Infectious Diseases of Wild Mammals and Birds in Europe

To my dear husband Frederik and my wonderful children,
Alexander, Verónica and Emelie.
Dolores Gavier-Widén

To Sam, Kitt and Anna, my family, and colleagues especially
in the AHVLA, Europe and America.
The intricacies of infection add to the wonder of our world.
J. Paul Duff

To friends and colleagues in the wildlife field,
and to my family for their patience and support.
Anna Meredith

Infectious Diseases of Wild Mammals and Birds in Europe

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CONTENTS

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PREFACE

The aim of this book is to provide a reference text on infectious diseases which affect free-living wild mammals and birds in Europe. This is a broad field and currently involves many scientific disciplines including ecology, biology, wildlife management, epidemiology, animal and human health, molecular biology, evolutionary biology, genetics, virology, bacteriology, pathology, and diagnostics. A wildlife disease literature in journals covering these diverse disciplines exists in Europe; however there was a pressing need to review this, and bring the essential information together in one text.

A volume of knowledge on wildlife disease has been produced in Europe over recent decades and research on wildlife diseases has significantly increased in the last 15 years, partly due to the growing concern about new and emerging pathogens and partly through projects financed by the EU and other international organisations. This interest has also concentrated on investigations into the risks to human health (the zoonotic risks from wildlife). Several recent discoveries in the field have been published together with the gathering of large amounts of supportive information. However, this information is frequently published as specialised articles, or it deals only with local situations or individual studies rather than in the continental context. It is now apparent that these diseases must be considered from the perspective of the European continent.

This book describes each significant wildlife infectious disease. The Europe-wide information has been extracted, condensed and written by specialists in each topic. The book presents high-quality, accurate, clear and up to date information on the important aspects of the infectious diseases of wildlife. This type of information is frequently sought-after, but not always easy to locate or assimilate.

We believe that this book is needed now because there are changing situations and increased awareness in Europe with respect to:

1. The emergence or re-emergence of new diseases from wild animal reservoirs, such as avian influenza, classical swine fever, rabies, tuberculosis and foot and mouth disease.
2. The zoonotic implications of wildlife, for example, in highly-pathogenic avian influenza, rabies, West Nile Virus, hantavirus infections and food borne zoonoses (tuberculosis, hepatitis E).
3. Changes in the environment that wildlife may be a sensitive indicator for, in particular climatic change and its effects on disease ecology, and vector popula-

tions (ticks, midges, mosquitoes) resulting in spread of disease to new areas within and into Europe, for example bluetongue, tick borne encephalitis, Usutu and West Nile viruses.
4. The involvement of wild animals in the infection of livestock and pets. As wild animals move freely and certain populations increase, then contact between wildlife and domesticated animals will increase. For example salmonellosis in domestic cats and classical swine fever from wild boar to pigs.
5. The public concern for the effects of disease on the health of wild animals, particularly if causing mass mortality, for example morbillivirus in seals and outbreaks of botulism in birds.
6. The opening of markets within Europe results in movements of animals and a higher risk of spreading disease between livestock and wildlife, for example foot and mouth disease, classical swine fever.
7. Limited control of wild populations, encroachment by man on wildlife habitat and decreased hunting is producing high density and urban populations of roe deer, red fox and wild boar. As a consequence, there is increased contact with domesticated animals and humans, which results in increased transmission of disease between species.
8. The importance of the sub-clinical reservoir role of wild animals and the need for surveillance methodologies in detecting these covert infections, for example in rabies and tuberculosis.
9. The potential for migrating wildlife to introduce new and exotic pathogens to Europe.
10. There is also recognition that those assessing diseased wild animals require background information and guidance as to treatment and control strategies.
11. Disease may threaten wild populations, endangered species, and ultimately biodiversity.

This book is original because it is the first text to describe in detail the infectious diseases of wild mammals and wild birds in Europe. Its key features are that –

- It presents information on aspects of each disease or group of pathogens to provide the reader with a clear background and also covers the distinctive nature of these diseases as they occur in Europe (pathogen strains, insect vectors, reservoir species, climate, etc).
- It concentrates on the Europe-wide situation, including geographical distribution of the diseases, European wildlife species and European regulations for the diagnosis and control of the diseases.
- It describes the latest advances in veterinary diagnostics including molecular technology. Wildlife vaccination and disease surveillance techniques are described.
- It provides practical information, for example listing the animal species in which the disease has been recorded; the samples required for diagnostic examination, the diagnostic methods and the EU community reference laboratories.
- It provides guidance on disease control measures.

Our aim is to provide useful information for scientists trying to understand the health of wildlife populations. The One World-One Health concept shows that this is essential in understanding global health systems. If biologists, ecologists, veterinarians, epidemiologists and wildlife rehabilitators find the text helpful then the aim will be achieved.

COVER IMAGE ACKNOWLEDGEMENTS

Top: A brown hare leveret belonging to a litter of four orphans was found opportunistically in the wild. Subsequently it was demonstrated that it had antibodies against *European brown hare syndrome virus*, indicating for the first time that passive transfer of immunity to this virus, probably through colostrum, occurs in hares. Photograph: Bengt Ekberg.

Middle: Scanning electron micrograph of cultured bacterial cells of the intestinal spirochaete '*Brachyspira suanatina*' isolated from a pig. This pig enteropathogen colonizes the intestines of free-living wild mallards and domestic pigs and may be experimentally transferred between the two species. Photograph: Désirée S. Jansson and Leif Ljung.

Bottom: Microphotograph showing intracytoplasmic eosinophilic (pink) inclusion bodies in degenerating skin cells of a musk ox calf with contagious ecthyma (orf) caused by parapoxvirus infection. Photograph: Turid Vikøren.

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We acknowledge the inspiring gatherings of the Wildlife Disease Association and its section, the European Wildlife Disease Association, for working toward their shared mission 'to acquire, disseminate and apply knowledge of the health and diseases of wild animals in relation to their biology, conservation, and interactions with humans and domestic animals'. The solid scientific platform of these organisations and their welcoming atmosphere gave us the opportunity to learn about wildlife diseases, to keep our knowledge updated and to meet wildlife scientists, several of whom are contributors to this book.

We also acknowledge the OIE (the World Organisation for Animal Health), IUCN (The World Conservation Union), the European Association of Zoo and Wildlife Veterinarians and like-minded societies, which have contributed to the knowledge base of this book. Several of the chapters were reviewed anonymously and we thank those external reviewers.

SECTION

1

Viral Infections

CHAPTER

1

HERPESVIRUS INFECTIONS

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INTRODUCTION

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Herpesvirales is a vast order of currently approximately 130 large enveloped DNA virus species divided into three families. Herpesviruses have been isolated from most species investigated, including mammals, birds, reptiles, insects, molluscs and amphibians; and several animal species have been found to be infected with several herpesvirus species. Herpesviruses are evolutionarily old viruses that have co-evolved with their hosts for more than 250 million years.

