9 *Family* Paramyxoviridae

Avulavirus Newcastle Disease (Avian Paramyxovirus 1)

Virions are spherical or pleomorphic, 150 nm or more, with one molecule ss(−)RNA sized 15 kbp.

Source of infection (natural host range): domestic, wild and pet birds.
Animal disease: Newcastle disease (ND) of fowl and other birds, e.g. wild cormorants in North America, with the fatality rate of 10–90%.
Transmission mode: contact, aerogenic (workers in fowl farms, laboratory accidents).
Human disease: sporadic – painful conjunctivitis, regional lymphadenitis, pharyngitis, bronchitis, atypical pneumonia, affection of the CNS.
Bio-containment: BSL-2/3.
Diagnosis: isolation of the virus from ocular irrigations or from the blood and urine, serology (VNT, ELISA, HIT).
Prevention: fowl vaccination.
Geographical distribution: worldwide.

Henipavirus Hendra

Also called equine morbillivirus, or bat paramyxovirus. Virions are spherical or pleomorphic, about 150 nm, 1 molecule ss(−)RNA sized 15 kbp. The virus is distantly related to distemper morbillivirus.

Source of infection (natural host range): horse; fruit bats (reservoir: *Pteropus alecto, P. poliocephalus, P. scapulatus, P. conspicillatus*).
Animal disease: fruit bats asymptomatic; in horses fever and bronchopneumonia, haemorrhage, encephalitis; fatality rate high, 65%. The disease has been known since 1994/1995, when 23 horse cases (16 died) were recorded on 3 places of Queensland, 13 of them in Hendra (a suburb of Brisbane). Pigs are susceptible to Hendra virus in experiment and develop fever, depression, respiratory and CNS affection signs.
Transmission mode (Fig. 8.16): contact – exposition to infected horses body fluids.
Human disease: affection of the respiratory tract (haemorrhagic pulmonary oedema), headaches, myalgia, encephalitis. Three cases (two fatal) were described in owners and trainers of 22 ill horses (14 died) from Hendra
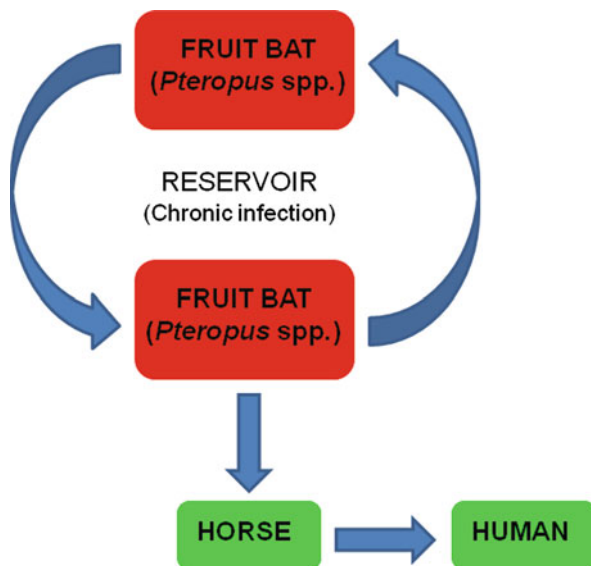
and Mackay in 1994, and similar 7 cases of horse owners and attending veterinarians (two were fatal) were documented in 2008–2009.

Bio-containment: BSL-4.

Diagnosis: isolation (urine, CSF, throat swab, nasal secretions) – risky procedure, RT-PCR, ELISA, VNT, IFA, immunohistochemistry.

Geographical distribution: Australia (Queensland), Papua New Guinea.

Henipavirus Nipah

Antigenically closely related to Hendra virus, the difference in nucleotide sequence of the genome is only 10–20%.

Source of infection (natural host range): pig (cat, dog, goat, man); the reservoir are fruit bats – *Pteropus hypomelanus*, *P. giganteus*, *P. lylei* and *P. vampyrus* (the virus was isolated from their guano in Thailand, Cambodia and Indonesia, and antibodies have been found in up to 30% of the fruit bats).

Animal disease: barking pig disease with fever, bronchopneumonia, secretion of mucus with blood from nasal and oral cavities, necrotic vasculitis, often encephalitis, pareses of hind limbs, and death; fatal rate nearly 100% in pigs. Asymptomatic in fruit bats. Bats contaminate fruit which have been used as pig feed. Experimental infection: fatal pneumonia in horse, cat, dog and guinea pig, and squirrel monkey *Saimiri sciureus*.

Transmission mode (Fig. 8.17): contact, food-borne, aerogenic (high contagiosity; in contrast to Hendra virus, this henipavirus is excreted in urine and by coughing of infected animals), and alimentary (consumption of raw

Fig. 8.17 Circulation of Nipah virus (drawing by Ivo Rudolf)
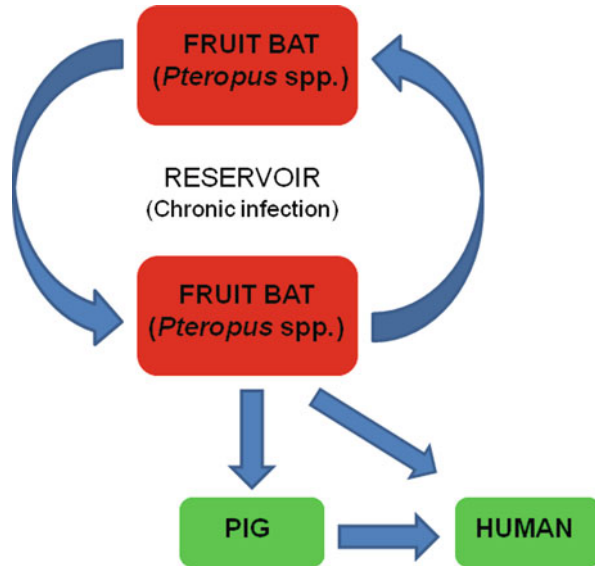
date palm sap – Bangladesh). Occupational disease: butchers (Singapore). In 2007, man-to-man transmission was documented for the first time – in Bangladesh (where more than half of the cases were nosocomial) and India.

Human disease: fever, headache, bronchopneumonia, vasculitis and thromboses, decreased consciousness, encephalitis; high fatality rate, 40%. Epidemics: 1998–1999 Malaysia 265 cases (41% fatal) and Singapore 11 cases (1 fatal); 2001–2007 Bangladesh several outbreaks with 192 cases in total and fatality rate of 69%; 3 additional fatal cases here (Faridpur district) have been reported in 2010.

Bio-containment: BSL-4.

Diagnosis: isolation (urine, CSF, throat swab, nasal secretions) – risky procedure, RT-PCR, ELISA, VNT, IFA, immunohistochemistry.

Prevention: liquidation of pig breeds affected (in Malaysia, 1999, 900,000 domestic pigs were slaughtered originating from 900 farms).

Geographical distribution: Malaysia, Singapore (introduced in 1999 with import of pigs from Malaysia), Thailand, Cambodia, Indonesia, India, Bangladesh, southern China. According to some serosurveys, similar virus also occurs on Madagascar.

Henipavirus **Menangle**

Antigenically related to Nipah virus. The virus was first identified in a piggery in Menangle near Sydney (Australia) in 1997 where they experienced a high number of pig stillbirths and deformities during farrowing.

Source of infection (natural host range): pig, man; the reservoir are fruit bats – *Pteropus conspicillatus* and *Aethalops alecto*.

Animal disease: in pigs abortions and teratogenic defects; mummified and stillborn piglets with severe degeneration of the brain and spinal cord (almost absent in some piglets), arthrogryposis, brachygnathia, and occasionally fibrinous body cavity effusions and pulmonary hypoplasia, occasionally nonsuppurative myocarditis. Asymptomatic infection in fruit bats.

Transmission mode: contact (wounds on hands and forearms) with bodily fluids from infected animals (blood and possibly foetal matter-e.g., amniotic fluid); aerogenic.

Human disease: a febrile illness with chills, malaise, headache, and a measles-like rash; only two human cases have been described in Australia (1997–1998).

Bio-containment: BSL-3.

Diagnosis: isolation (urine, CSF, throat swab, nasal secretions) – risky procedure, RT-PCR, ELISA, VNT, IFA, immunohistochemistry.

Geographical distribution: Australia.

8.2.10 *Family* **Bornaviridae**

Spherical virions (85–125 nm) with one molecule of ss(–)RNA, unsegmented, 9 kbp in size.

Bornavirus

Remark: many virologists do not regard that this is a zoonotic agent.

Source of infection (natural host range): horse; sheep, goat, cattle, rabbit, and other mammals (unclear). The natural virus reservoir is unknown at present (rodents?).

Animal disease: Borna disease, a fatal meningoencephalitis of horse and sheep (first epizootic described in Germany, 1885).

Transmission mode: contact with animal excreta (saliva, tears, nasal discharge), possibly also aerogenic and alimentary. Tenacity of the virus in the dry state is exceptionally long (1–3 years).

Human disease: neuropsychiatric disorders not sufficiently characterised, possibly also lymphocytic meningoencephalitis; bornavirus antibodies but also RNA were found in CSF and peripheral blood leucocytes of some psychiatric or immunocompromised patients in several studies.

Bio-containment: BSL-2.

Diagnosis: isolation in cell cultures; intracerebral inoculation of rabbit (fatal).

Geographical distribution: Germany, Austria, Switzerland, France; infrequently it occurs in Japan and USA.

8.2.11 *Family* **Coronaviridae**

Coronavirus **SARS**

Also called severe acute respiratory syndrome–associated coronavirus (SARS-CoV). Virions are spherical to oval (120–160 nm), enveloped, with a molecule of ss(+)RNA sized 28–31 kbp.

   Source of infection (natural host range): civet *Paguma larvata*, raccoon-like
      dog (*Nyctereutes procyonoides*), Chinese ferret-badger (*Melogale moschata*)
      and other medium-sized carnivores on South-Asian animal markets; man.
      However, probable reservoir are bats (SARS-like coronaviruses have been
      isolated from *Rhinolophus* spp., *Miniopterus magnater*, and *Pipistrellus*
      spp.).
   Animal disease: asymptomatic infection.
   Transmission mode: aerosol and contact. Man-to-man transmission is common.
      The virus has a considerable tenacity in environment – it survives up to
      1 week on surface of various utensils, or in faeces.
   Human disease: severe acute respiratory syndrome (SARS), which also affects
      gastrointestinal tract, and has high fatality rate (40–50%). SARS in chil-
      dren has a less severe course than in adults. The proportion of asymptomatic
      infections in humans is low. First outbreak was reported from the Chinese
      province Guangdong in November 2002; the epidemic then jumped to
      Hongkong (hotel Metropol etc.) and to many other localities and countries
      over the world, becoming a dreadful pandemic. In the period to May 2003, a
      total of 20,791 patients were reported (9,662 of the cases were fatal) from 30
      countries in 5 continents. One of the victims was also epidemiologist Carlo
      Urbani who put WHO on the alert at beginning of the epidemic.
   Bio-containment: BSL-3.
   Diagnosis: RT-PCR, isolation of the virus, serology (ELISA).
   Geographical distribution: China, Hongkong, Taiwan, Singapore; exported on
      airplanes also to Vietnam, Canada (Toronto), USA and 22 other countries.

8.2.12 *Family* Picornaviridae

Virions are spherical (30 nm), not enveloped, with one molecule ss(+)RNA sized
7–8 kbp.

Cardiovirus Encephalomyocarditis

Some virologists doubt that EMC virus is zoonotic in that an antigenically very
similar Saffold cardiovirus is anthroponotic.

   Source of infection (natural host range): rodents (reservoir – but it is uncertain
      whether EMC virus from rodents is transmissible to humans), pig and other
      domestic mammals, lagomorphs, non-human primates.
   Animal disease: encephalomyocarditis (EMC) or inapparent course; sporadic
      cases and outbreaks of myocarditis, encephalitis, and abortions in pigs.
   Transmission mode: aerogenic.
   Human disease: an uncommon disease with fever, headache, malaise, sweats,
      chills, myalgia, pallor, nausea, vomiting, and abdominal pain, weight
      loss, arthralgia, retro-ocular pain, photophobia, sometimes meningitis to
      encephalomyelitis; no mortality reported.
   Bio-containment: BSL-2.
   Geographical distribution: worldwide, but sporadic.

Parechovirus **Ljungan**

The virus is related to cardioviruses, it was isolated and named after a river in central Sweden in 1998. Scandinavian and American strains differ, and there are known three genotypes of Ljungan virus at present.

> Source of infection (natural host range): rodents, especially voles (*Myodes glareolus*, *Microtus montanus*) – reservoir; also detected in *Apodemus flavicollis*.
> Animal disease: *diabetes mellitus* (usually during overpopulation; the virus attacks pancreas) and myocarditis in *Myodes glareolus*, or inapparent course.
> Transmission mode: probably aerogenic.
> Human disease: intrauterine foetal death (the virus infects the CNS of embryo) – 2 cases have been described (Sweden, 2007, and USA 2008). Ljungan virus is suspect of causing also sudden infant death syndrome, CNS embryonal malformations (hydrocephalus, anencephaly – which has been confirmed in mouse), and it has been detected in adult patients with diabetes and myocarditis.
> Bio-containment: BSL-2.
> Geographical distribution: Sweden, Denmark, Italy, USA, but probably worldwide.

8.2.13 Family Caliciviridae

Virions are spherical (30–40 nm), not enveloped, with one molecule ss(+)RNA sized 8 kbp.

Swine *Norovirus*

> Source of infection (natural host range): domestic pig (but only the strains of the genomic group II are zoonotic); oysters.
> Animal disease: gastroenteritis, or asymptomatic infection.
> Transmission mode: alimentary (pork, oysters, other seafood etc.).
> Human disease: gastroenteritis. Simultaneous outbreaks occurred in UK, Norway, France, Sweden, and Denmark after consumption of contaminated oysters in 2009 and 2010 (334 cases in 65 clusters).
> Bio-containment: BSL-2.
> Geographical distribution: probably worldwide.

8.2.14 Family Hepeviridae

(**) *Hepevirus* **Hepatitis E**

Virions spherical (30 nm), not enveloped, with one molecule ss(+)RNA sized 7.2 kbp; antigenically uniform, but four genotypes (types I and II are anthroponotic, while the types III and IV are zoonotic).

Source of infection (natural host range): domestic pig, wild boar (reservoir), probably also red deer and rodents (brown rat, black rat); fowl, wild mongoose, horse, primates including man; shellfish.

Animal disease: inapparent course (piglets 2–3 months old) or gastroenteritis. Experimental infection successful in pig, monkey, sheep and rat.

Transmission mode: alimentary (food-borne: raw or undercooked swine liver and intestine, deer liver meat contaminated with the virus; or water-borne: drinking of water contaminated with contaminated sewage disposal); person-to-person transmission (among household members during outbreaks); parenteral (blood transfusion during a viraemic period), mother-to-child transmission (scarcely documented).

Human disease: hepatitis E – after a long incubation period (15–60 d., median about 40 d.) starts severe fatigue, gastroenteritis and acute hepatitis (increased levels of liver enzymes ALT and AST); fatality rate <1%. The infection is dangerous for pregnant women (with an up to 25% fatality rate). Big epidemics have been repeatedly reported in so-called endemic areas (and caused by anthroponotic strains): 1955 India 30,000 cases; 1976 Myanmar 20,000; 1978 Kashmir 52,000 (1,500 patients died); 1986 China 100,000 and Somalia 11,000; 1989 Mexico 4,000. The zoonotic hepatitis E, which occurs mainly in so-called non-endemic areas (Europe, North America, Japan), is probably an underdiagnosed disease (a number of subclinical cases occur). For instance, 116 cases of hepatitis E were diagnosed in Hungary from 2001 to 2006.

Bio-containment: BSL-2.

Diagnosis: isolation on cell cultures, IF; serology – VNT, ELISA (IgM anti-HEV).

Geographical distribution: zoonotic genotype III probably worldwide, it has been demonstrated in Japan, Korea, Indonesia, China, France, Great Britain, Sweden, Spain, the Netherlands, Czechland, Hungary, Greece, USA and Africa, while the other zoonotic genotype IV is restricted to India and East Asia. Anthroponotic genotype I extensively circulates in Asia (including India, Pakistan, Nepal, Bangladesh, China, Kyrgyzstan, and Uzbekistan) and Africa (including Egypt, Algeria, Morocco, Namibia, Sudan and Chad); genotype II occurs in Mexico and in some African countries (Nigeria, Namibia, Chad, and Sudan).

8.2.15 *Family* Retroviridae

**Lentivirus HIV-1, HIV-2

Virions spherical (110 nm), enveloped, contain 2 identical molecules (dimer) ss(+)RNA, reverse transcriptase, and 2 surface proteins.

Source of infection (natural host range): man. HIV-1 ("human immunodeficiency virus") evolved from SIV ("simian immunodeficiency virus") of

chimpanzee *Pan troglodytes troglodytes* (reservoir), and HIV-2 from SIV of sooty mangabey *Cercocebus torquatus.* HIV-1 and HIV-2 have therefore zoonotic origin, connected with crossing the species barrier (a host-jumping event).

Animal disease: decreased immunity with a milder course than is AIDS in humans.

Transmission mode: as the originating zoonosis by contact with the blood of killed wild primates ("cut-hunter hypothesis"); as anthroponosis transmitted sexually, by inoculation or transfusion (blood), transplantation of organs, and vertically from mother to child.

Human disease: acquired immune deficiency syndrome (AIDS) – first infections demonstrated sporadically already before 1959 (DR Congo – antibodies); start of AIDS epidemics were revealed in homosexual communities of New York and California in 1981, the number of patients increased continuously resulting in a pandemic: in the year 2001, 40 million persons were infected with HIV or had symptoms of AIDS, of this number 28 million in sub-Saharan Africa (local HIV prevalence rate was in the age 15–25 years 10%, in adults 20%) and 8 million in southeast Asia; 3 million patients succumbed to AIDS worldwide in the same year.

Bio-containment: BSL-2.

Treatment: zidovudin, Retrovir, Combivir.

Prevention: safe sexual behaviour, use of disposable injection syringes and needles in drug addicts.

Geographical distribution: worldwide.

8.2.16 *Family* Herpesviridae

Virions approximately spherical (100–230 nm), enveloped, contain a linear molecule dsDNA 125–240 kbp.

Varicellovirus Herpesvirus Suis 1 (pseudorabies virus, suid herpesvirus 1)

Source of infection (natural host range): pig (reservoir), ruminants, rodents, dog, cat.

Animal disease: pseudorabies (morbus Aujeszky) – encephalomyelitis with bronchopneumonia, fatal for piglets; in adult pigs the course is inapparent.

Transmission mode: direct contact, percutaneous (biting).

Human disease: nettle-rash, salivation, pharyngitis, affection of the CNS. However, the possibility of a zoonotic infection of man with this virus is unclear and doubted by many virologists.

Bio-containment: BSL-2.

Diagnosis: isolation in cell cultures, IF; VNT, ELISA.

Geographical distribution: worldwide.

Macacine Simplexvirus 1 **(Herpesvirus B, Monkey B virus)**

> Source of infection (natural host range): monkeys of the Old World (reservoir *Macaca* spp., mainly *M. mulatta* as well as *M. fascicularis*, but also *M. fuscata, M. arctoides, M. cyclopsis* and *M. radiate*): they excrete the virus with saliva and other secrets.
>
> Animal disease: inapparent course or stomatitis (small vesicles on tongue and mucosa), rarely encephalomyelitis.
>
> Transmission mode: contact – percutaneous (biting, scratching), also aerogenic; most commonly after a stress (caused e.g. by transport of the animals); there were reported also several laboratory infections during the work with monkey cell cultures.
>
> Human disease: simian herpes B (*herpes simiae*) – vesicles, lymphadenitis, severe encephalomyelitis with the fatality rate of 80%. Up to now a total of 40 cases have been reported. Risk groups: people getting into close contact with macaques (attendants, hunters, etc.).
>
> Bio-containment: BSL-3.
>
> Diagnosis: isolation on cell cultures, IF; VNT, ELISA.
>
> Treatment: acyclovir (Zovirax).
>
> Geographical distribution: southeastern Asia (also temples in Nepal and India), North Africa; secondarily other regions by transport of monkeys.

8.2.17 *Family* **Poxviridae**

Orthopoxvirus simiae **(Monkeypox Virus)**

Large subspherical or moderately pleomorphic virions (about 250×200 nm), enveloped, containing one linear molecule dsDNA sized 170–250 kbp.

> Source of infection (natural host range): monkeys of the Old World, rodents (African rope squirrels *Funisciurus congicus, F. anerythrus,* red-legged sun squirrel *Heliosciurus rufobrachium*, and rats *Cricetomys emini, C. gambianus*), as well as some other wild mammals and domestic pig.
>
> Animal disease: pox.
>
> Transmission mode: contact with wild animals (especially in hunters); the man-to-man transmission is relatively rare, but after 1995 the proportion of secondary man-to-man infection has obviously increased.
>
> Human disease: monkeypox, similar to smallpox, with fever, hyperhidrosis, headaches, myalgia, backaches, extreme fatigue, rash, lymphadenitis (the latter symptom differentiates both diseases); fatality rate can attain 10% (reports show 4–33%) – most lethal is monkeypox for small children up to 4 years old. Monkeypox in humans was described in 1970, and to 1999 nearly 1,000 cases (40 fatal) were reported in Africa: e.g., 398 cases in DR Congo between 1981 and 1986, an outbreak in the same country in 1996/1997 (88 cases, fatality rate 4%), and a total of 51 laboratory-confirmed cases between 2001 and

2004. In 2003, monkeypox was imported with wild rodents of several genera (e.g. *Cricetomys*, *Graphiurus*) from Ghana to Wisconsin (USA) where several captive American prairie dogs (*Cynomys ludovicianus*) were infected and unintentionally distributed to other US states; 35 attending persons (12 veterinarians and several animal shop assistants) acquired the infection as well, and the number of humans infected with monkeypox increased then to 82 in USA.

Bio-containment: BSL-3.

Diagnosis (valid also for other poxvirus infections): symptomatology; histopathology and electron microscopy of the vesicular lesions, IF, and isolation (on chick embryos or cell cultures Vero, LLC-MK2) from skin lesions; serology (HIT, ELISA, RDPA).

Treatment: cidofovir.

Prevention: vaccine against smallpox protects against monkeypox.

Geographical distribution: tropical forests in central and western Africa (DR Congo, Cameroon, Ghana, Gabon), Southeast Asia.

Orthopoxvirus bovis (Cowpox Virus)

By passaging this virus originated the vaccinia virus *O. officinalis*, [Lat. *vacca* = cow], used for vaccination against smallpox (variola, i.e. human pox). [This virus is also used for preparation of recombinant vaccines against different viruses, for instance the oral vaccine against fox rabies].

Source of infection (natural host range): cattle, camel, rabbit; brown rat including pet rats (*Rattus norvegicus*), and other rodents (reservoir), cat.

Animal disease: cowpox, *variola vaccina*. Zoo and circus mammals (elephants, rhinoceros, felids – often fatal), wild rodents.

Transmission mode: direct contact; infrequently also aerogenic and alimentary (risk – pet rodents).

Human disease: cowpox (uncommon) – papulae and pustulae on the skin, lymphatic changes on the hands, lesions on eyelids (the Netherlands 2002); exceptionally a generalized illness. Several human cases of cowpox have been reported since 2008 as acquired from pet rats in Germany and France. Occupational disease in farmers, milkers and their close contacts, also in zoo and circus animal attendants.

Bio-containment: BSL-2.

Geographical distribution: worldwide.

Parapoxvirus bovis 1 (Bovine Papular Stomatitis Virus)

Virions of parapoxviruses are oval, $220–300 \times 140–170$ nm. Size of DNA is $130–150$ kbp.

Source of infection (natural host range): cattle.

Animal disease: papular stomatitis (bovine pustular stomatitis) – papulae and pustulae on the muzzle and in oral cavity.

Transmission mode: direct contact (percutaneous).

Human disease: not commonly, bovine papular dermatitis – red-violet papulae and pustulae on fingers and forearm, the course is protracted.

Bio-containment: BSL-2.

Diagnosis: symptoms; isolation on cell cultures, electron microscopy of the lesion samples.

Geographical distribution: North America, Europe, Africa, Australia.

Parapoxvirus bovis 2 (Pseudocowpox Virus)

Source of infection (natural host range): cattle, sheep, pig.

Animal disease: paravaccine (pseudocowpox) – lesions on the udder of cows (papulae to pustulae).

Transmission mode: direct contact (percutaneously).

Human disease: milkers' nod(ul)es, paravaccinia – a disease milder than cowpox: small painful not suppurative papulae and nodules on the hands and forearms for up to 6 weeks.

Bio-containment: BSL-2.

Diagnosis: isolation on cell cultures, electron microscopy.

Geographical distribution: Europe, North America.

Parapoxvirus ovis (Orf parapoxvirus)

Source of infection (natural host range): sheep, goat.

Animal disease: contagious ecthyma, "orf" – pustulae and papulae on the face and udder.

Transmission mode: direct contact (percutaneous); the virus is resistant in the milieu (in scabs and crusts it can survive for years).

Human disease: contagious pustular dermatitis – painful dermatitis (papulae to pustulae) on fingers and arms and in the oral cavity, the course is protracted. The disease is rare, it occurs in sheep breeders and among butchers.

Bio-containment: BSL-2.

Diagnosis: electron microscopy of the pus from pustules and scabs.

Geographical distribution: worldwide.

Yatapoxvirus tanapox (Tanapoxvirus)

Virions 200×300 nm, DNA size is 145 kbp.

Source of infection (natural host range): monkeys (possibly also other wild and domestic animals).

Animal disease: papulae on the face.

Transmission mode: mosquitoes (*Mansonia*) – a mechanical transmission.

Human disease: fever with headaches and 1–2 pustular skin lesions for a period of up to 6 weeks.

Bio-containment: BSL-2.

Diagnosis: electron microscopy, and virus isolation from skin lesions.
Geographical distribution: tropical Africa (Kenya).

8.3 Bacteria

8.3.1 *Family* Chlamydiaceae [*Order* Chlamydiales*, Class* Chlamydiae]

Members of the order *Chlamydiales* are very small Gram-negative spherical bacteria with a diameter of 0.3–0.5 μm, obligate intracellular parasites preferentially of the mucosal epithelium. They do have solid cell wall but without muramic acid (in contrast to other bacteria peptidoglycan between the wall and plasmatic membrane is absent). They do not posses their own energetic metabolism and depend on the ATP system of the host cell (they are therefore called "energetic parasites"); thanks to this adaption, their genome is markedly reduced. The peculiar life cycle of chlamydiae differs greatly from that of other bacteria, and involves small extracellular non-metabolising but infectious elementary bodies (0.3–0.5 μm) that produce in the infected cell bigger and non-infectious reticulate bodies (also called "initial bodies", 0.7–1.1 μm); those divide by binary fission and form new elementary bodies which are able to survive extracellularly and also infect new hosts. Cell inclusions visible microscopically in chlamydial infections are, in fact, conglomerates (colonies) of replicating reticulate bodies together with the daughter elementary bodies. Finally, the infected cells crack or the bodies escape by exocytosis. Chlamydiae are well stained for microscopy according to Macchiavelli (initial bodies are stained blue, and reticulate bodies red) or Giemsa (blue and purple, respectively). The tenacity of chlamydiae in the milieu is very limited, especially for reticulate bodies (however, avian strains of *Chlamydophila psittaci* can survive in bird droppings for a long time). Chlamydiae are sensitive to diethyl ether because 50% of the cell wall content is formed by lipids, and are readily inactivated by certain antibiotics, largely those that inhibit proteosynthesis (chloramphenicol or tetracycline). The only typical zoonotic species among chlamydiae is *Chlamydophila psittaci*, while two other species cause anthroponotic infections: *Chlamydophila pneumoniae* causes respiratory infections (possibly also atherosclerosis and coronary disease), and *Chlamydia trachomatis* trachoma, but its serotypes D to K very frequent sexually transmissible infections of the human urogenital tract (and proctitis), and serotype L is the agent of *lymphogranuloma venereum*. In contrast to rickettsiae, chlamydiae are not transmitted by arthropod vectors.

Chlamydophila psittaci

A heterogenous species with a number of genotypes (A to F) differentiated according to the *ompA* gene (expressing outer membrane protein).

Source of infection (natural host range): domestic, wild and exotic (pet) birds – in psittacines genotype A – psittacosis; in other birds ornithosis: genotype

C prevails in anseriforms; genotype B in fowl ((hens, turkeys), feral pigeons (but also the genotype E, at least 500 human cases reported) and passerines; further gulls, egretts etc. – the disease was observed in 230 avian species belonging to 10 orders).

Animal disease: usually latent, sometimes subclinical or manifest – ornithosis of young fowl and pigeons, especially under stress conditions, with fluffed feathers, conjunctivitis (closed eyes), rhinitis, pneumonia, anorexia, lethargy, diarrhoea, pericarditis and enteritis, at a high fatality rate.

Transmission mode: aerogenic (inhalation of dust containing infected avian droppings); often occupational infectious disease (breeders, fowl farmers, butchers). Tenacity of the elementary bodies is relatively high.

Human disease: ornithosis/psittacosis ("parrot disease") – clinical symptoms are highly variable, from mild flu-like symptoms to severe pneumonia or systemic disease; the pneumonia is atypical (radiologic findings are substantially more pronounced than those physical – a nonproductive cough) with high prolonged fever that reveals relapses, headaches, myalgia and arthralgia, exhaustion, sometimes endocarditis, hepatosplenomegaly, encephalitis; fatality rate used to be high (e.g. it was 20% during the outbreaks transmitted by birds imported from Argentina in 1929/1930, but as high as 80% in pregnant women), today is only about 1%. The highest incidence rate of psittacosis is reported from Australia, UK, Denmark, Sweden and the Netherlands.

Bio-containment: BSL-2/3.

Diagnosis: anamnesis (contact with ill birds); serology (CFT, ELISA – there might occur cross-reactions with Q fever); isolation on chick embryo and McCoy cells, PCR, detection by microscopy of chlamydiae in sputum samples or in inoculated cells using IF or staining (Giemsa, Macchiavelli, Giménez, Lugol's iodine); PCR.

Treatment: doxycycline, tetracycline (adults), erythromycin (children, 3 weeks).

Geographical distribution: worldwide.

Chlamydophila abortus

Source of infection: sheep, goat, cattle; occasionally pig, horse, deer.

Animal disease: ovine enzootic abortion; in cattle, abortion, stillbirth, mastitis, metritis, epididymitis, orchitis, pneumonia, and occasionally encephalomyelitis (transplacental transmission of the bacteria to embryo); in young domestic animals (sheep, cattle, pig, horse) enteritis, conjunctivitis, pneumonia, and polyarthritis. This disease has very high economic impacts.

Transmission mode: inhalation, less often by contact or alimentary.

Human disease: mammalian chlamydiosis is milder than psittacosis, with fever, headache, conjunctivitis, arthralgia, affections of urogenital tract; in severe cases pneumonia, renal failure, miscarriage but also women sterility as a consequence; rarely fatal. Risk procedures: assistance with lambing (especially women).

Bio-containment: BSL-2.
Diagnosis and treatment: as for *C. psittaci*.
Geographical distribution: probably worldwide.

Chlamydophila felis, C. caviae

Source of infection: cat (*C. felis*), and guinea pig (*C. caviae*).
Animal disease: conjunctivitis, swollen eyelids, ocular and nasal discharge, pneumonia, occasionally chronic salpingitis.
Transmission mode: contact.
Human disease: conjunctivitis, in *C. felis* infection sometimes endocarditis and liver failure. A few zoonotic human infections have been reported.
Bio-containment: BSL-2.
Diagnosis and treatment: as for *C. psittaci*.
Geographical distribution: probably worldwide.

8.3.2 *Family* Parachlamydiaceae [*Order* Chlamydiales]

*Parachlamydia acanthamoebae

An obligate intracellular bacterium – endosymbiont of amoebae free-living in water.

Source of infection: river water, drinking water treatment plants, hospital water supplies.
Animal disease: abortions in ruminants.
Transmission mode: water-borne.
Human disease: pneumonia (purulent and interstitial), miscarriage, stillbirth and premature birth.
Bio-containment: BSL-2.
Diagnosis: PCR, isolation on cell cultures or by amoebal co-culture, serology (ELISA).
Treatment: specific treatment unknown, the bacterium is resistant to fluoro-quinolones and other antibiotics.
Geographical distribution: probably worldwide.

8.3.3 *Family* Simkaniaceae [*Order* Chlamydiales]

*Simkania genevensis

An endosymbiont of free-living amoebae.

Source of infection: river water, drinking water treatment plants.
Animal disease: unknown.
Transmission mode: water-borne.

Human disease: pneumonia.

Bio-containment: BSL-2.

Diagnosis: PCR, isolation on Vero cells or by amoebal co-culture, serology (ELISA).

Treatment: specific treatment unknown, the bacterium is resistant to fluoro-quinolones and other antibiotics.

Geographical distribution: Israel, but probably worldwide.

8.3.4 *Family* Waddliaceae [*Order* Chlamydiales]

Waddlia chondrophila

Source of infection (natural host range): domestic ruminants.

Animal disease: abortions.

Transmission mode: aerogenic.

Human disease: miscarriage (described in 2007).

Bio-containment: BSL-2.

Geographical distribution: USA, Germany.

8.3.5 *Family* Rickettsiaceae [*Order* Rickettsiales, *Class* Alphaproteobacteria]

Rickettsial microorganisms of the families *Rickettsiaceae* and *Anaplasmataceae* are very small Gram-negative bacteria, coccobacilli to short rods about 0.6×0.3 µm, intracellular parasites (in either nucleus – rickettsiae of spotted fever group, RSFG – or cytoplasma of endothelial cells or leucocytes) and replicate by cross section. They possess most of enzymes the other bacteria have, form ATP (contrary to chlamydiae), exhibit a normal bacterial cell wall with muramic acid, and are stained with Giemsa, Giménez or Macchiavelli stains, poorly according to Gram. A natural reservoir of rickettsiae are arthropods of diverse systematic groups, in them they live usually as commensals, while in the organism of an "unnatural" host, e.g. man, they often cause an illness with fever, vasculitis (they attack selectively endothelial cells) and often also a rash or erythema (spotted fevers and typhus). According to the vector they can be divided into:

(a) louse-borne: *Rickettsia prowazekii*
(b) flea-borne: *R. typhi*, *R. felis*
(c) tick-borne: rickettsiae of the spotted-fever group (SFG): *R. rickettsii*, *R. conorii*, *R. africae*, *R. sibirica*, *R. slovaca*, *R. japonica*, *R. australis*; further *Anaplasma phagocytophilum*, *Ehrlichia chaffeensis* and other ehrlichiae
(d) mite-borne: *R. akari*, *Orientia tsutsugamushi*
(e) cercaria-borne (trematode larvae): *Neorickettsia sennetsu*.

(**) *Rickettsia prowazekii*

Its genome is fully sequenced.

> Source of infection (natural host range): man; infrequently the flying squirrel *Glaucomys volans.*
>
> Animal disease: inapparent course.
>
> Transmission mode: body louse (*Pediculus humanus* – TST demonstrated, TOT not) – rubbing infectious louse excreta into the skin; aerogenic (inhallation of dust containing louse excreta). About 15 cases of human infection from flying squirrel have been described in USA recently (*R. prowazekii* was also isolated from the rodent) – it means that a limited sylvatic cycle of this rickettsiosis exists.
>
> Human disease: epidemic typhus, with severe headaches, pains in limbs, high fever for 10–20 days, chills, rash on the trunk and limbs (4–7 days), meningeal signs, bronchopneumonia, haemorrhagies and a fatality rate in non-treated cases 10–50%. Sometimes a recidiva even after 10–20 years, so-called Brill-Zinsser's disease, which is a re-activation of the agent persisting in lymphatic nodes or in adipose tissue. Big epidemics have been reported during wars, famin and natural disasters, especially in winter and early spring. Examples of the extensive outbreaks are "plague in Athens" 430 BC (most probably it was epidemic typhus, or combined with ergotism), 1490 Granada, 1528 Naples, 1542 Hungary, 1546 Beograd, 1618–1648 central Europe during 30-year War, 1640 England, 1914–1918 the Balkans during 1st WW (150,000 soldiers get infected solely in Austrian army), 1917–1922 Russia (30 million persons fell ill and 3 millions died), 1941–1945 Russia (about 20 million sick persons); 1997 sanatorium Lipeck in Russia (29 patients with Brill-Zinsser's disease); 1997–1998 Burundi and Rwanda: 50,000 to 100,000 patients in refugee camps, fatality rate up to 15%; 1998, an outbreak in Peru, and sporadic cases in Algeria, western Europe and USA (usually in homeless persons).
>
> Bio-containment: BSL-3.
>
> Diagnosis: symptoms; serology: CFT; AR (earlier also Weil-Felix AR with heterologous antigen used: *Proteus* OX-19), CFT, RIHA, ELISA, isolation (guinea pig), detection of rickettsiae in samples (IF, PCR).
>
> Treatment: tetracycline, doxycycline, chloramphenicol.
>
> Geographical distribution: Africa (Ethiopia, Algeria; Rwanda and Burundi, Zaire), Asia (e.g., Kazakhstan, 2000), South America, USA, Russia, western Europe.

Rickettsia typhi (Syn. *R. mooseri*)

> Source of infection (natural host range): synanthropic rodents (brown rat, black rat), cat; dog; in the sylvatic cycle opossum (*Didelphis marsupialis*).
>
> Animal disease: inapparent course.

Transmission mode: fleas (TOT demonstrated) *Xenopsylla cheopsis*, *Ctenocephalides felis* and other species; aerogenic (fleas' excrements).

Human disease: murine typhus, with symptoms similar to epidemic typhus but milder – fever, heavy headaches, pains in limbs, dry cough for 2 weeks, usually a 6-day rash on chest and abdomen, elevated transaminases; fatality rate in untreated cases is 1–4%; long convalescence (several months). For instance, 33 human cases of murine typhus were identified in USA in 2008.

Bio-containment: BSL-2/3.

Diagnosis: inoculation of blood samples in male guinea pig (scrotal reaction) or chick embryo; detection by PCR; serology (CFT, IFA, ELISA, in the past Weil-Felix with *Proteus* OX-19 and OX-2 antigen, latex agglutination), WB.