Morphologically, herpesviruses are distinct from all other viruses, with a linear, double-stranded DNA genome of 125–250 kbp contained within an icosadeltahedral capsid of 100 to 110 nm and containing 162 capsomers. This capsid is surrounded by an amorphous-looking, protein matrix, with variable thickness, called the tegument and then by a trilaminar envelope containing lipids and proteins, bringing the total size of the virion from 120 nm up to almost 300 nm. The presence of lipids in the envelope has practical implications, as it renders herpesviruses sensitive to detergents and lipid solvents. There are numerous spikes of glycoproteins protruding from the envelope. These spikes are more numerous and shorter than in other virus families. The variation in the size of the genome is to some extent attributed to the presence of internal and terminal repeats. Common to all herpesviruses is that they are complex and contain genes for a large number of enzymes necessary for their replication, that viral DNA synthesis and capsid formation takes place in the nucleus of the infected cell, and that infected cells are destroyed owing to the virus replication and release of virus progeny, together with the ability of herpesviruses to establish latent infections. During latency no virus progeny is produced and the genome remains in a circular form.

The order *Herpesvirales* can be divided into three families: the family *Herpesviridae* contains the viruses of mammals, birds and reptiles; the family *Alloherpesviridae* contains fish and frog viruses; and the family *Malacoherpesviridae* contains the bivalve virus. The family *Herpesviridae*, which includes approximately 79 known virus species so far, is further subdivided into three subfamilies: *Alphaherpesvirinae*, *Betaherpesvirinae* and *Gammaherpesvirinae*.

Alphaherpesviruses are characterized by a rather broad host range, short replication cycle, rapid destruction of

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infected cells and a rapid spread in the host. Furthermore, they have the ability to establish life-long latent infection in sensory ganglia, or sometimes in other ganglia. Alphaherpesviruses are known to cause several acute diseases of veterinary importance.

By contrast, betaherpesviruses, often called cytomegaloviruses, have a restricted host range, long replication cycle and a slow spread of infection, with latent or persistent infections possible in a range of tissues, e.g. lymphoreticular cells, secretory glands and kidneys. Infection usually results in significant enlargement of certain cell types, known as cytomegaly. Infections are often widely distributed in the host population and usually not clinically apparent, except when such a virus appears in a previously uninfected herd.

Gammaherpesviruses usually have a host range restricted to the host's family or order. Viruses of this subfamily have specificity for either B- or T-lymphocytes and may cause lymphoproliferative disease. Latency of gammaherpesviruses may be established in lymphoid tissue. Infections with viruses from this subfamily generally cause few clinical signs in the main host but may cause severe disease in other related species, as exemplified by malignant catarrhal fever.

The ability of herpesviruses to cause latent infections is of great epidemiological importance, as it is generally not possible to determine or confirm if an animal is latently infected owing to the almost complete absence of gene expression, viral replication or host immune response during latency. Thus, diagnostic assays usually do not detect latent infections. A latent infection can, however – under certain conditions such as in the presence of concurrent disease, stress, immunosuppression or hormonal changes – reactivate, resulting in a productive infection with excretion of viral particles, transmission and infection of susceptible animals.

HERPESVIRUS INFECTIONS IN WILD MAMMALS

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It is believed that most animal species can harbour at least one, if not more, endemic herpesviruses. With more than 5000 mammalian species and only around 200 herpesviruses identified so far, one can easily speculate on the many more yet to be found and added to the order *Herpesvirales*, already the biggest order of viruses in existence.

Phylogenetic studies show co-speciation between herpesviruses and their hosts, with divergences in viral taxonomy mimicking those of animal species. Whereas herpesviruses of mammals and birds have shared a common ancestor, divergence seems to have happened over 220 million years ago, with speciations within sublineages in the last 80 million years as mammalian radiation took place[1,2].

Although many herpesviruses are well adapted to their natural host, there are several that can cross the species barrier and infect other animals. This is the case for many herpesviruses that can circulate between wild animals and domestic animals (e.g. *Alcelaphine herpesvirus 1* and *2*). Some others can have zoonotic potential, such as herpesviruses from primates that infect and cause severe disease in humans (e.g. *Macacine herpesvirus 2*). Human-specific herpesviruses also have the potential to infect wild animals.

Table 1.1 summarizes some of the most important herpesviruses relevant to European wildlife.

AUJESZKY'S DISEASE, OR PSEUDORABIES

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Aujeszky's disease (AD), pseudorabies or 'mad itch' is a neurological/respiratory disorder that affects a wide range of animals, except humans and some primates. It is caused by porcine or suid herpesvirus type 1, also known as pseudorabies virus or Aujeszky's disease virus (ADV), which belongs to the family *Herpesviridae* in the genus *Varicellovirus*.

AETIOLOGY

ADV is a 150–180 nm virion composed of a 145 Kb linear double-stranded DNA genome within an enveloped

TABLE 1.1 Important mammalian herpesviruses for European wildlife. Viruses are presented according to their taxonomic distribution within the three subfamilies of the order *Herpesvirales*.

	Name	Acronym	Common name
Subfamily	***Alphaherpesvirinae***		
Genus	***Simplexvirus***		
Species in the genus	*Bovine herpesvirus 2*	BoHV2	Bovine mammilitis virus
	Human herpesvirus 1	HHV1	Herpes simplex virus type 1
	Macacine herpesvirus 1	McHV1	Herpes simian B-virus
Genus	***Varicellovirus***		
Species in the genus	*Bovine herpesvirus 1*	BoHV1	Infectious bovine rhinotracheitis virus
	Bubaline herpesvirus 1	BuHV1	Water buffalo herpesvirus*[a]
	Canid herpesvirus 1	CaHV1	Canine herpesvirus
	Caprine herpesvirus 1	CpHV1	Goat herpesvirus
	Cervid herpesvirus 1	CvHV1	Red deer herpesvirus
	Cervid herpesvirus 2	CvHV2	Reindeer herpesvirus
	Felid herpesvirus 1	FeHV1	Feline rhinotracheitis virus
	Phocid herpesvirus 1	PhoHV1	Harbour seal herpesvirus
	Suid herpesvirus 1	SuHV1	Pseudorabies virus
Unclassified in the subfamily	*n/a*	n/a	Bottlenose dolphin herpesvirus
	n/a	n/a	Tursiops truncatus alphaherpesvirus
Subfamily	***Betaherpesvirinae***		
Genus	***Cytomegalovirus***		
Species in the genus	*Macacine herpesvirus 3*	McHV3	Rhesus macaques cytomegalovirus*[b]
Unclassified in the subfamily	*Suid herpesvirus 2*	SuHV2	Porcine cytomegalovirus
	n/a	n/a	Bat betaherpesvirus
Subfamily	***Gammaherpesvirinae***		
Genus	***Lymphocryptovirus***		
	Human herpesvirus 4	HHV4	Epstein–Barr virus*[c]
Genus	***Macavirus***		
	Alcelaphine herpesvirus 1	AlHV1	Malignant catarrhal fever virus*[d]
	Alcelaphine herpesvirus 2	AlHV2	Hartebeest malignant catarrhal fever virus*[d]
	Caprine herpesvirus 2	CpHV2	Caprine herpesvirus 2
	Ovine herpesvirus 2	OvHV2	Sheep-associated malignant catarrhal fever virus
Genus	***Percavirus***		
	Mustelid herpesvirus 1	MusHV1	Badger herpesvirus
Unclassified in the genus	*Phocid herpesvirus 2*	PhoHV2	Phocid herpesvirus 2
Genus	***Rhadinovirus***		
Unclassified in the genus	*Leporid herpesvirus 2*	LeHV2	Herpesvirus cuniculi
Unclassified in the subfamily	*n/a*	n/a	Rupicapra rupicapra gammaherpesvirus 1
Unclassified in the family	*Erinaceid herpesvirus 1*	ErHV1	European hedgehog herpesvirus
	Sciurid herpesvirus 1	ScHV1	Ground squirrel cytomegalovirus
	Sciurid herpesvirus 2	ScHV2	Ground squirrel herpesvirus

n/a – not available

*[a] Most buffalos in Europe are semi-domesticated

*[b] Mostly only at zoos, only monkey wild population in Europe living in Gibraltar is free from McHV3

*[c] Shown experimentally to infect dog cells and also found in seroscreenings of canids

*[d] Present in Europe only in zoos but represent the type species in the genus

nucleocapsid. The 105–110 nm wide nucleocapsid is formed by different structural proteins and its envelope is a lipidic membrane composed of nine different enclosed glycoproteins used in the life cycle of the virus, immune modulation and pathogenicity.

EPIDEMIOLOGY

ADV is widely distributed in European wild boar populations (Figure 1.1)[3]. Some countries, where AD has not been identified in wild boar populations, have reported

FIGURE 1.1 European countries where reports of ADV surveillance in wild boar populations have been published in the scientific literature[3] or reported in national wildlife surveillance programmes between 1987 and 2011. Countries shaded in green represent ADV-positive wild boar populations, whereas countries shaded in blue represent ADV-negative surveyed wild boar populations. Countries shaded in orange have reported pseudorabies outbreaks in hunting dogs associated with wild boar hunting, but where the status of wild boar populations is unknown. Seroprevalence/prevalence reported range of the within-country surveyed European wild boar populations are shown.

pseudorabies outbreaks in dogs used in boar hunting, i.e. Austria[4,5], Belgium[6], Hungary and Slovakia[7]. Several European countries where ADV has not been reported or where it was eradicated from domestic pigs have not assessed the status of ADV in their wild boar populations (e.g. Denmark, Norway, Finland or the UK). Thus, the current known distribution of ADV in European wild boar populations may not be accurate.

ADV is able to infect a wide range of mammals, including ungulates, carnivores, lagomorphs, rats and mice. Infection in mammals is usually fatal; however, in some species subclinical infection is possible[8]. In suids, the only natural host species for ADV, the infection may cause disease or be subclinical.

Many European wild boar populations have had laboratory assessments for the presence of ADV or anti-ADV antibodies (Figure 1.1); however, the basic understanding of ADV epidemiology in boar is poor. Pan-European serological studies on ADV in wild boar have shown that the probability of contact with the virus increases with age. ADV causes life-long latent infection in suids and naturally infected animals remain seropositive, and potentially infective, for life. A similar viral exposure risk occurs for males and females; however, sex-related differences, with higher exposure of females to ADV, is seen in some European and North African wild boar populations (9). This may be related to behavioural differences between the sexes. Intra-group transmission is higher in all-female groups of wild boar, whereas males tend to be solitary. The probability of wild boar acquiring ADV in endemic areas also seems to be dependent on population density and the extent to which the animals aggregate[10], both of which

are highly variable factors across Europe, and this gives rise to regional/local variations in prevalence. Additionally, wild boar population structure, female group size, management or predation may influence the rate of transmission of ADV within and between groups. This could be the reason for the similar viral infection risk of males and females observed in many wild boar populations in Europe. Movement of individuals between infected and susceptible wild boar groups or populations is likely to be important for virus spread.

ADV survival rate in the environment is low. Transmission by the aerosol route is also low in hot and dry weather conditions, which are unfavourable for the virus, but is enhanced if weather conditions are cool and wet.

The European wild boar is currently considered as a true ADV reservoir, because the virus can infect, replicate and be excreted in this species, which is sufficiently abundant to be a wild reservoir. Other mammalian species are dead-end hosts in which death occurs before viral excretion. In the USA, some experimentally infected raccoons (*Procyon lotor*) have been found to behave as short-term reservoirs of ADV when infected at low doses[8], which would suggest a transient reservoir role.

Currently the main routes of ADV transmission in the European wild boar are not known; however, they are suspected to be by direct contact between individuals. There is little information as to whether aerosol infection is an efficient transmission pathway between wild boar. The oronasal route is suspected to be the usual means of ADV transmission between European wild boar, but the precise importance of aerosol transmission even over short distances is not known.

Venereal transmission is considered of primary importance for ADV transmission in American feral pig populations[11], and it may be an important route in European wild boar as well. An increase in seroprevalence after the mating season was found in wild boar in Spain[9], which, apart from suggesting an increasing contact rate between individuals, may perhaps also reflect the occurrence of venereal transmission. Additionally, ingestion of infected meat via cannibalism is considered a possible route of transmission.

Wild boar females usually live in groups with their offspring and juvenile animals. This may give rise to closer contact within female groups, and oronasal transmission is thought to predominate in these groups. Wild boar males are usually solitary for most of the year except during the mating season, when they make contact with female groups. Venereal transmission could be linked with reactivation of latent infections due to mating stress. Behavioural patterns of wild boar depend to a large extent on the availability of food resources, and it is believed that these food-based behaviours may be an important influence in determining ADV prevalence. The threshold infective dose for ADV in wild boar may vary according to the virulence of the circulating strain and the immune status of the infected animal, as occurs in the domestic pig.

ADV is excreted in suids by nasal exudates, saliva, vaginal mucus, sperm, milk, faeces and occasionally urine. Different routes of infection by ADV are potentially possible because there is some, unquantified, survival of the virus in the environment, particularly in organic material, and some persistence in aerosols. Wild carnivores acquire infection after consumption of ADV-infected wild boar meat, as may happen to dogs that eat or bite infected wild boar during hunting. Direct contact with ADV-excreting boar or indirect contact with infected fomites or aerosols are assumed to be the main ways of infection for wild ungulates.

PATHOGENESIS, PATHOLOGY AND IMMUNITY

Following primary infection, viral replication of ADV takes place in the nasal or genital mucosa and in the tonsilar epithelium. Later, ADV invades the nervous system via the nerve endings present in the genital, oral and nasal mucosae and progresses by moving along the nerves into the central nervous system (CNS). At this stage of infection, ADV can be detected in oropharyngeal tonsils, nasal cavity, genital mucosa, sacral ganglia or trigeminal ganglia. At this stage the virulence of the ADV strain and the immune status of the host (in the case of true reservoirs) determine whether there is invasion of the CNS, or establishment of a latent infection in the trigeminal or sacral ganglia. Infection progresses rapidly into the CNS in dead-end hosts. The virus can be detected in association with blood cells after infection but peripheral blood mononuclear cells do not carry ADV in latent infections. The virus may also replicate in lung and pharyngeal respiratory epithelia and in endothelium.