Treatment: doxycycline, tetracycline, chloramphenicol.

Geographical distribution: worldwide, but tropical and subtropical regions prevail; southern Europe, the Netherlands, Australia; especially harbours and coastal areas with markets (where rats are frequently present).

Rickettsia rickettsii

A prototype SFG rickettsia.

Source of infection (natural host range): rodents (*Spermophilus*, *Marmota*, *Eutamias, Hydrochaeris*), opossum, leporids, canids, occasionally birds.

Animal disease: inapparent course, but clinical disease in dogs.

Transmission mode (Fig. 8.18): ixodid ticks – in North America *Dermacentor andersoni*, *D. variabilis* (reservoir – TST and TOT demonstrated already
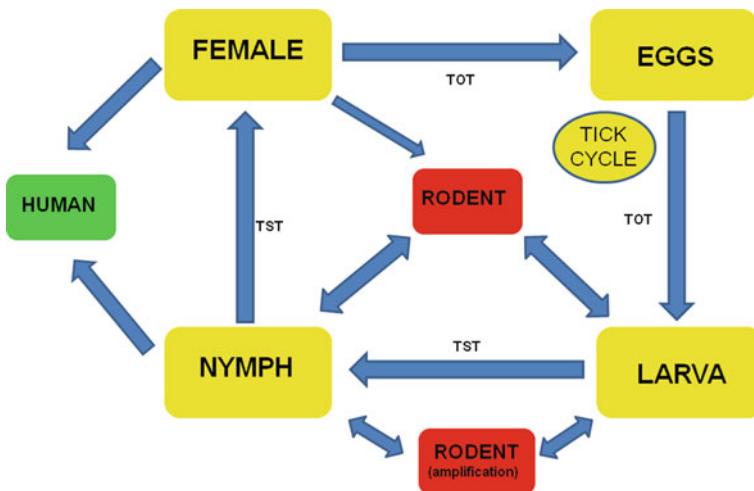
Fig. 8.18 Circulation of *R. rickettsii* in a natural focus of Rocky Mountain spotted fever (modified from Beaty and Marquardt 1996) (drawing by Ivo Rudolf)

by H.T. Ricketts), also *D. albipictus, D. parumapterus,* infrequently
Rhipicephalus sanguineus (Mexico, Brazil)*, Haemaphysalis leporispalustris*
(Costa Rica), *Amblyomma cajennense* and *A. aureolatum* (Brazil, Mexico);
aerogenically (laboratory infections).

Human disease: Rocky Mountains spotted fever (RMSF), and Brazilian
  spotted fever – severe illness with symptoms similar to epidemic typhus,
  with a sudden and longterm fever, severe headache, myalgia, abdominal
  pains, vomiting, rash on ankles and wrists, bronchopneumonia, photophobia,
  CNS affection (meningism), haemorrhages of limbs and genitalia (gangrene
  of scrotum), hepatosplenomegaly, myocarditis, renal lesions (up to renal
  failure); thrombocytopenia, increased transaminases; fatality rate is about
  20% in untreated sick persons (but in west Montana and in Brazil higher,
  30–42%), while in treated patients only 1–5%. The annual incidence of
  RMSF in the USA increased five times from 0.14 to 0.70 per 100,000
  population between 2000 and 2007.

Bio-containment: BSL-3.

Diagnosis: inoculation of blood samples in male guinea pig (scrotal reac-
  tion) or chick embryo; serology: in the past Weil-Felix AR with *Proteus*
  OX-19, OX-2; CFT, IFA, while at present microagglutination, IFA, PCR or
  immunohistochemistry of skin biopsies.

Treatment: doxycycline, tetracycline, chloramphenicol, ciprofloxacin.

Prevention: inactivated vaccine is slightly effective, it is not in use.

Geographical distribution: North America, Brazil (states Sao Paolo and Minas
  Gerais).

Rickettsia slovaca

A SFG rickettsia, genomically related to *R. rickettsii*. First described in *D. margina-tus* ticks collected in Slovakia in 1968.

Source of infection (natural host range): rodents, cattle.

Animal disease: inapparent course.

Transmission mode: ixodid ticks *Dermacentor marginatus*, infrequently *D.
  reticulatus* and *Rhipicephalus bursa.*

Human disease: TiBoLa ("**ti**ck-**bo**rne **l**ymph**a**denitis") or DeBoNEL
  ("**De**rmacentor-**Bo**rne-**N**ecrosis-**E**rythema-**L**ymphadenopathy"), with an
  eschar (an infiltrate with a dark scar) in the place where the vector tick
  fed – often in the scalp, with alopecia and erythema around the eschar,
  fever, headache, often painful regional (usually cervical) lymphadenitis and
  maculo papular rash. Laboratory findings include thrombocytopenia and
  increased transaminase levels. The illness usually occurs in early spring
  (February to May) when the vector ticks start to be active. The first human
  case of TiBoLa was reported by T. Mittermayer et al. in eastern Slovakia in
  1980.

Bio-containment: BSL-2.

Diagnosis: symptoms, serology (ELISA, IFA), PCR.

Treatment: tetracycline, doxycycline.

Geographical distribution: Europe (Slovakia, Hungary, Poland, France, southern Germany, Austria, Switzerland, Slovenia, Croatia, Spain, Portugal, Italy, Greece, Bulgaria, Romania, Ukraine, Russia), Armenia, Africa.

Rickettsia honei

A SFG rickettsia, with a subtype called "***R. marmionii***".

Source of infection (natural host range): rodents (*Rattus rattus*).

Animal disease: inapparent course.

Transmission mode: ixodid ticks *Haemaphysalis novaeguineae, Bothriocroton* (*Aponoma*) *hydrosauri* and *Ixodes granulatus.*

Human disease: Flinders Island spotted fever. *R. m.* causes Australian spotted fever with headache, pharyngitis, cough, maculopapular rash, and arthralgia.

Bio-containment: BSL-2.

Diagnosis: symptoms, serology (ELISA, IFA), PCR.

Treatment: tetracycline, doxycycline.

Geographical distribution: Australia and Oceania, Thailand.

Rickettsia conorii

A SFG rickettsia with several subspecies: *R. conorii conorii* (its full genomic sequence is known), and closely related rickettsiae of Astrakhan fever (*R. conorii caspia*), Israeli tick typhus (*R. conorii israelensis*) and Indian tick typhus (*R. conorii indica*).

Source of infection (natural host range): dog and other canids; rodents, wild rabbit.

Animal disease: usually inapparent course, but fever, anorexia, lethargy, splenomegaly, and polyneuritis in dogs.

Transmission mode: ixodid ticks *Rhipicephalus sanguineus* (TOT), *R. appendiculatus*, *R. pumilio* (*R. c. caspia*), *Haemaphysalis leachi* (TOT), *Hyalomma* spp.

Human disease: Mediterranean spotted fever, boutonneuse fever (French: "*fievre boutonneuse*") was first described in 1910 – an infiltrate in the place where the vector tick fed with a dark scar (primary necrotic skin lesion – "eschar", French "*tache noir*"), fever 1–2 weeks, headaches, arthralgia, myalgia, sometimes maculopapular rash, and regional lymphadenopathy in half of patients; increased transaminases AST and ALT; the complications might appear as pneumonia or intravenose coagulation; fatality rate is in untreated persons 1–5% but in places higher (e.g. in Portugal 32% in 1997). The geographical forms of the disease are Israeli tick typhus and Indian tick typhus, and Astrakhan fever (maculopapular rash, petechiae and

haemorrhagies due to thrombocytopenia) – the third geographical variant started to appear in south Russia in the 1970s, until 1983 >2,000 cases were recorded (allegedly in connection with building of a petrochemical plant with a high level of CO_2 emissions). In 1992, there was a large outbreak among American soldiers in Botswana.

Bio-containment: BSL-2.

Diagnosis: symptoms, serology (CFT, Weil-Felix *Proteus* OX-2 and OX-19 antigens, AR, IFA, WB), PCR, isolation on guinea pig.

Treatment: tetracycline, doxycycline, chloramphenicol.

Geographical distribution: the Mediterranean, Switzerland, the Balcans (Kosovo), the Black Sea region, North Africa, Uganda, Botswana, Asia (the Near East, India).

Rickettsia sibirica

SFG rickettsia related to *R. conorii*, and first detected in Siberia, 1939. ***R. mongolitimonae*** is newly classified as subspecies of *R. sibirica* (*R. sibirica mongolitimonae*).

Source of infection (natural host range): rodents, leporids, hedgehogs, cattle.

Animal disease: inapparent course.

Transmission mode: ixodid ticks (reservoir) *Dermacentor* (*D. nuttallii*, *D. silvarum*, *D. marginatus*), *Haemaphysalis* (*H. concinna*) and *Hyalomma (H. asiaticum, H. truncatum*).

Human disease: North-Asian (Siberian) tick typhus with fever, headache, regional lymphadenitis and papular rash (eschar).

Bio-containment: BSL-2/3.

Diagnosis: symptoms, serology (ELISA, IFA), PCR.

Treatment: tetracycline, doxycycline, minocycline.

Geographical distribution: northern Asia, Kazakhstan, Kirghizia, Japan, China, Korea, eastern Europe, Armenia, Pakistan; *R.s. mongolitimonae* was detected in Mongolia and China, but recently also as an autochthonous agent (several human cases) in Spain, Portugal, France, northern Africa, and Nigeria.

Rickettsia africae

A SFG rickettsia related to *R. conorii* and *R. parkeri*.

Source of infection (natural host range): canids, rodents, cattle; (equids?).

Animal disease: inapparent course.

Transmission mode: ixodid ticks *Amblyomma hebraeum, A. variegatum* (TST and TOT demonstrated) and *Boophilus decoloratus.*

Human disease: African tick bite fever (Kenyan, South-African, Nigerian) – eschar (quite often multiple eschars, fever for about 1 week, headaches, arthralgia, myalgia, maculo-papular rash, regional lymphadenopathy; the course is usually milder than in the Mediterranean spotted fever). It occurs

quite often in travellers to Africa (European hunters, safari participants, visitors of game parks) – the second most frequently identified cause for systemic febrile illness among travellers, following malaria.

Bio-containment: BSL-2.

Diagnosis: symptoms, serology (IFA, ELISA, WB), PCR from the skin biopsy (eschar), isolation in guinea pig.

Treatment: doxycycline (tetracycline, chloramphenicol).

Geographical distribution: sub-Saharan Africa, introduced in the Caribbean.

Rickettsia parkeri

A SFG rickettsia, closely related to *R. africae*, described in 1939 and previously thought to be non-pathogenic to man.

Source of infection (natural host range): rodents, domestic animals, birds.

Animal disease: inapparent course.

Transmission mode: ixodid ticks *Amblyomma maculatum, A. triste, A. americanum, Dermacentor variabilis*.

Human disease: American boutonneuse fever with eschar (area of necrosis) or multiple eschars at the site of tick bite, and rash. Other symptoms include mild headache, malaise, diffuse myalgia and arthralgia. The illness is emerging in southern and south-central states of USA since the beginning of twenty-first century.

Bio-containment: BSL-2.

Diagnosis: symptoms, serology (IFA, ELISA), PCR from the skin biopsy (eschar), isolation in guinea pig.

Treatment: doxycycline (tetracycline, chloramphenicol).

Geographical distribution: southern USA, South America (Uruguay, Brazil, Argentina).

Rickettsia raoultii

SFGR.

Source of infection (natural host range): probably mammals.

Animal disease: unknown.

Transmission mode: ixodid tick *Dermacentor reticulatus, D. marginatus,* and other *Dermacentor* spp.

Human disease: similar to TiBoLa – tick typhus, with an eschar, fever, headache, arthralgia, myalgia, maculopapular rash, regional lymphadenopathy.

Bio-containment: BSL-2.

Diagnosis: symptoms, serology (ELISA, IFA), PCR.

Treatment: tetracycline, doxycycline.

Geographical distribution: Africa – Morocco, Mali, Niger, Zimbabwe, Europe (Italy).

Rickettsia aeschlimannii

SFGR.

> Source of infection (natural host range): mammals (?).
> Animal disease: unknown.
> Transmission mode: ixodid ticks *Hyalomma marginatum* (*H. m. marginatum, H. m. rufipes*)*, Haemaphysalis punctata, Rhipicephalus appendiculatus, R. sanguineus, R. bursa, Ixodes ricinus.*
> Human disease: similar to the Mediterranean fever (tick typhus), with an eschar, fever, headaches, arthralgia, myalgia, maculopapular rash, and regional lymphadenopathy. Only four cases of *R. aeschlimannii* fever have been reported up to now; they were confirmed by IFA, WB, and cross-absorption studies.
> Bio-containment: BSL-2.
> Diagnosis: symptoms, serology (ELISA), PCR.
> Treatment: doxycycline; resistant to rifampin.
> Geographical distribution: southern Europe (Portugal, Spain, Croatia, Corsica, Sicily), Africa (Algeria, Morocco, Egypt, Mali, Niger, Zimbabwe, southern Africa), Kazakhstan.

Rickettsia massiliae

SFGR, first isolated in Marseille (France) from ticks in 1992.

> Source of infection (natural host range): probably mammals.
> Animal disease: unknown.
> Transmission mode: ixodid ticks *Rhipicephalus sanguineus, R. pusillus, R. turanicus* (TOT in the latter species), *R. muhsamae*, *R. lunulatus*, *R. sulcatus.*
> Human disease: several cases of a spotted fever-like illness with fever, eschar, and maculopapular rash due to *R. massiliae* have been reported in France and Spain since 2005.
> Bio-containment: BSL-2.
> Diagnosis: symptoms, serology (ELISA), PCR.
> Treatment: tetracycline, doxycycline; resistant to rifampin.
> Geographical distribution: France, Spain, Portugal, Italy (Sicily), Switzerland, Greece, Mali, Central Africa, USA.

Rickettsia japonica, *R. heilongjiangensis*

SFG rickettsiae, very similar to each other.

> Source of infection (natural host range): rodents, hedgehog; dog, cattle (?).
> Animal disease: inapparent course.
> Transmission mode: ixodid ticks (reservoir) *Haemaphysalis concinna* and *Dermacentor silvarum* (*R. heilongjiangensis*), *H. japonica*, *H. longicornis, H. flava* and *Ixodes ovatus* in *R. japonica.*
> Human disease: Japanese (Oriental) spotted fever, increasingly occurring in Japan since 1984.

Bio-containment: BSL-2.

Treatment: tetracycline, doxycycline.

Geographical distribution: Japan, China, the Far East (Khabarovsk); *R. heilongjiangensis* occurs in northern Asia.

Rickettsia helvetica, R. monacensis

Closely related SFG rickettsiae (*R. monacensis* was originally labeled as IRS3 strain).

Source of infection (natural host range): rodents, cattle.

Animal disease: mostly inapparent course, but pathogenic for *Microtus* and *Apodemus* spp.

Transmission mode: tick *Ixodes ricinus, I. ovatus, I. persulcatus, I. monospinus,* occasionally *Dermacentor reticulatus* and *Haemaphysalis inermis* (*R. helvetica*, Croatia and Hungary, respectively).

Human disease: fever with headache, myalgia, arthralgia, rarely chronic perimyocarditis or meningitis (*R. helvetica*); rash not reported. *R. helvetica* was the cause of a subacute meningitis case in Sweden in 2006 (confirmed by nucleotide sequencing). With *R. monacensis*, two human cases were reported in Spain, 2006.

Bio-containment: BSL-2.

Treatment: tetracycline, doxycycline.

Geographical distribution: *R. helvetica*: Europe (Switzerland, France, Germany, Poland, Slovenia, Portugal, Italy, Austria, Hungary, Sweden, Denmark). *R. monacensis*: Spain, Italy, Germany, the Netherlands, Slovakia, Hungary, Albania, Bulgaria, Morocco, Tunisia, Japan.

Other SFG Rickettsiae

Presumptively associated with human illness are the tick-borne species and candidates:

Rickettsia canadensis (the vector is *Haemaphysalis leporispalustris*) in Canada; "**R. amblyommii**" (vectors *A. americanum, A. cajennense, A. coelebs*) in USA (Tennessee); "**R. texiana**", the probable agent of <u>Bullis fever</u> (vector *A. americanum*) in USA (Texas); and possibly also **R. peacockii**, **R. rhipicephali**, **R. montana**, and **R. belli**.

Rickettsia australis

This species is close to *R. akari*.

Source of infection (natural host range): rodents, marsupials (bush rats, bandicoots, opossum), canids.

Animal disease: inapparent course.

Transmission mode: ixodid ticks *Ixodes holocyclus*, *I. tasmani, I. cornuatus*.

Human disease: Queensland tick typhus with fever up to 2 weeks, headache, eschar, regional lymphadenitis, and sometimes a generalized rash.

Bio-containment: BSL-2/3.

Diagnosis: isolation from the blood; serology (Weil-Felix AR, specific AR, CFT, RIHA).

Treatment: tetracycline.

Geographical distribution: Australia.

Rickettsia akari

Genomically similar to *R. felis* and *R. australis.*

Source of infection (natural host range): rodents – synanthropic (*Mus*, *Rattus*), less exoanthropic species.

Animal disease: lethal for mice.

Transmission mode: the mite *Liponyssoides* (*Allodermanyssus*) *sanguineus* (a mouse ectoparasite occasionally feeding also on man); laboratory infections.

Human disease: rickettsial-pox or vesicular rickettsiosis, with the course similar to African tick fever, but usually milder (however, sometimes lethal) – at first a primary ulcerating skin lesion (eschar) and regional lymphadenopathy, after a week follows fever with chills, headaches, myalgia, photophobia, and papulo-vesicular rash over the trunk and extremities (sometimes generalized) rash. Largely an urban zoonosis.

Bio-containment: BSL-2/3.

Diagnosis: symptoms, inoculaton of mice (the blood from febrile status), CFT, AR (Weil-Felix AR is negative).

Treatment: doxycycline, tetracycline (chloremphenicol).

Geographical distribution: towns in North America (New York: first described in 1946), Central America (Mexico), Eurasia (Russia, Ukraine, Croatia, southern Europe, Turkey, Korea) and South Africa.

Rickettsia felis

A SFG rickettsia originally labeled as "ELB agent", known since 1994, genomically closely related to *R. akari.*

Source of infection (natural host range): cat (dog, rodents), opossum (USA).

Animal disease: inapparent course.

Transmission mode: fleas *Ctenocephalides felis* (reservoir, TOT), *Archaeopsylla erinacei* (Germany), less often *C. canis, Pulex irritans*.

Human disease: flea-borne spotted fever – a febrile illness with rash, nausea, headache, joint pain, myalgia, and fatigue, occasionally skin rash, hepatitis; sometimes hospitalisation is necessary. In the years 1994–2006, 68 cases were reported, most frequently (40 cases) in Spain.

Bio-containment: BSL-2.

Treatment: doxycycline, tetracycline (chloramphenicol).

Geographical distribution: Mexico, USA, Caribbean islands, Brazil, Argentina, Spain (also Canarian Islands), France, Germany (2 cases), Great Britain, Tunisia, Kenya, DR Congo, South Korea, Taiwan, Laos, Thailand.

Orientia tsutsugamushi

Source of infection (natural host range): exoanthropic rodents (wild rats), insectivores and other small mammals.

Animal disease: septicaemia in mice.

Transmission mode: larvae ("chiggers") of the mites, especially of the genus *Leptotrombidium* (reservoir – TST as well as TOT): *L. deliense* (in the whole geographical range; also a non-bacteraemic transmission by co-feeding has been described), *L. akamushi* (in Japan), *L. pallidum* (Japan, Korea, Russian Far East), *L. scutellare* (Japan, China, Malaysia), *L. pavlovsky* (the Far East), *L. imphalum, L. chiangraiensis*, *L. fletcheri, L. aernicola*, etc.

Human disease: scrub typhus, tsutsugamushi – sudden high fevers for up to 2 weeks, hyperhidrosis, severe headaches, lymphadenopathy, often eschar (e.g. in 90% of patients in China), macular rash on face, chest and abdomen, cough to pneumonia, gastrointestinal symptoms, splenomegaly, conjunctivitis, photophobia, CNS affection, often temporary deafness; average fatality rate 15% (range, 1–40%, but up to 60% in untreated cases); Allied troops recorded a total of 20,000 tsutsugamushi cases during operations in 2ndWW, with a fatality rate of 25%. In the 1970s, scrub typhus was a frequent infectious disease among American soldiers also in the Vietnamese War. In South Korea, a total of 23,930 cases (68% serologically confirmed) were reported between 2001 and 2006.

Bio-containment: BSL-2/3.

Diagnosis: mouse inoculation (blood samples), serology (Weil-Felix AR, CFT, IFA, ELISA).

Treatment: tetracycline, doxycycline (chloramphenicol).

Prevention: effectivity of inactivated vaccine is low, it is not used.

Geographical distribution: southeastern, eastern and southern Asia (among others also the Himalayas in 2006), northern China, northern Australia, and Oceania, typically in grassy and bushy habitats that arose after cut down of tropical forests (Photo 5.32).

8.3.6 Family Anaplasmataceae [*Order* Rickettsiales]

Ehrlichia chaffeensis, *E. ewingii*

Genomic group *E. canis* s.l., also involves very similar *E. muris* and veterinary important *Ehrlichia* (*Cowdria*) *ruminantium*, the agent of heartwater disease of ruminants in Africa.

Source of infection (natural host range): whitetail deer (a competent host, with ehrlichaemia, and reservoir as well), sika deer, dog (also *E. ewingii*), coyote, cattle, goat; rodents (possibly only in east Asia, while not demonstrated as competent hosts in America).

Animal disease: chronic pancytopenia (thrombocytopenia) and a long-term ehrlichaemia in dogs infected with *E. ewingii*; *E. canis,* that is not transmissible to man, causes a severe disease in dogs while *E. chaffeensis* is only pathogenic to puppies.

Transmission mode: metastriate ixodid ticks *Amblyomma americanum* (*E. chaffeensis* – principal competent vector, *E. ewingii*), *A. cajennense* (*E. chaffeensis*), *A. parvum* (*E. chaffeensis* in Argentina), *Dermacentor variabilis* (*E. chaffeensis* in USA), *Rhipicephalus sanguineus* (*E. chaffeensis* in Cameroon), *Haemaphysalis longicornis* and *H. flava* (*E. chaffeensis* in Korea), *H. yeni* (*E. chaffeensis* in China).

Human disease: human monocytic ehrlichiosis (HME) – fever with chills, arthralgia, myalgia, headache, nausea, anorexia, diarrhoea, vomiting, aseptic meningitis (in about one-fifth of patients), rash similar to that in RMSF (30% of cases – only with *E. chaffeensis*); thrombocytopenia, leucopenia, anaemia, increased level of AST; fatality rate 1–5%. *E. ewingii* occurs more often in immunosuppressed individuals.

Bio-containment: BSL-2.

Diagnosis: blood smear (Giemsa: characteristic "morulae" of ehrlichiae in monocytes and macrophages); serology (IFA, ELISA, WB) with the antigen from *E. canis*; PCR with the blood samples, isolation on cell cultures (Vero, DH82 – canine histiocytes).

Treatment: doxycycline, tetracycline.

Geographical distribution: North America, Mexico, South America (Brazil, Chile, Venezuela, Argentina – *E. chaffeensis*), Japan, China, Korea, sporadically Europe (Portugal, Spain – *E. chaffeensis*), Africa (Cameroon, Mozambique, Mali, Burkina Faso).

Anaplasma phagocytophilum s.l.

The group of genomically very similar *A. phagocytophilum*, *A. equi* and HGE agent was earlier classified in the genus *Ehrlichia* as *E. phagocytophila* s.l. Another synonym is *Cytoecetes phagocytophila*. More distantly related species are *A. platys* and *A. marginale.* Within *A. phagocytophilum* s.l., there is a great variety of antigenic types (due to considerable variation of the outer membrane protein), genotypes, ecotypes and pathotypes.

Source of infection (natural host range): roe deer, red deer, fallow deer, sika deer, white-tailed deer (the deer are competent, amplifying hosts for variants such as Ap-1 that are probably not pathogenic for humans), moose, chamois; forest rodents like bank vole *Myodes glareolus*, yellow-necked mouse *Apodemus flavicollis*, wood mouse *A. sylvaticus*, white-footed

mouse *Peromyscus leucopus* (amplifying host and reservoir of the human-pathogenic variant Ap-ha), field vole *Microtus agrestis*, woodrat *Neotoma fuscipes,* squirrels, yellow-checked (redwood) chipmunk *Tamias ochrogenys* (a competent host).

Animal disease: <u>tick-borne fever</u> (TBF, first described in sheep and cattle in Scotland, 1932, although known in Norway for at least 200 years) – a severe febrile reaction accompanied with thrombocytopenia, leucopenia and anaemia of sheep, cattle, goat, dog, and horse. Infected hosts are more susceptible to other infections, e.g. to lameness caused by *Staphylococcus aureus* and called tick pyaemia. Persistent *A.p.* infections in lambs have been reported in Europe.

Transmission mode: ixodid ticks *Ixodes ricinus, I. scapularis* (TST, TOT), *I. pacificus*, *I. spinipalpis*, *I. trianguliceps*, *I. persulcatus*, *I. ovatus*.

Human disease: <u>human granulocytic anaplasmosis/ehrlichiosis</u> (HGA/HGE) with fever, headaches, myalgia, arthralgia, anorexia; bacteraemia, leucopenia, thrombocytopenia, increased levels of transaminases; fatality rate 5–10%; convalescence long, usually 1 month (total weakness, fatigue). In addition, infected persons are more susceptible to other infections (due to immunosuppression). The disease was first described and elucidated in USA, 1994. Probably a majority of *A. phagocytophilum* strains are nonpathogenic for man (they are endosymbionts of ticks and their host is deer, e.g. the variant Ap-1), and only some strains belong to the pathogenic genotype (e.g., the variant Ap-ha). The situation is confusing especially in Europe, where often disproportionately high prevalence rates (detected by PCR) of *A. phagocytophilum* have been reported in ixodid ticks approaching those of *Borrelia burgdorferi* s.l., whereas incidence of HGA in Europe is substantially lower than for instance that of LB.

Bio-containment: BSL-2.

Diagnosis: blood smear (Giemsa stain: the bacteria form micro-colonies visible as morulae in neutrophil granulocytes), serology (IFA, ELISA with the antigen from *A. equi*); PCR, cultivation in human leukemic cells HL-60, vertebrate endothelial cells or in tick cell lines.

Treatment: doxycycline (or other oxytetracyclines) up to 3 weeks, children and pregnant women rifampicin.

Geographical distribution: North America, Europe (Great Britain, Ireland, Scandinavia, Switzerland, Italy, Slovenia, the Netherlands, France, Spain, Germany, Austria, Poland, Denmark, Slovakia, Czechland), Madeira, Siberia, China, Japan.

Neoehrlichia mikurensis

First isolated in Japan, 2004.

Source of infection (natural host range): rodents (*Rattus norvegicus*).
Animal disease: unknown.

Transmission mode: ixodid ticks *Ixodes ovatus*, *I. ricinus*.
Human disease: a case of septicaemia in Switzerland, 2009.
Bio-containment: BSL-2.
Diagnosis: blood smear, PCR.
Treatment: doxycycline.
Geographical distribution: Japan, China, Asian Russia, Slovakia, Switzerland, the Netherlands.

Neorickettsia sennetsu (Syn. *Ehrlichia sennetsu*)

Forms a genomic group with the nearly identical veterinary important *N. risticii.*

Source of infection (natural host range): parasites of freshwater fishes and molluscs – larvae (cercaria) of trematodes (for instance *Nanophyetus salmincola* in the USA).
Animal disease: <u>Potomac horse fever</u> – monocytic ehrlichiosis with fever and colitis (*N. risticii*).
Transmission mode: ingestion of cercariae that have as intermediate hosts freshwater molluscs and fish.
Human disease: <u>sennetsu fever</u> with lethargy, lymphadenopathy, haematological changes similar to those present in mononucleosis; mortality has not been recorded.
Bio-containment: BSL-2.
Diagnosis: serology (IFA, ELISA), inoculation in mouse (blood samples), blood smear (monocytes, macrophages), PCR. Cultivation impossible.
Treatment: tetracyclines.
Geographical distribution: Japan and SE. Asia; *E.r.* in USA and Europe.

8.3.7 *Family* Bartonellaceae [*Order* Rhizobiales*, Class* Alphaproteobacteria]

Six species have been documented to cause endocarditis in humans: *Bartonella quintana*, *B. henselae*, *B. elizabethae*, *B. vinsonii* subsp. *berkhoffii*, *B. koehlerae*, and *B. alsatica*.

(**) *Bartonella quintana*

Bartonellae were formerly classified as rickettsiae. However, they differ from them in many respects, e.g. by the ability of extracellular growth in vitro on (special) bacteriological media. *B. quintana* is genomically closely related to *B. henselae*, in fact its "descendant" that aroused by a reductive evolution of the genome.

Source of infection (natural host range): man (reservoir); exceptionally cat
and dog.

Transmission mode: excrements of body louse (*Pediculus humanus*); *B. quin-
tana* replicates intensively in its intestinal epithelium extracellulary. In Africa
(Gabon, 2005), DNA of *B. quintana* was detected in the flea *Pulex irritans*.

Human disease: trench fever (*febris quintana*) – chills, headaches, backaches
and pains in limbs; 4–5 (or more) relapses at intervals of 4–6 days, some-
times even after years; endocarditis, bacillary angiomatosis (cutaneous and
subcutaneous vascular lesions), rash (infrequently), long-term bacteraemia.
During the 1st WW, >1 million cases in the battlefields. Today the disease
occurs occasionally among homeless people, alcoholics, drug abusers and
AIDS patients.

Bio-containment: BSL-2.

Diagnosis: cultivation of blood samples in vitro (chocolate BA, 5% CO_2, 35°C),
serology, xenodiagnostics (experimental infection of lice with the blood or
urine from the patient).

Treatment: tetracycline, chloramphenicol.

Geographical distribution: Europe (Poland, Ukraine during the 1st WW, the
Far East, north Africa, Mexico, the Andes (villages)); Seattle 1995, Moscow
1997–1998, Marseille 1998–2001, Utrecht 2001 (homeless people), central
Africa (refugee camps).

**Bartonella bacilliformis*

Very small coccobacilli 0.3–1.5×0.2–0.5 μm.

Source of infection (natural host range): man.

Transmission mode: sandflies (especially *Lutzomyia verrucarum*).

Human disease: bartonellosis, in two clinical phases: (1) Oroya fever (Carrión's
disease – acute form – anaemia, with fatality rate 10–40% when untreated);
(2) *verruga peruana* (Peruvian wart – papular to verrucose secondary cuta-
neous form, with a low mortality) [Daniel Carrión autoinfected himself with
the blood of a patient, and documented the two stages in 1885; he succumbed
to the disease]. A total of about 7,000 workers died due to this disease during
the building of the railway connecting Lima with Oroya in 1870.

Bio-containment: BSL-2.

Diagnosis: blood smear – Giemsa (intraerythrocytic localisation) in acute
phase; skin biopsy in the secondary phase; haemocultivation (chocolate
BA or agar with rabbit serum, 5–10% CO_2, the growth is slow); serology
(CFT, AR).

Treatment: chloramphenicol, ampicillin, penicillin, streptomycin, tetracycline
(in verruga peruana mainly against secondary microflora).

Geographical distribution: South America (valleys of the Cordillera mountains
in Peru, Ecuador and Columbia).

Bartonella henselae, B. clarridgeiae, B. koehlerae, B. rochalimae, B. alsatica

B. henselae is genomically closely related to *B. quintana*, in fact its "ancestor".

Source of infection (natural host range): cat (reservoir, 10–25% of cats in USA and New Zealand are bacteraemic), rodents. In *B. rochalimae,* also red fox and gray fox (reservoir?), coyote, raccoon, rural domestic dogs. In *B. alsatica*, wild rabbit is a natural host.

Animal disease: usually inapparent course, but may cause reproductive failure in female cats, and endocarditis in dog (*B. rochalimae*).

Transmission mode: percutaneous by cats (scratching, biting); the cat flea *Ctenocephalides felis* (a competent vector, but flea-to-human transmission is obviously rare). [Often discussed possible transmission of *B. henselae* by ixodid ticks has not been proven, there are no clear supportive data].

Human disease: <u>cat scratch disease</u> (CSD) or <u>felinosis</u>, benign lymphoreticulosis, regional lymphadenopathy – especially in children, high fever with headaches, pharyngitis, exhaustion, sometimes conjunctivitis, nodular syndrome, arthralgia, a long-term rash (4–24 months), endocarditis, bacillary (epitheloid) angiomatosis (cutaneous, subcutaneous and other vascular lesions – especially in AIDS patients), persistent bacteraemia, infrequently chronic osteomyelitis, aseptic meningitis; no mortality. In the USA up to 20,000 cases annually, and from that number >2,000 need hospitalisation (mean incidence rate is 9/100,000 population annually). In the Netherlands, the CSD incidence estimates are 2,000 per year, and 12/100,000 population. *B. alsatica* was first identified in 1999 in Alsace, France, as an agent of bacteraemia in healthy wild rabbits. In 2006, interest in *B. alsatica* increased when it caused a blood culture-negative endocarditis in a patient with lymphadenopathy who had contact with rabbits.

Bio-containment: BSL-2.

Diagnosis: clinical symptoms (primary cutaneous lesion – a papule, enlarged lymph nodes, etc.), anamnesis, biopsy of lymphatic glands, serology (IFA, CFT, ELISA – IgM: but there might occur cross reactions with *B. quintana*, *Coxiella burnetii* and *Chlamydophila pneumoniae*), intradermal test (Hanger-Rose); detection of antigen (IF, ELISA) or DNA (PCR) of the agent; the cultivation is quite difficult (BHI agar with 5% blood or chocolate BA, 5% CO_2) because the growth is slow – 10–40 days/35°C.

Treatment: symptomatic; long-term antibiotic treatment (rifampicin, ciprofloxacin, azithromycin, gentamicin, or trimethoprim + sulphamethoxazole) is not always very effective.

Geographical distribution: *B. henselae* worldwide; *B. clarridgeiae* in USA, Chile, Israel, Japan, the Philippines, Indonesia, Thailand, France, Germany, the Netherlands and Spain; *B. koehlerae* in USA; *B. rochalimae* in Peru, Chile, USA and France; *B. alsatica* in France and Spain.

Bartonella elizabethae, *B. vinsonii*, *B. grahamii*

Source of infection (natural host range): brown rat (reservoir for *B. elizabethae*) and other rodents (e.g., white-footed mice – reservoir, and groundhog in *B. vinsonii,* bank vole – reservoir, and yellow-necked mouse in *B. grahamii*); dog, coyote (*B. vinsonii* ssp. *berkhoffii*).

Animal disease: largely asymptomatic or subclinical course, but endocarditis in dogs infected with *B. vinsonii*.

Transmission mode: fleas and mites of rodents. For instance, main vector of *B.g.* is the flea *Ctenophthalmus nobilis*.

Human disease: endocarditis, neuroretinitis and other ocular manifestations (e.g. occlusion). *B. elizabethae* occurs in intravenous drug users and homeless people.

Bio-containment: BSL-2.

Diagnosis: serology (CFT), detection of antigen (IF, ELISA) or DNA (PCR) of the agent; the cultivation is quite difficult (BHI agar with 5% blood or chocolate BA, 5% CO_2), the growth is slow.

Treatment: symptomatic; the effect of antibiotics (doxycycline, rifampicin) is debatable.

Geographical distribution: the Americas (*B. elizabethae, B. vinsonii*), Sweden (*B. elizabethae*), Portugal (*B. elizabethae*), *B. vinsonii* also occurs in Europe. *B. grahamii* occurs in Eurasia (e.g., UK, Poland, Czechland, Sweden etc.), within the range of its reservoir host, *Myodes glareolus.*

Bartonella schoenbuchensis, Candidatus **Bartonella melophagi**

Source of infection (natural host range): deer (*B. schoenbuchensis*), sheep (*B. melophagi*).

Animal disease: asymptomatic course.

Transmission mode: in *B. schoenbuchensis*, deer keds (hippoboscids) *Lipoptena cervi* (Europe)*, L. mazamae* (North America), while sheep keds *Melophagus ovinus* in *B.* melophagi.

Human disease: <u>deer ked dermatitis</u> (speculation at present, the aetiology has not yet been proved). *B.* melophagi was isolated from the blood of two women with skin lesion, myalgia, fatigue and dry cough (and pericarditis in one of them) in the USA. However, the clinical relevance of these findings remains to be established.

Bio-containment: BSL-2.

Diagnosis: serology (CFT), detection of antigen (IF, ELISA) or DNA (PCR) of the agent.

Treatment: symptomatic; the effect of antibiotics is debatable.

Geographical distribution: Germany and some other European countries, USA.

8.3.8 *Family* **Brucellaceae [***Order* **Rhizobiales,** *Class* **Alphaproteobacteria]**

Brucella abortus, *B. melitensis*, *B. suis*, *B. canis*

Non-motile Gram-negative coccobacilli or short small rods $0.6–1.5\times0.5–0.7$ μm without bipolar staining, not encapsulated. Several biotypes (biovars) in each species.

Source of infection (natural host range): domestic and wild ruminants – e.g., bison (*B. abortus*), elk (*B. abortus*), reindeer and caribou (*B. suis* biotype 4), domestic pig and wild boar (*B. suis* biotypes 1–3), brown hare (*B. suis* biotype 2), goat and sheep (*B. melitensis*), dog (*B. canis*).

Animal disease: Bang disease, brucellosis (*B. abortus*) – infectious abortions of cattle; arthritis; orchitis and epididymitis, sterility; chronic forms (often inapparent infections). Also other wild ruminants involved in the disease: bison, elk, reindeer. Porcine brucellosis (*B. suis* biovars 1, 2, or 3) causes reproductive failure in domestic and wild sows, orchitis in boars, arthritis or paralysis. *B. suis* biovar 2 is also the agent of the hare brucellosis – necrosis of spleen, liver and genital organs. Newly described species of brucellae cause illness in marine mammals (***B. pinnipedialis, B. ceti***) or in terrestrial rodents (***B. microti***), but their pathogenicity for man is as yet unknown (although there are 3 reports on naturally acquired infection of humans with brucellae originating from marine mammals to date).

Transmission mode: alimentary (unpasteurized milk and cheese), percutaneous, aerogenic, per conjunctivae, or by contact; quite high tenacity in animal products, but not in external milieu. A number of laboratory infections have been described.

Human disease: brucellosis – a serious disease with acute or undulating fever (relapses are especially common with *B. melitensis*), hyperhidrosis, headaches, myalgia, arthralgia, lymphadenitis, hepatosplenomegaly, pericarditis, formation of granulomas, arthralgia, synovitis, myalgia, oedema of legs (*B. melitensis*), osteomyelitis, damage of kidneys and some other organs (orchitis, endocarditis); fatality rate in untreated cases is low, 2–5%. Relatively frequent are chronic forms leading to permanent disability. Occupational disease: veterinarians, animal keepers and attendants, meat industry workers, hunters, medical and veterinary microbiologists are at risk.

Bio-containment: BSL-2/3.

Diagnosis: cultivation of the blood and tissue samples (liver agar, glucose agar with serum, 5% CO_2), inoculation of guinea pig (increased laboratory risk), PCR, serology (AR – cross reactions with *Francisella tularensis* and *Yersinia enterocolitica* serovar O9, sometimes it occurs so-called prozonal effect – the serum sample does not react with the brucella antigen at low dilutions but starts to react at higher dilutions, Rose Bengal AR; CFT – specific, Coombs test for incomplete antibodies, etc.), intradermal test. Some species of the

genus *Brucella* can be misidentified as *Ochrobactrum* (e.g., *O. intermedium*) when using commercial biochemical test kits because their reactions are similar.

Treatment: difficult – doxycycline (tetracycline) + streptomycin; rifampicin + doxycycline combination for ≥6 weeks; cotrimoxazole. No steroid administration.

Prevention: eradication programs in domestic animals (cattle), milk pasteurization.

Geographical distribution: nearly worldwide (in Europe especially the Mediterranean), in some places Bang disease was eradicated (e.g. in Czechland).

8.3.9 *Family* Francisellaceae [*Order* Thiotrichales, *Class* Gammaproteobacteria]

Francisella tularensis

Non-motile very small spherical to ovoid (0.3–1.0×0.2–0.5 µm) Gram-negative and facultatively intracellular bacteria. The species consists of several subspecies: *F. tularensis tularensis* (type A – "*nearctica*", the most virulent one for mammals), *F. t. holarctica* (type B), *F. t. mediasiatica*, and the low virulent *F. t. novicida* (only 5 patients with suspected infection with this subspecies have been reported). Another species of the genus *Francisella* is *F. philomiragia*, living saprophytically in water, being of low pathogenicity for man. There are also ichthyopathogenic francisellae recorded in marine fish farms in USA, Japan and Norway which are not pathogenic for endothermic vertebrates and are unable to grow at 37°C. Very interesting are the francisellae living as endosymbionts in metastriate ixodid ticks especially of the genus *Dermacentor* in the Holarctis. The new findings indicate two probable trajectories of evolutionary adaptation from saprophytism to parasitism within the genus *Francisella*: (a) via water organisms; (b) via ixodid ticks.

Source of infection (natural host range): leporids, rodents (*Microtus*, *Arvicola*, *Ondatra*, in Canada also *Castor* – especially during overpopulation of the rodents and tularaemia epizootic in process within them); but never a tularaemic patient. Very recent data show that *F. tularensis* could also be an endosymbiont or commensal of water amoebae of the genus *Acanthamoeba,* the bacterium being able to replicate in these protozoa and survive in their cysts for several weeks. The interaction between aquatic and terrestrial cycles of *F. tularensis* is largely unknown.

Animal disease: tularaemia (usually lethal epizootics of hares, rabbits and rodents; sometimes sheep – only with the type A; subcutaneous nodules in dogs).

Transmission mode (Fig. 8.19): unusually varied – direct contact (e.g. skinning the infected animals); bites of ixodid ticks *Dermacentor* (reservoir,

Fig. 8.19 Natural cycles of *Francisella tularensis* (drawing by Ivo Rudolf)
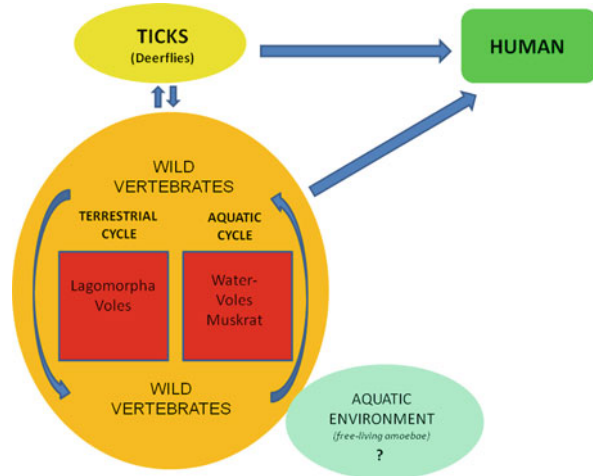
TST and TOT demonstrated) – *D. variabilis*, *D. andersoni*, *D. reticulatus*, *Amblyomma americanum*, rarely *Ixodes ricinus* complex, and in certain areas also mechanical transmission by bites of tabanids *Chrysops* spp. ("deerfly disease" – USA, Russia) while reports on transmission by mosquito bites are unsubstantiated at present (Scandinavia); alimentary (water or food contaminated by infected rodents); aerogenic (inhalation of contaminated aerosol or dust during the work with hay, straw or other agricultural products including sugar beet – outbreaks in sugar refineries in central and eastern Europe: Austria, Czechland, Slovakia, Ukraine). An unusual air-borne outbreak of tularaemia occurred among hunters (39 cases, 1 person died) in Hesse, Germany, 2005: the infection occurred when aerosol was generated during rinsing with a water hose of disemboweled hares (some of them being infected) after the hunt. High tenacity of the microbe at low temperatures – at 4°C *F. tularensis* survives up to 4 months. Interestingly, *F. tularensis* survives in *Acanthamoeba castellanii* cysts for up to several weeks; trophozoites of this amoeba encyst rapidly in response to infection with *F. tularensis*.

Human disease: <u>tularaemia</u> – sudden fever, chills, headache, backache, hyperhidrosis, diarrhoea, anorexia; then according to the entrance gate different clinical forms: (1) (ulcero-/oculo-)glandular (bubonic) with the primary ulcer on the skin and regional painful lymphadenitis; (2) pulmonary (pneumonia); (3) abdominal (typhoid, oropharyngeal, gastrointestinal); or (4) generalized (septic); fatality rate is about 5% (but up to 30% in the typhoid form) in USA; while in Eurasia <3% (due to absence of the highly virulent type A tularaemia); convalescence is long (2–3 months or more), especially in improperly treated illness. Occupational disease: farmers, hunters or trappers of leporids and big rodents (caught for skin, e.g. muskrats), workers in veterinary laboratories. There was a big outbreak of hare/rodent-borne tularaemia in Central Europe (Austria, Czechoslovakia) in 1936/1937, with

many hundreds of patients. A recent (1999–2000) outbreak of largely alimentary tularaemia in Kosovo involved 327 confirmed cases; the main source were rodents, overpopulated during the Civil War in this region. The first two outbreaks of tularaemia in Spain occurred in 1997 and 1998, and were associated with hare hunting (585 patients) and crayfish (*Procambarus clarkii*) fishing in a contaminated freshwater stream, respectively. In the USA from 1990 to 2000, the average annual number of human cases of tularaemia was 124 (the range, 86–193); however, prior to 1950 there were many hundreds of cases annually (maximum in 1939: 2,291 reported patients). For comparison, in the Central-European Czechland (about 10 million population), the annual incidence averaged in the same decade 75 cases (the range, 12–225); an absolute maximum of tularaemia incidence in Czechland was recorded in 1962, with a total of 1,468 reported (serologically confirmed) patients.

Diagnosis: symptomatology, anamnesis, serology (AR, ELISA etc. – possible cross-reactivity with brucellae), subcutaneous inoculation of adult laboratory mouse (MLD is 1 cell!), cultivation of the blood or ulcer secreta samples on TGKA (glucose BA with thioglycollate) or cysteine BA, PCR; past infection could well be detected by an intradermal test with tularin; IF microscopy of lesion secreta, immunohistochemistry.

Bio-containment: BSL-2/3. (MID for man is as low as 10 viable cells applied intradermally or by inhalation). Considered as a bioterrorist agent (but only the type A tularaemia might be dangerous).

Treatment: streptomycin, gentamicin (tetracycline, chloramphenicol); exstirpation of enlarged lymph nodes.

Prevention: vaccination by scarification (the attentuated Russian strain, LVS) – but routinely the vaccine is inaccessible.

Geographical distribution: holarctic (Eurasia and North America). *F. t. tularensis* (A) only occurs in North America, and *F. t. mediasiatica* only in Central Asia. For a natural focus of tularaemia, see Photo 5.42.

8.3.10 *Family* Legionellaceae [*Order* Legionellales, *Class* Gammaproteobacteria]

*Legionella pneumophila (L. micdadei, L. bozemanii, L. longbeachae, L. anisa etc.)

Poorly stainable Gram-negative aerobic rods 2–6(15)×0.3–0.9 μm, motile and facultatively intracellular. Up to now about 15 species (with a number of serotypes) of legionellae pathogenic to man have been described, the most important being the serotypes 1 and 6 of *L. pneumoniae* causing 80–90% of cases. The group of so-called "legionella-like amoebal pathogens" (LLAP) is formed by parasites of amoebae; interestingly, antibodies to LLAP were detected in up to 20% of patients hospitalized with pneumonia of unclear aetiology in USA and Canada in 1995.

Source of infection: water (for industrial purposes, heated water; water in air-conditioning systems, cooling towers in industrial plants, gas turbines, buildings' water systems, shower heads, whirlpools, warmwater mains, water sprayers, rainwater in puddles on asphalt roads during warm weather); a saprophytic and thermophilic microbe (the growth optimum is about 40°C), often associated with other thermophilic bacteria and protozoa, e.g. cyanobacteria, *Flavobacterium,* amoebae (legionellae are endosymbionts of *Acanthamoeba,* capable to replicate in them). They often form biofilms in water habitats (water mains, cooling towers, etc.) and are resistant to standard disinfection compounds and measures. Potting soil is an alternative infections source of *L. pneumophila* and other *Legionella* species (especially in Australia and New Zealand, but rarely in Europe or North America).

Transmission mode: aerogenic – inhalation of aerosol (water droplets) containing the bacteria.

Human disease: two clinical forms – (1) legionellosis (Legionnaires' disease, LD) – pneumonia with high fever (up to 41°C), chills and dry to spasmodic cough, headache, neurological and psychotic disturbances (confusion, anxiety), somnolence, vomiting, diarrhoea, damage to visceral organs (liver, kidneys); fatality rate 10–25%; the disease is most dangerous for smokers and immunosuppressed persons; (2) Pontiac fever (the agent is, e.g., *L. anisa*), a flu-like illness with a sudden onset, myalgia and headache, but without any pulmonary affliction; mortality has not been reported. Very susceptible to both forms of legionellosis are immunosuppressed persons. Epidemics of legionellosis: first known in 1976 during a meeting of American legionnaires in Philadelphia: 221 patients (34 died; the agent, *L. pneumophila* was isolated and identified from the air-conditioning system in the meeting hotel); retrospectively (serologically), another outbreak of atypical pneumonia with 19 cases including 14 fatal in a psychiatric clinic in Washington was resolved as legionellosis; 1977, five smaller epidemics of legionellosis (total about 135 cases) in Ohio, Vermont, Tennessee, Los Angeles (USA) and Nothingham (UK); 1985, an outbreak (14 cases, 3 fatal) of LD after a banquet in Michigan; 1989, 34 LD cases (2 fatal) in Louisiana; 1998, 45 cases of Pontiac fever in Wisconsin (caused by a whirlpool); 1998, Institute of Clinical and Experimental Medicine in Prague 13 patients with transplanted organs (8 died; the source of LD was contaminated warm water in the institution); 1999, Bovenkarspel (the Netherlands) 233 LD cases (22 died) after a visit of exhibition of consumer goods including warmwater whirling baths (the source of infection); 1999, 22 cases of Pontiac fever in a hotel in Georgia, USA; 2000, 20 cases of Pontiac fever (from a hotel whirlpool) in Wisconsin; 2000, Melbourne 101 LD patients (5 died) after a visit of sea aquarium; 2000, Vigo (Spain) 28 LD patients were infected during hospitalisation due to other disease, and 3 died; 2002, 117 cases of Pontiac fever caused by *L. anisa* at a restaurant in Tennessee; 2004, 66 cases of Pontiac fever among visitors of a hotel in Oklahoma; 2010, Ulm

(Germany) 65 patients (5 deaths; a community outbreak of LD associated with a water cooling system), and Barcelona (43 cases of Pontiac fever).

Bio-containment: BSL-2.

Diagnosis: serology (paired serum samples: IFA, AR, ELISA); detection of the agent in sputum and other excreta or by bronchoalveolar lavage (IF; PCR could detect viable, but non-cultivable cells); cultivation on a special BCYE agar medium containing yeast extract, cysteine, iron ionts and charcoal in 5% CO_2 (LLAP need the presence of amoebae for growth); antigen detection in urine (ELISA, PCR).

Treatment: clarithromycin, erythromycin, tetracycline, levofloxacin, azithromycin, rifampicin (penicillin and ampicillin are ineffective).

Geographical distribution: worldwide, but (surprisingly) mainly in the temperate zone. *L.l.* occurs in Australia and USA while in Europe sporadically.

8.3.11 *Family* Coxiellaceae [*Order* Legionellales]

Coxiella burnetii

Pleomorphic minute coccobacilli or short rods 0.4–1.0×0.2–0.4 µm. Obligate intracellular parasite with a greatly reduced genome, able to multiply also in phagolysosomes of vertebrates. It reveals a phase variation: virulent phase I (S), and avirulent II (R). During a passage in the chick embryo, the coxiellae undergo the transformation I → II (which corresponds to a deletion mutation expressed by a surface lipopolysaccharide change), but the reverse transformation II → I is possible by passaging through a mammal or a tick. Only the phase I occurs in nature. The strains of *C. burnetii* obviously differ substantially in their virulence. (In the past, the genus *Coxiella* was assigned to rickettsiae.)

Source of infection (natural host range): all domestic ruminants (cattle, sheep, goat), less rodents, insectivores, and other wild mammals (including ruminants, and marsupials – kangaroos in Australia), occasionally pigeons.

Animal disease: coxiellosis – inapparent persistent infection; sometimes abortions or pneumonia.

Transmission mode (Fig. 8.20): aerogenic – by aerosol (foetal fluid and placenta of infected cows, ewes, and goats contain up to 109 coxiellae per gram – the infection exacerbates before delivery) and dust (straw, hay; dispersal by wind), often also alimentary (raw milk of goat and sheep: *C. burnetii* may resist even pasteurization 30 min/60°C), percutaneous (by bites of metastriate ticks, e.g. *Rhipicephalus sanguineus, Dermacentor marginatus, Hyalomma lusitaniae* – more often in subtropical regions; tick excrements contain up to 1010 coxiellae per gram; TOT in the ticks was demonstrated). *C. burnetii* has a very high tenacity in milieu: it resists drying and light exposure for many months: for instance on wool, cotton or in tick excrements it remains viable for a number of months, and in the soil for up to
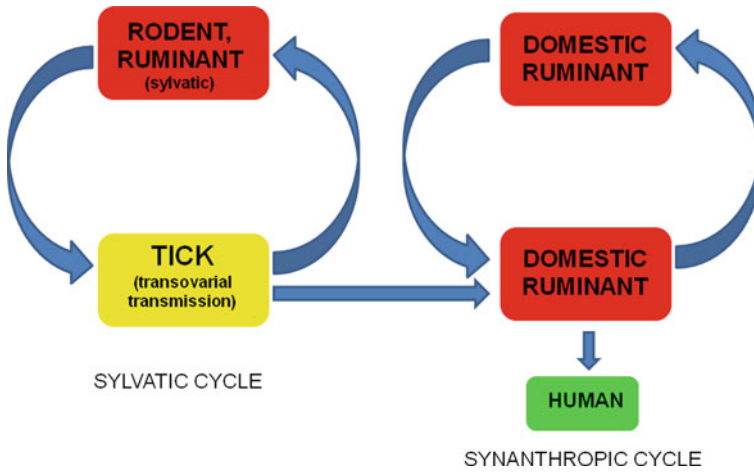
Fig. 8.20 The cycles of *Coxiella burnetii* (drawing by Ivo Rudolf)

20 days. The agent is extremely contagious and man is highly susceptible to
infection. Exoanthropic cycle: rodents and wild ruminants (deer) + ixodid
ticks (Metastriata). Synanthropic cycle: among domestic ruminants (con-
tact). Some flies feeding on the faeces, milk, carcasses, or blood of infected
domestic animals can spread *C. burnetii* mechanically.

Human disease: Q fever (Q meaning "query"), first described (by Edward
Derrick) in butchers working in a slaughterhouse in Brisbane (Queensland,
Australia) in 1935–1937 – sudden fever and chills, often recurrent, hyper-
hidrosis, weakness, intense headaches, fatigue, myalgia, arthralgia, atypical
pneumonia (infiltrate, but not always), weight loss, myo- and endocardi-
tis, rash does not occur (contrary to rickettsioses), sometimes meningitis;
hepatitis (increased levels of transaminases); fatality rate ≤1%; often inap-
parent or abortive infections (one-third to one-half of cases), on the other
hand also chronical course occurs in 1–3% of cases, accompanied with a
latency period of several years (the agent persists in liver and spleen) and
a characteristic subsequent endocarditis, sometimes granulomatous hepati-
tis (granulomas could also appear in the bone marrow). Reactivation of a
latent or chronic infection can occur in gravidity (coxiellae are then present
in placenta). Many smaller epidemics occur often in some Balkan countries,
e.g. in Bulgaria have been reported hundreds of cases in conjuction with
a rapid expansion of goat breeding since the 1990s. A very similar story
is the recent (2008–2010, still ongoing) major outbreak of Q fever in the
Netherlands where many farmers moved from cattle and pig breeding to goat
(and sheep) breeding in recent years (there were >350,000 goats present in
the Netherlands in 1999); 86 commercial dairy goat farms (i.e. 24% of all
commercial goat farms) have been involved as of April 22, 2010, and the inci-
dence of Q fever in humans has been unusually high. This epidemic started

sneakingly, with only 5 human cases in 2005, 12 cases in 2006, 127 cases in 2007, but as many as 1,014 cases were reported in 2008 (with one fatal case), 2,357 cases (6 fatalities) in 2009, and 421 cases in 2010 (as of end July); the situation resulted in a mass culling of goats (>10,000 animals). Recently, new cases of Q fever also occur in Belgium, Germany (848 human cases were reported in 2006–2009), France, UK, Canada, USA, and Australia. In 2009, several patients with Q fever endocarditis were described in a settlement in eastern Greenland – the likely animal source included sled dogs and seals. Q fever is an occupational disease with a risk for breeders and attendants of small domestic ruminants (goat, sheep), veterinarians (delivery in ruminants), butchers; also many laboratory infections are known. For instance, a teacher and 33 students (of 48 exposed) acquired Q fever during veterinary practice in a Slovenian sheep farm in 2007.

Bio-containment: BSL-3.

Diagnosis: serology (micro-AR, IFA, CFT; antigen phase II indicates acute infection, while antigen I confirms the chronic stage of Q fever; in ELISA there can occur cross-reactions with *Chlamydophila psittaci*), intraperitoneal inoculation of the blood sample in guinea pig, mouse or on chick embryo (patients have intense bacteraemia during acute phase of the illness, but the isolation is a risky operation); staining after Giménez or Giemsa, immunohistochemistry; PCR.

Treatment: doxycycline, tetracycline, chloramphenicol (they shorten febrile period, but do not kill the intracellularly localized coxiellae), cotrimoxazole in children and pregnant women; a recommended combinations in chronic cases of Q fever are doxycycline with hydroxychloroquine, rifampicin or cotrimoxazole.

Prevention: Australian vaccine is approved for humans (it is prepared from the phase I); the protection is assumed to last 10 years. Culling of infected small ruminant herds according to the "test (bulk milk samples tested by PCR) and slaughter" policy. In addition, mandatory vaccination of small ruminants started in the Netherlands in 2009.

Geographical distribution: worldwide, most often in steppe habitats with brush of the Mediterranean and Black Sea regions (Photo 5.31), or in analogous Australian habitats.

8.3.12 *Family* Enterobacteriaceae [*Order* Enterobacteriales, *Class* Gammaproteobacteria]

Salmonella enterica

Motile facultatively anaerobic Gram-negative rods not fermenting lactose and producing hydrogen sulphide; the only for man pathogenic species of the genus, with 6 subspecies; virtually all serovars pathogenic for man are in the subspecies *S. e. enterica*, including also anthroponotic *S.* Typhi and *S.* Paratyphi. The serovars (today a

total of *c.* 2560 but those pathogenic for man are only about 100 from the groups B, C, D and E) are differentiated according to the Kauffmann-White scheme based on thermostable somatic antigens O (polysaccharides of cell wall) and thermolabile antigens H (from German "Hauch") of two phases (flagellar proteins); sometimes is also present thermolabile capsular Vi-antigen, masking the O antigen.

> Source of infection (natural host range): fowl (especially *S.* Enteritidis: it contaminates the eggs in the ovary and sometimes even penetrates eggshell), rodents (*S.* Typhimurium), pig (*S.* Derby), cattle (*S.* Typhimurium, *S.* Dublin, *S.* Enteritidis), sheep (*S.* Montevideo), domestic mouse, synanthropic birds (gulls, house sparrow, collared dove, feral pigeon: *S.* Typhimurium, *S.* Enteritidis and other serovars), some ectothermic vertebrates.

> Animal disease: usually inapparent course, or enteritis acute (young animals) or chronic (adults); abortions. Salmonellae are able to persist for a long time in visceral organs of mammals (spleen, liver, kidney, biliary duct) and in oviducts of fowl. *S.* Dublin and *S.* Pullorum (both not transmissible to man) cause severe disease in cattle and in chickens, respectively.

> Transmission mode: alimentary – typical "food-borne disease" (mainly via contaminated eggs with *S.* Enteritidis and meat; MID for man is quite high, usually 106, exceptionally 102–103, but in some highly virulent strains allegedly 101 cells will do; some salmonellae easily replicate in foods at room temperature); direct contact (nosocomial infections). Great tenacity of *S.* Enteritidis (and other zoonotic salmonellae): e.g., in chicken droppings it survives viable ≥1 year.

> Human disease: salmonellosis, one of the most frequent and important zoonoses – gastroenteritis with a very short incubation period (6–48 h), watery diarrhoea (about 10 days), headache, abdominal pain, nausea, vomiting, fever, usually associated with haematogenic and lymphogenic dissemination of the agent, sometimes resulting in meningitis (especially in children); fatality rate is low, about 0.1% (mortality only occurs in toddlers and old patients – via critical dehydratation). The most frequent serovars are *S.* Enteritidis (frequent phagotypes are European PT4 and American PT8; – they have been panzootic and pandemic in the 1990s) and *S.* Typhimurium, less common are e.g. *S.* Virchow, *S.* Agona, and a number of others. Incidence of salmonellosis in the USA: 1956 reported 1,700 cases, while 1965 already 20,865. A similar increase of incidence was recorded in European countries; for instance in Czechland: 1,981 reported cases in 1955, 4,975 in 1965, 7,678 in 1975, 8,492 in 1985, and 52,588 in 1995. A considerable increase of the incidence in Czechland was reported in 1989, from 11 to 24 thousands of cases (it was caused by import of pandemic strain of the serovar *S.* Enteritidis from west Europe and USA), and the subsequent mean salmonella morbidity in the years 1990–2000 was 43,450 cases annually. While the incidence rate of salmonellosis per 100,000 population in Czechland was 385 in 1997, the average for Europe in the same year was 20–130, but it could be biased by underreporting (or no obligatory reporting) of

the disease in some European countries. An instructive outbreak of salmonellosis occurred in Minnesota in 1994 with 224,000 patients (the largest salmonellosis outbreak in the U.S.A.): infective vehiculum was ice cream, and the mechanism was contamination with *S.* Enteritidis of a tank transporting egg melange used for preparation of that ice cream. The economical costs of salmonellosis can be high: it has been calculated that diagnosis and therapy of one human case of this disease in EU countries will cost on average 1,100€. [Remark: typhoid fever (*typhus abdominalis*) and paratyphoid fever, caused by *S.* Typhi and *S.* Paratyphi, respectively, are anthroponoses characteristic by a subsequent chronic asymptomatic carriership in 1–10% of patients].

Bio-containment: BSL-2.

Diagnosis: cultivation of faecal samples in semiselective enrichment fluid media, e.g. tetrathionate or selenite enrichment broth, with a subsequent inoculation on selective solid agar media with deoxycholate, brilliant green etc. (MAL, XLD, SS, Rambach, Wilson-Blair and others); serotypization and phagotypization (PT), PCR (RFLP).

Treatment: rehydration (antibiotics prolong the carriership stage; they are used only in necessary cases and after sensitivity testing, especially in toddlers); Endiaron N.

Geographical distribution: worldwide.

Escherichia coli – Enteropathogenic and Enterohaemorrhagic Strains

Straight rods 1–6×0.3–1.0 µm with flagella (peritricha), fermenting lactose, with a number of serovars (160 antigenic types O, 56 types H, 80 types K/Vi). The strains pathogenic for man are labeled as *E. coli* enteropathogenic (EPEC), enterohaemorrhagic (EHEC), enterotoxigenic (ETEC), enteroinvasive (EIEC) or enteroaggregative (EAEC); usually formed toxins include verotoxin (VTEC: e.g. in O157) or thermostable "shiga-like toxin" Stx 1 and Stx 2 (STEC); the most frequent antigenic types of these bacteria are O157:H7, further O111 and O26.

Source of infection (natural host range): cattle (reservoir, about 1% of the animals has EHEC in faeces); less often sheep, goat, pig, domestic dog and cat (pet contact), game animals (deer); VTEC isolates O157 also found in fowl. Seagulls can be competent hosts of multi-drug resistant *E. coli*. In addition, asymptomatic human carriers of some of these pathogenic strains have been revealed.

Animal disease: inapparent course or colibacillosis – diarrhoea in young domestic mammals (calf, lamb, piglet) and fowl.

Transmission mode: alimentary – contaminated hamburgers and other meat, contaminated vegetables (lettuce, spinach, salads from biofarms, vegetable sprouts) and fruit, contaminated water or milk; MID very low, only 70 cells during an epidemic in USA 1992/1993; by contact. EHEC strains tolerate

even very acid conditions (pH 2), temperature of 75°C, and have marked tenacity in excreta (they survive >100 days). Infectious disease in man by EHEC strains from domestic animals was firstly reliably documented as a zoonosis in 1967. Mechanical transmission of *E. coli* O:157 by flies was also observed.

Human disease: <u>colibacillosis</u> – severe bloody diarrhoea (<u>haemorrhagic colitis</u> in VTEC), in 5–15% of patients (STEC, VTEC), the infection sometimes progresses to renal failure (<u>haemolytic- uraemic syndrome</u>, HUS: most often with strains O157, O26, O111, O103 and O145, causing anaemia, thrombocytopenia, renal insufficiency to anuria brain oedema, and fatality rate of 3–5%). In USA, EHEC has been observed since 1982, but the first patient was described already in 1975; an epidemic with >500 cases (4 children died) occurred in 1992/1993. In the years 1982–1998, 4,384 cases were recorded in USA. Annual incidence rate of EHEC in USA approximates 1–6 per 100,000 population (the average is 2, and 250 patients die); in Czechland is the mean 12 per 100,000, and about 100 patients are infected with O157 annually. The number of VTEC cases in the European Union was 2,905 in 2007. A major outbreak of EHEC occurred in Toronto area in Canada in 2000 when >2000 people got infection (and 6 children died) – the infection vehiculum was a contaminated water from a farm.

Bio-containment: BSL-2.

Diagnosis: cultivation of faecal samples (differentiating MacConkey agar with sorbitol, so-called SMAC, or even more selective Rainbow O157 agar), with a subsequent typization and toxigenicity testing.

Treatment: rehydration, exceptionally antibiotics (ampicillin, cephalosporins).

Geographical distribution: worldwide.

Yersinia enterocolitica

Slowly motile (but non-motile at 37°C) ovoid bacterium 1–2×0.8 μm with bipolar staining; 34 O-antigens and 19 H-antigens, a great number of serovars.

Source of infection (natural host range): pig, dog, cat (these species are reservoir of the serovar O3), leporids, rodents (*Rattus*, *Ondatra*, *Castor*), wild mammals, bats, birds, reptiles, frogs, molluscs, fish.

Animal disease: colitis in hares and pigs, in young animals sometimes septicaemia.

Transmission mode: alimentary (replicates in meat and in tins at temperature of 4°C – contrary to salmonellae and campylobacters; milk, water); blood transfusion (at least 17 fatalities have been described).

Human disease: <u>yersiniosis</u> – peracute gastroenteritis (main serovar O3, less often O8 [USA] and O9) usually in winter period; pseudoappendicitis in >10% cases, fever, sometimes a subsequent polyarthritis, Reiter syndrome, erythema; rarely septicaemia (1%). For instance, an outbreak due to contaminated pork brawn occurred in Norway, 2005.

Bio-containment: BSL-2.

Diagnosis: cultivation (faeces, pus from lymph nodes: selective media – e.g. CIN), serotypization, serology (AR, ELISA: serovar O9 of *Y. enterocolitica* cross-reacts with brucellae).

Treatment: in septical forms tetracycline, cotrimoxazole, doxycycline, chloramphenicol, streptomycin (but not penicillin).

Geographical distribution: largely holarctic, especially in the colder climatic zone (Scandinavia); incidence in Europe is moderately increasing.

Yersinia pseudotuberculosis

Slowly motile (at 22–25°C, but not at 37°C) ovoid to short rod $0.6–1.2 \times 0.4–0.8\,\mu m$ with bipolar staining; 10 serovars.

Source of infection (natural host range): cat (carrier host), rodents (reservoir), leporids, dog, beaver (Canada), ruminants, bats, birds; also soil, water (it can replicate in water at 18–20°C), foods, vegetables (*Y. pseudotuberculosis* replicates in vegetables at 4°C).

Animal disease: pseudotuberculosis (necroses and granulomas on liver, spleen, lymphatic nodes), especially in rabbit, sheep, birds; cattle, horse, dog.

Transmission mode: alimentary, less often direct contact, via conjunctivae and aerogenic; the agent has considerable tenacity in external milieu.

Human disease: pseudotuberculosis – fever, purpura, acute gastroenteritis, abdominal pain, mesenteric lymphadenitis, hepatosplenomegaly and abscesses in liver, sometimes pseudoappendicitis, bronchopneumonia; occasionally severe invasive septical illness with a considerable mortality.

Bio-containment: BSL-2.

Diagnosis: cultivation of the blood and pus from lymph nodes (at 22–25°C), serology (AR), intradermal test.

Treatment: only in severe forms – ampicillin, tetracycline, streptomycin.

Geographical distribution: worldwide.

Yersinia pestis

Short Gram-negative rods $1.5 \times 0.5–0.7\,\mu m$ with bipolar staining, pleomorphic (occasionally thread-like, large spherical and other involution forms), non-motile, producing a capsule. A facultative intracellular parasite. The whole genome of *Y. pestis* was sequenced in 2001: it contains 4.7 million nucleotides and 3 plasmids sized 96, 70, and 10 kbp. According to phylogenetic analyses *Y. pestis* originated from *Y. pseudotuberculosis* roughly 10,000 years ago as a clone capable of transmission by fleas and lost about 13% of the original genes. The biovars of *Y. pestis* Antiqua, Medievalis, Orientalis, and Microtus differ in the ability of nitrate reduction, and glycerol and arabinose fermentation.

Source of infection (natural host range): rodents (reservoir) – in the natural cycle gerbils and jirds (*Rhombomys opimus* in central Asia; *Meriones libycus* in north Africa and Arabia; *Tatera* spp.), marmots in high mountain steppes of Asia and North America (reservoir – *Marmota sibirica* in Mongolia, *M. himalayana* in China), pikas *Ochotona pallasi* and *O. daurica* in Mongolia, chipmunks *Tamias* spp. in California, ground squirrels (*Spermophilus* spp., e.g. *S. beecheyi* in California, *S. undulatus* and *S. erythrogenys* in Mongolia) and prairie dogs (*Cynomys* in USA); in the secondary urban cycle rats (*Bandicota*, *Mastomys*, *Rattus*, *Arvicanthis*); *Y. pestis* is able to survive for long periods in the soil of the rodent burrows (for at least 1 month) and in carrions of mammals (including man). In North America the source of human infection is occasionally cat that hunted infected rodents in a natural focus.

Animal disease: sometimes latent; but more often a general illness, e.g. in brown rats and other rodents (MID in them is about 10 cells), cats (may be severely affected), goats and camels.

Transmission mode (Fig. 8.21): fleas of the genera *Xenopsylla* (especially *X. cheopsis*, that after death of its rat host easily moves to man as an alternative host; also *X. brasiliensis*), *Oropsylla* (*O. montana* in California, the marmot flea *O. silantiewi* in Mongolia), *Monopsyllus*, *Nosopsyllus* (China), possibly also the human flea *Pulex irritans* (Tanzania). Bacteraemia in mammalian donors of agent should be at least 10^8 cells/ml blood. The imbibed plague bacilli replicate and form a "block" in the flea's *proventriculus* that
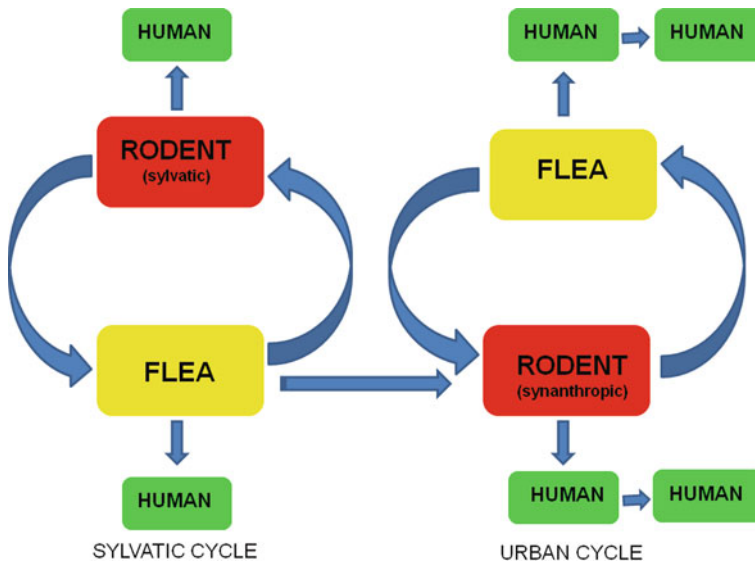
Fig. 8.21 The cycles of *Yersinia pestis* (drawing by Ivo Rudolf)

limits the ability of the flea of further blood feeding (Fig. 6.14), and leads to regurgitation. TOT has not been demonstrated in fleas. Other means of plague transmission are aerogenic (person-to-person, droplets during cough) which results in the most feared epidemics, and alimentary – e.g. 18 plague cases were described after consumption of uncooked liver of infected camels in Libya and Saudi Arabia. The plague sometimes occurs as an occupational infectious disease – hunters of marmots and other rodents: e.g. 19 marmot hunters (handling infected animals or their carcasses) got plague in north China in 2004, and 8 died. An alternative (but speculative) mode for *Y. pestis* transmission during outbreaks might be via the body lice *Pediculus humanus*.

Types of disease foci: (1) primary, natural, sylvatic (the hosts of the agent are exoanthropic rodents) in steppe, semidesert or tropical forest ecosystems; (2) secondary, urban – largely in sea ports (the main hosts of the agent are rats – "urban" plague).

Human disease: plague (*pestis*) starting with fever and fatigue. The clinical forms are bubonic (bubonic plague, with lymphadenitis: swollen and extremely painful lymph nodes called *buboes*), pulmonary (severe bronchopneumonia and respiratory distress syndrome – usually in the anthroponotic infection), oroglandular (after an alimentary infection, associated with abdominal pain and diarrhoea), and primarily septic (without lymphadenitis, but with meningitis, endotoxic shock, organ failure and a disseminated intravascular coagulation); occasional severe peripheral tissue necrosis and gangrene remind of medieval epithet ("Black Death"). Mean fatality rate is 2–10% in treated and 50–95% in untreated cases (the most lethal are septic and pulmonary forms). Historically, the first major epidemic of plague was reported in China in 224 BC. Later on, three pandemics of plague were recorded: (1) "Justinian's plague" in the sixth century (with roughly 100 million victims out of the 142 million infected); (2) "black death" in the fourteenth century killed nearly whole populations of Tatars and Saracens, then the disease encompassed nearly whole Europe where died one-fourth of the population (about 25 millions), and further roughly 25 millions died before in Asia and Africa; (3) "modern" plague in the nineteenth and twentieth centuries (30 million people got the disease, 12 millions of them died; the pandemic started in Hongkong after introduction from the continental China; rats and their fleas dispersed plague on ships to many seaports of Japan, India, Europe, Africa, America and Australia). According to new molecular data, probably only the biovar Orientalis of *Y. pestis* participated in all three pandemics. The last outbreak of pulmonary plague was recorded in Los Angeles in 1924, with 32 cases (30 were fatal). In India, there were 52 fatal cases of plague in 1994. Another outbreak with 18 cases (the bubonic form) occurred in Oran (Algeria) in 2003 (here after more than 50 years). Sporadic cases of plague still occur in a limited number of natural foci in the world. For instance, on average 10 human cases are reported annually from western USA. However according to WHO, there are 1,000–2,500 cases of plague reported each year worldwide; for instance, a total incidence of plague in

the years 1987–2001 was 36,876 persons, and 2,847 (7.7%) died. Recent outbreak of plague in Peru (2010) involved 31 cases including 3 deaths (the last outbreak of bubonic plague in northern Peru before was in 1994, which affected more than 1,100 persons and killed 35 of them).

Bio-containment: BSL-3.

Diagnosis: microscopy (methylene blue) and cultivation (BA, agar with gentianviolet at 27°C) of the pus from enlarged lymph nodes, blood, CSF, bone marrow or sputum samples, subcutaneous inoculation of guinea pig, laboratory rat or mouse; serology (IFA, CFT, RIHA).