Very little is known about natural disease development in wild boar, but recent natural AD cases in wild boar piglets in Germany[12] show similarities with domestic pigs. Clinical disease in the domestic pig ranges from fatal

nervous disease usually seen in piglets, respiratory problems in post-weaning pigs and respiratory and reproductive manifestations in adults. Encephalitis has been found in wild boar naturally infected with ADV[12,13] and in animals that have been experimentally infected with ADV of moderate virulence[14]. The pathological outcome of ADV infection depends on the virulence of the strain. It is hence probable that low-virulence strains present in European wild boar populations may cause no lesions in this species. The histopathological findings consist of non-suppurative meningoencephalitis and ganglioneuritis with neuronal degeneration, focal gliosis, perivascular mononuclear cuffing and lymphocytic inflammation. Intranuclear inclusion bodies may be observed in neurons of the CNS or in ganglionar neurons. The viral tropism for epithelial tissues in the respiratory tract leads to alveolar, bronchiolar and bronchial epithelial degeneration and mononuclear cell infiltration. Degeneration and necrosis, often with intranuclear inclusion bodies, may occur in the liver, spleen, kidneys, pancreas, adrenal gland, thymus, lymph nodes, tonsils and intestinal epithelium. Oedema and haemorrhages are frequently observed.

Disease in dead-end hosts progresses rapidly, usually with a fatal outcome within 24 to 72 hours following infection. The tropism of ADV for endothelial cells leads to extravasations and oedema in the lungs, nasal and oral cavities. ADV pathogenesis is broadly similar for different dead-end host species, except for mink, in which vasculopathy is predominant to neuropathy[15]. Gross and microscopic lesions of AD in dead-end hosts and domestic pigs reflect the neurotropic nature of this herpesvirus. Many of the affected dead-end hosts may show no gross lesions because of the rapidly fatal outcome of infection, or they may show skin lesions caused by self-trauma due to the intense pruritus (see the Clinical Signs section below). Fibrinoid vasculitis, with haemorrhages and myocardial necrosis, is inconsistently described but appears to be typical in farmed mink[16]. Cardiac alterations in dogs may cause sudden death due to arrhythmias. Lesions in abdominal organs have been also found in different species of North American carnivores such as bears, coyotes (*Canis latrans*) and a Florida panther (*Puma concolor coryi*).

ADV infection evokes both humoral and cell-mediated immune responses in suids, but the immune response is unable to completely clear infection, and reinfection and activation of latent infections may occur. The cellular immune response to ADV has been the subject of little research in wild boar. Outer envelope ADV glycoproteins stimulate the production of neutralising antibodies, particularly those directed against glycoproteins (g) C and D (gC and gD). During the early stages of infection, neutralizing antibodies block virus attachment and invasion of cells. Infection of wild boar with low-virulence ADV strains (those circulating in European wild boar populations) induces a long-lasting active humoral immunity, which can be passed on to the offspring and confer protection to boar piglets during the first 15 weeks of life[17].

A characteristic of herpesviruses is their ability to evade the host immune response by producing long-term latent infections in specific tissues. Subsequent immunosuppression in the host may allow the infection to reactivate with viral replication and then dissemination throughout the body. Virus can then be excreted in high titre and is able to infect other susceptible individuals. ADV mainly establishes latency in neuronal cells, such as the trigeminal or sacral nervous ganglia. Reactivation of latent infections does not usually lead to overt clinical disease. Reactivation of latent infections should be carefully considered when planning ADV eradication from domestic pigs. It should also be considered when studying ADV epidemiology in wild boar populations.

CLINICAL SIGNS

ADV strains circulating in some European wild boar populations are attenuated and as a result have low virulence. The majority of the wild boar infected with ADV show no clinical signs. Experimental infection of wild boar with virulent strains has resulted in fatal disease[14], similar to that following experimental infection in domestic pigs. Experimental infection of immune-compromised wild boar with ADV strains of wild boar origin has resulted in clinical disease[14]. An outbreak of AD was reported in European wild boar in Spain, where juveniles and adults showed nervous clinical signs and the mortality was 14%[13]. Two wild boar with signs of neurological disturbance have been diagnosed with AD in Germany[12]. These findings indicate that clinical disease cases in free-living boar in Europe may occur but are infrequently observed.

Mild clinical signs including mild pyrexia, sneezing, nasal discharge and conjunctivitis were observed in wild boar experimentally infected with an ADV isolate from wild boar origin[14]; however, following steroid-induced immunosuppression, when these animals were reinfected

using the same strain, they developed severe clinical disease with pneumonia and death.

In wild dead-end host species the clinical outcome of AD is usually fatal, resulting in death within a few days after infection. The first signs are appetite loss and diminished activity, but later the animal develops mild nervous signs. A sero-mucoid nasal discharge may appear, as well as respiratory distress and fever. The affected animals often develop pruritus, which may lead to self-mutilation. Later excitement and hyperaesthesia become greater and convulsions can occur before the animal collapses and dies. In some cases the clinical course is very short and death is rapid, with only minimal clinical signs observed.

DIAGNOSIS

Aujeszky's disease should be considered when neurological disease is seen in European wild mammals; however, some countries, such as the UK, are free of ADV. Detection of virus is by isolation in cell cultures or molecular detection of ADV genomic material in tissues. PCR testing utilizes the glycoprotein encoding genes, which are highly conserved between different ADV strains (gB/gD) and constitute the main target of polymerase chain reaction (PCR) tests.

Viral isolation and/or viral genome detection by PCR are used for the diagnosis of ADV infection in the European wild boar. The trigeminal ganglia (TG) are considered the best site to detect latent infections in domestic pigs. The attenuated nature of European wild boar ADV strains may lead to the establishment of latent infections in sacral ganglia after venereal transmission as has been recorded in feral pigs in North America. Hence, absence of ADV in TG does not exclude latent infection in European wild boar[18]. In preparation for PCR testing, both sets of ganglia require dissecting out and removal from dead animals.

Serological methods for detection of anti-ADV antibodies are of limited diagnostic use in non-suid species because of the rapid course of the infection. Viral neutralization tests, western blot and enzyme-linked immunosorbent assay (ELISA) may be useful techniques for the detection of antibodies against ADV in suids. The ELISA is a sensitive and specific test in the domestic pig. Owing to its low cost, high reproducibility and rapidity of use it is also a useful tool for epidemiological studies in European wild boar. However, 45% of European wild boar with viral ADV DNA did not have antibodies detectable with ELISA[18]. As a result of suspicions that the currently used ELISA may not detect all wild boar ADV antibodies, new serological tests may be necessary in particular to identify latently infected wild boar in Europe. Further research is required in Europe to ensure that diagnostic tests used for wild boar are reliable.

MANAGEMENT, CONTROL AND REGULATIONS

Management of ADV in wild boar populations first requires surveillance for the disease. Where presence of ADV is identified, management of the disease in free-living European wild boar is difficult because: i) ADV is widely distributed across European wild boar populations; ii) its prevalence is high in some wild boar populations and seems to be increasing, while the geographical range of ADV infected boar is also extending in some regions; iii) there is little relevant information on the efficacy of preventive management strategies such as vaccination, reduction of population densities (through targeted hunting) and avoiding supplementary feeding, which results in concentration of animals[19]. Risk assessment is particularly important when considering ADV control in wild boar. A limited amount of work has been done in Europe on the testing of an Aujeszky's disease vaccine for wild boar; however, currently no validated vaccine is available. There is no reporting regulation of ADV in wild boar in Europe. Aujeszky's disease is notifiable to the World Organisation for Animal Health (OIE).