Treatment: streptomycin, tetracycline, doxycycline, chloramphenicol, gentamicin.

Prevention: vaccine (inactivated or attenuated – strain EV76 produced in the former Soviet Union, or heat-killed *Y. pestis* vaccine manufactured in Australia) produces a short-term protection; rat control in endemic areas.

Geographical distribution: natural foci of plague occur in parts of Asia (India – Photo 5.39, Burma, Iraq, central Asia, Mongolia – Photo 5.40, northern China), Africa (including Madagascar – here recently 45% of all African cases), South America and western North America (USA: Oregon, California, Nevada, Arizona, Colorado, Utah, New Mexico, and Texas).

8.3.13 *Family* Pasteurellaceae [*Order* Pasteurellales*, Class* Gammaproteobacteria]

Pasteurella multocida, *Mannheimia haemolytica*

Short Gram-negative rods to coccobacilli 0.5–1.2×0.3–0.6 μm with a marked bipolar staining, facultatively anaerobic, non-motile, forming capsula; serovars A, B, D, E in *P. multocida*.

Source of infection (natural host range): serovar A birds, serovars B and D domestic (cattle, sheep, pig, dog, cat, rabbit) and wild (rodents, hare) mammals.

Animal disease: pasteurellosis, called avian cholera in fowl – bronchopneumonia, haemorrhagic septicaemia. Asymptomatic in cats and dogs.

Transmission mode: contact (percutaneous: biting or scratching by a cat, less often a dog; insect bite or sting), sporadically alimentary or aerogenically.

Human disease: pasteurellosis (infrequent illness) – local inflammation, abscesses, lymphadenitis; respiratory illness; sometimes meningitis; rarely appendicitis.

Bio-containment: BSL-2.

Diagnosis: cultivation of pus or tissue samples (BA; does not grow on Endo agar), microscopy of the blood or tissue smears (methylene blue: bipolar staining; IF microscopy), inoculation of mouse or rabbit.

Treatment: penicillin (higher doses); ceftriaxone, ciprofloxacin, amoxicilin, doxycycline, tetracycline, chloramphenicol, erythromycin, cotrimoxazole, cefuroxim.
Geographical distribution: worldwide, sporadic.

8.3.14 *Family* Vibrionaceae [*Order* Vibrionales*, Class* Gammaproteobacteria]

(*) *Vibrio cholerae*

Short motile (monotricha) slightly crescent-shaped Gram-negative rods; facultatively anaerobic; one H antigen, but 80 somatic O-antigens. The serogroups pathogenic for man are O1 (with two biotypes: classical, and haemolytic "El Tor"; and with two main serotypes – Inaba and Ogawa), and O139 (Bengal); infrequently certain others called "non-O1/O139 *V. cholerae*".

Source of infection: man; brackish water and marine animals (crustaceans – mainly *Copepoda*, shellfish including raw oysters, and fish) in endemic areas.
Transmission mode: alimentary (municipal or surface water, food); *V. cholerae* non-O1 quite often after consumption of raw "seafood" or street vendors' beverage. The agent survives in water for up to several weeks.
Human disease: <u>cholera</u> – sudden vomiting without nausea, intense longterm watery diarrhoea (excrements look like rice water and have fish odour) causing rapid dehydratation and hypovolemic shock, anuria, muscle cramps; death can occur in severe cases sometimes within 1 or 2 days. The cholera enterotoxin stimulates hyperproduction of cAMP that inhibits in the cells of intestinal villi influx of ions Na+ and Cl–and at the same time stimulates hypersecretion of Cl–and HCO_3– causing a blockade of intestinal water influx. *V. cholerae* non-O1 causes less severe but sometimes bloody diarrhoea and abdominal spasms. A total of 10 explosive pandemics of cholera occurred in the nineteenth and twentieth century, and most of them were initiated in the Indian subcontinent. For instance the first known pandemic of cholera (1817) started in the Gangus river valley, and spread up to southern Russia. A new serotype O139 Bengal caused first outbreak in Bangladesh in 1992, and reemerged there in 2002 (about 30,000 cases). The risk of cholera always increases during great humanitarian disasters, civil wars, in refugee camps. [Extensive anthroponotic epidemics in Zimbabwe and Tanzania occurred in 2008–2009].
Bio-containment: BSL-2.
Diagnosis: microscopy (darkfield) and cultivation of faecal samples (enrichment in alkalic peptone water and a subsequent isolation on agar medium TCBS with thiosulphate, citrate, deoxycholate and saccharose), combined with agglutination of the grown colonies.

Prevention: inactivated oral vaccines (DUKORAL, OROCHOL); the use of packaged water in endemic areas, appropriate hygiene practices.

Treatment: intense rehydration and influx of ions; antibiotics (gentamicin, chloramphenicol, tetracycline).

Geographical distribution: tropics and subtropics. Endemic area is, e.g., Bangladesh.

Vibrio parahaemolyticus

A halophilic marine bacterium, pathogen of fish. Pandemic strain (spreading since 1996) posses antigenic structure O3:K6.

Source of infection: marine coastal water (especially at higher temperatures, often coincidence with algal overpopulation), marine fish, crabs and oysters.

Transmission mode: alimentary ("seafood-borne disease": oysters etc.). In addition, asymptomatic human carriers of these strains have been revealed.

Human disease: vibrio gastroenteritis – an about 3-day, often bloody, diarrhoea, abdominal pains, nausea, vomiting. In Japan the agent causes about 50% of all patients with bacterial gastroenteritis. Sometimes epidemic occurrence. For instance, during a recent outbreak in Chile (2004–2007), the total number of reported cases was 10,783 (as of March 2005), making this the largest documented occurrence of *V. parahaemolyticus* diarrhoea in the world. The bacterium also causes ear and wound infections.

Bio-containment: BSL-2.

Diagnosis: cultivation of stools and food remnants (TCBS agar with 3% NaCl); serotypization, Kanagawa's test for detection of thermostable haemolysin.

Treatment: rehydration.

Geographical distribution: worldwide – coastal areas, very often in Japan and Singapore, but also other parts of southeast Asia, further India, Spain, USA and South America.

Vibrio vulnificus

A halophilic bacterium, serious pathogen of eel.

Source of infection: coastal water, marine fish, crabs and oysters (a halophilic bacterium).

Transmission mode: contact (skin injuries, especially with fish bones), alimentary (e.g., raw oysters, fish).

Human disease: skin wound lesions with necrotizing fasciitis, primary septicaemia sometimes accompanied with a septic shock (fatality rate 30–50%); or acute diarrhoea (fatality rate low, but in the biotype 3 up to 10%). Usually sporadic cases, sometimes epidemics (e.g. in Israel, 1996/1997: 134 cases in fishers and fish consumers, biotype 3). In the Los Angeles area (California), a

total 252 cases were reported between 1980 and 2004, and in Japan 94 cases between 1999 and 2003.

Bio-containment: BSL-2.

Diagnosis: cultivation (TCBS agar, with 3% NaCl).

Treatment: rehydration in intestinal form of the disease, antibiotics in severe cases (cephotaxin + minocycline); surgery (amputation occasionally necessary in fasciitis cases).

Geographical distribution: Mexico, southern USA, India, southeastern Asia (Taiwan), Japan, Israel, Baltic Sea, Spain.

Vibrio metschnikovii

Source of infection: coastal marine or brackish water, fish, oysters (a halophilic microorganism).

Animal disease: pathogenic for birds, illness in birds similar to cholera.

Transmission mode: contact, alimentary.

Human disease: diarrhoea, pneumonia, infection of wounds etc. – low incidence.

Bio-containment: BSL-2.

Geographical distribution: southern USA, Southeast Asia, Germany.

Other "Non-cholera" Vibrios

Pathogenic for man (causing wound infections or gastroenteritis) but with a lower risk, they involve *Vibrio mimicus, V. fluvialis, V. furnissii, V. alginolyticus, Grimontia hollisae*, and *Photobacterium damselae.*

8.3.15 *Family* Aeromonadaceae [*Order* Aeromonadales]

Aeromonas hydrophila, A. caviae

Gramnegative rods to coccobacilli, facultatively anaerobic, usually motile (with one terminal flagellum). Some human isolates posses Shiga toxin genes (*stx1*, *stx2*) similar to those in pathogenic *Escherichia coli* strains.

Animal disease: pathogenic for frogs and fish.

Source of infection: water.

Transmission mode: contact, alimentary (e.g., fish). Sometimes associated with disasters (hurricanes, tsunami, earthquakes).

Human disease: gastroenteritis, bacteraemia, prostatitis, HUS. Usually sporadic cases.

Bio-containment: BSL-2.

Diagnosis: cultivation and biochemical tests.

Treatment: antibiotics in severe cases.

Geographical distribution: worldwide.

8.3.16 *Family* Campylobacteraceae [*Order* Campylobacterales, *Class* Epsilonproteobacteria]

Campylobacter jejuni (*C. coli*, *C. laridis*, *C. foetus*)

Mildly bended, S-shaped to shortly screw-like Gram-negative rods 0.5–1.2×0.2–0.5 µm with two polar flagella (*amphitricha*), aerobic to microaerophilic, thermophilic (optimum growth temperature 42°C, do not grow at <30°C); 65 serovars differentiated according to O and H antigens.

Source of infection (natural host range): domestic fowl (>50% human cases; in broilers commonly up to 10^7 cells/g) and other birds (reservoir), mammals (cattle, sheep, pig – *C. coli*, dog, cat); man (about 1% asymptomatic carriers); oysters, crabs; surface water (contaminated by birds).

Animal disease: usually asymptomatic, or diarrhoea (calves); fowl hepatitis. *C. foetus* causes septic abortions and fertility disturbances in sheep and cows.

Transmission mode: alimentary – e.g. grilled fowl (especially chicken broilers), hamburgers ("fast-food"), processing raw meat, drinking raw milk, eating fresh cheese, water (campylobacters survive in it for >4 months/5°C); contact. Mechanical transmission by flies has been repeatedly recorded. MID for man is 500–800 cells.

Human disease: campylobacterosis (commonly used term "campylobacteriosis" is imprecise because the agent is not a "*Campylobacterium*", but *Campylobacter*), together with salmonellosis probably the most frequent zoonosis and a major health problem worldwide. After a short incubation period (usually 3–5 days) appears fever (with chills), headache, myalgia, anorexia, nausea, acute enteritis (abdominal pains are stronger than in salmonellosis), prolonged diarrhoea (sometimes bloody) for up to 10 days, pseudoappendicitis, arthritis; sometimes meningoencephalitis (*C. foetus*). The arthritic and neurologic sequelae of infection with *C. jejuni* involve in <1% of patients Guillain-Barré syndrome (demyelinizing peripheral neuropathy with acute neuromuscular paralysis: symmetrical flaccid effect on limbs and loss of tendon reflexes; up to 30% cases of this polyradiculoneuritis syndrome is attributed to *C. jejuni*) or Reiter syndrome (reactive arthropathy); mortality is rare. Campylobacterosis, in contrast to salmonellosis, occurs more often sporadically than in epidemics. Incidence rate was 20–110 per 100,000 population in Europe in 1996–1999, while 7–32 in the USA (that of salmonellosis was 13–26 in the same period). In Great Britain the annual incidence of campylobacterosis attained 50,000 cases in the years 1997–1999 and surpassed that of salmonellosis (51,800 cases in 2007). A similar trend was observed in Czechland (EPIDAT Report on incidence of infectious diseases): while the mean annual incidence was 8,630 cases in the decade 1990–2000, as many as 23,480 cases (annual average; the range, 20–30 thousands) were reported in the period 2001–2008, i.e. not much

less than in salmonellosis in the same period (annual average 25,800, the range 11,000–33,000 cases). However, this obviously increasing trend could be biased by a much better and more widespread laboratory diagnosis of campylobacterosis.

Bio-containment: BSL-2.

Diagnosis: microscopy of native stool samples in darkfield, cultivation of faeces (sampled in a special transport medium), blood or CSF on selective Preston agar with charcoal and cefoperazon or on other special agar media (Karmali; Butzler) at 42°C in 5–10% CO_2 and 85% N_2; serology (ELISA, CFT), PCR.

Treatment: rehydration; antibiotics should be used only in severe cases (erythromycin, tetracycline, azithromycin, clarithromycin, ciprofloxacin). Campylobacters are usually resistant to quinolones (these drugs used to be occasionally added to chicken food).

Geographical distribution: worldwide.

8.3.17 *Family* Helicobacteraceae [*Order* Campylobacterales]

Helicobacter bizzozeronii, H. felis

Small, mildly bended and motile Gram-negative rods, microaerophilic. In the past, helicobacters were classified in the genus *Campylobacter*, and the two mentioned species formed a group *Helicobacter* "heilmannii", observed in animals and man. [Anthroponotic species is *H. pylori*, causing gastritis and peptic ulceration].

Source of infection (natural host range): dog (*H. bizzozeronii*), cat (*H. felis*), pig.

Animal disease: probably usually asymptomatic.

Transmission mode: alimentary or contact.

Human disease: gastritis, severe dyspeptic symptoms. Only few cases have been described (e.g., one in Finland recently).

Bio-containment: BSL-2.

Diagnosis: microscopy of native stool specimens in darkfield, cultivation from faeces (very difficult).

Treatment: unknown (metronidazole unsuccessful).

Geographical distribution: Europe, possibly worldwide.

8.3.18 *Family* Leptospiraceae [*Order* Spirochaetales*, Class* Spirochaetes]

In addition to saprophytic leptospirae (e.g., *L. biflexa*, *L. parva*) living in water, 13 spp. are regarded as pathogenic, with more than 260 serovars at present. However, the most important is one species – *L. interrogans*.

Leptospira interrogans

A very motile obligately aerobic spirochaete 6–20×0.1–0.2 μm with many dense coils, two periplasmic flagella and usually hooked ends. Twenty-three serogroups with more than 200 serovars have been described in this species.

Source of infection (reservoirs) with particular serovars: brown rat (Icterohaemorrhagiae), voles and other rodents (Grippotyphosa, e.g., *Microtus agrestis*, *M. arvalis*: persistent infection in these species, the agent lives in renal tubules and is excreted in urine that contains up to 106 leptospi-rae per ml) and Sejroe (largely *Mus musculus*); pig (Pomona – also *Apodemus agrarius,* and Australis), cattle (Tarassovi, Hardjoe), dog (Canicola), hedge-hog and horse (Bratislava). Leptospirae are very resistant in water milieu; they can also be transported by waterbirds (from and to rice fields etc.).

Animal disease: inapparent, or (cattle, pig) abortions, meningitis and dying of some young animals.

Transmission mode: contact (percutaneous; bathing, water sports, work in water or in sewers), alimentary (water or food contaminated with rodent urine, e.g. after floods; uncooked meat of infected animals).

Human disease: leptospirosis is one of the most widespread zoonoses in the world – a flu-like illness, often with biphasic body temperature curve, aseptic meningitis and nephritis (albuminuria). The course of the disease varies according to serovar; the most severe is Weil's disease (*L. interro-gans* Icterohaemorrhagiae) with hepatitis, icterus, myalgia, necroses, cardiac and ocular involvement, nephritis (damage to glomerulae; leptospiruria), haemorrhagies, encephalitis, and fatality rate in untreated cases 10–20%; milder but most frequent is the "harvest fever" in central Europe (caused by *L. interrogans* Grippotyphosa); also other anicteric leptospiroses usu-ally have milder symptoms, e.g. "fever of swine breeders" (*L. interrogans* Pomona). The incidence of leptospirosis is not very high in most countries; for instance in Czechland, on the average 82 (the range, 12–226) cases were reported annually in the years 1970–1999, while 38 (the range, 11–88) in the decade 1990–2000 (0.4 cases per 100,000 population); in France, an average of about 250 cases annually have been reported since 1980 (0.5 cases/100,000). Occupational disease: up to 30% of cases form butchers in slaughterhouses, farmers, meat inspectors, workers in sewerage system, miners, soldiers, gamekeepers, hunters, rodent control workers, sugar cane cutters, ricefield workers, animal attendants in zoological gardens and work-ers in sewerage system; leptospirosis also often occurs as a "recreational" (leisure time) infectious disease (about 30% of all cases) – during outdoor activities like water sports (canoeing, swimming), or camping. Epidemics also follow natural disasters, such as floods, hurricanes or earthquakes. For instance, 167 Philippinos died from leptospirosis after storm Ondoy flooded Manila and Rizal in 2009.

Bio-containment: BSL-2.

Diagnosis: microscopy (darkfield or phase contrast) and cultivation (EMJH [Ellinghausen-McCullough-Johnson-Harris] medium, Korthoff's fluid peptone medium supplemented with rabbit serum or bovine albumin and Tween 80, or Fletcher, Noguchi and Stuart liquid media; however, the growth of leptospirae is slow) of the blood (acute stadium) or urine (convalescence) samples; intraperitoneal inoculation of young guinea pig or hamster; serology (CFT, slide AR, IHA, ELISA; the most specific is the micro-agglutination-lysis test, when a viable culture is mixed with antiserum, and then observed under darkfield microscope); RFLP, ribotyping, PFGE.

Treatment: penicillin, ampicillin, doxycycline, tetracycline, streptomycin, erythromycin, ceftriaxone, cefotaxime, ciprofloxacin; haemodialysis is sometimes necessary during renal insufficiency.

Prevention: vaccination of domestic animals (cattle, pigs, dogs) is possible with serovar-specific vaccines. Vaccines for humans are produced in France (serovar Icterohaemorrhagiae), China and Japan (serovars Canicola, Icterohaemorrhagiae and Pomona).

Geographical distribution: worldwide (mainly humid ecosystems: Photo 5.38).

8.3.19 *Family* Spirochaetaceae [*Order* Spirochaetales]

**Borrelia recurrentis*

A spirochaete up to 30 μm long, genomically very closely related to *B. duttonii* as found by DNA sequencing and molecular phylogenetic analyses. In fact, it should be regarded as a population subset or an ecotype of *B. duttonii* rather than a separate species.

Source of infection (natural host range): man.

Transmission mode: body louse (*Pediculus humanus*; the lice, when infected, remain infectious longlife) – by swating and rubbing in skin (or conjunctiva).

Human disease: epidemic louse-borne relapsing fever (*typhus recurrens*) – high fever for 3–9 days with 1–5 relapses (they are associated with the change of surface antigens of the spirochaetes and their reappearance in the blood; afebrile intervals use to be 5–10 days), chills, severe headaches, myalgia, arthralgia, abdominal pain, anorexia, hepatosplenomegaly, sometimes icterus, CNS affection (photophobia, dizziness); fatality rate in untreated cases up to 40%, in treated patients 4–7%. The spirochaetes in the human blood first observed and described Obermaier in 1867. Epidemic of recurrent typhus (on many occasions together with rickettsial epidemic typhus) are determined by socioeconomic factors, they occur during wars, famine and big disasters, usually in winter and early spring. Examples: in 1919–1923, epidemics in southern Europe (the Balkans) and Russia (5 million victims); recent cases and outbreaks have been reported in Ethiopia (1,000–5,000 cases annually), Sudan, Burundi, Rwanda, Uganda, China, Peru, and Russia.

Bio-containment: BSL-2.

Diagnosis: darkfield microscopy of the blood (in the acute phase patients have up to 106 spirochaetes/ml blood), blood smear or thick drop (stained by Giemsa, IF); inoculation on suckling mice or chick embryo; cultivation *in vitro* is difficult (fluid BSK medium); serology (Weil-Felix reaction with antigen *Proteus* OXK).

Treatment: tetracycline, erythromycin, doxycycline, penicillin.

Geographical distribution: Africa (Ethiopia, Rwanda, Senegal), Asia Minor and Central Asia, foothills of the Andes.

Borrelia duttonii, B. hispanica, B. crocidurae, B. persica, B. caucasica, B. latyschevi, B. hermsii, B. parkeri, B. turicatae, B. theileri, B. coriaceae, B. venezuelensis, B. mazzottii, B. miyamotoi

A large group of relapsing fever zoonotic borreliae. *B. duttonii* is phylogenetically closely related to *B. recurrentis* (even more than to *B. hispanica*) and *B. crocidurae*.

Source of infection (natural host range): rodents (mice, hamsters, gerbils), porcupine, hedgehogs, canids and other wild mammals (armadillo, opossum, bats), and some reptiles (agama); man (*B. duttonii*).

Animal disease: usually inapparent course (arthritis in dogs).

Transmission mode: argasid ticks of the genus *Ornithodoros* (reservoir – TOT), living in burrows of medium-sized rodents and other mammals, in small caves, crevices of cottages and buildings: *O. moubata* (*B. duttonii* in eastern Africa), *O. marocanus* (*B. hispanica*), *O. sonrai* (*B. crocidurae* in western Africa), *O. erraticus* (*B. crocidurae, B. hispanica*), *O. tholozani* and *O. papilidog* (*B. persica*), *O. verrucosus* (*B. caucasica*), *O. hermsi* (*B. hermsii*), *O. turicata* (*B. turicatae*), *O. rudis* (*B. venezuelensis*), *O. parkeri* (*B. parkeri*), *O. coriaceus* (*B. coriaceae*), *O. rudis* (*B. venezuelensis*), *O. tartakovskyi* (*B. latyschevi*), *O. talaje* (*B. mazzottii*) – by blood feeding, also via coxal fluid of the soft ticks. Sometimes transmit the borreliae also ixodid ticks: *Boophilus microplus* (*B. theileri*), *Ixodes persulcatus, I. ricinus* and *I. scapularis* (*B. miyamotoi*).

Natural cycle: mammal → argasid → mammal.

Human disease: endemic tick-borne relapsing fever (endemic *recurrens*) – papula, rash (in half of patients), recurrent fever with chills (2–10 relapses going on for 2–7 days as a reaction to the antigenic "shift" of the agent, afebrile periods last 2–5 days or up to several weeks), hyperhidrosis, headaches, myalgia, arthralgia, abdominal pain, cough, conjunctivitis, lymphadenopathy, miscarriage, in a part of patients also affection of CNS, myocarditis, hepatosplenomegaly; fatality rate 2–10%.

Bio-containment: BSL-2.

Diagnosis: darkfield microscopy of the blood smear or a thick drop from the febrile phase (Giemsa or Wright staining, IF); intranasal (or conjunctival)

inoculation of guinea pig or intraperitoneal inoculation of suckling mouse; serology (CFT), detection of the antigen (immunohistochemistry), cultivation (BSK medium).

Treatment: penicillin, tetracycline, erythromycin, doxycycline, chloramphenicol.

Geographical distribution: subtropics and tropics (steppe to semidesert habitats) – the Mediterranean (Pyrenean Peninsula, Greece), North Africa (*B. hispanica* is frequent in Morocco), tropical east Africa (Tanzania etc.), western Africa (*B. crocidurae* – Senegal), the Caucasus, the Near East, Central Asia (*B. latyschevi* – Tadjikistan, Uzbekistan), India, China, Japan, America (*B. parkeri* – Colorado, *B. hermsii*, *B. turicatae*, *B. coriceae*), Mexico (*B. mazzottii*).

Borrelia lonestari

A spirochaete belonging to the group of relapsing fever zoonotic borreliae.

Source of infection (natural host range): rodents.

Animal disease: not observed.

Transmission mode: bites of the Lone Star tick *Amblyomma americanum*.

Human disease: **S**outhern **t**ick **a**ssociated **r**ash **i**llness (STARI) – similar to erythema migrans in Lyme borreliosis (see below).

Bio-containment: BSL-2.

Diagnosis: similar as in the previous borreliae, but cultivation *in vitro* is difficult.

Treatment: antibiotics.

Geographical distribution: southern USA.

Borrelia burgdorferi **s.l.**

Spirochaetes 4–25×0.2–0.3 µm with 3–10 coils and 7–11 periplasmic flagella. Genomic species ("genomovars", called often "genospecies" in discrepancy with standard bacteriological nomenclature) of *B. burgdorferi* s.l. (described as late as 1982), pathogenic for man, are *B. burdorferi* s.s. (its full genome was already sequenced), *B. afzelii* and *B. garinii*, less often *B. valaisiana*, *B. lusitaniae*, while genomic species with not yet sufficiently demonstrated pathogenicity are *B. bissetii*, *B. spielmanii* and a few others. In the host, some borreliae are able to produce persisting 'cystic forms' or 'blebs'.

Source of infection (natural host range): forest rodents (in Eurasia *Apodemus* and *Myodes* spp. – competent hosts and the reservoir especially for *B. afzelii*; in America *Peromyscus leucopus*), squirrels, chipmunks (e.g., *Tamias striatus*), leporids, turdid and other ground-foraging forest birds (the reservoir for *B. garinii*); also lizards (*B. lusitaniae*).

Animal disease (dog, ruminants, horse): borreliosis with arthritis, affection of the kidneys and CNS.

Transmission mode: ixodid ticks of the *Ixodes ricinus* complex (reservoir – TOT, TST): *I. ricinus*, *I. persulcatus*, *I. pavlovskyi*, *I. scapularis*, *I. pacificus*, also *I. uriae* (*B. garinii,* in seabird colonies: Photos 5.37, 7.77).

Human disease: Lyme borreliosis (LB, or Lyme disease in America, described in Old Lyme, Connecticut, in 1975) – a multisystem illness with 3 clinical phases (the course of untreated LB can remind that of the syphilis): (1) in 60–90% of infected persons appears a localized erythema (*erythema migrans*, EM) that was already described by Afzelius (1910) and Lipschütz (1913); (2) when not treated with antibiotics, the spirochaetes disseminate and secondary lesions may appear such as new EM lesions or lymphocytoma (*lymphadenosis benigna cutis*), sometimes increased temperature, headaches and myalgia, fatigue, regional lymphadenopathy, conjunctivitis, affection of CNS (meningitis), polyneuritis (*n. facialis* – Bell's palsy, radiculoneuritis), arthralgia and carditis; (3) the late phase (usually after 2–3 years) is manifested by chronic relapsing polyarthritis affecting big joints (synovitis, erosion of cartilage), chronic neuroborreliosis (peripheral neuropathies, affection of CNS), chronic affection of the skin (*acrodermatitis chronica atrophicans*, ACA: described by Buchwald already in 1883). *B. burgdorferi* can sometimes persist in the human body for long periods (up to 10 years), e.g. in synovial fluid or in the CNS (often in changed morphological forms – cystic, circular or granular). LB is the most frequent ixodid tick-borne human disease in the world, with an estimated 85,000 patients annually (Europe 65,000, North America 16,500, Asia 3,500). For instance in Czechland was reported on average 3,330 (the range, 1,446–6,302) cases annually in the years 1990–2006, which presents an incidence rate of 32 (the range, 16–61) per 100,000 population (EPIDAT).

Bio-containment: BSL-2.

Diagnosis: symptoms (EM) and anamnesis (tick bites), serology (ELISA IgM and IgG, optimally with confirmation using WB; IF); isolation of spirochaetes from the skin biopsy or from CSF fluid complex BSK (or MPK) medium at 33°C (the growth is very slow); also electron or IF microscopy of CSF; PCR. The 'cystic forms' or 'blebs' are impossible to diagnose in darkfield microscopy.

Treatment: doxycycline, amoxicilin, penicillin, tetracycline, erythromycin, deoxymykoin in the acute phase; in the chronic phase cephalosporins (cefotaxim, ceftriaxon), or big doses of penicillin.

Prevention: suitable human vaccine against LB does not exist at present. The lately released US vaccine "LYMErix" was removed from the market several years ago. Examination by darkfield microscopy or PCR of ixodid ticks feeding on a person may be helpful – in positive cases, prophylactic treatment of the person with one or few doses of doxycycline should be recommended.

Geographical distribution: holarctic (Europe; northern Asia – including Siberia, China, Japan, Korea; and North America). For different habitats of LB agents, see Photos 5.33 to 5.37.

8.3.20 *Family* Serpulinaceae [*Order* Spirochaetales]

Brachyspira pilosicoli

An anaerobic spirochaete.

Source of infection (natural host range): pig – some strains of swine brachyspi-
   rae are pathogenic for man.
Animal disease: enteritis.
Transmission mode: contact, alimentary.
Human disease: enteritis, intestinal spirochaetosis (colon epithelium is
   affected) – abdominal pain, chronic (sometimes bloody) diarrhoea; the ill-
   ness occurs mainly in immunosuppressed persons. In Bangladesh, often
   associated with cholera. Occupational risk (butchers, vets, animal breeders).
Bio-containment: BSL-2.
Diagnosis: cultivation *in vitro* is difficult.
Treatment: antibiotics (optimally after differential susceptibility test).
Geographical distribution: worldwide.

8.3.21 *Family* Flavobacteriaceae [*Order* Flavobacteriales*, Class*
   Flavobacteria]

Capnocytophaga canimorsus

Motile (gliding) Gram-negative fusiform rods to filaments, facultatively anaerobic,
and fastidious.

Source of infection (natural host range): dog (a commensal in the oral cavity),
   less often cat, and rodents.
Animal disease: usually asymptomatic infection.
Transmission mode: percutaneous (by bites, scratches, or licking of scratched
   skin by pet animals).
Human disease: fever, chills, myalgia, vomiting, septicaemia (disseminated)
   intravascular coagulation, peripheral gangrene, purulent meningitis, endo-
   carditis. Infection can be fatal (sepsis) especially in immunosuppressed
   individuals. Since the first description in 1976, about 200 human cases have
   been reported. Risk groups are animal keepers, breeders, veterinarians and
   pet owners.
Bio-containment: BSL-2.
Diagnosis: cultivation *in vitro* is difficult (BA or chocolate agar in the atmo-
   sphere of 5% CO_2, or anaerobically), the growth is slow; microscopy
   (bacteria present within neutrophils).
Treatment: penicillin G, doxycycline, imipenem, clindamycin (or other antibi-
   otics optimally after differential susceptibility test).

Geographical distribution: worldwide (North America, Australia, South Africa, Europe).

8.3.22 *Family* Burkholderiaceae [*Order* Burkholderiales*, Class* Betaproteobacteria]

Burkholderia mallei

A non-motile, slightly bended, strictly aerobic, polymorphic Gram-negative rod 2–5×0.5–1 μm.

Source of infection (natural host range): equids (horse, donkey, mule), rodents (*Rattus*), cat, dog; man.

Animal disease: glanders (malleus) – very dangerous, acute (donkey, mule) or also chronic (horse – carriers) infectious illness of odd-toed ungulates, producing nodules and ulcerations in the respiratory tract, occasionally also skin ulcerative lesions called "farcy".

Transmission mode: percutaneous (skin abrasions) or aerogenic, less frequently alimentary, via conjunctiva. The agent is highly contagious, but its tenacity is low.

Human disease: glanders (malleus) – multiple nodules, abscesses or flat ulcers in subcutis, muscles, visceral organs and on mucosa, high fever with headaches, photophobia, lacrimation, lymphadenitis, purulent haemorrhagic bronchopneumonia and septicaemia with a high fatality rate (40–100%) in untreated cases; less common is a chronic form.

Bio-containment: BSL-3. Considered to be a serious bioterrorist threat (against animals and humans; it was used during the WWI and WWII against military horses).

Diagnosis: microscopy and cultivation (media with 3–5% glycerol) of the pus from scars and nasal secret; intraperitoneal inoculation of male hamster or guinea pig (scrotal oedema – so-called Strauss effect), serology (CFT, ELISA, RIHA, AR), intradermal test for hypersensitivity (malein), PCR.

Treatment: streptomycin, tetracycline, chloramphenicol, gentamicin; sulphonamides sulphadiazin, cephtazidim, carbapenem.

Geographical distribution: Asia (Mongolia, Turkey, Middle East, Iran, Pakistan), north and eastern Africa, Mexico, South America (Brazil). In the past worldwide, but eradicated from most countries, lately reemerging in some areas (e.g., Arab Peninsula).

*Burkholderia pseudomallei

A motile polymorphic aerobic rod 1.5–6×0.5–1 μm with bipolar staining, growing also at 42°C.

Source of infection: stagnant water (in rice fields, wetlands), potable water, soil/mud (a soil-borne bacterium); infrequently it occurs in rodents, sheep, cattle, pig, horse, leporids; occasionally primates, deer, dogs, cats, koalas, kangaroos, camels, crocodiles.

Animal disease: inapparent course, or acute melioidosis (in sheep).

Transmission mode: percutaneous (traumatic inoculation from the soil), less often aerogenic (inhalation of contaminated dust or water droplets, with a severe course) or alimentary (e.g. water ingested or swallowed during swimming); the agent has considerable tenacity in the milieu.

Human disease: melioidosis (pseudomalleus, also called Whitmore disease or Nightcliff gardener's disease in Australia) – fever, a fierce diarrhoea of an extraordinary frequency (20 per h), disseminated disease with multiple abscesses and granulomas (similar to those in tuberculosis) in different organs (liver, lungs, spleen, kidney, prostate) and also in subcutis, lymphadenopathy, pneumonia, sometimes also septicaemia with a very high fatality rate (80–95%); also chronic forms occur with relapses even after a number of years (despite therapy). It is primarily an infection of humans with underlying diseases such as alcoholism, malnutrition, cirrhosis, and immunosuppression, but can also affect healthy individuals. An increased incidence of melioidosis is usually recorded in endemic areas after cyclones, heavy rains or floods. This is also a "tourist infectious disease" during the travel or adrenaline sports in wet tropical areas (importation to e.g. Europe has been repeatedly recorded).

Bio-containment: BSL-2/3.

Diagnosis: cultivation of the blood, sputum, urine and pus samples, inoculation of young male hamster or guinea pig (scrotal reaction), serology (HA, IFA, CFT, AR, ELISA).

Treatment: long-term (6–12 months) application of doxycycline + amoxicilin, or large doses of sulphonamides (cephtazidim, cotrimoxazole, imipenem, meropenem, carbapenem).

Geographical distribution: tropics and subtropics (largely wetland habitats) – Southeast Asia (Singapore, Indonesia) and northern Australia; also China, Taiwan, India, Bangladesh, Iran, Madagascar, Ecuador, Peru, Brazil, and Central America (Caribbean islands).

*Burkholderia cepacia Complex

About 10 species, pathogenic are mainly *B. cepacia*, *B. multivorans*, *B. cenocepacia*, and *B. cocovenenans*.

Source of infection: soil, water, hospital environment (nosocomial infectious disease); also man.

Transmission mode: percutaneous, less often aerogenic or alimentary.

Human disease: bronchitis to pneumonia especially in person with cystic fibrosis and vascular transplantates. *B. cocovenenans* causes alimentary, often fatal intoxications (2007 Java: 10 patients died due to this illness).
Bio-containment: BSL-2.
Diagnosis: *in vitro* cultivation of the sputum samples.
Treatment: piperacilin, cotrimoxazole, chloramphenicol, carbopenem.
Geographical distribution: worldwide.

8.3.23 *Family* Neisseriaceae [*Order* Neisseriales, *Class* Betaproteobacteria]

*Chromobacterium violaceum

Gram-negative, facultatively anaerobic rods.

Source of infection: soil and water (saprophytic growth).
Animal disease: pathogenic for birds, their illness reminds cholera.
Transmission mode: contact, alimentary.
Human disease: chromobacteriosis with fever, headache, vomiting, cutaneous, hepatic and pulmonary abscesses, sometimes fatal septicaemia. The illness is infrequent (about 150 cases have been described), but serious.
Bio-containment: BSL-2.
Diagnosis: cultivation – the bacterium produces violet pigment due to tryptofan oxidation on usual agar media.
Treatment: gentamicin, trimethoprim + sulphamethoxazole, meropenem, ciprofloxacin, chloramphenicol.
Geographical distribution: (sub)tropical regions – Asia, Australia, USA, South America (Argentina, Brazil), Africa.

8.3.24 *Family* Spirillaceae [*Order* Nitrosomonadales, *Class* Betaproteobacteria]

Spirillum minus

A short coiled pleomorphic Gram-negative rod 2–5×0.2–1 μm, with several (up to 7) polar flagellas (*amphitricha*); sometimes it forms long and disintegrating filaments.

Source of infection (natural host range): rodents (largely *Rattus* spp.) – they excrete the agent by saliva.
Animal disease: inapparent course.
Transmission mode: percutaneous (biting).

Human disease: <u>sodoku</u> [Japanese "so" = black rat; "doku" = poison], <u>rat-bite fever</u> – papular rash (on face and arms), ulceration, regional lymphadenitis, high recurrent fever (usually 6–8 relapses within 2 months, but sometimes prolonged over a number of months or even years), without arthritis; fatality rate 5–10%.

Bio-containment: BSL-2.

Diagnosis: intraperitoneal inoculation of laboratory rat, mouse or guinea pig (bacteraemia); darkfield microscopy, thick drop or blood smear (Giemsa stain); non-cultivable *in vitro*.

Treatment: penicillin, aureomycin.

Geographical distribution: eastern Asia (Japan), Australia.

8.3.25 *Family* Fusobacteriaceae [*Order* Fusobacteriales, *Class* Fusobacteria]

Streptobacillus moniliformis

A non-motile pleomorphic Gram-negative rod 1–3 μm long, sometimes producing very long and disintegrating filaments up to 100 μm; facultatively anaerobic.

Source of infection (natural host range): rodents (*Rattus* etc. – *S. moniliformis* is a part of their oral microflora; it is excreted via saliva and urine); cat.

Animal disease: usually asymptomatic infection, but sometimes polyarthritis, oedema of limbs and fingers.

Transmission mode: percutaneous (bites); alimentary (ingesting food or drink such as milk or water contaminated with rat excrement).

Human disease: <u>Haverhill fever</u> (<u>rat-bite fever</u>, streptobacillosis) with headache, sometimes rash of rubella type, photophobia, lymphadenitis, pharyngitis, septic mono- or polyarthritis, occasionally endocarditis and pneumonia; fevers can be recurrent for a period of months; fatality rate 10% in untreated cases.

Bio-containment: BSL-2.

Diagnosis: cultivation of the blood or synovial fluid samples (BA with serum or ascitic fluid, 5–10% CO_2), intraperitoneal inoculation of mouse, agglutination.

Treatment: large doses of penicillin, tetracycline (streptomycin).

Geographical distribution: worldwide but sporadic.

Fusobacterium necrophorum

Anaerobic non-motile Gram-negative rods 2–5×0.5–1.5 μm or filaments up to 80–100 μm long.

Source of infection (natural host range): domestic ruminants.

Animal disease: necrobacillosis (necrotic lesions on limbs, diphteroid stomatitis, arthritis, abscesses on liver) in calves, lambs, piglets and young rabbits, with a high fatality rate; necrosis of genital organs in male bisons (Bialowieža, Poland).

Transmission mode: contact; tenacity of the agent is low.

Human disease: <u>necrobacillosis</u> – necrotic ulcers and regional lymphadenitis.

Bio-containment: BSL-2.

Diagnosis: cultivation (BA, 5% CO_2), intradermal inoculation of laboratory animals (mouse in the tail, rabbit in the auricle).

Treatment: penicillin + streptomycin, tetracycline, chloramphenicol, sulphonamides, locally potassium iodide; surgery.

Geographical distribution: worldwide, sporadic.

8.3.26 *Family* Erysipelotrichaceae [*Order* "Incertae sedis", *Phyllum* Firmicutes]

(*) *Erysipelothrix rhusiopathiae*

Non-motile pleomorphic non-sporulating Gram-positive rods $0.8–2.5 \times 0.1–0.3\ \mu m$.

Source of infection (natural host range): pig (*E. rhusiopathiae* var. *suis*) and other domestic and wild mammals (especially rodents – *E. rhusiopathiae* var. *murisepticum*), also domestic birds (turkey, chickens), fish, cuttlefish, molluscs, crabs, lobsters; water.

Animal disease: swine erysipelas with acute or chronic course (rash, septicaemia, endocarditis, arthritis), sometimes clinical disease in domestic and wild birds.

Transmission mode: contact (percutaneous at injury: occupational disease in butchers, veterinarians, fishers, pickers and shope assistants of seafood, farmers); exceptionally haematophagous arthropods (isolation of *E. rhusiopathiae* from ixodid ticks – experimentally confirmed mechanical (?) transmission by *Ixodes persulcatus*, mites, fleas and flies); considerable tenacity of the agent (in water and soil it survives at 5°C up to 970 days, in animal cadavers up to 9 months, it also resists higher concentrations of NaCl).

Human disease: <u>erysipeloid</u> [*vs*. erysipel caused by *Streptococcus pyogenes*] – characteristic erythema on hands, oedema of finger joints; infrequently a generalized (septic) form with polyarthritis and endocarditis (in that case the fatality rate is high).

Bio-containment: BSL-2.

Diagnosis: symptoms and anamnesis (haemo)cultivation (BA with glucose, 5–10% CO_2), subcutaneous inoculation of mouse.

Treatment: penicillin, erythromycin (tetracyclines).

Prevention: vaccination of animals.
Geographical distribution: worldwide.

8.3.27 *Family* Listeriaceae [*Order* Bacillales, *Class* Bacilli]

(*) *Listeria monocytogenes* (*L. ivanovii*)

Short, motile ("tumbling" at lower temperatures of 20–25°C), non-sporulating aerobic Gram-positive rods 0.5–2×0.4–0.6 µm. A total of 13 serotypes have been described, in humans occur most frequently the serovars 1/2a, 1/2b, and 4b.

Source of infection (natural host range): (1) domestic and wild mammals (sheep, goat, cattle, pig, rodents), birds (corvids, gulls, fowl), and man (up to 5% of the human population are asymptomatic carriers); (2) soil, water, fodder, silage, household rubbish (*L. monocytogenes* grows even in fridge at 4°C).

Animal disease: often inapparent infection, sometimes encephalitis, abortions (especially in sheep), and fatal septicaemia in young fowl, lambs and calves.

Transmission mode: nearly exclusively alimentary ("food-borne" infection: soft maturing cheese sorts, raw milk, smoked tongue, ham, paté, raw cabbage, salads, sea fish and other seafood), less often contact (farmers, fishers), aerogenic, via conjunctivae, and transplacentary. The agent's tenacity is remarkable: it survives at a lower temperature 1 year or more, and tolerates, e.g., 10% NaCl. *L. monocytogenes* also survives in macrophages, and its virulence is activated by contact with gastric fluid of the host.

Human disease: <u>listeriosis</u> – often inapparent infection. Incubation period of listeriosis is very long, on the average one month but sometimes up to 70 days. The symptoms of listeriosis are fever, headaches, chills, pharyngitis, nausea, mononucleosis, purulent meningitis (in up to a quarter of cases), encephalitis, endocarditis, granulomas and abscesses in liver, subcutis and other organs, septicaemia (bacteraemia) in half of the cases; fatality rate is up to 20–30% (mortality usually occurs in old persons, at immunosuppression or at a large infectious dose). Characteristically, there is no enteritis. A dangerous disease is the listeriosis in pregnant women usually leading to miscarriage or pre-term delivery of babies associated with septicaemia, severe damage to CNS and visceral organs; fatality rate is high, 30–80%. *L. monocytogenes* has namely a considerable tropism for the CNS, placenta and uterus of mammals. Extensive epidemics of listeriosis after consumption of contaminated food (cheese etc.) occurred in USA, Switzerland, France, Denmark and Germany (serotype 4b, a few hundreds of persons) in the years 1985–1990. A total of 1,566 persons (292 of them hospitalized) were infected with the serotype 4b of *L. monocytogenes* in northern Italy in 1999; the source of infection was a maize salad with tuna fish. An increased incidence of listeriosis was also observed in UK and other European countries in 2001 and 2003. In the

European Union the incidence of listeriosis has been growing moderately since 2004: from 1,264 total cases, to 1,427 cases in 2005, 1,583 in 2006, and 1,554 in 2007; it corresponds to the incidence rate of 0.3 per 100,000 population; the mortality rate remains high, about 20%. *L. ivanovii* is an enteric opportunistic pathogen, causing occasionally gastroenteritis and bacteraemia in man; the isolates are indistinguishable from ruminant strains.

Bio-containment: BSL-2.

Diagnosis: cultivation of the blood, CSF, placenta samples (MPA, BA; optimally after a semi-selective replication in enrichment broth at 4°C and subsequently at 37°C), CAMP test with *Rhodococcus equi* or *Staphylococcus aureus*, inoculation of mouse (treated with cortisone); serology less useful (CFT), PCR.

Treatment: ampicillin, azithromycin, gentamicin, large doses of penicillin plus kanamycin, tetracycline, chloramphenicol.

Geographical distribution: worldwide (less often in tropics), ubiquitous. Within Europe, listeriosis occurs more frequently in countries that produce a lot of maturing cheese (e.g. France, Switzerland, Germany, the Netherlands, Italy).

8.3.28 *Family* Bacillaceae [*Order* Bacillales]

(*) *Bacillus anthracis*

Greek "ανθραξ" (anthrax) means "glowing coal" (cutaneous form of the disease is characterized by a black skin lesion). An aerobic, non-motile Gram-positive rod 4–8×1–1.5 μm with a mucous polyglutamate capsule and a spore located in the cell centrally, as observed already by Robert Koch in 1876 who cultivated the anthrax bacillus first. Virulence is determined by a plasmid.

Source of infection (natural host range): domestic and wild ruminants (especially cattle, sheep and goat), equids; soil (on pastures contaminated with the agent or its spores mainly in alluvial areas of rivers, or in deltas), animal products (manure, skins, fur, wool).

Animal disease: anthrax (herbivores, much less pigs and carnivores) – a peracute septic illness with a sudden death. For instance, ten thousands of livestock died in Zimbabwe in 1979–1982; about 70 cattle, horses and pigs died in Romanian Danube delta in 2000. An epizootic of bisons in the USA, 2007.

Transmission mode: contact (percutaneous, e.g. at sloughter of an infected animal), aerogenic, inoculative by biting insects or in heroin addicts (many recent cases in the UK), alimentary (water); the spores of *B. anthracis* are very resistant (in the soil they remain viable for tens of years: enzootic foci in inundated pasture areas).

Human disease: anthrax – according to the transmission mode there are the forms cutaneous (*pustula maligna* – a necrotic black scare); pulmonary;

abdominal; and septic. The number of anthrax cases in the world varies between 5 and 100,000 yearly (WHO), with a considerable mortality (25–100%) especially in non-cutaneous forms of the disease with septicaemia (the fatality rate in cutaneous forms is 5–20%). Epidemics: e.g., Zimbabwe *c.* 10,000 cases in 1979–1985 (the source: infected cattle); 64 persons died in Yekaterinburg (former Sverdlovsk) after an unintentional release of *B. anthracis* spores in 1979 from a plant producing bacteriological weapons; 2000 Volgograd: 25 persons were infected after consuming infected meat; 2000 Romanian Danube delta: 2 persons died. In 2001, anthrax spores were used as a bacteriological weapon in letters in USA: 22 persons were infected, and 5 of them died (11 cases were the inhalational clinical form, and 11 were the cutaneous form). Occupational disease: the wool, skin and fur proceeding workers (carpets etc.), veterinarians, butchers, farmers, diggers in burial ground with the anthrax contaminated bodies.

Bio-containment: BSL-2/3. Potential bioterrorist agent (used, e.g., in USA).

Diagnosis: symptoms (in cutaneous form), blood smear (Gram, IF) with detection of capsules (Giemsa or polychromic methylene blue stain, or phase contrast); cultivation (MPA, BA: colonies have a characteristic shape *caput Medusae,* without haemolysis; selective media such as PLET agar inhibiting *Bacillus cereus*); inoculation of laboratory mouse (or guinea pig, rabbit); Ascoli's precipitation reaction (overlaying of anti-anthrax precipitation serum with the filtered extract of the tested organ); serology (IFA), PCR.

Treatment: large doses of penicillin, amoxicilin, ampicillin, penicillin combined with streptomycin; tetracycline, chloramphenicol, erythromycin.

Prevention: vaccine (immunization with a toxoid; for humans and animals, a non-virulent strain; developed Louis Pasteur in 1880, who cultivated the strain for 6 weeks/42–43°C); (a vaccine licensed in USA – AVA/Biothrax – is applied by skin scarification). In leather manufacture, inactivation of *B. anthracis* spores with peracetic acid (Persteril) is used for raw materials (skins).

Geographical distribution: worldwide, but sporadic (enzootic foci) – Asia (Siberia, Turkey, Iran, Iraq), Europe (Romania, southern Russia, Hungary), Africa, South America.

8.3.29 *Family* Staphylococcaceae [*Order* Bacillales]

(**) *Staphylococcus aureus*, *S. epidermidis*, *S. haemolyticus*, *S. intermedius* and Other spp.

Non-motile Gram-positive cocci of about 1 μm, in clusters, facultatively anaerobic and producing catalase. *S. aureus* and *S. intermedius* also produce plasmacoagulase, haemolysin, and ferment mannitol.

Source of infection (natural host range): cattle, dog (*S. intermedius*), pig and horse (MRSA zoonotic strains) and other mammals; man. The MRSA strains associated with livestock are called LA-MRSA.

Animal disease: mastitis in cows (an economically very important illness), dermatitis, arthritis, abscesses, and abortions.

Transmission mode: contact, alimentary (milk and dairy products: e.g., 15,000 patients were infected after drinking infected milk in Japan in 2000; meat). Occasionally nosocomial infections. Some staphylococcal strains have a considerable tenacity.

Occupational risk: farm workers.

Human disease: staphylococcosis – dermatitis (3–11% isolates are of animal origin, e.g. sequence type *S. aureus* ST 398 in the Americas; *S. intermedius* rarely – after dog bites) and other suppurative processes, abscesses, osteomyelitis, sinusitis, respiratory illness, endocarditis, arthritis. Staphylococcal enterotoxicosis with enterotoxigenic strains (gastroenteritis) with an incubation period as short as 2–6 h; the incidence of this illness is generally increasing. Sometimes septic shock, especially with MRSA possessing so-called superantigen; many of these strains are zoonotic, originating from domestic animals. Staphylococci (including zoonotic) produce a number of exotoxins (hyaluronidase, coagulase, α- and β-haemolysin, enterotoxins, exfoliatins etc.) that together with other virulence factors (peptidoglycan, protein A etc.) very strongly affect pathogenesis of the infection.

Bio-containment: BSL-2.

Diagnosis: cultivation (e.g. on BA, or selective media with mannitol and 10% NaCl), coagulase test, phagotypization, molecular typization; serological detection of toxin.

Treatment: antibiotics (optimally after the susceptibility testing) – oxacilin, methicillin (but some strains are MRSA – methicillin resistant *S. aureus*), chloramphenicol, tetracycline, ampicillin, erythromycin; junction of abscesses. There is no specific therapy for staphylococcal enterotoxicosis.

Geographical distribution: worldwide.

8.3.30 *Family* Streptococcaceae [*Order* Lactobacillales*, Class* Bacilli]

Streptococcus suis, *S. equi* ssp. *zooepidemicus*

Pyogenic streptococci of the α-haemolytic group D (*S. suis*), and β-haemolytic group C (*S. equi* ssp. *zooepidemicus*). Non-motile cocci 1 μm, arranged in chains, do not produce catalase.

Source of infection (natural host range): domestic and wild swine (*S. suis,* reservoir, the agent occurs mainly on tonsils and in vagina), horse (*S. equi* ssp. *zooepidemicus*).

Animal disease: purulent inflammation of upper respiratory tract, with lymphadenitis (in horse, *S. equi* ssp. *zooepidemicus*), vaginitis and uterus inflammation (mare, *S. equi* ssp. *zooepidemicus*), less often mastitis in cattle and arthritis in lambs (*S. equi* ssp. *zooepidemicus*); bronchopneumonia, endocarditis, polyarthritis, septicaemia and meningitis in swine with fatality rate up to 50% (*S. suis*, mainly serotype 2).

Transmission mode: alimentary (raw milk and cheese, undercooked pork – *S. suis*) or aerogenic (*S. equi* ssp. *zooepidemicus*), contact (percutaneous – skin injury).

Occupational risk: farmers, swine breeders, butchers, slaughterhouse workers, cooks (*S. suis*).

Human disease: streptococcosis (purulent meningitis, septicaemia); in *S. equi* ssp. *zooepidemicus* pneumonia, septicaemia, endocarditis, nephritis; arthritis, meningitis, osteomyelitis; in *S. suis* fever, meningitis, deafness, (poly)arthritis, uveitis, diplopia, endocarditis, pneumonia, peritonitis, septicaemia, ataxia, toxic shock syndrome, with the fatality rate about 8–20%. The first case of human infection with *S. suis* was described in Denmark in 1968, later a few cases in other European countries (the Netherlands, England, Czechland, Slovakia, Sweden, Belgium, Germany, France, Hungary, Austria, Croatia, Italy, Spain, Greece), Canada, Hawaii, China (1998: 25 cases including 14 deaths), etc.; up to 2005, the total number of documented cases in the world was 410. However, a major outbreak of *S. suis* infections occurred in Sechuan (China) with 218 severe cases (in farmers) in 2005, mostly as the toxic shock syndrome and fatality rate of 19%. Another outbreak occurred in Vietnam, 2007, involved 45 cases (at least two patients died).

Bio-containment: BSL-2.

Diagnosis: anamnesis; cultivation (BA) of throat swabs, CSF and blood samples; biochemical tests (API 20 Strep System); serotypisation; MLST. *S. suis* might be misidentified as a member of the viridans group streptococci.

Treatment: penicillin.

Geographical distribution: worldwide, *S. suis* is more common in southeast Asia, and China sporadic in mild climatic zone (UK, the Netherlands**,** Germany, Czechland, Slovakia, Croatia, Greece, New Zealand, USA, Canada).

Streptococcus iniae

Non-motile streptococci 1 μm in diametre, do not produce catalase. Virulence factors similar to those found in group A streptococci (*S. pyogenes*).

Source of infection: fish.

Animal disease: fish pathogen (sporadic infections in tilapia, yellowtail, rainbow trout, and coho salmon as well as disease outbreaks with high mortality rates in aquaculture farms) – skin lesions, necrotizing myositis, meningoencephalitis, panophthalmitis; sometimes asymptomatical course.

Transmission mode: percutaneous (throw soft tissue injuries that may occur during the preparation of fresh fish from wet markets).

Human disease: streptococcosis, usually results in bacteraemic cellulitis of the hand, accompanied with one or more of these symptoms: endocarditis, meningitis, arthritis, sepsis, pneumonia, osteomyelitis, and toxic shock. First human case of *S. iniae* infection was reported in 1996, and since 2000, hundreds of cases have been reported from southern Asia and Australia. Most of infections occur in elderly people who commonly have underlying conditions such as diabetes mellitus, chronic rheumatic heart disease, cirrhosis, etc.

Bio-containment: BSL-2.

Diagnosis: anamnesis, cultivation (BA), PFGE, MLST (multi-locus sequence typing).

Treatment: penicillin, ampicilin, cloxacillin, cefazolin, and/or gentamycin, doxycycline, and trimethoprim/sulphamethoxazole.

Geographical distribution: probably worldwide (global distribution among fish population), largely North America, Israel, and Asia (Japan, Taiwan, China).

Enterococcus faecalis (VRE, HLGRE)

Enterococci are gut commensals in humans and other endothermic vertebrates. But the emerging vancomycin-resistant (VRE) and high-level gentamicin-resistant (HLGRE) enterococci (multilocus sequence type 16) can cause serious diseases, e.g. endocarditis.

Source of infection: some HLGRE and VRE isolates from pigs are identical with those from humans suffering from endocarditis.

Animal disease: not observed.

Transmission mode: alimentary, by contact. Nosocomial infections.

Human disease: endocarditis, sometimes severe.

Bio-containment: BSL-2.

Diagnosis: by cultivation (BA, Slanetz-Bartley or kanamycin-aesculin-azid medium) and antibiotic sensitivity testing.

Treatment: normally combinations of antibiotic such as one affecting cell wall of bacteria (ampicillin, penicillin, or vancomycin) plus gentamicin or vancomycin. But the HLGRE and VRE strains pose now great problems. The alternative use is linesolid, quinupristin or some fluoroquinolones.

Geographical distribution: USA, Europe (e.g., Denmark), but probably also many other geographic areas.

8.3.31 *Family* Clostridiaceae [*Order* Clostridiales, *Class* Clostridia]

*Clostridium tetani

Anaerobic slim Gram-positive rod 4–8×0.4–0.8 μm with a terminal spore of the *plectridium* type.

Source of infection: soil (especially manured); horse and other mammals.

Animal disease: tetanus (dogs, cats and birds are resistant).

Transmission mode: by contact (percutaneous – injury: e.g., farmers, gardeners); spores are very resistant, they survive (e.g. in soil >10 years, and in boiling water up to 3 h).

Human disease: tetanus (lockjaw); classical neurotoxicosis (main toxin is tetanospasmin, its MLD for mouse is 0.0001 μg; another one is tetanolysin), with the fatality rate of 30–70%; first clinical signs are the blockade (painful contraction) of maxillary muscles, *trismus* (sardonic grimace) and abdominal rigidity, later convulsions of back muscles (*opisthotonus*: bow-shaped deflection of body to the back, leading sometimes to fractures of vertebrae and muscles), respiratory and other muscles; no fever, consciousness is conserved.

Bio-containment: BSL-2/3.

Diagnosis: clinical symptoms (tonic spasms); microscopy and anaerobic cultivation of wounded tissue, subcutaneous inoculation of mice (combined with toxin neutralization test).

Treatment: surgical treatment of the wound, application of specific human (TEGA) or horse immunoglobulin (antitoxin, antitetanic serum) within 24 h; pulmonary ventillation or tracheotomy, myorelaxants and sedatives; penicillin and metronidazole.

Prevention: immunization with anatoxin (toxoid).

Geographical distribution: worldwide (less frequent in cold areas).

*Clostridium perfringens Type A, *C. septicum*, *C. novyi* etc.

Anaerobic Gram-positive rods 4–6×0.6–1 μm, non-motile (*C. perfringens*) or motile (*C. septicum*, *C. novyi*).

Source of infection: soil (contaminated with animal excrements).

Animal disease: gastritis in lambs (*C. septicum*); necrotic hepatitis in sheep (*C. novyi*).

Transmission mode: by contact (injury, trauma – car accidents and other harms; iatrogenic).

Human disease: gas gangrene – cellulititis, myositis, fasciitis, necrosis of tissue with gas production (main toxin is lecithinase – phospholipase C); fatality rate is 30–90%. A total of 35 (out of 104 infected drug-users) died of sepsis

after syringe application of contaminated drugs in Great Britain in 2000 (*C. novyi*, *C. perfringens*).

Bio-containment: BSL-2/3.

Diagnosis: clinical symptoms, microscopy and anaerobic cultivation of the tissue, detection of lecithinase.

Treatment: surgical treatment of wounds; antibiotics (penicillins) in high doses; hyperbaric box.

Geographical distribution: worldwide.

(*) *Clostridium difficile*, *C. perfringens*

Anaerobic Gram-positive rods. Highly virulent ribotypes O17, O27, and O78 of *C. difficile* emerged lately.

Source of infection: soil, water, digestive tract of vertebrates (pig, cattle, poultry); man. Ribotypes of many human isolates have been shown to be identical with bovine strains.

Animal disease: calf diarrhoea, infectious enterotoxemia of domestic animals.

Transmission mode: alimentary (food-borne: e.g., undercooked and longer weaned meat, salads); nosocomial – man to man transmission (about 5% of adults act as carriers of *C. difficile* occurring in the gut).

Human disease: intestinal clostridiosis – infection or intoxication (toxin production in the gut – enterotoxins A and B) – pseudomembraneous colitis with diarrhoea, occasionally occurrence of megacolon with risk of gut perforation and subsequent peritonitis; gastroenteritis with diarrhoea and abdomen convulsions, and quite high lethality. The disease frequently occurs after long-term treatment with various antibiotics (so-called "post-antibiotic colitis"), when *C. difficile* replicates abundantly in the gut and produces toxins (the most virulent are the strains with type V toxin). *C. difficile* forms considerable portion of bacterial enteric infections in old peoples' homes, hospices and some clinics (a hospital ecological niche); the incidence rate of this disease, sometimes called CDAD (*C. difficile*-associated disease) has increased tenfold recently – it is an emerging nosocomial infectious disease, spreading as anthroponosis especially in North America, Europe and Singapore. People older than 65 years are very susceptible to this illness.

Bio-containment: BSL-2.

Diagnosis: anaerobic cultivation of stool samples and foods (selective media with cefoxitin and cycloserine), detection of enterotoxin in the stool and blood specimens on tissue cultures or by ELISA.

Treatment: rehydration, vancomycin, metronidazole. However antibiotic treatment increases incidence of *C. difficile* infections in healthcare settings.

Geographical distribution: worldwide, with increasing incidence in countries associated with high hygienic standard (Canada, Great Britain, Belgium, Netherlands, France, Austria).

(*) *Clostridium botulinum*

An anaerobic motile rod 4–6(10)×1 μm, with a subterminal spore.

> Source of infection (natural host range): pig, ruminants, horse, poultry; fish (type E); soil, vegetables, fruit, silage.
> Animal disease: botulism (death caused by diaphragm paralysis) in mammals and birds (chicken, wild waterfowl).
> Transmission mode: alimentary (gastric trypsin activates the toxin) – ham, sausages, home-made tins of meat, vegetable (green beans, peas, root vegetables) and fish origin, liver pate; wound botulism (drug users).
> Human disease: <u>botulism</u> (alimentary intoxication of humans by the types A, B, E, F and G, the most effective neurotoxins at all causing after an incubation period of 12 h to several days irreversible neuromotoric paralysis by blockade of acetylcholine release on synapses, with loss of motility, diplopia, strabism, paralysis of respiratory muscles); fatality rate 15–75%. MLD of botulinum toxin ("sausage poison") for man is 0.0625 μg. Very dangerous is <u>infant botulism</u> with replication of clostridia and toxin production in the gut of babies (honey as an addition to baby food is the most frequent source of *C. botulinum*).
> Bio-containment: BSL-2.
> Diagnosis: detection of botulotoxin in serum, faeces, vomitus or food residues by toxin-neutralization test on mice (ELISA is 10–100× less sensitive); cultivation of the stool or of food residues is less important.
> Treatment: stomach and intestine lavage; botulinum antitoxin poly- or monovalent.
> Prevention: vaccination with toxoid (in occupations with risk, and animals).
> Geographical distribution: worldwide.

8.3.32 *Family* **Mycobacteriaceae** [*Order* **Actinomycetales***, Class* **Actinobacteria**]

** *Mycobacterium bovis***

Non-motile, chromophobic by Gram (but de facto Gram-positive) acidoresistant irregular rods 3.0×0.3 μm. Diaminopimelic and mycolic acids (waxes) are included in cell wall of mycobacteria. Genomically very closely related are **M. microti, M. caprae**, and **M. pinnipedii**, jointly belonging to the *M. tuberculosis* complex, and occasionally transmissible to humans.

> Source of infection (natural host range): cattle and other domestic (occasionally pig, cat, camel) and free-living mammals (e.g., badger *Meles meles* is reservoir in England; wild boar *Sus scrofa* is reservoir in some countries of continental Europe, African buffalo *Syncerus caffer*, red lechwe *Kobus leche*,

greater kudu *Tragelaphus strepsiceros* and warthog *Phacochoerus africanus* in Africa; American bison *Bison bison* and deers in North America; or common brushtail *Trichosurus vulpecula* in New Zealand). Source of infection in *M. microti* are small terrestrial mammals (voles).

Animal disease: tuberculosis of domestic (bovine tbc) and free-living ruminants (bison in Canada), pig, cat, dog – chronic disease with tubercles in the lungs and other tissues.

Transmission mode: aerogenic, alimentary (raw milk and milk products, undercooked meat); high tenacity of the agent.

Human disease: bovine tuberculosis (clinically indistinguishable from the human tuberculosis) – pulmonary, more often extrapulmonary (urogenital, gastrointestinal, bone, meningeal or skin), slow progressive disease accompanied by fever and cervical lymphadenopathy (scrophula); incubation from 1 month up to several years. It could lead in lethal disease without sufficient therapy. Immunocompromised persons seem to be the most susceptible (e.g., with HIV infection). Zoonotic tuberculosis constitutes in different regions 3–10% of overall tuberculosis cases in humans. Disease caused by *M. microti* occurs only rarely in human (a total of 11 cases have been described), usually in patients with HIV, but also two immunocompetent patients suffered from pulmonary tuberculosis due to *M. microti* in Germany in 1999. Vaccine strain (BCG) occasionally causes postvaccination infections in immunosuppressed patients. [*M. tuberculosis* sensu stricto or "BK", Koch's bacillus, is causative agent of human tuberculosis (an anthroponosis)].

Bio-containment: BSL-3.

Diagnosis: X-ray computed tomography, microscopy (Ziehl-Neelsen stains of acidoresistant microbes: hot karbolfuchsin, decolouration with alcohol-3% HCl, another staining with malachite green or methylene blue) and difficult, up to 8-week cultivation of sputum (after treatment of the sputum samples with 4% NaOH or HCl and subsequent neutralization), CSF, urine or pus on Löwenstein-Jensen egg medium, Šula's or Ogawa media with glutamate; also tuberculin skin test (allergic response). Multiplex PCR. Molecular identification by means of RFLP typing; inserted sequence IS6110 is characteristic for this complex.

Treatment: isoniazid + rifampicin, pyrazinamide, ethambutol; formerly streptomycin and other tuberculostatics (p-aminosalicylic acid, cycloserine, ethionamide etc.).

Prevention: attenuated vaccine *M. bovis* BCG (bacillus Calmette-Guérin: effectiveness not optimal, about 80%); eradication programs in domestic animals. Attenuated vaccine from strain VO166 *M. microti* has been formerly used in Czech Republic and Great Britain. Vaccine strain (BCG) occasionally causes post-vaccination infections in immunosuppressed patients.

Geographical distribution: worldwide (but eradicated in many countries).

Mycobacterium avium Complex

Rods 1–2×0.5 μm. Non-pigmented mycobacteria with subspecies *M. a. **avium*** (causative agent of avian tuberculosis), *M. a. **silvaticum*** (the wood pigeon strains, pathogenic for wild and domestic birds), *M. a. **paratuberculosis*** (99.9% similarity of 16S rRNA with *M. a. avium*, causative agent of paratuberculosis in ruminants and other mammals), and probably non-zoonotic *M. a. **hominissuis*** (causes avian mycobacteriosis in pigs, cattle and other animals including human) and *M. a. **intracellulare*** (the latter is sometimes recognized as distinct species because DNA–DNA hybridization studies demonstrate that *M. avium* and *M. intracellulare* strains only share approximately 50% similarity). A new, sapronotic mycobacterium species is ***M. chimaera***, closely related to *M. intracellulare*.

Source of infection (natural host range): poultry (*M. a. avium*) and other birds (rook, pheasant, sparrow, woodpigeon, feral pigeon – *M. a. avium, M. a. silvaticum*), pig, rarely cattle in *M. a. avium*; cattle, sheep, goat in *M. a. paratuberculosis* (also wild mammals like red deer, roe deer, mouflon, chamois, wild boar, rabbit, etc.).

Animal disease: avian tuberculosis; lymphadenitis (*M. a. avium*), mesenteritis (*M. a. intracellulare*) in pigs; Johne's disease (*M. a. paratuberculosis*) – paratuberculosis, a veterinary important chronic hypertrophic enterititis in cattle with diarrhoea and anorexia, leading to death.

Transmission mode: alimentary (milk, less often meat), aerogenic; high tenacity of the agent in environment (*M. a. avium* is viable in water for about 113–417 days, in humid excrements up to 400 days; *M. a. paratuberculosis* usually survives in excrements of cattle and also in the soil up to 1 year). *M. chimaera* is present in water-cooling towers and circuits, the transmission is aerogenic.

Human disease: avian tuberculosis – pulmonary lesions, lymphadenitis, gastrointestinal lesions, after dissemination even fatal; incidence is increasing (frequent in immunocompromised persons with AIDS, etc.). Lesions are typically without necroses, calcification and fibroses. *M. a. paratuberculosis*: paratuberculosis, perhaps also Crohn's disease (multifactorial chronic granulomatous enteritis or ulcerative colitis with relapses). Infectious aetiology of Crohn's disease has not been proven reliably until present, however an association with paratuberculosis exists: presence of antibodies and enhanced detection of *M. a. paratuberculosis* in the patients. The prevailing opinion is that Crohn's disease has a multifactorial aetiology.

Bio-containment: BSL-2.

Diagnosis: as in the previous species; microscopy and histopathology; cultivation seems difficult, PCR detection, serology (CFT) in *M. a. paratuberculosis*. Molecular differentiation within the *M. avium* complex is based on DNA fingerprinting by RFLP typing-inserted DNA sequences IS900 (present only in *M. a. paratuberculosis*), IS901/902 (only present in *M. a. avium*) and IS1245 (in *M. a. avium* and *M. a. hominissuis*).

Treatment: difficult (number of strains are resistant) and long-term (at least 24 months), relapses of the diseases occur quite often. Therapy with isoniazid + rifampicin, pyrazinamide, ethambutol.

Geographical distribution: *M. a. avium* worldwide. *M. a. paratuberculosis* in New Zealand, Australia, Europe (Great Britain, France, Netherlands, Denmark, Germany, Belgium, Italy, Austria, Switzerland, Czechland, etc.), USA. *M. chimaera* has been detected in Germany and Oman.

*Mycobacterium kansasii

First described as "yellow bacilli"; "atypical", slow-growing "non-tuberculous" saprophytic mycobacteria, however potentially pathogenic for human. Optimum growth temperature is 30–33°C, but it is capable of growth at 40–45°C.

Source of infection: industrial, technical, and tap water (forming biofilms in water piping, shower heads, aquaria), soil and dust; amoebae, cockroaches, and some mammals (goat, monkey, dog, cattle).

Transmission mode: aerogenic, alimentary; iatrogenic.

Human disease: mycobacteriosis – chronic pulmonary disease (risk factor for acquiring infection: occupational exposure in coal miners; e.g., in Ostrava (Czechland), a total of 1,034 patients were recorded in 1968–1999), more often in immunocompromised persons (AIDS, chronic bronchitis, history of tuberculosis, pneumoconiosis, or people with lung parenchyma damage). Extrapulmonary forms of the disease involve skin lesions, cervical lymphadenitis, and uterine infections. A case of fatal septicaemia was also demonstrated. During an epidemiological survey in Spain in 2000–2003, a total of 598 cases were identified, with distinct clinical (mainly pulmonary) manifestation in 74% of them.

Bio-containment: BSL-2.

Diagnosis: see other mycobacteria; the optimum temperature for its in vitro isolation ranges between 30 and 33°C.

Treatment: clarithromycin + ciprofloxacin.

Geographical distribution: worldwide.

*Mycobacterium xenopi

"Atypical", mostly slow-growing "non-tuberculous" saprophytic mycobacteria, however potentially pathogenic for human; maximum growth temperature is between 42 and 45°C. *M. xenopi* was found to be more heat-resistant than *Legionella pneumophila*.

Source of infection: potable warm water systems (households, hospitals, dental units), animals (domestic and wild pig, cat, cattle), soil (?).

Transmission mode: aerogenic, alimentary.

Human disease: <u>mycobacteriosis</u> – chronic pulmonary and extrapulmonary disease, especially in immunocompromised persons (AIDS). Usually sporadic disease, but outbreaks of pulmonary mycobacteriosis due to *M. xenopi* may rarely occur.

Bio-containment: BSL-2.

Diagnosis: see other mycobacteria.

Treatment: clarithromycin + ciprofloxacin.

Geographical distribution: worldwide.

M. marinum, M. haemophilum

"Atypical" photochromogenic (*M. haemophilum* is not photochromogenic), mostly slow-growing "non-tuberculous" saprophytic mycobacteria, however potentially pathogenic for human. *M. haemophilum* demands special growth conditions (ferric ions).

Source of infection: water (aquaria – water, plants, sediment, biofilm, aquatic invertebrates, swimming pools, coal mine water, surface water, sewage, drinking water systems, soil, peat); fish (*M. marinum* – this species is also ichtyopathogenic); fish (zebrafish), insects, reptiles in *M. haemophilum.*

Transmission mode: by contact – skin injury.

Human disease: <u>mycobacteriosis</u> – skin disease characterized by rash, tubercles and raddish scabs 2 weeks after infection, then granulomas, nodules and ulceration evolve (*M. marinum*: "swimming pool granuloma" or "fish tank granuloma"); often in fish breeders and aquarists – usually after injury – also *M. haemophilum*. Typical location of skin lesions are elbows, knees, lower legs, hands and fingers in amateur fishermen and aquarists; cervical lymphadenitis was recorded mostly in Dutch children (*M. haemophilum*).

Bio-containment: BSL-2.

Diagnosis and therapy: biopsy of damaged tissue for laboratory testing (culture, histology); optimum growth temperature for in vitro isolation ranges between 30 and 33°C; for other methods see previous species.

Geographical distribution: worldwide.

Mycobacterium abscessus, M. chelonae, M. fortuitum, M. phocaicum

"Atypical", mostly fast-growing "non-tuberculous" saprophytic mycobacteria, however potentially pathogenic for human; optimum temperature growth usually at 28–30°C.

Source of infection: drinking water (hospital water supplies, showers, cooling towers, consumption of ice, ground water (*M. chelonae*, *M. fortuitum*), treated surface water, biofilms (bronchoscopes, dialysers)).

Transmission mode: aerogenic, alimentary, iatrogenic. Some of these mycobacteria can survive in amoebae.

Human disease: <u>mycobacteriosis</u> – chronic pneumonia, abscesses; most infections that have been reported are nosocomial – contaminated instruments and fluids (in conjuction with cardiac bypass, dialysis, plastic surgery, postinjection abscesses), or in immunocompromised patients (AIDS etc.).

Bio-containment: BSL-2.

Diagnosis: see other mycobacteria; patterns of large restriction fragments by PFGE, patterns of mycolic acids separated by HPLC, sequencing of 16S rRNA genes (gold standard).

Treatment: klaritromycin + ciprofloxacin, sulphonamides, cefoxitin, amikacin, ciprofloxacin, imipenem in *M. fortuitum*, tetracycline.

Geographical distribution: worldwide.

*Mycobacterium ulcerans

This microorganism evolved by means of ecological specialization from *M. marinum* (reduction of genome caused by DNA deletions from 6.6 to 5.8 Mb), as found by molecular analyses. Ten genotypes associated with geographical origin have been recognised until today. It does not grow at temperatures above 32°C.

Source of infection: aquatic environment (stagnant water in swamps, less in rivers): aquatic plants (*Echinochloa pyramidalis*), insects and snails, small fish and other vertebrates (koala, possum and captive alpaca in Australia).

Transmission mode: by contact (injury) and inoculative (mechanically by aquatic insects and mosquitoes – during one Autralian outbreak in 2004 several strains of *M. ulcerans* were detected in mosquitoes in the endemic area).

Human disease: "<u>Buruli ulcer</u>" (Bairnsdale or Searls's ulcer in Australia, Kumusi ulcer in Papua New Guinea) – a chronic necrotizing infection of the skin, deep dermal and subcutaneous tissue, and bone. The clinical forms are either localized (nodules, minor ulcer, major ulcer) or disseminated (plaques, oedematous form, metastatic disease-may enter lymphatic system or bloodstream). Incidence in endemic areas is high; for instance in Benin, 2,598 new and recurrent cases were reported and treated in the years 2003–2005. Annual number of new cases is estimated in Africa as at least 4,000. Higher rates of infection with *M. ulcerans* are usually documented after flooding.

Bio-containment: BSL-2.

Diagnosis: see other mycobacteria.

Treatment: rifampicin, moxifloxacin, amikacin; in severe cases surgical excision of large ulcers, even amputations, are necessary.

Geographical distribution: tropics and subtropics – Africa (Angola, Benin, Burkina Faso, Cameroon, Democratic Republic of Congo, Gabon, Ghana, Guinea, Ivory Coast, Liberia, Nigeria, Sierra Leone, Sudan, Togo, Uganda), less often in (sub)tropical areas of Asia, Australia and Papua New Guinea, and Central and South America.

(*) *Mycobacterium leprae*

This microbe has lost at least 2,000 genes after adaptation to human host (drastic reduction of genome, whose complete sequence of nucleotides has been described recently).