PUBLIC HEALTH CONCERN

ADV is considered as a non-zoonotic pathogen, but mild pruritus may appear in humans when handling the virus in the laboratory[20].

SIGNIFICANCE AND IMPLICATIONS FOR ANIMAL HEALTH

Aujeszky's disease is common to both wild boar and domestic pigs, and it has been eradicated from the domestic pig in many European countries. Contact between wild boar and domestic pigs, especially in extensive production,

may lead to outbreaks in the domestic pig as a consequence of ADV circulating in wild boar populations.

The effect of ADV on the population dynamics of wild boar appears to be limited to reduced reproductive output and reduced survival of neonates, with little measurable effect in reducing the overall numbers of animals in boar populations. However, there is insufficient information on the disease in wild boar populations to properly assess the effects of ADV.

There are several reported incidents of Aujeszky's disease causing deaths in dogs used in boar hunting across Europe. Carnivores, including threatened species, that consume wild boar are at risk of acquiring ADV. Wolves are active predators of European wild boar and may be at a high risk of contracting Aujeszky's disease; however, there is little published evaluation of the effects of the disease on wild animals other than boar. The seroprevalence of ADV in wild boar has increased substantially in the remaining habitat for the IUCN critically endangered Iberian lynx (*Lynx pardinus*) in Spain, and the disease poses a risk to reintroduction programmes in these areas.

MALIGNANT CATARRHAL FEVER

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Malignant catarrhal fever (MCF) is a generally fatal disease of artiodactyla, primarily affecting ruminants of the subfamily Bovinae and family Cervidae[21]. It is caused by closely related rhadino herpesviruses, which characteristically infect their natural host in the absence of any recognized clinical signs but which are capable of transmission to other species, causing a catastrophic immunological dysfunction and resulting in dramatic clinical and pathological disease[22].

AETIOLOGY

Worldwide, the principle cause of MCF is the rhadino herpesvirus, *Ovine herpesvirus 2* (OvHV2), which infects domestic sheep and may infect other species of the subfamily Caprinae, in the absence of recognised disease[23]. The other principle cause of MCF is *Alcelaphine herpesvirus 1* (AlHV1), which inapparently infects wildebeest (*Connochaetes* spp.). This form of the disease primarily affects cattle in Africa but has also affected other ruminant species in zoological collections elsewhere. In addition, *Caprine herpesvirus 2* (CpHV2) of domestic goats and the so-called virus of 'white-tailed deer' have also been implicated as causal agents in a few cases.

In the context of European wildlife, the only known potential causes of disease are OvHV2 and CpHV2, neither of which have been isolated in conventional culture systems. Infection with either agent can, however, be confirmed through PCR or detection of antibody that cross-reacts with the AlHV1 antigens.

These viruses, together with those of large African antelope *(Alcelaphinae* and *Hippotraginae)* form a complex of viruses referred to as the MCFV complex (Figure 1.2)[24].

EPIDEMIOLOGY

GEOGRAPHICAL DISTRIBUTION AND HOSTS

Initially MCF was described as a disease of domestic cattle in Europe, but a very similar disease of cattle was recognized in southern Africa shortly thereafter and subsequently has been reported in a variety of species worldwide[25]. Cattle of Asiatic origin (*Bos javanicus* and *Bos gaurus*), water buffalo (*Bubalus bubalis*), many species from the family Cervidae, excluding fallow deer (*Dama dama*), and North American bison (*Bison bison*) are particularly susceptible to infection.

Despite the normally dramatic fatal presentation of the disease and high incidences in deer and bison when managed as farm animals, there are relatively few reports of the disease affecting free-living animals[26–28]. In addition, as it is now recognized that OvHV2 can cause MCF in domestic pigs[29], it is probable that wild boar would also be susceptible, although no disease has been reported in Europe or elsewhere. It should also be noted that experimentally both AlHV1 and OvHV2 can be transmitted to laboratory rabbits, producing characteristic MCF[30]. It is therefore theoretically possible that wild rabbits could be affected, although no such cases have been reported.

Both sheep and goats appear to be able to act as natural hosts for OvHV2, whereas only goats have been identified in the case of CpHV2. In the natural host, infection appears to transmit efficiently with all, or most, adults

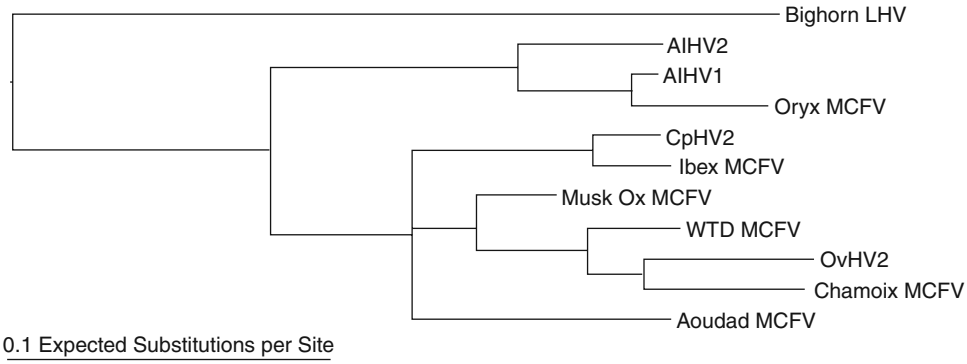

FIGURE 1.2 Phylogenetic analysis of MCF viruses was based on a 177 bp fragment of the DNA polymerase gene, for which the widest range of sequences were available. The DNA sequences were aligned using ClustalV, based on the translated amino acid sequences, and the phylogenetic analysis was done using TOPALi[24]. Model selection was used to define the appropriate parameters for analysis by Mr Bayes using codon position models. The sequence from the bighorn sheep lymphotropic herpesvirus was included as an outgroup. The analysis was performed by Dr George Russell, Moredun Research Institute. AlHV = alcelaphine herpesvirus; CpHV = caprine herpesvirus; LHV = lymphotropic herpesvirus; OvHV = ovine herpesvirus; WTD = white-tailed deer.

carrying latent infection. Transmission of OvHV2 in Europe would appear to be essentially perinatally among lambs, establishing a life-long latent infection, probably as with other herpesvirus infections, with periodic recrudescence and virus excretion[31]. All sheep and goats should thus be regarded as potential sources of infection. It is probable that native European species of sheep and goats and related species of the subfamily Caprinae are carriers of these, or similar, viruses, and evidence of infection with either virus is not normally associated with pathological changes.

In addition, the quantity of viral DNA detected in affected tissues is trivial, and there is no evidence of productive viral replication in any MCF-affected animals. It is concluded that MCF-susceptible species are not responsible for the spread of the virus, nor do they act as carriers. Disease in European wildlife has only been described in species of deer, although the susceptibility of North American bison to MCF suggests the potential susceptibility of European bison, while rare cases in domestic pigs does raise the possibility that wild boar could also be susceptible. The most convincing evidence of MCF in free-living wildlife is from a report from Norway in which disease was confirmed in moose (*Alces alces*), roe deer (*Capreolus capreolus*) and red deer (*Cervus elaphus*) over a 23-year period. Evidence of MCF in these animals on both histological and molecular virological grounds is compelling, and both OvHV2 and CpHV2 appear to have been involved. MCF in farmed deer is a relatively common disease, and in the early years of deer farming substantial outbreaks occurred both in the UK and in New Zealand. Disease in free-living animals has, however, never been reported in either country, despite the very substantial numbers of deer and sheep in both countries. It is noteworthy that, compared with the high incidence of MCF experienced in the first 10 years of deer farming in the UK, the disease is now sporadic and relatively uncommon. Spectacular outbreaks of MCF in farmed North American bison have also been reported in herds that have only recently been subjected to relatively intense management[32].

It is tempting to speculate that the susceptibility of certain species may therefore be related to exposure to management systems that have not been optimized in favour of animal welfare.

It is also noteworthy that reports of MCF of pigs have most frequently been associated with Scandinavia, although there are also reports of the condition from Germany, Switzerland and the USA. In these cases the causal virus has been OvHV2, and there is no evidence that a variant form of the virus with greater infectivity for pigs has been involved. In addition, the breeds of pigs affected in these outbreaks have been varied, which suggests that susceptibility is unlikely to be determined by breed. It is thus concluded that as-yet unidentified environmental factors result in pigs becoming apparently more susceptible to infection in Scandanavia. In the absence of any other explanation, such unidentified factors may be impacting similarly on free-living deer in Norway.

PATHOGENESIS, PATHOLOGY AND IMMUNITY

The most likely route of entry of the MCF viruses is the mucosa of the upper respiratory tract and the tonsils. The virus infects lymphocytes (CD8+ T cells); their role in the pathogenesis is unclear. Lymphoproliferation is likely to be the result of dysfunction of T-lymphocytes. Disturbed cytotoxic T-cell activity is probably involved in the development of vascular and epithelial lesions.

Gross pathological changes reflect the variable clinical signs and may involve most systems. MCF is characterized by erosions and ulcerations in the mucosae and in the skin, vasculitis and lymphoproliferation. Skin lesions are not infrequent in deer and may involve extensive alopecia, erosions and crusting dermatitis, primarily of the limbs and perineum. Bilateral corneal opacity and conjunctivitis are frequently present and catarrhal encrustation of the nares and oral cavity are often a feature, together with erosion of the epithelium. Lymph nodes are generally enlarged and oedematous, and may be haemorrhagic. Haemorrhage of the intestinal mucosa is frequently present and can affect the abomasum and most sections of the large and small intestine. Characteristic lesions of the urinary bladder include petechiae and ecchymosis and the kidney frequently has raised white nodules, which are the result of lymphocytic accumulations.

Presumptive diagnosis has relied on the detection of histological lesions characterized by epithelial degeneration, vasculitis, hyperplasia and necrosis of lymphoid organs and widespread accumulations of lymphoid cells in non-lymphoid organs. All epithelial surfaces may be affected and are characterized by erosion and ulceration with sub- and intra-epithelial lymphoid cell infiltration, which may be associated with vasculitis and haemorrhage.

Vasculitis affecting veins, arteries, arterioles and venules, but most typically medium-sized arterioles, is generally present and most pronounced in the brain (Figure 1.3). It is characterized by perivascular accumulation of lymphoid cells, and fibrinoid degeneration or necrotizing vasculitis, and there may be endothelial damage, which may lead to occlusion of vessels.

Lymph nodes characteristically are affected by lymphoblastoid cell expansion in the paracortex and degeneration of follicles, and oedema and inflammation are present in the perinodal tissue. Interstitial accumulation of lymphoid cells, particularly in the renal cortex and periportal areas of the liver, are commonly present and may be extensive. Non-suppurative meningoencephalitis with lymphocytic perivascular cuffing is frequently present in the brain. Histological lesions of the cornea are characterized by lymphoid cell infiltration originating in the limbus and progressing centrally, and vasculitis, hypopyon and iridocyclitis may also be present.

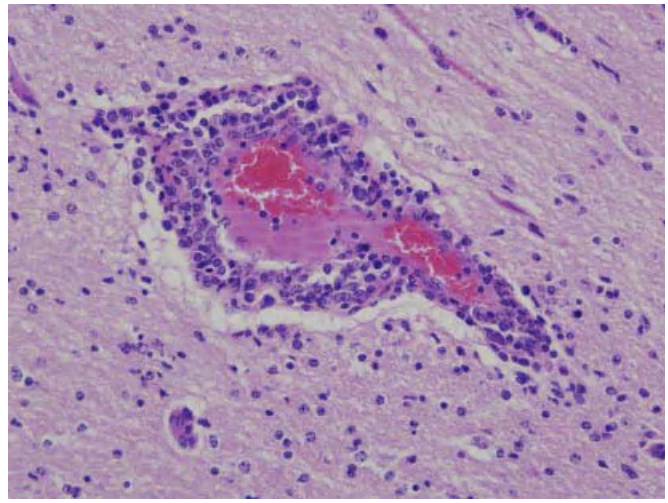

FIGURE 1.3 Histological section of farmed red deer brain with MCF, showing non-suppurative encephalitis. Note the characteristic accumulation of predominantly lymphoid inflammatory cells around the blood vessel (peri-vascular cuff) along with those free in the neuropil (gliosis, to the left of the blood vessel). Haematoxylin and eosin, original magnification ×100.

The serological response of MCF-affected animals may be undetectable or directed at only a few viral epitopes, implying that there is only limited virus antigen expressed in diseased animals[33]. The development of antibodies does not prevent a lethal outcome.

CLINICAL SIGNS

The clinical presentation of MCF is very variable and can involve most systems, ranging from peracute to chronic. In the peracute cases that have been observed in farmed deer, high fever, depression and profuse diarrhoea, which may be haemorrhagic, are the principle clinical signs. Generally, the course is more protracted and involves nasal and ocular discharges, which may be profuse and catarrhal, bilateral corneal opacity, enlarged lymph nodes, erosions in the oral cavity, erosion and/or hyperkeratosis of the skin and/or neurological signs involving blindness and behavioural changes. In chronic cases in deer, alopecia has also

been a feature. In the cases involving wild deer, they were thin, often recumbent and showed a variety of clinical signs, including diarrhoea, abnormal behaviour, incoordination, blindness and convulsions[26]. Thus in light of the variability of the clinical presentation of MCF, this disease should be considered in any unexplained condition observed in deer.

DIAGNOSIS

In suspected cases of MCF in wildlife, examination of tissues for evidence of characteristic histological lesions, especially in the brain, is the most appropriate method of achieving an initial presumptive diagnosis.

Of the viruses that have been associated with MCF, only AlHV-1 has been recovered in conventional tissue culture, although lymphoblastoid cell lines with limited productive virus replication have been propagated from animals affected with both AlHV1 or OvHV2 forms of the disease. Despite not being applicable as an aid to diagnosis, these lymphoblastoid cell lines have proved valuable in understanding the pathogenesis of disease and have provided a source of viral DNA. Such DNA has facilitated the sequencing of the genome of both viruses and permitted the selection of suitable PCR reactions for amplifying DNA sequences that detect either the MCF group of agents or are virus-specific[23]. Such PCR reactions are now the method of choice for reaching a definitive diagnosis of MCF and identifying potential carrier animals.