> Source of infection: human; water (peatbogs and bryophytes growing in the sur-roundings of waterfalls and on fiord slopes with south exposition in Norway, Portugal, southern USA etc.), open water bodies (bathing) and contamination of clothes by washing in streams in endemic regions; systemic leprosy occurs occasionally in feral ninebanded armadillos.
>
> Transmission mode: airborne – human to human transmission (thus anthro-ponosis); according to J. Kazda a L.W. Irgens transmission from environment either by ingestion of infected water (conducted by wood pipe-lines down-hills in Norway) or by direct contact (scratches, from mother to child) is also possible.
>
> Human disease: leprosy (Hansen's disease) – a chronic infection character-istic by skin, nervous or even general manifestations, without appropriate treatment leading in mutilations.
>
> Bio-containment: BSL-2.
>
> Treatment: long-term (6–12 months), with a combination of rifampicin, Dapsone, and clofazimine.
>
> Diagnosis: microscopy of skin scrapings (staining according to Ziehl-Neelsen); it does not grow in vitro, while replicating in armadillo *Didelphis* and "nude" laboratory mice.
>
> Geographical distribution: formerly worldwide, very limited at present.

8.3.33 Family Corynebacteriaceae [*Order* Actinomycetales]

Corynebacterium ulcerans*, *C. pseudotuberculosis

Short irregular Gram-positive rods 1–3×0.4 µm, aerobic and non-motile.

> Source of infection (natural host range): cattle, sheep, horse, dog, cat, pig and monkey (only *C. ulcerans*), occasionally other mammals; human.
>
> Animal disease: pneumonia, abscesses and lymphadenitis.
>
> Transmission mode: alimentary (raw milk), percutaneous (biting by monkey), by contact. Occupational risk in farmers and vets.
>
> Human disease: pharyngitis (*C. ulcerans*) similar to diphtheria, wound infec-tions (*C. pseudotuberculosis*) and regional lymphadenitis, pneumonia; a rare disease.
>
> Bio-containment: BSL-2.
>
> Diagnosis: cultivation of swabs (resp. smears) originated from wounds, milk (BA, selective agar with tellurate, Loeffler medium slants,

Lowenstein-Jensen agar, some other media for isolation of mycobacteria); inoculation of guinea pigs; detection of toxin.

Treatment: erythromycin, antitoxin.

Prevention: vaccination (diphtheric toxoid).

Geographical distribution: Great Britain, but obviously worldwide.

8.3.34 *Family* **Actinomycetaceae** [*Order* **Actinomycetales**]

Arcanobacterium pyogenes

Syn. *Corynebacterium pyogenes.* Slim Gram-positive rods fragmenting gradually into small coccobacilli, aerobic and non-motile.

Source of infection (natural host range): cattle, sheep, goat, pig, horse and other mammals; human.

Animal disease: abscesses, polyarthritis, lymphadenitis.

Transmission mode: by contact (via biting).

Human disease: purulent wound infections after biting and painful regional lymphadenitis, arthritis, sometimes sepsis; a quite rare disease.

Bio-containment: BSL-2.

Diagnosis: cultivation of scumming from wounds (BA, selective agar with tellurate); inoculation of guinea pig; detection of toxin.

Treatment: erythromycin.

Geographical distribution: probably worldwide.

8.3.35 *Family* **Nocardiaceae** [*Order* **Actinomycetales**]

**Rhodococcus equi*

Synonym *Corynebacterium equi*. Pleomorphic coryneform Gram-positive, partially acidoresistant, aerobic cocci to rods (coccobacilli), forming short filaments with symptoms of branching.

Source of infection: soil enriched with faeces of domestic mammals (*R. equi* is a commensal of their gastrointestinal tract), in which is the rhodococcus able to replicate.

Animal disease: acute or chronic bronchopneumonia with lung abscesses (sometimes lethal) of foals; lymphadenopathy in pigs.

Transmission mode: aerogenic, less often alimentary, contact with horses.

Human disease: rhodococcosis – fever, nausea, vomiting, diarrhoea, dizziness, granulomatous bronchopneumonia (opportunistic infection in immunocompromised patients), often lethal (fatality rate about 50%); sometimes lymphadenitis and abscesses in the CNS, liver, kidneys and other organs, skin lesions in immunocompetent persons (mainly childrens) after injury may

also occur. In patients with AIDS, the disease is often chronic and recurrent, because R. equi can survive intracellularly in macrophages. Incidence is quite low, about 200 cases have been described since the disease description in 1967.

Bio-containment: BSL-2.

Diagnosis: cultivation of blood, sputum, bronchial and tissue samples (BA, Czapek-Dox agar; pink colonies) and detection of "equi" factor (CAMP test: synergistic haemolysis against *Staphylococcus aureus* and other bacteria: partial haemolysis of *S.a.* turns into complete in proximity of *R.e.* colonies due to the effect of rhodococcal cholesteroloxidase); histopathology, acidoresistant staining (partially acidoresistant).

Treatment: long-term (2–9 months or even lifelong treatment) – rifampicin, vancomycin, erythromycin, gentamicin, imipenem, carbapenem, meropenem (often in combination of two drugs, e.g. sulbactam + cefoperazone; confirmation of in vitro sensitivity is needed).

Geographical distribution: probably worldwide.

Nocardia asteroides, *N. brasiliensis*

Aerobic filaments of mycelial character, fragmenting into pleomorphic Grampositive rods to cocci, forming also aerial mycelium on solid substrates.

Source of infection: soil, plant remnants.

Animal disease: nocardiosis – infection of subcutis and lymph nodes (dog, cat), sporadically mastitis and pneumonia in cattle.

Transmission mode: aerogenic, percutaneous.

Human disease: nocardiosis pulmonary, cutaneous (actinomycetoma), visceral, even CNS affection (by dissemination from lungs, about 30% of cases); fatality rate 80% in CNS forms.

Bio-containment: BSL-2.

Diagnosis: microscopy of sputum, pus or CSF (Gram and acidophilic staining), cultivation (BA etc.); X-ray computed tomography.

Treatment: cotrimoxazole, sulphonamides in long-term usage; some strains sensitive to ampicilin, amikacin, cephalosporines and other antibiotics; surgical intervention (abscesses).

Geographical distribution: worldwide, however sporadic; *N. brasiliensis* occurs more frequently in (sub)tropics.

8.3.36 *Families* Thermomonosporaceae, Streptomycetaceae [*Order* Actinomycetales]

Actinomadura madurae, *A. pelletieri*, *Streptomyces somaliensis*

Source of infection: soil, vegetation.

Animal disease: (actino)mycetoma.

Transmission mode: percutaneous (injury – thorns etc.).

Human disease: actinomycetoma predominantly on lower extremities. It is distinguishable from (eu)mycetoma caused by fungi.

Bio-containment: BSL-2.

Diagnosis: microscopy of pus or biopsy (Gram and acidophilic staining), cultivation, X-ray examination.

Treatment: cotrimoxazole, streptomycin, amikacin; surgical intervention.

Geographical distribution: America, Africa, Arabic Peninsula – semiarid habitats in areas with dry and warm climate.

8.3.37 *Family* Dermatophilaceae [*Order* Actinomycetales]

Dermatophilus congolensis

Facultatively anaerobic microbe with heavily septate hyphae, dividing both transversally and longitudinaly, forming characteristic packages of Gram-positive coccoid cells; motile cells (spores) are also recognized, 0.3–0.5 µm.

Source of infection (natural host range): cattle, sheep, horse, mammals in ZOO, less often dog and cat (soil source of infection is disputable).

Animal disease: dermatophilosis (cutaneous streptothrichosis, sometimes falsely regarded as mycotic dermatitis) – pustules and papules, occasionally lethal (mainly in tropics). Symptoms increase in severity with infestation of the affected animal by ticks of the genus *Amblyomma*.

Transmission mode: percutaneous (by direct contact); possibly also by arthropods (ticks – *Amblyomma variegatum*, diptera – mechanically).

Human disease: dermatophilosis – acute to chronic pustules and ulcers on hands.

Bio-containment: BSL-2.

Diagnosis: microscopy of epidermal scales (Giemsa staining), histology, cultivation (without antibiotics – BHI, BA, while SGA not).

Treatment: streptomycin, penicillin.

Geographical distribution: areas with humid climate in Africa, Australia, USA and Europe (Scotland, the Netherlands, Bulgaria).

8.4 Fungi

In this chapter filamentous (with mycelium) and yeastlike eukaryotic microorganisms are dealt with. The taxonomic nomenclature of fungi differentiates two forms according to their life cycle: the anamorph is the asexual phase with asexual spores (conidia), or sterile; the teleomorph is the sexual phase with fruiting bodies and sexual spores (ascospores, basidiospores or zygospores). The complex of all developmental forms of the fungus (i.e., the teleomorph including all its anamorphs) is regarded as a holomorph, the binomical determination of which is identical with a teleomorph.

Fungi (micromycetes) that are pathogenic for vertebrates cause two basic clinical syndromes of mycoses: (1) superficial diseases with lesions on the skin and its adnexes (nails, hairs etc.), and (2) visceral (deep, organ, systemic) diseases. Among the most common agents causing skin mycoses belong dermatophytes of the teleomorph genus *Arthroderma* (with the anamorphs *Trichophyton*, *Epidermophyton* and *Microsporum*); these dermatomycoses are differentiated according to the source of infection being antropophilic (corresponding to the adjective "anthroponotic" used throughout this book), zoophilic (corresponding to the adjective "zoonotic"), and geophilic (corresponding to the adjective "sapronotic"). Noteworthy, many agents causing visceral mycoses are dimorphic: their saprophytic phase is filamentous, but the parasitic phase appearing in tissues of warm-blooded vertebrates is morphologically entirely different – most often yeast-like, or spheruliform.

Among the first signs indicating that a patient could be suffering from a visceral mycosis is usually failure of antibiotic therapy (especially when virus infections have been excluded). Immunocompromised patients (AIDS, cancer, malnutrition, post-transplantal and post-operation stages are among the main risk factors) present the highest risk of acquiring visceral mycoses.

Several microfungi (especially of the genera *Aspergillus*, *Fusarium,* etc.) produce mycotoxins often causing dangerous mycotoxicoses affecting diverse organs of vertebrates (including man): liver, intestine, CNS, kidneys or the reproductive system.

The traditional diagnosis of fungal diseases is based on microscopic examination of samples originating from lesions (crusts, scrapings, scales of skin and nails) after their partial digestion in 10% KOH. The microscopy is supplemented with cultivation of the samples, frequently on Sabouraud agar (SGA) with chloramphenicol and cykloheximide simultaneously at temperatures 25–30°C and 37°C for up to 2–3 weeks; some yeasts or visceral fungi require richer media such as BA or BHI agar. The macroscopic pattern of the fungal colony and microscopic morphology of the fungus is evaluated. In addition, biochemical identification sets are generally used for the determination of pathogenic yeasts (*Candida* spp., *Cryptococcus neoformans*). Histological examinations are also applied in invasive visceral mycoses (e.g. histoplasmosis, blastomycosis) – the sections are usually stained according to Schiff's or the more specific Grocott and Gomori methods. Serological examinations of the patients include RDPA, counter immunoelectrophoresis, agglutination and ELISA. Latex agglutination or ELISA is generally used for the detection of antigen. Molecular biology methods (PCR) have evolved into progressive diagnostic tools in recent years.

8.4.1 Family Arthrodermataceae [*Order* Onygenales*, Class* Ascomycetes]

Trichophyton mentagrophytes*, *T. quinckeanum*, *T. erinacei

Teleomorph: *Arthroderma benhamiae*.

Source of infection (natural host range): rodents (mouse, black rat, brown rat, muskrat, nutria, squirrel), hedgehog (*Erinaceus europaeus*, *Atelerix albiventris* – *T. erinacei*) and other mammals.

Animal disease: inapparent course, or trichophytia (favus) in some animals.

Transmission mode: by direct or indirect contact (zoophilic dermatophytes).

Human disease: dermatophytosis (trichophytia, favus).

Bio-containment: BSL-2.

Diagnosis: symptoms; microscopy of affected epidermis, hair and nails samples (preparate in 10% KOH), cultivation (SGA with chloramphenicol and cykloheximide).

Treatment: topical fungicide ointments; oral fungicides (griseofulvin, itraconazole).

Prevention: vaccine for animals, e.g. Trichopelen (Czech Bioveta).

Geographical distribution: worldwide.

Trichophyton verrucosum

Source of infection (natural host range): cattle (a typical zoophilic dermatophyte).

Animal disease: trichophytia (ringworm).

Transmission mode: by contact (arthrospores in hairs may be viable for more than 1 year).

Human disease: dermatophytosis (trichophytia).

Bio-containment: BSL-2.

Diagnosis: see prior species.

Treatment: griseofulvin perorally, topically imidazoles (econazole, ketoconazole etc.), Hexadecyl spray.

Prevention: vaccine Trichoben (Bioveta) for calves.

Geographical distribution: worldwide.

Trichophyton simii

Teleomorph: *Arthroderma simii*.

Source of infection (natural host range): primates, occasionally domestic fowl.

Animal disease: dermatophytosis.

Transmission mode: by contact.

Human disease: dermatophytosis (trichophytia).

Bio-containment: BSL-2.

Diagnosis and therapy: see prior species.

Geographical distribution: India, Africa, South America (Brazil).

Microsporum canis

Teleomorph: *Arthroderma otae*.

Source of infection (natural host range): cat, dog.
Animal disease: inapparent course (symptomless carriage), or else dermatophy-
    tosis.
Transmission mode: by contact (zoophilic dermatophyte).
Human disease: dermatophytosis.
Bio-containment: BSL-2.
Diagnosis and therapy: see prior species.
Prevention: vaccine Biocan M (Bioveta).
Geographical distribution: worldwide.

Microsporum persicolor

Teleomorph: *Arthroderma persicolor.*

Source of infection (natural host range): rodents, insectivores (hedgehog).
Animal disease: sporadically dermatophytosis.
Transmission mode: by contact.
Human disease: dermatophytosis (microsporia).
Bio-containment: BSL-2.
Diagnosis and therapy: see prior species.
Geographical distribution: Europe, Canada.

Microsporum gypseum, *M. fulvum*

Teleomorph: *Arthroderma gypsea*, *A. fulva*, *A. incurvata*.

Source of infection: soil (a typical geophilic dermatophyte).
Animal disease: sporadic dermatophytosis.
Transmission mode: contact with contaminated soil (e.g., in gardeners).
Human disease: dermatophytosis (microsporia).
Bio-containment: BSL-2.
Diagnosis and therapy – see prior species.
Geographical distribution: worldwide.

8.4.2 *Family* Gymnoascaceae [*Order* Onygenales]

Coccidioides immitis, *C. posadasii*

Closely related species. The teleomorph has not yet been recognized, but according
to recent molecular studies of the anamorph it could belong to this family (because
the close genomic relationship with *Uncinocarpus reessi,* a gymnoascacean fungus).
A dimorphic fungus: filamentous form with arthrospores 4–6×2–3 μm; but
spherules with diameter 20–60 μm containing endospores 2–5 μm in the tissues.

Source of infection: soil in arid and semi-arid areas (mainly in and around rodent burrows).

Animal disease: inapparent course or bronchopneumonia.

Transmission mode: aerogenic – by inhalation of arthrospores (released from the soil during strong winds, dusty storms, ground works, earthquakes), highly contagious agent. Frequent infections in persons occurring in natural foci of the disease (farmers, military personnel, tourists). Positive intradermal tests have been found in inhabitants of endemic areas at high prevalence rates (up to 90%).

Human disease: coccidioidomycosis – primarily febrile pulmonary disease with skin erythema; about 60% of cases are subclinic, but dissemination (progressive form) may occur in approximately 1% of patients with the fungus dissemination into different organs including skin, bones or CNS, with fatality rate of 40% in the treated disseminated form. Very severe course of infection is described in immunocompromised patients; increased susceptibility and higher risk for disseminated coccidioidomycosis is also observed in Philippinos, Afroamericans and Indians. A total of 4,500 new cases have been recorded in California in 1992 (majority of cases in San Joaquin Valley: the disease is sometimes called "Valley fever" in America). Human incidence rate in the area of Tulare (California) has been evaluated as 41/100,000 population in 1991, and up to 54/100,000 population in Arizona during 1998–1999. A total of c. 900 out of 5,300 inmates at the central Californian "Pleasant Valley State Prison" contracted coccidioidomycosis in the years 2004–2006, and at least 12 died from it. The whole California reported c. 3,000 cases of Valley fever in 2006. Several imported cases of coccidioidomycosis have been reported annually in Europeans returning from a visit to endemic US areas (probably underreported due to underdiagnosis).

Bio-containment: BSL-3 (a high risk of laboratory-acquired infection).

Diagnosis: microscopy (biopsy, sputum, pus, CSF: spherules), serology (CFT, precipitation), intradermal test (coccidioidin); cultivation (SGA).

Treatment: amphotericin B, ketoconazole (not in the CNS forms), fluconazole, itraconazole, triazole.

Prevention: vaccine, but insufficiently available.

Geographical distribution: southwestern USA (xerothermic, mainly desert areas – California, Nevada, Arizona, Utah, New Mexico and south Texas), Mexico, less often semiarid areas of Central and South America (e.g. Venezuela, Brazil).

8.4.3 *Family* Ajellomycetaceae [*Order* Onygenales]

Histoplasma capsulatum, *H. duboisii*

Teleomorph: *Ajellomyces capsulatus*. A dimorphic fungus with a filamentous conidial form in the saprophytic phase, while a yeast-like budding form in the host's tissue; intracellular parasite.

Source of infection: soil with droppings of birds (starling, American blackbirds, cowbirds, oilbird) and bats in their communal roosting sites and gathering places (caves, parks, groves etc.).

Animal disease: inapparent course or lymphoreticulitis, enteritis; sporadically death in dogs. A closely related species, *H. farciminosum*, causes histoplasmosis in horses.

Transmission mode: aerogenic (ground works on bird roosting sites, demolition of old buildings, visits of caves contaminated with bat and oilbird droppings).

Human disease: histoplasmosis – primarily a pulmonary disease characterized with pneumonia and lymphadenopathy, similar to tuberculosis (lung calcification), with a good prognosis; however, generalized or progressive clinical forms (approximately 1% of all cases) may result in hepatosplenomegaly, the CNS infection, ulceration of digestive tract and other severe manifestations with high lethality. Pulmonary histoplasmosis might be chronic, and with relapses. The course of disease is severe in immunocompromised patients (e.g., AIDS) as well. *H.d.* is the agent of African histoplasmosis with predominantly subacute course and formation of cutaneous or bone granulomas without lung affection.

Bio-containment: BSL-3.

Diagnosis: chest X-ray, microscopy (Gram and Giemsa staining) and cultivation of sputum or tissue lesions (SGA, 25–30°C: production of characteristic conidia, and demonstration of conversion of isolate M → Y at 37°C), liver or lung biopsy in severe forms (microscopy-silver stain); serology (RDPA, ELISA, CFT, IFA, latex agglutination), intradermal test (histoplasmin – retrospective demonstration of exposition).

Treatment: amphotericin B, ketoconazole itraconazole.

Geographical distribution: nearly worldwide. *H. capsulatum* is relatively frequent in the USA (natural foci are present in e.g. the basin of Mississippi and Ohio rivers), Central and South America, Africa, India and southeastern Asia (e.g., first indigenous case reported in Taiwan in 2005), while infrequent occurrence has been reported in Europe where, e.g., 118 cases were documented in 1995–1999 (but only eight obviously autochthonous in Italy, UK, Turkey and Germany); *H. duboisii* has been recorded in Africa only.

*Blastomyces dermatitidis

Teleomorph: *Ajellomyces dermatitidis*. A dimorphic fungus: normally filamentous, but yeast-like forms are produced in the tissue at 37°C.

Source of infection: soil; dog (equids). The specific reservoir (ecological niche) unknown, but the fungus habitat seems to be associated with waterways in North America.

Animal disease: ulcerative changes.

Transmission mode: aerogenic, by contact.

Human disease: (North American) blastomycosis – pulmonary, occasionally cutaneous, mucocutaneous as well as generalized (disseminated) forms with high lethality.

Bio-containment: BSL-2/3.

Diagnosis: chest X-ray, microscopy of sputum, pus and biopsy (yeast-like forms), cultivation (SGA), serology (RDPA, ELISA).

Treatment: amphotericin B, ketoconazole itraconazole.

Geographical distribution: North America. Some cases reported in Africa, Asia and Europe.

Emmonsia crescens

Teleomorph: *Ajellomyces crescens*. A dimorphic fungus, very closely related to genera *Histoplasma* and *Blastomyces* (based on 18S rRNA sequences), with small spherical to oval conidia (2–3 µm) in saprophytic phase, and with thick-walled spherules called adiaspores with a diameter up to 500 (700) µm (whereas close species *Emmonsia parva*, with unproven pathogenicity for man, has spherules only up to 40–50 µm) in the lung tissue; granulomas.

Source of infection: soil (rhizosphere), burrows of mammals.

Animal disease: pulmonary emmonsiosis (adiasporomycosis, adiaspiromycosis) of mammals, mainly in rodents. Emmonsiosis caused by *E. parva* occurs in steppe and desert rodents.

Transmission mode: aerogenic (from the soil). Enhanced occupational risk of infection (farmers and persons handling with soil–diggers).

Human disease: pulmonary emmonsiosis (adiasporomycosis, adiaspiromycosis), occasionally with fever, cough, dyspnoea, exceptionally with lethal course. A number of severe cases of the disease have been recorded in Brazil and Argentina. Several benign cases have been documented in the former Czechoslovakia.

Bio-containment: BSL-2.

Diagnosis: chest X-ray, lung biopsy (adiaspores), cultivation (SGA).

Treatment: amphotericin B, ketoconazole.

Geographical distribution: worldwide (*E. crescens*).

Paracoccidioides brasiliensis

Teleomorph: supposedly *Ajellomyces* (based on 18S rRNA sequences of the anamorph). A dimorphic fungus: filamentous, but multipolarly budding yeast-like form in the tissue.

Source of infection: soil, plant substrates. A number of isolates have been obtained from the armadillo *Dasypus novemcinctus* in Brazil; armadillos obviously play an important role in the ecology of this fungus (hosts, reservoir?), but they are uncertain as the source of human infection.

Transmission mode: aerogenic (farmers), by contact, alimentary.

Human disease: paracoccidioidomycosis (South American blastomycosis) – the fungus invades either nasal and oral mucosa and skin (mucocutaneous form: ulcerative granulomas) or predominantly lymphatic system (the lymphatic form), eventually disseminate (the visceral form, affecting the lungs, intestine, liver).

Bio-containment: BSL-2/3.

Diagnosis: microscopy (tissue, pus, sputum), biopsy, cultivation (SGA); RDPA.

Treatment: amphotericin B, ketoconazole.

Geographical distribution: South and Central America.

*Lacazia loboi

The fungus is genomically closely related to *P. brasiliensis*.

Source of infection: marine ecosystem – vegetation, soil; dolphins (?).

Transmission mode: contact – percutaneous (skin traumatized by plant thorns or insect bites).

Animal disease: lobomycosis in bottlenose dolphins (*Tursiops truncatus, T. aduncus*) and Guiana dolphin (*Sotalia guianensis*).

Human disease: lobomycosis – chronic, granulomatous lesions on the skin and in subcutaneous tissues, similar as in dolphins.

Bio-containment: BSL-2.

Diagnosis: microscopy (biopsy and histopathology: spherical, thick-walled and yeast-like cells in the tissue); the organism has not yet been cultured *in vitro*.

Treatment: surgery; clofazimine with or without itraconazole, and with fluorocytosine.

Geographical distribution: coastal areas in the South and Central America, less common in southern USA (Florida, Carolina), South Africa, and exceptionally in Europe.

8.4.4 *Family* Ophiostomataceae [*Order* Ophiostomatales]

*Sporothrix schenckii

Teleomorph: *Ophiostoma*. A dimorphic fungus: filamentous, but usually yeast-like forms occur in the tissue.

Source of infection: soil, plant remnants, cornstalks, woody plants; sphagnum moss, less often cat.

Animal disease: furunculous dermatitis, lymphadenitis, sometimes disseminated form.

Transmission mode: by contact (injury by thorns and splinters; or scratching/biting by infected cats: 24 cases in Brazil); less often aerogenic (an outbreak in South-African mines).

Human disease: sporotrichosis – chronic pyogenic granulomatous disease of the skin, subcutis, mucosa and lymph nodes. A number of cases of miners in South African (diamond and gold) mines (occupational disease). A total of 41 human cases have been reported in Australia between 2000 and 2003 (the source was contaminated hay). In 1998, a large epidemic occurred in Brazil which continued to 2004 with a total of 759 human cases (the source were infected cats).

Bio-containment: BSL-2.

Diagnosis: microscopy (biopsy), cultivation (SGA).

Treatment: potassium iodide (very effective by local use), amphotericin B (in disseminated forms, i.v. application).

Geographical distribution: mainly (sub)tropics (America, Africa), Australia, rarely in Europe.

8.4.5 *Family* Eurotiaceae [*Order* Eurotiales]

Penicillium marneffei

It is the only dimorphic (yeast-like arthrospores in the tissue) and pathogenic species of *Penicillium*.

Source of infection (natural host range): large bamboo rat (*Rhizomys sumatrensis*) and lesser bamboo rat (*Cannomys badius*).

Animal disease: inapparent course.

Transmission mode: aerogenic.

Human disease: disseminated penicillosis (high fever, skin lesions, intestinal, pulmonary etc. symptoms), more often in immunocompromised patients (HIV); very high fatality rate – 75% in treated patients, 100% in untreated cases.

Bio-containment: BSL-2/3, highly contagious.

Diagnosis: microscopy and cultivation of samples from biopsy.

Treatment: amphotericin B, itraconazole.

Geographical distribution: Southeast Asia, India (state Manipur), China (Guangdong province).

**Aspergillus fumigatus*, *A. flavus*

Source of infection: plant remnants, hay, compost (thermotolerant fungi).

Animal disease: pulmonary aspergillosis (pneumomycosis); abortion of cattle; aflatoxicosis.

Transmission mode: aerogenic; alimentary intoxicosis (aflatoxins: *A. flavus* – contaminated nuts, dried tropical fruit, succade etc.).

Human disease: pulmonary aspergillosis (*A. fumigatus*), or aspergilloma (colonization of lung cavities with mycelium, encapsulation); secondary dissemination and invasion of the kidney, heart (endocarditis), CNS and paranasal cavities (granulomas), spleen, liver, stomach, mainly in immunocompromised persons (e.g., with tuberculosis, AIDS or cancer); allergic aspergillosis (hypersensitivity in persons with atopic eczema and asthma, then "syndrome of maltster's lungs" – infection originates from barley); aspergillosis is the most frequent human visceral mycosis, with fatality rate of about 10%; further aflatoxicosis (especially *A. flavus* – aflatoxins damage the liver and are cancerogenic).

Bio-containment: BSL-2.

Diagnosis: microscopy (sputum, biopsy), cultivation (SGA without cycloheximide, Czapek-Dox agar), serology (RDPA, latex agglutination, electrophoresis, ELISA), intradermal test.

Treatment: potassium iodide, amphotericin B, ketoconazole, itraconazole, voriconazole and newly posaconazole; surgical resection of wounded tissue (aspergilloma); corticosteroids (allergic aspergillosis).

Geographical distribution: worldwide.

8.4.6 *Family* Hypocreaceae [*Order* Hypocreales]

*Fusarium solani, F. oxysporum, F. moniliforme

Characteristically curved (crescent-shaped) septal macroconidia. Teleomorphs belong to ascomycetous genera *Gibberella*, *Nectria* etc.

Source of infection: plant remnants.

Animal disease: fusariosis; also fusariotoxicosis (the toxins are fumonisine B1, zearalenone, deoxynivalenon – they damage nervs, kidney, lungs, heart, uterus).

Transmission mode: by contact (percutaneously, *via conjuctivae*).

Human disease: fusariosis – keratomycosis (ocular infection), onychomycosis. For instance, about 100 cases of *Fusarium* keratitis were reported in people using contact lenses from Singapore and Hongkong in 2005–2006, and a similar disease (130 cases) was notified in USA in the same 2 years.

Bio-containment: BSL-2.

Diagnosis: microscopy of the affected tissue, cultivation.

Treatment: amphotericin B.

Geographical distribution: worldwide.

8.4.7 *Order* Dothideales [*Class* Ascomycetes]

Madurella mycetomi*, *M. grisea*, *Scedosporium apiospermum*, *Exophiala jeanselmei*, *Leptosphaeria senegalensis*, *Phialophora* spp., *Neotestudina rosatii

Teleomorph of *S. apiospermum* is *Pseudallescheria boydii* (*Microascaceae*). Teleomorph of *L. senegalensis* belongs to *Dothideales*.

Source of infection: soil, plants.

Animal disease: maduromycosis.

Transmission mode: by contact, percutaneously (injury) – people working on fields (farmers).

Human disease: maduromycetoma, eumycetoma (mycotic mycetoma) – granulomas and abscesses with fistula in skin and subcutis, rarely on bones, sometimes dissemination; it is necessary to differentiate this disease from mycetoma caused by actinomycetes (i.e. actinomycetoma).

Bio-containment: BSL-2.

Diagnosis: microscopy (pus from fistula, biopsy), cultivation (SGA without cycloheximide etc.).

Therapy (less effective): ketoconazole, amphotericin B; resection of the affected tissue.

Geographical distribution: tropics and subtropics (America, Africa, Asia, Australia).

8.4.8 *"Family"* Dematiaceae [*Class* Hyphomycetes]

Teleomorphs mostly unknown at present.

Phialophora verrucosa*, *Fonsecaea compacta*, *F. pedrosoi*, *Cladophialophora carrionii*, *Exophiala jeanselmei*, *Rhinocladiella aquaspersa

Teleomorphs unknown at present.

Source of infection: plant remnants.

Animal disease: chromoblastomycosis.

Transmission mode: by contact (percutaneously) – farmers.

Human disease: chromoblastomycosis (chromomycosis) – chronic disease of skin and subcutis characterized by verrucose lesions covered with scurfs (mainly on extremities, especially on legs).

Bio-containment: BSL-2.

Diagnosis: microscopy of the tissue (typical brown spherical multiseptate cells and brown hyphae), cultivation (SGA).

Treatment: amphotericin B, fluorocytosine.
Geographical distribution: worldwide, predominantly areas with warm climate.

Exophiala dermatitidis, *E. spinifera*, *Ochroconis gallopavum*, *Phialophora* **spp.**, *Cladophialophora bantiana*, *Scedosporium proliferans*, *Ramichloridium mackenziei*, *Drechslera* **spp.**, *Curvularia* **spp.**, *Alternaria alternata*, *Aureobasidium pullulans*

Teleomorphs, if known, belong to ascomycetes.

Source of infection: plant remnants.
Animal disease: phaeohyphomycosis.
Transmission mode: percutaneous.
Human disease: phaeohyphomycosis – nonspecific solitary lesions in sub-
cutis, rarely inside the affected tissue, keratitis, occasionally a disseminated
infection (cerebral abscesses).
Bio-containment: BSL-2.
Diagnosis: microscopy of wounded tissue, cultivation (SGA).
Treatment: amphotericin B, ketoconazole; surgical intervention (excision).
Geographical distribution: worldwide.

Hortaea (Exophiala) werneckii

Source of infection: soil (a halophilic saprophyte).
Animal disease: unknown.
Transmission mode: percutaneous.
Human disease: tinea nigra palmaris – dark lesions localized on the palm of one
or both hands, occasionally on the sole.
Bio-containment: BSL-2.
Diagnosis: microscopy of wounded tissue, cultivation (SGA).
Treatment: ketoconazole.
Geographical distribution: only in tropical and subtropical areas.

Acremonium kiliense, *A. recifei*, *Scytalidium hyalinum*

Source of infection: plant remnants.
Animal disease: hyalohyphomycosis.
Transmission mode: percutaneous.
Human disease: hyalohyphomycosis – usually ulceratose and nodulose lesions
in skin and subcutis, keratitis (an uncommon disease).
Bio-containment: BSL-2.
Diagnosis: microscopy of wounded tissue, cultivation (SGA).
Treatment: ketoconazole.
Geographical distribution: worldwide.

8.4.9 *Family* Mucoraceae [*Order* Mucorales*, Class* Zygomycetes]

*Absidia corymbifera, Apophysomyces elegans, Rhizopus oryzae, Rhizomucor pusillus, Mucor spp.

Source of infection: dead plant remnants and other abiotic substrates.
Animal disease: mucormycosis of gastrointestinal tract, abortion.
Transmission mode: alimentary, aerogenic.
Human disease: mucormycosis (zygomycosis) – cutaneous (wound infec-
    tions), gastrointestinal, cranial, pulmonary or disseminated; the most serious
    and often fatal is rhinocerebral zygomycosis (spreading from nasal mucosa
    through nasal cavities and orbit into the brain; predisposition factors: dia-
    betes, leukaemia, lymphoma, uraemia, immunosuppression).
Bio-containment: BSL-2.
Diagnosis: microscopy (biopsy: wide non-septate hyphae), cultivation (SGA);
    often diagnosed only post mortem at autopsy.
Treatment: amphotericin B (itraconazole, posaconazole, caspofungin); iron
    chelation, hyperbaric oxygen, cytokine therapy; resection of the necrotic
    tissue.
Geographical distribution: worldwide, ubiquitous.

8.4.10 *Family* Entomophthoraceae [*Order* Entomophthorales]

*Basidiobolus ranarum, Conidiobolus spp., Entomophthora

Source of infection: plant remnants, water, soil; reptiles, amphibians.
Animal disease: entomophthoromycosis (phycomycosis).
Transmission mode: by contact (traumatic inoculation), exceptionally also
    alimentary.
Human disease: entomophthoromycosis (phycomycosis) – subcutaneous and
    mucosal (nasal) tumor-like changes.
Bio-containment: BSL-2.
Diagnosis: microscopy (biopsy: hyphal elements sparse and often fragmen-
    tary; occasional septations within hyphae – contrary to mucormycosis),
    cultivation.
Treatment: potassium iodide, amphotericin B.
Geographical distribution: worldwide, but mostly in Africa, India and South
    America.

8.4.11 *Family* Filobasidiaceae [*Order* Filobasidiales *(Sporidiales), Class* Heterobasidiomycetes]

*Cryptococcus neoformans, C. gattii

Teleomorph: *Filobasidiella neoformans. C. neoformans* complex produces spherical
yeast-like cells 2–10 μm with a mucopolysaccharide capsule; serotypes A to D.

Earlier regarded as two closely related subspecies of *C. neoformans,* today they are treated as separate species; *C. gattii* corresponds to the serotypes B and C. The species *C. neoformans* is now differentiated into two variants: *C. neoformans* var. *grubyi* (serotype A), and *C. neoformans* var. *neoformans* (serotype D, and hybrid serotype AD).

> Source of infection: soil contaminated with bird droppings in *C. neoformans* (faeces of pigeons and some ornamental birds present a nutritive substrate for the growth of serotypes A, D, and AD; pigeon excreta may contain as many as about 5.10^7 of *C. neoformans* cells per gram). In *C. gattii*, some species of trees (mainly eucalyptus) – leaves, bark, wood chips, decaying wood or rhizosphere present the fungus habitat, i.e. reservoir of infection.
>
> Animal disease: cryptococcosis – pneumonia, meningitis; *C. gattii*: disease in koalas, cats, dogs, ferrets, and other mammals.
>
> Transmission mode: aerogenic by inhalation; less often by contact. Increased risk of transmission in persons, who come in contact with contaminated bird (largely feral pigeon) droppings (occupational exposure: road sweepers, workers on roofs, lofts and garrets, diggers).
>
> Human disease: cryptococcosis – chronic to subacute pulmonary, skin or disseminated form (meningoencephalitis), with the fatality rate about 20–30%; often in patients suffering from AIDS and other immunocompromising diseases. Small discrete nodules, scarification and encapsulation of focuses similar to those in tuberculosis may occasionally occur in pulmonary form of the disease. While cryptococcosis caused by *C. neoformans* commonly occurs in immunocompromised patients, the disease due to *C. gattii* largely occurs in immunocompetent persons and causes headache, persistent cough, shortness of breath, and the case fatality rate is about 9%. For instance, 272 cases (as of July 2010) of predominantly pulmonary cryptococcosis caused by *C. gattii* highly virulent genotype VGII have been reported on the Vancouver Island and adjacent inland areas in British Columbia (Canada) since 1999; and after 2003, the disease has spread to northwest U.S. states Washington, Oregon, California and Idaho, causing 60 human cases (15 ended in death), as reported in July 2010, in 2005–2006.
>
> Bio-containment: BSL-2.
>
> Diagnosis: microscopy (Indian ink negative preparation for detection of capsula) and cultivation (SGA or selective agar with *Guizotia*) of CSF, pus, urine, prostatic excretion; serological demonstration of polysaccharide antigen in CSF (CFT, latex test, ELISA).
>
> Treatment: combination of amphotericin B (the most effective is its liposomal form) + flucytosine, ketoconazole, fluconazole.
>
> Geographical distribution: *C. neoformans* worldwide. *C. gattii* is mainly distributed in tropical and subtropical areas: Australia, New Zealand, Papua New Guinea, southeastern Asia, India, central and southern Africa, South America (Brazil), Mexico, south California, but lately also in British

Columbia and northwestern USA, occasionally southern and central Europe (Spain, France, Italy, Greece, Austria, Germany).

8.4.12 *Order* Ustilaginales [*Class* Heterobasidiomycetes]

(**) *Pneumocystis jirovecii* (Synonym *P. carinii*)

Formerly classified within *Protozoa*, but the rRNA analysis revealed close similarity with smut fungi.

Source of infection (natural host range): brown rat and other rodents, domestic animals (dog); man (there are anthroponotic and zoonotic strains of this species).

Animal disease: inapparent course (sometimes massive findings of cysts in lungs of rabbits and rodents).

Transmission mode: aerogenic, by contact.

Human disease: pneumocystosis, often in immunocompromised persons (leukaemia, AIDS, malnutrition); generally subfebrile. [Interstitial pneumonia of newborns is an anthroponosis with fatality rate 0–40% (aetiology of this disease was elucidated by Vaněk and Jírovec in 1952)].

Bio-containment: BSL-2.

Diagnosis: microscopy of sputum (IF determination of antigen), bronchoalveolar lavage, lung biopsy. Giemsa or silver staining of the samples: mature "cysts" (in fact basidia) are 5–8 µm in diameter with double wall and contain eight uninuclear bodies, "sporozoites" (in fact basidiospores), and spots of eosinophilic substance with aggregations of very small spherical forms 1–4 µm, called "trophozoites"; PCR, sometimes serology (CFT, IFA).

Treatment: cotrimoxazole, trimethoprim + sulphamethoxazole, pentamidine, sulphadiazine + pyrimethamine.