All serological tests rely on AlHV1 antigens, as none of the other viruses can be productively replicated in tissue culture to provide virus specific reagents. The only critical report employing immunoblotting indicated that the sera of sheep and cattle infected with OvHV2 reacted erratically with AlHV1 antigens compared with sera of wildebeest[33]. It is also known that serological tests for herpesviruses as a group can cross-react. Thus, despite a number of serological tests being available, caution in interpreting results when employing them with sera from novel species, which are almost certainly infected with their own specific herpesviruses, is essential. In addition, sera from free-living animals may be of variable quality, which has the potential to impact on the reliability of tests. The merit of surveys for evidence of infection with MCF viruses employing sera from free-living species of wild animals is thus questionable and the results should not be assumed to indicate evidence of the incidence of infection.

PUBLIC HEALTH CONCERN

There are no indications that MCF can infect humans.

MANAGEMENT AND CONTROL

Control of MCF is based on preventing contact between susceptible hosts and the natural carriers (sheep and goats).

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RUMINANT ALPHAHERPESVIRUS INFECTIONS

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The subfamily *Alphaherpesvirinae* includes several viruses that cause a range of diseases in members of the suborder Ruminantia. With the exception of *Bovine herpesvirus 2* in the genus *Simplexvirus*, a virus with little impact in European wildlife, all other relevant ruminant alphaherpesviruses are in the genus *Varicellovirus*, as shown in Table 1.2. Of these, *Bovine herpesvirus 1* (BoHV1) is by far the most studied, serving as model for this group of ruminant viruses.

BOVINE HERPESVIRUS 1

BoHV1 is the aetiological agent of infectious bovine rhinotracheitis (IBR), and infectious pustular vulvovaginitis (IPV) or infectious pustular balanoposthitis (IPB). BoHV1 causes significant economic losses for the cattle industry worldwide, for which programmes of eradication and/or control of the disease have long been in place.

Although there are several differences in genomic organization and sequences between the different ruminant alphaherpesviruses, mechanisms related to gene expression

TABLE 1.2 Ruminant alphaherpesviruses in the genus *Varicellovirus* (with permission from Das Neves, 2009[34]).

Virus	Natural host	Disease	Geographic distribution	Status in European wildlife
Bovine herpesvirus 1 BoHV1	Bovine (*Bos taurus*)	Bovine rhinotracheitis, pustular vulvovaginitis and balanoposthitis	Europe, America, Asia and Oceania	Suspected but virus never isolated
Bovine herpesvirus 5 BoHV5	Bovine (*Bos taurus*)	Bovine encephalitis	Europe, America, Oceania	Not described
Bubaline herpesvirus 1 BuHV1	Water buffalo (*Bubalus bubalis*)	No clinical disease	Europe, Australia	Not described
Caprine herpesvirus 1 CpHV1	Goat (*Capra aegagrus*)	Vulvovaginitis, abortion, neonatal systemic infection, conjunctivitis	Europe, America, Australia	Suspected but virus never isolated
Cervid herpesvirus 1 CvHV1	Red deer (*Cervus elaphus*)	Ocular syndrome	Europe	Virus isolated
Cervid herpesvirus 2 CvHV2	Reindeer (*Rangifer tarandus*)	Ocular syndrome, respiratory disease, mucosal lesions, abortion	Europe	Virus isolated
Elk herpesvirus 1 ElkHV1	Elk (*Cervus canadensis*)	No clinical disease	America	Not described

or viral replication and latency, as well as pathogenesis, have been shown to be common to all of them.

The BoHV1 genome consists of a double-stranded linear DNA sequence with 135 301 nucleotides, comprising 67 unique genes. Some of these genes encode envelope proteins commonly called glycoproteins. Of these, gB not only plays an essential role in virus attachment and entry, but is also highly immunogenic, representing a dominant viral antigen that can lead to a protective immune response.

Despite its worldwide spread in domestic cattle and being the target of intense study, BoHV1 has not been reported to naturally cause disease in wildlife. Wildlife species have been screened using serological kits for BoHV1 based on gB as antigen. These tests, however, do not enable discrimination between the various ruminant alphaherpesviruses, so it is not possible to rule out the possibility that many wildlife species may harbour herpesviruses closely related to BoHV1 rather than BoHV1 itself.

Seropositive results against BoHV1 have been described throughout Europe in ibex (*Capra ibex*), chamois (*Rupicapra rupicapra*), red deer (*Cervus elaphus*), roe deer (*Capreolus capreolus*), reindeer (*Rangifer tarandus*), fallow deer (*Dama dama*), mouflon (*Ovis musimon*), European bison (*Bison bonasus*) and water buffalo (*Bubalus bubalis*)[35].

Several studies have focused on BoHV1 infections of heterologous hosts. Goats can be infected with BoHV1, leading to high excretion titres and latency. Experimental reactivation has also been successful, but these studies focused on domestic goats and little is known about BoHV1 infections of wild goats, for example. BoHV1 infections of deer and reindeer lead to minimal or no excretion, and latency does not occur. Altogether, studies seem to demonstrate that although BoHV1 can infect some wild ruminants it cannot be maintained over time, and wildlife seem therefore not to be important reservoirs for this virus.

In primary infections, BoHV1 has potential ports of entry in the nasal cavity, oropharynx, eyes and genital tract. Replication normally takes place in the epithelial cells, and high titres of BoHV1 are excreted at those ports of entry within 4–5 days post-infection. Although, as for most ruminant alphaherpesviruses, viraemia is possible, it seems that BoHV1 shows little systemic spread and is often restricted to the local ports of entry in primary infections. Nonetheless, BoHV1 can spread by association with mononuclear blood cells and reach the digestive tract, ovaries and fetus, where it can lead to abortion.

A strong humoral and cell-mediated response develops within 5 days post-infection, with maximum antibody titres around days 10–12 days post-infection. Residual antibody titres can be detected for up to 2 to 3 years post-infection. Besides the viral lytic cycle, in which active replication takes place, BoHV1 can become latent when viruses migrate to the CNS ganglia (e.g. TG or sacral ganglia) and enter a dormant stage. Different stimuli such as transport stress, calving, and other concurrent infections may lead to reactivation of BoHV1, with the virus returning to the port of entry or spreading to other organs and replicating. Although reactivation can lead to re-excretion,

reactivation episodes are often subclinical and hence difficult to identify.

In cattle, IBR can cause clinical signs such as high fever, anorexia, coughing, excessive salivation, nasal discharge, conjunctivitis, dyspnoea and nasal lesions that often consist of clusters of necrotic lesions on the mucosa. In the IPV/IPB form, clinical signs often start with frequent urination and mild mucosal irritation, and can progress to swelling of the vulva with small papules, followed by erosion and ulcers on the mucosal surface of either the vagina or the penis and prepuce. Respiratory infection can result in abortion or neonatal death, especially in calves deprived of colostrum. Infections with BoHV1 alone do not usually cause death in healthy mature cattle, unless in situations where the virus causes a generalized, systemic infection. No clinical signs or pathology related to BoHV1 infections have been reported in wildlife in Europe.