Geographical distribution: worldwide.

8.4.13 *Family Pythiaceae* [*Order* Peronosporales, *Class* Oomycetes]

*Pythium insidiosum

A plant pathogen (in nature, biflagellate zoospores attach the water plants), not a true fungus.

Source of infection: water.

Animal disease: a cutaneous and pulmonary disease in domestic mammals (cat, dog, horse, cattle), with chronic ulcerated lesions on the limbs, chest, and abdomen.

Transmission mode: by contact (skin abrasions – e.g., in rice fields).

Human disease: pythiosis – ocular form (keratitis), cutaneous and subcutaneous form, and arterial form with chronic ulcers on the legs, ischaemia, thrombosis of major arteries, and necrosis (fatality rate of c. 40% in the arterial form).

Bio-containment: BSL-2.

Diagnosis: microscopy (biopsy: hyaline, thin-walled hyphae and hyphal fragments resembling zygomycetes – the cell walls are non-parallel), cultivation (e.g., SGA), serology (IFA, immunodiffusion).

Treatment: surgery combined with antifungal compounds (amphotericin B, azoles); in ocular pythiosis, keratoplasty is usually necessary but not always it is successful.

Geographical distribution: tropical and subtropical regions (reported from Thailand, Malaysia, Australia, New Zealand, Haiti).

8.5 Protozoa

8.5.1 Family Trypanosomatidae [Order Kinetoplastida, Class Kinetoplasmidea]

Trypanosoma cruzi

Flagellates of two genotypes (I and II), and two life forms: trypomastigotes 15–20×2 µm with an undulating membrane (operates as a traction propeller) and kinetoplast (a specialized mitochondrion near the base of flagellum); replicating spherical amastigotes (2–6 µm) occur in the tissue (RES and muscle cells) most of them generate new trypomastigotes that disseminate via the blood.

Source of infection (natural host range): dog, cat, pig, goat, cattle, rabbit, rodents (*Neotoma* spp. woodrats, guinea pig), armadillo (*Dasypus*), opossum (*Didelphis albiventris*, reservoir), punaré (*Trichomys apereoides,* reservoir), racoon (*Procyon*), bats, monkeys; man (sporadically).

Animal disease (dog): chronic myocarditis, meningoencephalitis, anaemia.

Transmission mode: bites of tropical haematophagous insects – kissing bugs (*Reduviidae*): *Triatoma infestans*, *T. sanguisuga*, *Rhodnius prolixus, Meccus longipennis, T. rubida* (USA), etc. (trypanosomes develop in their gut and are rubbed with the bugs' excrements into wounds or conjunctiva). Other means of transmission include blood transfusion, organ transplantation, or congenital infection in childbirth, and also alimentary route – 58 food-borne human cases were reported in Brazil in 2007 and 2009, after eating "acai", a food prepared from palm tree of the family *Aracaceae* (fruit, juice). A similar large outbreak also occurred in children from a school in Venezuela: the palm

juice was apparently contaminated with triatomid bugs (their excrements). Most of the Amazonian population consumes acai juice daily.

Feral cycle: exoanthropic (wild) mammals → kissing bugs → mammals.

Domestic cycle: synanthropic mammals, human → kissing bugs.

Peridomestic cycle: involves domestic animals.

Human disease: Chagas disease, American trypanosomiasis – a chronic general disease with cardiomyopathy and CNS affection, it starts after an 1–2 week incubation period; initial symptoms include occuloglandular syndrome called Romaña's sign in 50% of infected people (one-sided conjuctivitis and swelling of the eyelid), enlargement of regional lymph nodes, irregular febrile periods, myalgia; the fatality rate is about 8% in children (myocarditis, meningoencephalitis), lower lethality in adults. Chronic forms (sometimes latent period of 10–20 years): heart arhythmia, dilatation of heart, esophagus ("megaoesoophagus"), gut ("megacolon"), and hepatosplenomegaly; the death usually occurs after perforation ("explosion") of the dilated gut or heart. An estimated 18 million persons are infected in Central and South America, and 15,000–50,000 of them die annually. For example, an average of 50% of Bolivian population was infected in 1994, while the infection rate in endemic areas was as high as 80–90%. Morbidity due to Chagas disease in Honduras was estimated as 5,172/100,000 population in 1996. In 2007, a major alimentary outbreak occurred in Venezuela (1,000 exposed persons; 75% of the cases were observed among students 16-year old or younger; 75% were symptomatic, 28% with cardiac involvement, one child died of acute myocarditis). The disease is ancient: DNA of *T. cruzi* was detected in 4,500–9,000-year old mummies of Indians.

Bio-containment: BSL-2.

Diagnosis: microscopy – blood smear, thick drop (Giemsa staining: flagellar promastigote or trypomastigote phase), biopsy (lymphatic glands: oval unflagellar amastigote phases in tissues and cells of RES – pseudocysts 5×1.5 μm with spherical nucleus and rod-shaped blepharoblasts), intraperitoneal inoculation of infected blood to guinea pig (or to Syrian hamster or suckling mouse), cultivation (enriched BA, so-called NNN agar Novy-McNeal-Nicoll), serology (CFT, RIHA, IFA, ELISA, latex agglutination), intradermal test. Xenodiagnostics may also be used (uninfected kissing bugs from a laboratory rear are fed on patients, after 3–4 weeks the gut content of the bugs is examined for trypanosomes).

Treatment (difficult – no satisfactory drugs are available): nitrofurfuryliden (Nifurtimox), benzonidazole, isometamidium derivates.

Prevention: vaccine unavailable. Control of triatomine bugs (spraying the walls and roofs of houses, self-protection).

Geographical distribution: Central and South America, Mexico, southern USA (a rare human disease in Arizona – a total of 7 cases were reported, but 41% of kissing bugs collected in Tucson were found infected with *T. cruzi* in 2006; occasionally also Louisiana, Texas, Tennessee, and California).

Trypanosoma brucei

Flagellates (15)20–30(40) × 1.5–3 μm with an undulating membrane. Two sub-species: zoonotic *T. b. rhodesiense*, and anthroponotic **T. b. gambiense*, formerly classified as two separate species. They are characteristic by their "immune evasion" strategy due to antigenic variation within a host, caused by switching of surface glycoproteins (VSG genes are responsible).

- Source of infection (natural host range): in *T. b. rhodesiense* antelopes (*Tragelaphus*, *Sylvicapra*), cattle, sheep, goat, swine, dog, African buffalo, warthog, elephant, rhinoceros, hippopotamus, lion, hyena and other wild mammals (reservoir), crocodile (*T. b. rhodesiense*); in *T. b. gambiense* mainly human; but also domestic pig, dog, cattle, sheep, putty-nosed monkey (*Cercopithecus nictitans*), some rodents and carnivores.
- Animal disease: inapparent course [the closely related *T. b. brucei* causes disease of cattle called "nagana", and *T. congolense* fever of cattle named "gambia"; these both trypanosomes are however non-pathogenic for man].
- Transmission mode: tsetse flies (via infected saliva: *Glossina morsitans* [savannah ecosystem] – *T. b. rhodesiense*; *G. palpalis* and *G. tachinoides* [river habitats] – *T. b. gambiense*). Tsetse flies remain infective during their whole life (up to 6 months), the development of trypanosomes into infective stage takes about (12)20–30(40) days.
- Human disease: African sleeping disease, African trypanosomiasis (East-African – *T. b. rhodesiense*, or West-African – *T. b. gambiense*) – irregular high fever attacks without chills, hyperhidrosis, headache, tachycardia, respiratory distress, skin rash, anaemia at the beginning, later lymphadenitis (swollen lymph nodes in the hind trianguloid part of the neck and axillae, called Winterbottom's sign), swollen eyelids, oliguria, neurologic signs (hypersensitivity for touch, hypersomnia, meningoencephalitis); hepatosplenomegaly; acute course (2–4 months: *T. b. rhodesiense*) or chronic (1–6 years: *T. b. gambiense*). Fatality rate is high (90%), 1–2 years after primary infection without specific therapy the patient dies. Re-emergence of the disease has occurred during last years (DR Congo etc.: *T. b. gambiense*). The annual incidence of sleeping sickness in sub-Saharan Africa is estimated as 50,000–70,000 cases.
- Bio-containment: BSL-2.
- Diagnosis: microscopy – thick drop, blood smear, smear of punctate from swollen lymphatic nodes or from CSF (native preparation, Giemsa, IF), serology (IFA, CFT, AR, RDPA, ELISA, RIHA), cultivation (agar), inoculation of laboratory rat or mouse (*T. b. rhodesiense*); novel molecular techniques such as PCR and proteome fingerprinting.
- Treatment: pentamidine (ineffective in late-stage of disease, less effective against *T. b. rhodesiense*) in acute phase or as a prophylaxis; suramin (a trypanocidal agent that is however toxic, causing degeneration of the liver,

kidney and adrenal glands), arsenic preparates (toxic) in chronic phase (CNS affection) such as melarsoprol (the effectivity of this drug has been reduced by some 20% in the last years as demonstrated in DR Congo), tryparsamide, and the latest α-difluoromethylornithine (DFMO), being relatively nontoxic and effective in combination with nifurtimox even in therapy of the CNS phase of the disease.

Geographical distribution: tropics of East Africa – savannah (*T. b. rhodesiense*), West and Central Africa – river basins (*T. b. gambiense*); both subspecies occur simultaneously only in Uganda.

Leishmania major, L. tropica, L. aethiopica, L. donovani, L. infantum, L. mexicana, L. braziliensis, L. chagasi, L. peruviana, L. killicki

Flagellates without the undulating membrane (promastigote form 14–25 × 1.5–3 µm, of the morphological type of "leptomonas"), intracellular parasites of RES cells (dividing spherical amastigote form 2–5–5 × 1.5–2 µm, with a nucleus and kinetoplast) of vertebrates. The taxonomy of leishmaniae is complicated: *L. donovani* complex includes three species (*L. donovani, L. infantum*, and *L. chagasi*); *L. mexicana* complex also encompasses three main species (*L. mexicana, L. amazonensis*, and *L. venezuelensis*); *L. tropica; L. major; L. aethiopica*; and the subgenus *Viannia* is represented with four main species: *L. (V.) braziliensis, L. (V.) guyanensis, L. (V.) panamensis*, and *L. (V.) peruviana*.

Source of infection (natural host range): in cutaneous leishmaniasis rodents (gerbils *Rhombomys*, *Meriones*, *Psammomys* in desert; rats *Mastomys*, *Arvicanthis,* etc. in savannah; punaré *Trichomys apereoides* in Brazil, hyrax *Procavia capensis* in Jordan, Israel and Kenya), sloth, kinkajou, also dog, jackal, fox, goat, buffalo, cattle, horse and human in urban cycle; in visceral leishmaniasis canines, rats (*Rattus rattus*), opposoms and man in the urban cycle.

Animal disease: ulcerative inflammations accompanied with changes on skin and mucosa; in dogs also marked cachexia, alopecia, depigmentation of nose and overgrown claws in the cutaneous form, and swollen lymph nodes, ocular signs, epistaxis and kidney failure in the visceral form; canine leishmaniasis is an emerging zoonotic infection in the Mediterranean region, and in North America.

Transmission mode: sandflies (*Phlebotomus* in the Old World; *Lutzomyia* in the Americas) – by biting and infectious excrements; in the vector, there are flagellar forms (leptomonas), replicating in its midgut. Within the human host, the promastigote forms are ingested by macrophages where they change into amastigote forms. Main vectors of leishmaniasis in the Mediterranean region are e.g., *P. neglectus*, *P. perfilievi*, *P. perniciosus*, *P. papatasi* (*L. major*), *P. arabicus*; elsewhere in the world *P. martini*, *P. orientalis*, *P. chinensis*, *P. sergenti* (*L. tropica*), *P. duboscqui* (*L. major*), *P. alexandri*, *P. argentipes*,

P. longipes (*L. aethiopica*), *P. pedifer* (*L. aethiopica*), *Lutzomyia longi-palpis*, *L. olmeca* (*L. mexicana*), *L. wellcomei* (*L. braziliensis*), *L. carrerai* (*L. braziliensis*). New experimental molecular data surprisingly indicate possible transmission of *L. infanti* by the dog tick *Rhipicephalus sanguineus*, including TOT in the tick (Exp. Parasitol. 125: 184–185, 2010). The transmission of leshmaniasis is also possible from man to man by the blood transfusion, inoculation (sharing contaminated needles), or congenitally.

Cycles: exoanthropic (deserts, savannah, tropical rainforests), and synanthropic (domestical, urban).

Human disease: leishmaniasis, a chronic disease with incubation period from a week up to 1 year (sometimes longer in visceral form) and manifesting in several clinical forms: (1) visceral (*L. infantum*, *L. donovani* ["kala-azar"], in America *L. chagasi*) – disease of RES, with fever, hepatosplenomegaly, lymphadenopathy, cachexia, thrombocytopenia, anaemia, leucopenia, hyperglobulinemia and if untreated with a high fatality rate; (2) cutaneous with skin ulcers on face, arms, and legs (*L. tropica* causes anthroponotic urban form, *L. major* causes zoonotic form, *L. aethiopica* – restricted to East Africa; in America *L. mexicana* causes a disseminated cutaneous form Chicler's ulcer, destructive disease of *otis media*, *L. donovani* in Sri Lanka, *L. peruviana* – Uta disease); two types of cutaneous leishmaniosis are differentiated: (a) *urbanus* ("dry form") – dry ulcer, late ulcerating, with synanthropic cycle (*L. tropica*, *L. aethiopica*); (b) *rusticus* ("wet form") – large acute ulcer, early necrotizing, with exoanthropic cycle (*L. major*). (3) The mucocutaneous "espundia" form occurs in America and affects nasopharyngeal mucosa with deforming lesions and tissue damage in the mouth, ears or nose that resemble leprosis (*L. braziliensis*). *Leishmania*/HIV co-infection is getting an emerging problem worldwide. According to WHO report, 12 million people in the world suffer from leishmaniasis, the number of new cases is about 1.5 million annually and *c.* 50,000 die. In Africa, the mean yearly number of new cases of visceral leishmaniasis is 19,000–24,000. However, 100,000 persons died during a massive outbreak of visceral leishmaniasis in south Sudan between the years 1985 and 1987. Ninety percent of visceral leishmaniasis cases occur in Bangladesh, Brazil, India, Nepal and Sudan; 90% of mucocutaneous leishmaniasis occurs in Bolivia, Brazil and Peru; and 90% of cutaneous leishmaniasis occur in Afghanistan, Iran, Saudi Arabia, Syria, Brazil and Peru. The annual incidence in Europe (southern only) is much lower, about 750 cases. Asymptomatic infections of humans with *Leishmania* are 30−100 times more frequent than the clinical cases. DNA of *L. donovani* was amplified from ancient Egyptian and Nubian mummies 4,000 years old.

Bio-containment: BSL-2.

Diagnosis: microscopy of amastigotes (biopsy – lymph nodes, scrapings, smears: amastigote forms $2.5–5 \times 2$ μm, replicating in monocytes, macrophages; punctate from spleen or sternum in visceral leishmaniasis), cultivation (NNN agar), inoculation of mouse or Syrian hamster (ears, tail),

serology (IFA, ELISA or haemagglutination test in visceral and mucocutaneous leishmaniasis), intradermal test, PCR.

Treatment: derivatives of pentavalent antimony (natrium stilbogluconate, megluminantimonate, pentamidine), pyrimethamine (Daraprim), amphotericin B (the most effective in liposomal form in visceral leishmaniasis: AmBiosome) or ketoconazole (in cutaneous leishmaniasis), cycloguanyl, miltefosine, N-chlorotaurin (*L. donovani*); local injections of ulcers, cryotherapy.

Prevention and risk avoidance: human vaccine unavailable at present. LEISHMUNE vaccine is the first licensed vaccine against canine visceral leishmaniasis caused by *L. donovani* in Brazil. Skin collar with deltametrine can be helpful in dogs. Dog travel to and from endemic areas poses a risk of introducing *L. infantum* to other areas.

Geographical distribution: (semi)arid areas – steppe and savannah in Asia (*L. tropica*, *L. major, L. donovani*), Africa (*L. tropica*, *L. aethiopica*, *L. donovani*) and the Mediterranean (*L. tropica*, *L. infantum*); forest ecosystems in Central and South America (*L. mexicana*, *L. donovani*, *L. braziliensis*, *L. peruviana, L. chagasi*). *L. major* and *L. killicki* occur in Tunisia. In 1999, an outbreak of canine leishmaniasis caused by *L. infantum* was reported in New York, and several other outbreaks followed in Foxhound kennels across the USA. For typical habitats of sandflies, see Photos 5.43 and 5.44.

8.5.2 *Family* Hexamitidae [*Order* Diplomonadida*, Class* Trepomonadea]

(**) *Giardia lamblia* (Synonyms *G. intestinalis*, *G. duodenalis*)

The two life forms are an inactive ovoid cyst (8–15×7–10 µm) and active trophozoite – bilaterally symmetrical pyrifom-shaped flagellate 9–21×6–12 µm with four pairs of flagella, two nuclei and an adhesive disc. Several genomic types: A (anthroponotic), B (zoonotic), and C–G (animal types probably not transmissible to humans). The protozoan was first described by Lambl in 1859, as *Cercomonas*.

Source of infection (natural host range): human; less often rodents (castor, muskrat etc.), dog; rabbit, sheep; contaminated water (e.g., swimming pools). However, the importance of zoonotic transmission in human giardiosis is not clear.

Animal disease: inapparent course.

Transmission mode: alimentary (water contaminated with resistant cysts of rodents), by contact, mechanically via houseflies (*Musca domestica*) and cockroaches; high contagiosity. The cysts have considerable tenacity, and are resistant even to common disinfectants.

Human disease: giardiosis – intestinal form (giardiae adhere on intestine and duodenum mucosa): enteritis with abdominal pain, nausea, fatigue,

diarrhoea, loss of weight and hepatobiliary damage. Extensive epidemics occurred in the 1970s (St. Petersburg, New York, Colorado). Giardiosis is a quite frequent human disease. For instance, the average annual incidence of giardiosis in Czechland was 645 in the period of 1990–2000, and *Giardia* was prevalent in about 1% adults and 4–5% children at the same time.

Bio-containment: BSL-2.

Diagnosis: microscopy of fresh stool using flotation method (4-nucleic cysts; flagellar trophozoites are usually present only in watery stool or in duodenal fluid), enteroscopy.

Treatment: albendazole, metronidazole, mepakrin, furazolidone; acridine dihydrochloride, paromomycin.

Geographical distribution: worldwide.

8.5.3 *Family* Vahlkampfiidae [*Order* Schizopyrenida, *Class* Heterolobosea]

*Naegleria fowleri

Amoeabe free-living in warm water, e.g. in indoor warmed swimming pools (the amoebae are able of replication in sand filters of the pool system). One isolate was recovered from a hot geyser in Yellowstone Park (USA), and a number of others from artesian wells in Arizona. They have two morphological forms: amoeba (a trophozoite) and a dormant cyst stage.

Source of infection: water (warm or warmed-up surface and well water).

Transmission mode: inhalatory – throw nasopharynx mucosa (amoeabe invade into brain along the olfactory nerves, and divide rapidly in the CNS); the cysts are extremely resistant in external milieu.

Human disease: primary amoebic meningoencephalitis (naegleriosis) – purulent, acute (3–10 days), causing haemorrhagic necrosis of the brain; the fatality rate is up to 100% even in young, immunocompetent persons. First epidemics have been observed in Australia in 1965 and also in Czechland in 1962–1965; in the latter country, 16 swimmers died, and other two fatal cases were recorded until 1984.

Bio-containment: BSL-2.

Diagnosis: microscopy of CSF under warm conditions (motility of trophozoites with a diameter of 7–20 μm) or smear (IF), cultivation. Often misdiagnosed as a bacterial meningoencephalitis.

Treatment: amphotericin B (intravenously or intrathecally administered), in combination with fluconazole (or ketoconazole) and rifampicin.

Geographical distribution: probably worldwide, but only sporadic cases (Australia, New Zealand, USA, Czechland, Great Britain, Belgium).

8.5.4 *Family* Acanthamoebidae [*Class* Lobosea*, Phyllum* Rhizopoda]

**Acanthamoeba castellanii*, *A. polyphaga*

Amoebae (trophozoites *c*. 30 μm; cysts 10 μm) free-living in soil and water (cooling, waste, or contaminated seawater – these amoebae are frequently used as waste indicators in polluted water, sometimes they are more specific indicators of pollution than coliform bacteria or *Clostridium perfringens*). Interestingly, these protozoa may serve as obligatory hosts of legionellae and novel endosymbiont species *Protochlamydia naegleriophila* (isolated from immunocompromised patient with pneumonia).

> Source of infection: water (thermal, polluted), soil.
> Transmission mode: inhalatory (nasal mucosa, airways), per conjuctivae (contact lenses), even percutaneously (skin scratches). The cysts are very resistant to dessication, freeze and most disinfectants (including chlorination). Interestingly, *Francisella tularensis* survives in the cysts of *A. castellanii* for at least 3 weeks, and this amoeba can also harbour *Legionella pneumophila* for a long period or may serve as obligatory host of this bacterium.
> Human disease: two main clinical forms – amoebic keratitis (usually in carriers of contact lenses), and chronic granulomatous amoebic meningoencephalitis (dissemination of the agent into CNS; most frequently in immunocompromised patients – e.g., with AIDS). Cutaneous lesions (hard erythematous nodules, papules or ulcers) caused by *Acanthamoeba* have also been described in immunocompromised persons.
> Bio-containment: BSL-2.
> Diagnosis: microscopy of CSF, scraping or rinsing slide prepartions from cornea (IF), confocal microscopy of corneal tissue, serology (IFA), cultivation (on 1.5% non-nutrient agar plates with a growth of e.g. *Escherichia coli* – amoebae feed on the bacteria, or on cell cultures – amoebae cause cytopathic effect), PCR.
> Treatment: amphotericin B, sulphonamides, dibromopropamide (liniment), izothionate (eye drops), neomycin, itraconazole, miltefosine (alcylphosphocholin).
> Geographical distribution: worldwide, ubiquitous.

8.5.5 *Family* Leptomixidae [*Class* Lobosea]

**Balamuthia mandrillaris*

Large amoebae free-living in the soil, 50–60 μm in diameter, forming thick-walled cysts 15–30 μm. First isolated from the brain of a mandrill baboon *Papio sphinx* that died of encephalitis in San Diego Zoo, 1989.

Source of infection: soil.

Transmission mode: inhalatory (nasal mucosa, airways), percutaneously (skin scratches), organ (kidney) transplantation (2 cases in USA); cysts are very resistant in external milieu.

Human disease: granulomatous amoebic encephalitis, a rare infection with lesions on skin and in nasopharynx, further hemipareses, ataxia, seizures, personality disturbances. A total of about 150 cases have been reported (half of them in the USA – predisposition was found in Hispanic and immunocompromised persons) since discovery of the disease in 1990, usually fatal. First case in Europe: Czechland, 1995.

Bio-containment: BSL-2.

Diagnosis: CT scan of the brain, microscopy of CSF, print (scraping, rinsing) of lesions (IF), cultivation, PCR; often post mortem.

Treatment: amphotericin B, sulphonamides, dibromopropamide (ointment), neomycin, itraconazole.

Geographical distribution: worldwide, sporadic.

8.5.6 *Family* **Thecamoebidae**

*Sappinia pedata

Two morphological forms as in other free-living amoebae (trophozoite and cyst stage).

Source of infection: soil contaminated with ruminant faeces.

Transmission mode: probably inhalatory.

Human disease: amoebic encephalitis, a rare infection (only one case has been described – in USA, 2001 – the patient was an immunocompetent and previously healthy man).

Bio-containment: BSL-2.

Diagnosis: as with other free-living amoebae.

Treatment: the patient survived after surgical excision of the tumour-like mass in the brain, and subsequent treatment with azithromycin, itraconazole, flucytosine and pentamidine.

Geographical distribution: worldwide.

8.5.7 *Family* **Eimeriidae [***Order* **Eucoccidiida,** *Class* **Coccidea,** *Phyllum* **Apicomplexa]**

**Cyclospora cayetanensis*

Source of infection: vegetables, herbs, strawberries, lettuce, red pepper, garlic, basil etc.

Transmission mode: food-borne and water-borne.

Human disease: protracted and relapsing diarrhoea (incubation period about 7 days) in children and adults; often as a traveller diarrhoea. First cases were diagnosed in the late 1970s, and the first outbreak was reported from USA and Canada in 1995. Recently, a total of 29 cases were described in British Columbia (Canada) in 2007.

Bio-containment: BSL-2.

Diagnosis: microscopy of oocysts in stool samples (staining by Lugol's iodine, safranin, or Ziehl-Neelsen), PCR.

Treatment: cotrimoxazole (sulphamethoxazole + trimethoprim), pyrimethamine, ciprofloxacin.

Geographical distribution: probably worldwide, but predominantly in tropical and subtropical areas.

8.5.8 *Family* Sarcocystidae [*Order* Eucoccidiida, *Class* Coccidea]

Sarcocystis bovihominis, *S. suihominis* (= *S. miescheriana*), *S. lindemanni*

They have a two-host parasitic cycle: intermediate hosts are herbivores, with the tissue cysts (sarcocysts) containing thousands of cystozoites (merozoites, bradyzoites) in muscles; sexual stage of the cycle occurs in definitive (final) hosts – carnivores (gametogony generates oocysts with sporocysts and sporozoites). Humans and other primates can serve as definitive hosts for both *S. bovihominis* and *S. suihominis*.

Source of infection (natural host range): domestic animals (cattle – *S. bovihominis*, pig – *suihominis*).

Animal disease: whitish cysts in muscles, called Miescher's vesicles; in rodents so-called M-organism in the brain.

Transmission mode: alimentary (water or food contaminated faeces from unknown carnivore or omnivore in muscular form of infection; or raw or undercooked meat in intestinal form of infection).

Human disease: sarcosporidiosis (sarcocystosis) with two clinical forms: intestinal coccidiosis, when human serves as the definitive host (*S. bovihominis*, *S. suihominis*), with nausea, anorexia, vomiting, abdominal pain, diarrhoea, tachycardia; and infrequent muscular coccidiosis (human serves as the intermediate host), with fever, musculoskeletal pain, rash, subcutaneous swelling, cardiomyopathy, occasional necrosis and atrophia of muscles, tongue, larynx, oesophagus, diaphragma, etc. (*S. lindemanni*).

Bio-containment: BSL-2.

Diagnosis: flotation of stool and microscopy (oocysts 20–33×10–16 μm with 2 sporocysts 15×9 μm each, containing 4 sporozoites) in intestinal form; biopsy in muscular form, serology (CFT).

Treatment: cotrimoxazole, furazolidone, albendazole, sulphonamides (Biseptol) + pyrimethamine (but with unclear outcomes sometimes), anti-inflammatories; no specific treatment in muscle coccidiosis.

Geographical distribution: worldwide, endemic, with predominance in the tropical areas.

Toxoplasma gondii

Forms: (1) (acute stage) vegetative crescent-shaped trophozoites (endo- or tachyzoites) 4–7×2–4 µm; in cell vacuoles often 2–32 together, forming "pseudocysts"; (2) (chronic stage) spherical tissue cysts 50–300 µm with thick membrane in muscles, heart, diaphragma and brain, with 1,000–60,000 crescent-shaped bradyzoites (cystozoites); (3) oocysts (10–12 µm) discovered in the 1970s, result of sexual reproduction of gametocytes (gametogony) occurring only in the gut epithelium of definitive, final host. Sporulation (excitation) occurs only after excretion of immature oocysts, 2 sporocysts with 4 sporozoites 8×2 µm may be released from each oocyst. There are four genomic lineages of *T. gondii*.

Source of infection (natural host range): cat and other felids (*Felis*, *Lynx*) are definitive hosts of *T. gondii* (they become infected with meat of intermediate host such as rodents containing bradyzoites, and excrete oocysts in faeces during 1–3 weeks: about 1% of cats in Europe); intermediate hosts (only with asexual stages of parasite, cysts) are rodents (reservoir: TOT), lagomorphs (e.g., 80% of domestic rabbits in Czechland have antibodies to *T. gondii* and 25% cysts), pig (cysts in 1–2%, antibodies in 10%), sheep (cysts in 5%, antibodies in 40–60%), cattle, and occasionally birds. In addition to the domestic cycle (domestic cat – mouse) there is a sylvatic cycle, observed e.g. in French Guyana: highly virulent strains of *T. gondii* circulate between free-living jaguars (*Panthera onca*, the definitive host) and their prey (intermediate hosts – deer, armadillos, pacas, peccaries). Sources of human infection is undercooked meat from hunted game and water containing oocysts excreted by wild cats.

Animal disease: inapparent course, sometimes fever or even fatal infection (hen, pig, rabbit, hare in winter, rarely cat); abortions in sheep, goats, and pigs (Japan).

Transmission mode: alimentary (rare meat: bloody steaks or slightly smoked meat with tissue cysts, fruit, vegetables, water and soil contaminated with cat excrements containing oocysts, which are very resistant – they survive in cat faeces and water for up to 17 months); transplacentary. Among risk areas are children playgrounds with sand, which are visited by cats excreting the oocysts.

Human disease: toxoplasmosis – pantropic chronic disease: (1) congenital toxoplasmosis (infection of foetus → pathologic gravidity [acquiring infection during second trimester seems to be critical], miscarriage, chorioretinitis +

hydrocephalus, microcephalia + CNS calcification of the foetus); (2) post-natal or acquired toxoplasmosis (lymphadenopathy, cough, fever, headache, fatigue, sometimes meningoencephalitis, chorioretinitis, various mental disorders), often latent (activation in AIDS patients etc.; 80–90% of infection cases are asymptomatic). Toxoplasmosis is a common zoonosis in many countries. For instance, on average 1,008 (range, 670–2,049) clinical cases were reported annually in Czechland in the years 1990–2000, and about 30% population revealed antibodies to *T. gondii*.

Diagnosis: serology (CFT, ELISA, Sabin-Feldman's test, which encompasses staining of trophozoites with alkaline methylen blue in the presence of examined sample and so-called activator, that is accesoric factor of native human serum; in the presence of antibodies have trophozoites characteristic original crescent-like shape with uncoloured plasma and blue nucleus, while without presence of antibodies they round off and stain deep blue; further serological tests include IFA, RDPA, RIHA etc.), intraperitoneal inoculation of mouse (and histology of its brain), biopsy of lymph nodes (IF, Giemsa: trophozoites have blue plasma, nucleus is red, plasmatic granula are red-brown); X-ray examination (calcification of the brain in newborns).

Bio-containment: BSL-2.

Treatment: sulphonamides for long-term usage, sulphadiazine (sulphamethoxy-din) + pyrimethamine (Daraprim); antibiotics – spiramycin (during gravidity), clindamycin and doxycycline.

Prevention: necessary in pregnant women (avoid contact with raw or undercooked meat, cats). Vaccine unavailable.

Geographical distribution: worldwide (very often in tropics).

8.5.9 *Family* Cryptosporidiidae [*Order* Eucoccidiida, *Class* Coccidea]

Cryptosporidium parvum, C. felis, C. canis, C. muris, C. suis, C. meleagridis

Forms: oval trophocytes 2–4 μm in the epithelial cells of respiratory and gastrointestinal tract of vertebrates including man. One-host developmental cycle (monoxenic), the host excretes oval thick-walled oocysts (sporocysts, with 4 sporozoites) about 5 μm.

Source of infection (natural host range): cattle (*C. parvum* bovine genotype II – a number of cases: e.g. major part of human isolates from Switzerland, also occurrence in Great Britain and Czechland), deer (*C. parvum* deer genotype), horse, sheep, cat (*C. felis* >30 cases), pig (*C. parvum* pig genotype), rodents (*C. parvum, C. muris* – sporadic cases), rabbit (*C. parvum* rabbit genotype), dog (*C. canis*), birds (*C. meleagridis* – several human cases); oysters and sea mussels; water – even after sand filtration. [Anthroponotic

C. parvum genotype I, nowadays classified as a separate species *C. hominis*, is the principal causative agent of human cryptosporidiosis].

Animal disease: enteritis mainly in young animals (e.g., calves). Pathogenicity for animals was proven only in 1955, earlier the cryptosporidia were regarded as nonpathogenic, commensal species of many animals.

Transmission mode: alimentary (ingestion of oocysts excreted in faeces) mainly by water "water-borne" – largely the *C. parvum* bovine genotype 2, but also food-borne; several epidemics reported after swimming in a pool (source: swimming pool filter backwash – 2000 Columbus, Ohio: 137 sick persons, 2000 Mallorca – a hotel swimming pool: 50 British tourists) or water parks. MID for man is only 30 oocysts, which are extremely resistant to chlorine (they survive at least 2 h in 5% of hypochlorite), they also survive in rotifers (*Rotifera*), and could be mechanically transmitted by house-flies. Many outbreaks of cryptosporidiosis occur in North America and Europe, and it is often unclear whether humans or animals are the source of contamination.

Human disease: cryptosporidiosis – enteritis lasting 1–2 weeks with fever, headache, abdominal pain, vomiting and diarrhoea (up to 20 times daily); relapses may occur. Disease was first described in 1976, it is common in immunocompromised persons e.g., with HIV infection (*C. parvum*, *C. felis, C. canis*, *C. meleagridis*, *C. muris*). A huge epidemic was recorded in Milwaukee (Wisconsin) in 1993: 403,000 persons were affected, 4,000 of them had to be hospitalised; the infection was spread by municipal water supply, which was contaminated by excrements of cattle.

Bio-containment: BSL-2.

Diagnosis: flotation of stool (in saturated solution of saccharose) and microscopy (oocysts), staining of stool specimens according to Miláček, or IF; serology (ELISA), PCR-RFLP.

Treatment: less effective – sulphonamides (trimethoprim + sulphomethoxazole, pyrimethamine), spiramycin, paromomycin; symptomatic treatment (Endiaron, Reasec).

Geographical distribution: worldwide.

8.5.10 *Family* Plasmodiidae [*Order* Haemosporida, *Class* Haematozoea, *Phyllum* Apicomplexa]

**Plasmodium falciparum*, P. vivax, P. ovale, P. malariae*

The developmental cycle is complicated, it involves alteration of asexual replication (schizogony) and sexual development (sporogony). From a mosquito female, where sporogony takes place, sporozoites migrate into vertebrate blood during mosquito feeding, and in liver (cells of the RES) they change to cryptozoites (EE stadium, exoerythrocytic), and by schizogony into merozoites; the latter are released into blood and form trophozoites (ring-like forms) in erythrocytes; new merozoites arise by schizogony (simultaneously with disruption of erythrocytes, clinically associated

with fever), but also part of trophozoites remain in erythrocytes and change themselves on <u>micro</u>-(male) and <u>macro</u>-(female) <u>gametocytes</u>. Only these last stages could infect the vector mosquito again, where they transform in micro- and macrogametes, which form ookinete (zygota) after copulation that penetrates the mosquito midgut and produce <u>oocysts</u> in body cavity; <u>sporozoites</u> then form in the oocysts and migrate into salivary glands. Gametogony and sporogony occur thus exclusively in the mosquito vector.

Source of infection (natural host range): man (thus de facto anthroponosis).

Transmission mode: anopheline mosquitoes *Anopheles maculipennis* s.l. (south Europe – *An. atroparvus*, *An. labranchiae*), *An. sacharovi* (Asia Minor and Turkey), *An. gambiae* (Africa), *An. darlingi* and *An. albimanus* (South and Central America), group *An. leucosphyrus* and *An. culicifacies* (southeast Asia); iatrogenic (blood transfusion).

Human disease: <u>malaria</u> with several forms: *tropica* (*P. falciparum*, the most serious – particularly in children and gravid women), *tertiana* (*P. vivax*, *P. ovale*), and *quartana* (*P. malariae*): period of schizogonic erythrocyte cycle is 48 h ("3-day malaria"), 72 h in *P. malariae* ("4-day malaria"); frequent relapses. Symptoms: chills, fever, hyperhidrosis, splenomegaly, sometimes affection of CNS (then often fatal course). WHO 1990: a total of 270 million people affected worldwide, approximately 1 million of which die annually. A total of about 20,000 cases of malaria are imported annually to Europe of them about 8,000 caused by *P. falciparum*. For example 918 cases in Germany in 1999 (60% formed tourists travelling to endemic areas). Air carriage of infected mosquitoes in airplanes can result in "<u>airport malaria</u>" (infection of human via mosquito biting in the surroundings of airport or during intermediate landing; >100 cases have been registered since 1977, only in Paris 25 cases).

Bio-containment: BSL-2.

Diagnosis: microscopy – blood smear or thick drop (Giemsa), serology (IFA, RIHA, etc.). ELISA is a suitable serologic marker for detecting malaria in areas of low endemicity (lack of sensitivity of commonly used methods).

Treatment: chloroquine (*P. falciparum* nowadays generally resistant; meflochine is an alternative), amodiachin, quinine + doxycycline, atebrin (uneffective on gametocytes); primaquine (only against parasites in liver and gametocytes); clindamycin, tetracycline.

Prophylaxis and prevention: proguanil, meflochine, chloroquine (resistance), pyrimethamine; repellents, and mosquito bed nets. Effective vaccine unavailable at present.

Geographical distribution: tropics and subtropics. Malaria has been extensively spread in Europe before WWR2 (commonly found in Mediterranean and Balkan) as well as in North America. Some hypotheses reveal that malaria could be one of the major causes of the fall of Roman Empire in fifth century. Italians controlled malaria in the neighbourhood of Rome by desiccation of lowland wetlands only in 1929. For some typical habitats of malaria, see Photos 5.45–5.48.

Plasmodium knowlesi, *P. simium*, *P. cynomolgi*

Zoonotic species causing malaria in man.

Source of infection (natural host range): simian reservoir – monkeys (*Aotus*, *Callithrix*, *Macaca fascicularis*, *M. nemestrina*, *Alouatta*) and higher primates (chimpanzee, gorilla, orang-utan).

Animal disease: simian malaria.

Transmission mode: mosquitoes (*Anopheles latens, An. cracens*).

Human disease: simian malaria – previously regarded as a rare zoonosis, first described in 1965 (*P. knowlesi*). Up to 2004, only 5 cases (*P. knowlesi*) were reliably described. However, a number of additional cases have been reported since 2004 (at least 7, a few fatal) and it seems that simian malaria is an underdiagnosed (or misdiagnosed as classical human malaria), widely distributed disease in tropical countries.

Bio-containment: BSL-2.

Diagnosis: *P. knowlesi* is morphologically very similar to *P. malariae* and *P. falciparum* which could result in misidentification. Modern rapid diagnostic tests (e.g. OptiMAL-IT for detection of parasite lactate dehydrogenase, or even PCR) could produce cross-reactive or nonspecific results and miss *P. knowlesi* in the blood samples.

Treatment: identical to human malaria.

Geographical distribution: largely in forested areas of southeast Asia (Thailand, Malaysia, Sarawak, Borneo, Myanmar, Singapore, Vietnam, China – Yunnan province, the Philippines); rare in West Africa, Brazil.

8.5.11 *Family* Babesiidae [*Order* Piroplasmida*, Class* Haematozoea*, Phyllum* Apicomplexa]

Babesia divergens, *B. microti*, *B. duncani* (WA1), *B. venatorum* (EU1)

Very small (1.5–2.0 μm) erythrocyte parasites of vertebrates (intermediate hosts) with final progression in ticks, their former designation piroplasmas has derived from latin "*pirus*" = pear, and it describes their characteristic shape in erythrocytes. According to up-to-date molecular studies (based on 18S rRNA gene sequencing) it has been shown that mentioned species are phylogenetically related more closely to the genus *Theileria* than to typical representatives of the genus *Babesia*, so we could expect some nomenclatural changes in foreseeable future.

Source of infection (natural host range): rodents of the genera *Microtus* and *Peromyscus* (*B. microti*), cattle (*B. divergens*), roe deer (*B. microti, B. venatorum*), and some other mammals.

Animal disease: piroplasmosis (babesiosis) with fever, haemoglobinuria, icterus, and haemolytic anaemia causing major losses in cattle (*B. divergens*, *B. major*, *B. bigemina*); haemolytic dog anaemia (*B. canis*).

Transmission mode: ixodid ticks *Ixodes scapularis* (*B. microti*), *I. ricinus* (*B. microti, B. venatorum* – TST and TOT confirmed, *B. divergens, B. duncani*), further *Rhipicephalus*, *Dermacentor*, *Haemaphysalis*, mainly by nymphs (inside them are present "vermicules" up to 16 μm long); blood transfusion (a number of cases have been recorded in the USA).

Human disease: <u>babesiosis</u> – the course similar to malaria, with incubation period about 1–12 months: high fever (chronic in untreated patient) with chills, hyperhidrosis, exudation, fatigue, anorexia, headache, myalgia, cough, arthralgia, hepatosplenomegaly, icterus, haemolytic anaemia, thrombocytopenia, increased level of lactate dehydrogenase and bilirubin, haemoglobinuria, kidney failure. The most susceptible are splenectomized or otherwise immunocompromised people (*B. divergens* – *c.* 40 fatal cases in Europe: the former Yugoslavia, Scotland, Russia, France, Spain, Finland); *B. microti* is responsible for severe disease even in immunocompetent persons with spleen; fatality rate is about 5%. Often longlife carriage of the parasite (blood of these persons can not be used for transfusion). Only few cases of the disease caused by *B. venatorum* have been recorded recently in Europe (Italy, Austria, Germany – all in splenectomized persons), and two symptomatic infections with *B. microti* (Switzerland – autochthonous, and Czechland – import from USA).

Bio-containment: BSL-2.

Diagnosis: blood smear, thick drop (Giemsa staining: oval or ring-like forms resembling trophozoites of *Plasmodium falciparum* in erythrocytes, sometimes in characteristic shape of Maltese cross); intraperitoneal inoculation of golden hamster; serology (IFA, ELISA; cross-reaction with *Plasmodium*), PCR of blood samples.

Treatment: a combination of quinine + clindamycin, the alternative is atovaquone + azithromycin; blood transfusion if necessary.

Geographical distribution: North America (except of *B. divergens*; *B. duncani* in west USA), Europe (except of *B. duncani*): Ireland, Scotland, France (*B. venatorum*), Switzerland, Italy, Austria, Germany (*B. venatorum*), Slovenia, Serbia, Russia, Czechland (evidence of *B. microti* in *Ixodes ricinus* ticks), Poland (*B. venatorum*), Finland (*B. divergens*), and China (*B. microti*). For a natural focus of babesiosis due to *B. microti*, see Photo 5.49.

8.5.12 *Family* Balantidiidae [*Order* Trichostomatida, *Class* Litostomatea, *Phyllum* Ciliophora]

Balantidium coli

The largest pathogenic protozoan and only one medically important ciliate: it forms motile trophozoites 50–300×40–70 μm, and spherical cysts 50–65 μm.

Source of infection (natural host range): pig, wild boar (reservoir: a commensal of the swine intestine); occasionally rats, monkeys, dog.

Animal disease: inapparent course.

Transmission mode: alimentary (cysts from pigs present in water).

Human disease: balantidiosis–chronic dysenteria, abdomen colics, occasionally peritonitis.

Bio-containment: BSL-2.

Diagnosis: microscopy of stool (motile trophozoites, less often cysts), biopsy (scraping of intestinal mucosa), serology (IFA, immobilization).

Treatment: tetracycline, metronidazole, hydroxychinolin, sulphaguanidine.

Geographical distribution: worldwide (more frequently in tropics and subtropics), a sporadic occurrence.

8.6 Other Eucaryotic Microorganisms

8.6.1 Algae

Family *Chlorellaceae* [Order *Chlorellales,* Class *Chlorophyceae*]

*Chlorella spp.

Unicellular green spherical algae, reproducing asexually by internal septation, producing up to 20 "endospores" within the parent "sporangium".

Source of infection: river water.

Animal disease: unknown.

Transmission mode: by contact with traumatized skin.

Human disease: chlorellosis – a rare disease (only one case has been described up to now) with wound lesion draining a greenish-yellow exudate.

Bio-containment: BSL-2.

Diagnosis: microscopy of the tissue (endosporulating cells 6–9 μm, with bright green granules), culture.

Treatment: surgical, amphotericin B.

Geographical distribution: USA (the human case).

**Prototheca wickerhamii, P. zopfii*

Oval to spherical cells, morphologically similar to *Chlorella*, but without the green pigment.

Source of infection: grass, soil, water; animals.

Animal disease: unknown.

Transmission mode: by contact (traumatic inoculation).

Human disease: protothecosis – an uncommon disease (but more frequent than chlorellosis), with skin lesions (nodules, papules), occasionally disseminated

(algaemia demonstrated) and then fatal (especially in immunocompromised patients).

Bio-containment: BSL-2.

Diagnosis: microscopy of the tissue (endosporulating hyaline cells 5–15 μm), culture.

Treatment: surgical excision of localized lesions, antifungal agents (amphotericin B, itraconazole, fluconazole, ketoconazole).

Geographical distribution: worldwide.

8.6.2 *Blastocystea* [*Class* Opalinata*, Phyllum* Chromista]

(**) *Blastocystis hominis*

Formerly classified in Protozoa. A total of 9 genomic types have been differentiated, based on 18S rRNA analyses. Four morphologic forms (ontogenetic phases) are present: vacuolar form (2–200 μm, with central vacuole); granular form (forming of granules in central vacuole); amoebic form (motile, with pseudopodia); thick-walled cyst (very resistant).

Source of infection (natural host range): human; less often pig, horse, cattle, brown rat, opossum, chickens (some animal genotypes have zoonotic potential – especially those from cattle and pig).

Animal disease: usually asymptomatic infection.

Transmission mode: alimentary (ingestion – water, food), tenacity of cysts is quite high – they resist even gastric fluid.

Human disease: blastocystosis – usually an acute gastrointestinal disease with diarrhoea, anorexia, abdominal pains and convulsions, sometimes with fever and fatigue.

Bio-containment: BSL-2.

Diagnosis: microscopy, serology (ELISA), PCR, cultivation is also possible.

Treatment: metronidazole.

Geographical distribution: worldwide, with majority in (sub)tropical countries.

8.6.3 *Microsporidia* [*Order* Microsporida*, Class* Microsporea*, Phyllum* Microspora]

Microsporidia, formerly classified as protozoans, are more related to fungi, but belong systematically probably to a separate class of microorganisms based on rRNA analyses. They are obligate intracellular parasites of invertebrates (especially arthropods) and vertebrates (mainly fishes) with ontogenic phases: schizogony (by another name merogony: binary fission in so-called parasitophoric vacuoles of host cells) and sporogony, followed by the release of spores from split host cell. Ovoid to pyriform small spores are very characteristic (1.5–4.0×1.0–3.0 μm) and contain

one (*Encephalitozoon*, *Enterocytozoon* etc.) or two nuclei (binucleate), a posterior vacuole (polaroplast) and ejection apparatus (a tubulous fibre, which is in the caudal part of spores helically stranded into 4–8 coils and after swelling of the polaroplast ejectable throw frontal polar cap); sporoplasma with nucleus is dislodged into host cell after the fibre ejection. Microsporidia do not contain mitochondria, only their residua (called mitosomes). The microsporidian *Nosema bombycis* is the first discovered microbial parasite of insects, namely of silkworm *Bombyx mori* (Louis Pasteur studied this disease). Except for the species mentioned below, zoonotic transmission of other microsporidia to man has been demonstrated only rarely, especially in the genera *Pleistophora*, *Tachipleistophora*, *Brachiola* and *Vittaforma*.

(**) *Encephalitozoon cuniculi*, *E. intestinalis*, *E. hellem*

Source of infection (natural host range): human; rarely rabbit (*E. cuniculi* I), hare (*E. intestinalis*, *E. hellem*), rodents (*E. cuniculi* II), dog (*E. cuniculi* III), parrots (*E. hellem*), waterfowl (*E. hellem*, *E. intestinalis*).

Animal disease: usually asymptomatic infection. Sometimes clinical illness can be observed in rabbits, rodents (laboratory), carnivores (canine encephalitis-nephritis syndrome), and monkeys (usually in young animals).

Transmission mode: ingestion (surface water or groundwater, food) or inhalation, by contact, man-to-man transmission (male homosexuality). Tenacity of spores, which are excreted via urine and by expectorating, is quite high. The parasite can naturally persist in the human population and environment (even for several months under humid conditions).

Human disease: microsporidiosis (intestinal, respiratory and ocular form) – chronic diarrhoea, frequently in immunocompromised persons (AIDS, organ transplantations), occasionally even necrosis of intestine, gallbladder, liver, kidney, pancreas, paranasal cavities and respiratory tract may evolve in these patients. Ocular microsporidiosis may occur in patients which were exposed to pet birds (oral or ocular autoinoculation throw contaminated fingers).

Bio-containment: BSL-2.

Diagnosis: light and transmission electron microscopy, IF and histochemical staining of samples (urine, stool and sputum), PCR, RFLP, sequencing; serology (IFA, ELISA: occasionally ambiguous outcomes).

Treatment: albendazole (only against *E. intestinalis*), metronidazole, cotrimoxazole, fumagilin.

Geographical distribution: worldwide.

(**) *Enterocytozoon bieneusi*

Very small spores, $1.5 \times 1.0\ \mu$m. A number of genotypes have been distinguished (A to K at present), and some of them are zoonotic.

Source of infection (natural host range): man (the genotype B); zoonotic transmission of some genotypes to humans has been occasionally confirmed e.g., from cat, pig, dog, rabbit, rhesus monkey, cattle, goat, llama, wild mammals (beavers, foxes, muskrats, otters, raccoons), and recently also birds (chickens, pigeons).

Animal disease: usually asymptomatic infection.

Transmission mode: see *Encephalitozoon*.

Human disease: microsporidiosis – chronic diarrhoea and malabsorption syndrome mainly in persons suffering from AIDS (worlwide mean prevalence of *E. bieneusi* used to be 15–30% in those persons); less often in immunocompetent persons, usually as so-called traveller's diarrhoea. Mostly asymptomatic in humans: e.g., 10% of healthy persons have been found seropositive in Czechland.

Bio-containment: BSL-2.

Diagnosis and treatment: similar as for *Encephalitozoon.*

Geographical distribution: worldwide, sporadic.

Brachiola (*Nosema*) *algerae*

A microsporidian parasite of anopheline mosquitoes.

Source of infection (natural host range): probably infected mosquitoes and other insects.

Transmission mode: mosquito bites?

Human disease: 3 human cases have been described, also in immunocompetent persons: corneal lesions; a cutaneous nodule; lesions in muscle tissue.

Bio-containment: BSL-2.

Diagnosis and treatment: similar as for other microsporidia.

Geographical distribution: probably worldwide, but sporadic.

8.6.4 *Dermocystida* [*Class* Mesomycetozoea*, Phyllum* Choanozoa]

**Rhinosporidium seeberi*

Systematic classification of this species was unclear for long times. However, it was found (based on 18S rRNA analyses) that rhinosporidia do not belong either to fungi, where were assigned formerly, or to typical protozoa; they are more closely related to animals. From phylogenetic point of view they are very close to the genera *Dermocystidium* and *Amphibiocystidium*, whose representatives are parasites of fishes and amphibians; corresponding order *Dermocystida* belongs to microbial Protista of the class *Mesomycetozoea* (earlier *Ichthyosporea*).

Source of infection: stagnant or lacustrine water.

Animal disease: rhinosporidiosis of fishes, amphibians and rarely waterfowl. It has been also documented in many mammalian species, including cats, dogs, cattle, and horse (equine cases are infrequent but have been reported from the southern United States, South America, South Africa, and Great Britain).

Transmission mode: inhalation, ingestion, per conjunctivae from water (swimming), or percutaneous (injury).

Human disease: rhinosporidiosis – chronic granulomatous polyps of raspberry appearance in nasal mucosa, nasopharynx, and conjuctiva. Occupational risk: farmers working on rice fields.

Bio-containment: BSL-2.

Diagnosis: macro- and microscopy, biopsy, histology (*R. seeberi* forms big thick-walled spherical sporangia in the affected tissue with diameter 50–1,000 μm containing endospores 5–10 μm); cultivation impossible.

Treatment: surgical excision of the lesions, but recurrences are common.

Geographical distribution: tropical areas (Sri Lanka, India, southeast Asia, South America), rare autochthonous cases recorded also in Egypt, Turkey, Congo, Canada and USA (Florida), and Europe (UK).

Photographs

Chapter 5. The Epidemic Process in Zoonoses and Sapronoses

Typical Habitats, Ecosystems, Natural Foci

Photo 5.1 Natural focus of sylvatic yellow fever in Kerio Valley, Kenya, 1993 – the main local vector was *Aedes africanus* mosquito (P. Reiter)

Photo 5.2 Natural focus of sylvatic yellow fever in Kerio Valley, Kenya, 1993 (P. Reiter)

Photo 5.3 Natural focus of West Nile fever in Camargue, southern France (Z. Hubálek)

Photo 5.4 Natural focus of West Nile virus (lineage Rabensburg) in south Moravia, Czechland (Z. Hubálek)

Photo 5.5 Overwintering site (a small 2nd WW bunker) for female mosquitoes *Culex pipiens*, *Anopheles maculipennis* s.l. and *Culiseta annulata*: WNV (Rabensburg lineage) area, South Moravia, Czechland (Z. Hubálek)

Photo 5.6 Area of a dengue outbreak in Velur, southern India (B. Rosický)

Photo 5.7 Theriodic (wildlife) focus of TBE: Cvilín near Krnov, Czechland (M. Pejčoch)

Photo 5.8 Theriodic (wildlife) focus of TBE in Mid-European broad-leaved forest: Pöls, Styrian Austria (Z. Hubálek)

Photo 5.9 Theriodic (wildlife) focus of TBE and Lyme borreliosis, with both *Ixodes ricinus* and *I. persulcatus* ticks as vectors: Kirsino at St. Petersburg, Russia (Z. Hubálek)

Photo 5.10 Theriodic (wildlife) focus of TBE in taiga (*Ixodes persulcatus* is the vector): Khabarovsk, Far East (B. Rosický)

Photo 5.11 Boskematic (pastoral) focus of TBE: Rožňava district, Slovak Karst (Z. Hubálek)

Photo 5.12 Natural focus of louping ill in Scottish highlands (Z. Hubálek)

Photo 5.13 Stands of the Common Bracken (*Pteridium aquilinum*) present a good microhabitat for *Ixodes ricinus* ticks in natural foci of louping ill in the UK and Ireland (Z. Hubálek)

Photo 5.14 Breeding place of *Aedes albopictus* and *Ae. aegypti* mosquitoes in La Réunion Island during the chikungunya outbreak in 2005–2007 (courtesy D. Fontenille)

Photo 5.15 Natural focus of Ťahyňa virus infections – floodplain forest ecosystem: south Moravia, Czechland (Z. Hubálek)

Photo 5.16 Natural focus of Ťahyňa virus infections – floodplain forest ecosystem: south Moravia, Czechland (Z. Hubálek)

Photo 5.17 Natural focus of CCHF: southern slopes of Stara Planina, Bulgaria (B. Rosický)

Photo 5.18 Boskematic (pastoral) focus of Bhanja virus infections: Kečovo, Slovak Karst (Z. Hubálek)

Photo 5.19 Boskematic (pastoral) focus of Bhanja virus infections: Campodimele near Fondi, Italy – the site of the first isolation of Bhanja virus in Europe (Z. Hubálek)

Photo 5.20 Boskematic (pastoral) focus of Bhanja virus infections: Croatian island of Brač (Z. Hubálek)

Photo 5.21 Boskematic (pastoral) focus of Bhanja virus infections in Sri Lanka (W. Sixl)

Photo 5.22 Peridomestic habitat of *Phlebotomus* spp., the vector of pappatasi fever: Croatian island of Brač (Z. Hubálek)

Photo 5.23 A shed in Cala Galera (Monte Argentario, Italy), the source of first isolation of Toscana virus from *Phlebotomus perniciosus* sandflies (Z. Hubálek)

Photo 5.24 Transmission site of Rift Valley fever in northern Senegal (V. Chevalier)

Photo 5.25 Transmission site of Rift Valley fever in northern Senegal (V. Chevalier)

Photo 5.26 Natural focus of HFRS (Dobrava hantavirus) in Fruška Gora, Serbia (B. Rosický)

Photo 5.27 Natural focus of HFRS (Dobrava hantavirus) in Ruská Poruba, eastern Slovakia (B. Rosický)

Photo 5.28 Natural focus of Marburg haemorrhagic fever — a communal roosting site of thousands of fruit bats *Rousettus aegyptiacus* in a cave: Maramagambo, Uganda (V. Patrovská-Vernerová)

Photo 5.29 Kikwit area (Zaire), the place of the Ebola haemorrhagic fever outbreak in 1995 (P. Reiter)

Photo 5.30 Kikwit area (Zaire), the place of the Ebola haemorrhagic fever outbreak in 1995 (P. Reiter)

Photo 5.31 Natural focus of Q-fever in Bulgaria (B. Rosický)

Photo 5.32 Natural focus of scrub typhus (tsutsugamushi) in Sri Lanka (B. Rosický)

Photo 5.33 Natural focus of Lyme borreliosis in south Moravia, Czechland (I. Rudolf)

Photo 5.34 Lyme borreliosis can be occasionally acquired even at higher elevations: Gaberl in Styria (Austria), 1,350 m above sea level − we found local *Ixodes ricinus* ticks infected with *Borrelia afzelii* and *B. garinii* (Z. Hubálek)

Photo 5.35 Natural focus of Lyme borreliosis in Old Lyme, Connecticut, USA (Z. Hubálek)

Photo 5.36 Natural focus of Lyme borreliosis in Old Lyme, Connecticut, USA (Z. Hubálek)

Photo 5.37 Natural focus of Lyme borreliosis – a huge breeding colony of the Common Murre, *Uria aalge*, the host of *Ixodes uriae* ticks, vectors of *Borrelia garinii*: Cliffs of Moher, Ireland (Z. Hubálek)

Photo 5.38 Focus of leptospirosis at Ostrava, Czechland (B. Rosický)

Photo 5.39 Natural focus of plague: Kolar, India – *Tatera indica* rat is the local host (B. Rosický)

Photo 5.40   Natural focus of plague in Mongolia – *Marmota sibirica* is the local host and reservoir (B. Rosický)

Photo 5.41   A burrow of *Marmota sibirica*, the local reservoir of plague in Mongolia (B. Rosický)

Photo 5.42 Natural focus of tularaemia and West Nile virus (lineage Rabensburg) in south Moravia, Czechland (Z. Hubálek)

Photo 5.43 Natural focus of cutaneous leishmaniasis in a semidesert habitat near Samarkand, Uzbekistan (B. Rosický)

Photo 5.44 A burrow of *Rhombomys opimus*, the reservoir of the cutaneous leishmaniasis agent in a semidesert habitat near Samarkand, Uzbekistan (B. Rosický)

Photo 5.45 Rice fields form an excellent breeding place for malaria mosquitoes: Madagascar (D. Fontenille)

Photo 5.46 A desert focus of malaria, breeding site of *Anopheles* mosquitoes: Mauretania (D. Fontenille)

Photo 5.47 Breeding site of malaria mosquitoes in Sri Lanka (B. Rosický)

Photo 5.48 A peridomestic focus of malaria (with *Anopheles stephensi* as the vector) near Madras, India (B. Rosický)

Photo 5.49 Peridomestic focus of babesiosis (*Babesia microti*) and Lyme borreliosis in Old Lyme, CT (USA) – several cases of babesiosis and many cases of LB have been acquired here (Z. Hubálek)

Epidemiological Surveillance

Photo 5.50 First descriptive epidemiological study was done by John Snow on cholera outbreak in Broad Street (now Broadwick Street) in London, 1854 (Z. Hubálek)

Photo 5.51 A local pump was found to be the source of cholera infection and recommended by J. Snow to be blocked for the use of drinking water (Z. Hubálek)

Photo 5.52 Investigation of Ebola haemorrhagic fever outbreak in Kikwit area (Zaire), 1995: a "BSL-4" field laboratory (P. Reiter)

Sampling of Arthropods

Photo 5.53 Light trap for mosquitoes in Kikwit, Zaire (P. Reiter)

Photo 5.54 CDC CO_2 minilight trap (I. Rudolf)

Photo 5.55 CDC CO$_2$ minilight trap placed in the canopy (I. Rudolf)

Photo 5.56 Goat-baited mosquito trap in Senegal (V. Chevalier)

Photo 5.57 Pigeon-baited mosquito trap (O. Šebesta)

Photo 5.58 Pigeon-baited mosquito trap exposed (I. Rudolf)

Photo 5.59 Collection of overwintering *Culex pipiens* mosquitoes in a cellar (I. Rudolf)

Photo 5.60 Collection of larval mosquitoes in an endothelm (tree hole) during yellow fever outbreak in Kerio Valley, Kenya, 1993 (P. Reiter)

Photo 5.61 Collection of mosquitoes by entomological net during their overpopulation in a floodplain forest ecosystem (I. Rudolf)

Photo 5.62 Flagging of ixodid ticks (*Dermacentor reticulatus*) in a natural focus of tularaemia (I. Rudolf)

Outbreak Control

Photo 5.63 Outbreak control: vaccination campaign against yellow fever during an outbreak in Kerion Valley (Kenya) in 1993 (P. Reiter)

Chapter 6. Haematophagous Arthropods

Hard (Ixodid) Ticks

Photo 6.1 The hard tick
Ixodes ricinus (female)
(I. Rudolf)

Photo 6.2 The hard tick
Ixodes ricinus (detail of
hypostome) (I. Rudolf)

Photo 6.3 The hard tick
Ixodes ricinus (females after
blood feeding) (J. Erhart)

Photo 6.4 The hard tick
Ixodes ricinus (male)
(J. Erhart)

Photo 6.5 The hard tick
Ixodes ricinus (in copula)
(J. Erhart)

Photo 6.6 The hard tick
Ixodes scapularis (female)
(J. Erhart)

Photo 6.7 The hard tick
Ixodes scapularis (male)
(J. Erhart)

Photo 6.8 The hard tick
Haemaphysalis punctata
(female) (S. Hornok)

Photo 6.9 The hard tick
Haemaphysalis punctata
(male) (S. Hornok)

Photo 6.10 The hard tick
Dermacentor marginatus
(female) (J. Erhart)

Photo 6.11 The hard tick *Dermacentor marginatus* (male) (J. Erhart)

Photo 6.12 The hard tick *Dermacentor reticulatus* (female) (I. Rudolf)

Photo 6.13 The hard tick *Dermacentor reticulatus* (male) (I. Rudolf)

Photo 6.14 The hard tick *Dermacentor reticulatus* (in copula) (S. Hornok)

Photo 6.15 The hard tick *Dermacentor variabilis*, female (K. Stafford)

Photo 6.16 The hard tick *Hyalomma marginatum* female (M. Madder)

Photo 6.17 The hard tick
Hyalomma marginatum
(male) (M. Madder)

Photo 6.18 The hard tick
Rhipicephalus sanguineus
(female) (J. Erhart)

Photo 6.19 The hard tick
Rhipicephalus sanguineus
(male) (J. Erhart)

Photo 6.20 The hard tick
Boophilus annulatus (female)
(M. Madder)

Photo 6.21 The hard tick
Boophilus annulatus (male)
(M. Madder)

Photo 6.22 The hard tick
Amblyomma americanum
(female) (K. Stafford)

Photo 6.23 The hard tick
Amblyomma hebraeum
(female) (M. Madder)

Photo 6.24 The hard tick
Amblyomma hebraeum
(male) (M. Madder)

Photo 6.25 The hard tick *Amblyomma variegatum* (molting female) (A. Nijhof)

Photo 6.26 The hard tick *Amblyomma variegatum* (molting male) (A. Nijhof)

Soft (Argasid) Ticks

Photo 6.27 The soft tick
Argas reflexus (imago, dorsal
side) (P. Rödl)

Photo 6.28 The soft tick *Argas reflexus* (imago, ventral side) (P. Rödl)

Photo 6.29 The soft tick *Argas reflexus* (imago, lateral side) (P. Rödl)

Photo 6.30 The soft tick *Ornithodoros moubata* (imago, dorsal side) (J. Erhart)

Photo 6.31 The soft tick
Ornithodoros moubata
(imago, ventral side)
(J. Erhart)

Lice

Photo 6.32 The body louse
Pediculus humanus (male and
female) (O. Sychra)

Bed Bugs

Photo 6.33 Adult bed bug *Cimex lectularius* – dorsal side (O. Šebesta)

Photo 6.34 Adult bed bug
Cimex lectularius – ventral
side (O. Šebesta)

Photo 6.35 Nymphal bed
bug *Cimex lectularius* (dorsal
side) (O. Šebesta)

Kissing (Triatomine) Bugs

Photo 6.36 The kissing bug *Dipetalogaster maximus* (imago) (J. Erhart)

Photo 6.37 The kissing bug *Dipetalogaster maximus* (nymph) (J. Erhart)

Photo 6.38 The kissing bug *Triatoma infestans* (imago) (J. Erhart)

Photo 6.39 The kissing bug
Triatoma infestans (nymph)
(J. Erhart)

Photo 6.40 The kissing bug
Rhodnius prolixus (imago)
(J. Erhart)

Photo 6.41 The kissing bug
Rhodnius prolixus (nymph)
(J. Erhart)

Mosquitoes

Photo 6.42 The mosquito
Aedes aegypti (P. Rödl)

Photo 6.43 The mosquito
Aedes albopictus (R. Eritja)

Photo 6.44 The mosquito
Aedes albopictus (R. Eritja)

Photo 6.45 Larvae of *Aedes albopictus* mosquito (D. Fontenille)

Photo 6.46 The mosquito
Aedes vexans (O. Šebesta)

Photo 6.47 The mosquito
Culex pipiens (O. Šebesta)

Photo 6.48 The mosquito
Anopheles maculipennis
(O. Šebesta)

Photo 6.49 The mosquito
Anopheles gambiae
(N. Rahola, courtesy
D. Fontenille)

Sandflies

Photo 6.50 The sandfly
Phlebotomus perniciosus
(R. Eritja)

Photo 6.51 The sandfly *Phlebotomus perniciosus* (R. Eritja)

Deerflies

Photo 6.52 The deerfly
Chrysops viduatus
(S. Hornok)

Photo 6.53 The deerfly
Tabanus sp. (S. Hornok)

Tsetse Flies

Photo 6.54 Tsetse-fly *Glossina palpalis* (M. Kozánek)

Photo 6.55 Tsetse-fly
Glossina palpalis (engorged
female) (M. Kozánek)

Photo 6.56 Tsetse-fly
Glossina morsitans
(M. Kozánek)

Photo 6.57 Tsetse-fly
Glossina morsitans (in
copula) (M. Kozánek)

Flatflies

Photo 6.58 The flatfly *Lipoptena cervi* (I. Rudolf)

Fleas

Photo 6.59 The flea
Xenopsylla cheopsis, female
– a total view (L. Kolářová)

Photo 6.60 The flea *Xenopsylla cheopsis,* female – head (L. Kolářová)

Photo 6.61   The flea *Ctenocephalides felis* (O. Sychra)

Chapter 7. Vertebrates as Hosts and Reservoirs of Zoonotic Agents

Mammals

Photo 7.1   European Hedgehog, *Erinaceus europaeus* (P. Rödl)

Photo 7.2   Eastern Hedgehog, *Erinaceus concolor* (P. Rödl)

Photo 7.3   Common Shrew, *Sorex araneus* (P. Rödl)

Photo 7.4   Water Shrew, *Neomys fodiens* (P. Rödl)
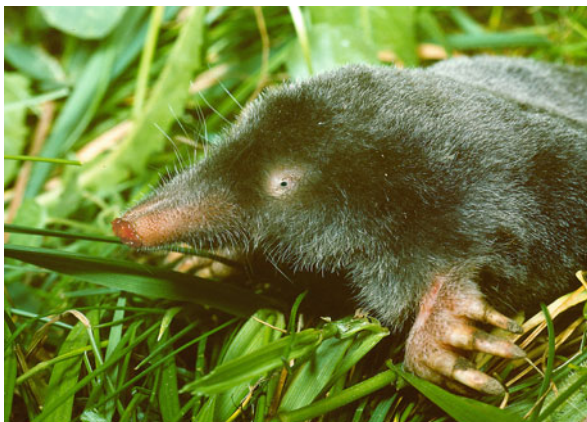
Photo 7.5 Common Mole, *Talpa europaea* (P. Rödl)

Photo 7.6 Egyptian Rousette, *Rousettus aegyptiacus* (M. Patrovská-Vernerová)

Photo 7.7 Epauletted Fruit Bat, probably *Epomophorus wahlbergi*, captured in Kikwit, Zaire 1995 − potential reservoir of Ebola filovirus as found recently (P. Reiter)

Photo 7.8 East African Epauletted Fruit Bat, *Epomophorus minimus,* captured in Kikwit, Zaire 1995 (P. Reiter)

Photo 7.9 Straw-coloured
Fruit Bat, *Eidolon helvum*
(P. Rödl)

Photo 7.10 Vampire Bat,
Desmodus rotundus (P. Rödl)

Photo 7.11 Greater Mouse-eared Bat, *Myotis myotis* (P. Rödl)

Photo 7.12 Noctule Bat, *Nyctalus noctula* (P. Rödl)

Photo 7.13 Serotine, *Eptesicus serotinus* (P. Rödl)

Photo 7.14 Schreiber's Bat, *Miniopterus schreibersi* (P. Rödl)

Photo 7.15 Green (Vervet)
Monkey, *Chlorocebus*
(*Cercopithecus*) *aethiops*
(P. Rödl)

Photo 7.16 Rhesus Monkey,
Macacca mulatta (P. Rödl)

Photo 7.17 Olive Baboon, *Papio anubis* (Z. Hubálek)

Photo 7.18 Red Fox, *Vulpes vulpes* (P. Rödl)

Photo 7.19 Raccoon Dog,
Nyctereutes procyonides
(P. Rödl)

Photo 7.20 Raccoon,
Procyon lotor (P. Rödl)

Photo 7.21   Weasel, *Mustela nivalis* (P. Rödl)

Photo 7.22   Stoat (Ermine), *Mustela erminea,* in winter coat (P. Rödl)

Photo 7.23   Polecat, *Mustela (Putorius) putorius* (P. Rödl)

Photo 7.24 Steppe Polecat, *Mustela* (*Putorius*) *eversmanni* (P. Rödl)

Photo 7.25 Beech (Stone)
Marten, *Martes foina*
(P. Rödl)

Photo 7.26 European Pine Marten, *Martes martes* (P. Rödl)

Photo 7.27 Badger, *Meles meles* (P. Rödl)

Photo 7.28 Mongoose, *Herpestes* sp. (P. Rödl)

Photo 7.29 Wild Cat, *Felis silvestris* (P. Rödl)

Photo 7.30 Pale-throated (Three-toed) Sloth, *Bradypus tridactylus* (P. Rödl)

Photo 7.31 African Elephant, *Loxodonta africana* (Z. Hubálek)

Photo 7.32 Rock Hyrax,
Procavia capensis (P. Rödl)

Photo 7.33 Blacktail Prairie Dog, *Cynomys ludovicianus* (P. Rödl)

Photo 7.34   Richardson Ground Squirrel, *Spermophilus richardsoni* (Z. Hubálek)

Photo 7.35   European Ground Squirrel (Souslik), *Spermophilus citellus* (P. Rödl)

Photo 7.36 Red (Spruce) Squirrel, *Tamiasciurus hudsonicus* (L. Mráz)

Photo 7.37 Small Chipmunk, *Tamias minimus* (archives IVB)

Photo 7.38 Siberian Chipmunk, *Tamias sibiricus* (P. Rödl)

Photo 7.39 (European) Red Squirrel, *Sciurus vulgaris* (P. Rödl)

Photo 7.40 Eastern Gray Squirrel, *Sciurus carolinensis* (Z. Hubálek)

Photo 7.41 Canadian Beaver, *Castor canadensis* (Z. Hubálek)

Photo 7.42 Great Jerboa, *Jaculus jaculus* (J. Bohdal)

Photo 7.43 Common
Hamster, *Cricetus cricetus*
(P. Rödl)

Photo 7.44   Golden Hamster, *Mesocricetus aureus* (P. Rödl)

Photo 7.45   Striped Dwarf
Hamster, *Cricetulus
barabensis* (P. Rödl)

Photo 7.46 White-footed Mouse, *Peromyscus leucopus* (J. F. Anderson)

Photo 7.47 Great Gerbil, *Rhombomys opimus* (B. Rosický)

Photo 7.48 Fat Sand Rat, *Psammomys obesus* (P. Rödl)

Photo 7.49 Bank Vole, *Myodes* (*Clethrionomys*) *glareolus* (P. Rödl)

Photo 7.50 Common Vole, *Microtus arvalis* (P. Rödl)

Photo 7.51 Field Vole, *Microtus agrestis* (P. Rödl)

Photo 7.52 Water Vole, *Arvicola terrestris* (P. Rödl)

Photo 7.53 Muskrat, *Ondatra zhibethicus* (P. Rödl)

Photo 7.54 Striped Field Mouse, *Apodemus agrarius* (P. Rödl)

Photo 7.55 Yellow-necked Mouse, *Apodemus flavicolis* (P. Rödl)

Photo 7.56 Natal Multimammate Rat, *Mastomys natalensis* (R. Makundi)

Photo 7.57 House Mouse, *Mus musculus* (P. Rödl)

Photo 7.58 Nile Grass Rat,
Arvicanthis niloticus
(M. Anděra)

Photo 7.59 Black Rat,
Rattus rattus (P. Rödl)

Photo 7.60 Brown Rat, *Rattus norvegicus* (P. Rödl)

Photo 7.61 Edible
Dormouse, *Glis glis* (P. Rödl)

Photo 7.62 Garden Dormouse, *Eliomys quercinus* (P. Rödl)

Photo 7.63   Coypu (Nutria) *Myocastor coypus* (O. Šebesta)

Photo 7.64   Brown
(European) Hare, *Lepus
europaeus* (P. Rödl)

Photo 7.65 Rabbit, *Oryctolagus cuniculus* (P. Rödl)

Photo 7.66 Black Rhinoceros, *Diceros bicornis* (Z. Hubálek)

Photo 7.67 Wild Boar, *Sus scrofa* (P. Rödl)

Photo 7.68 Warthog, *Phacochoerus aethiopicus* (Z. Hubálek)

Photo 7.69 Caribou, *Rangifer caribou* (Z. Hubálek – museum Edmonton)

Photo 7.70 Red Deer, *Cervus elaphus* (P. Rödl)

Photo 7.71 Roe Deer, *Capreolus capreolus* (P. Rödl)

Photo 7.72 American Bison, *Bison bison* (P. Rödl)

Photo 7.73 European Bison, *Bison bonasus* (B. Rosický)

Birds

Photo 7.74   White Egret, *Egretta alba*, in the Doud'j National Park, Senegal (V. Chevalier)

Photo 7.75   White Stork, *Ciconia ciconia* (archives IVB)

Photo 7.76 Bar-headed Goose, *Anser indicus,* a migratory host of H5N1 influenza virus in Asia (Z. Hubálek)

Photo 7.77 Puffin, *Fratercula arctica*, the host of *Ixodes uriae* ticks, vectors of *Borrelia garinii* (Z. Hubálek)

Photo 7.78 Herring Gull, *Larus argentatus* (Z. Hubálek)

Photo 7.79 Black-headed Gull, *Larus ridibundus* (Z. Hubálek), a common host of *Salmonella eneteritidis* var. Typhimurium

Photo 7.80 Feral pigeons in Barcelona, Spain (Z. Hubálková)

Photo 7.81 American Crow, *Corvus brachyrhynchos*, the most common vertebrate host and victim of West Nile disease in USA (G. Beaton, courtesy J. F. Anderson)

Photo 7.82   House Sparrow, *Passer domesticus* (Z. Hubálek)

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http://www.cdc.gov   Centers for Disesease Control and Prevention

http://www.cdc.gov/ncidod/EID   Emerging Infectious Diseases journal (online)

http://www.ecdc.europa.eu   European Centre for Disesease Prevention and Control (ECDC)

http://www.enivd.de   European Network for Imported Viral Diseases (ENIVD)

http://www.eurosurveillance.org   European journal for epidemiology of infectious diseases (ECDC)

http://www.sove.org   Society for Vector Ecology (SOVE)

http://www.promedmail.org   ProMED-mail, a program of the International Society for Infectious Diseases, monitoring epidemics in the world

Index