Definitive diagnosis is achieved by viral isolation, detection of viral DNA by specific PCR or restriction endonucleases analysis. Presence of virus in lesions can also be demonstrated by immunohistochemistry or electron microscopy. gB-based serological assays have been designed as an easy tool to identify the presence of BoHV1 infections, especially as antibody titres raised against gB tend to persist for long periods after infection, even though this method will also recognize antibodies against other closely related ruminant alphaherpesviruses. Seroneutralization assays or ELISA with less conserved antigens may be, in these cases, an important additional tool in discriminating among these ruminant alphaherpesviruses.

Controls and treatments for IBR/IPV in cattle vary among European countries depending, among other factors, on the status of the disease in a given country. Options may include the culling of seropositive animals in areas of low seroprevalence, or large-scale vaccination programmes in areas of higher seroprevalence. Identification of latent carriers also constitutes an important step in the control and prevention of outbreaks of BoHV1.

There are no known public health concerns associated with BoHV1. The impact of cross-infections between ruminant alphaherpesviruses is described in the sections on caprine and cervid herpesviruses.

CAPRINE HERPESVIRUS 1

Caprine herpesvirus 1 (CpHV1), previously known as *Bovine herpesvirus 6*, is an alphaherpesvirus closely related to BoHV1. It is widely distributed across Europe, can cause enteritis and generalized infections in neonatal kids, and induces vulvovaginitis, balanoposthitis, respiratory disease or abortion in adult animals.

This virus should not be confused with *Caprine herpesvirus 2* (CpHV2), a recently classified gammaherpesvirus closely related to *Ovine herpesvirus 2* and *Alcelaphine herpesvirus 1*, which is endemic in domestic goats and has been observed or is suspected to cause clinical MCF in certain species of deer in the USA[36] and Norway[26]. CpHV1 has a genomic organization similar to the genome of BoHV1, and there are several different strains.

CpHV1 probably has a worldwide distribution. In Europe, studies have reported this virus, or antibodies against it, in domestic goats in Italy, Spain, Norway, Greece, Germany and France (Corsica). CpHV1 is genetically and antigenically closely related to BoHV1, and conventional ELISA testing for antibodies usually cannot differentiate the virus to which the immune response has developed.

Few studies have been carried out in wild populations, but in France ibex (*Capra ibex*), chamois (*Rupicapra rupicapra*), red deer (*Cervus elaphus*), and roe deer (*Capreolus capreolus*) have tested seropositive for CpHV1[37].

Pathogenesis of CpHV1 is very similar to that of other ruminant alphaherpesviruses. CpHV1 can infect the animal by either the respiratory or the genital routes, quickly establishing viraemia and being detected in a variety of organs. Latency takes place at the TG or sacral ganglia, and upon reactivation (e.g. physiological stress), respiratory, genital tract and ocular re-excretion of the virus is possible. Experimental reactivation of CpHV1 has only been successful under high doses of dexamethasone. In kids the virus spreads very quickly, leading to a systemic disease with high morbidity. Vertical transmission from mother to fetus is also possible, and CpHV1 is associated with episodes of abortion in domestic goats, especially during the second half of pregnancy[38]. The impact of CpHV1-related abortions in wild goats and other members of the *Caprinae* subfamily remains unknown.

CpHV1 can lead to four main types of clinical situations.

1. Systemic form, often seen in young kids that exhibit progressive weakness and abdominal pain. There can be conjunctivitis and purulent nasal discharges, erosion in the oral and nasal cavities and oedema of the myocardium, among other signs. Animals usually die within 2–4 days after the onset of the infection[39].

2. Genital form, mostly in adult animals, where the infection can often be subclinical. Lesions in the genital mucosa can develop with hyperaemia, oedema and the appearance of papules, vesicles and ulcers[40].
3. Respiratory form, usually combined with a secondary bacterial infection, where animals develop an acute pneumonia[41].
4. Abortive form, especially during the second half of pregnancy, frequently without any other clinical ill-health in the dam, or lesions in the aborted fetus[38].

There is no known treatment for caprine herpesvirus infections. Confirmation of CpHV1 infection is usually achieved by viral isolation, or detection of viral DNA by specific PCR. Presence of virus in lesions can also be demonstrated by immunohistochemistry or electron microscopy. Conventional ELISA screening serology for BoHV1 will recognize antibodies against CpHV1 but not distinguish between the two viruses. Seroneutralization assays may be, in these cases, an important additional tool.

Given the potential of CpHV1 to cause fatal diseases in kids and also in adult animals, hygiene-based preventative control measures in the event of an outbreak are essential to avoid the rapid spread of the virus. The culling of seropositive animals and latent carriers may be necessary to prevent spread of disease. Vaccination studies are underway and, as for BoHV1, this might prove to be an important tool for the control of outbreaks.

There are no known public health concerns associated with this virus. CpHV1 has been shown to infect and establish latency in bovine calves, but reactivation was not successful and clinical signs were absent[42]. The impact of this virus in other species of the *Caprinae* subfamily remains unknown.

CERVID HERPESVIRUSES 1 AND *2*

Serological studies of cervids have long shown exposure of these animals to BoHV-1 or other closely related viruses. Two ruminant herpesviruses belonging to the same *Alphaherpesvirinae* subfamily as BoHV1 have so far been identified and isolated in Europe from members of the *Cervidae* family: *Cervid herpesvirus 1* (CvHV1) in red deer (*Cervus elaphus*) and *Cervid herpesvirus 2* (CvHV2) in reindeer (*Rangifer tarandus*).

CvHV1 and CvHV2 can cause outbreaks of infectious keratoconjunctivitis in red deer and reindeer, respectively[43]. CvHV2 has further been demonstrated to have the potential to be involved in respiratory disease and abortion[34,44].

Two different strains of CvHV1 have so far been identified in the UK and Belgium[45,46], while isolates from CvHV2 in Norway and Finland seem to represent the same strain[47,48]. Both viruses have a genomic organization similar to that of BoHV1[49].

Because of the close genetic and antigenic relationship between cervid herpesviruses and BoHV-1, serological cross-reactions are detected by conventional ELISA testing. Because of this, many surveys that have classified cervids as seropositive for BoHV1 may have actually detected antibodies against cervid herpesvirus. CvHV1 and CvHV2 may hence be much more common in cervid populations than previously thought.

CvHV1

Cervid herpesvirus was first identified during an outbreak of ocular disease in farmed red deer in the UK in 1982[45], but since then many serosurveys have identified the presence of alphaherpesviruses circulating among different deer species. Studies revealed high seroprevalences among red deer in England as well as in the Czech Republic (translocated animals tested by virus neutralization in the early 1990s). A serosurvey of wild animals in France and Belgium revealed higher neutralizing titres against CvHV1 than BoHV1[37]. Other studies have also identified seropositive red deer in Scotland, England, France, Norway and Germany. Some roe deer (*Capreolus capreolus*) were also found to be seropositive for either CvHV1 or a BoHV1-related virus in France, Germany, Norway and Hungary, where fallow deer (*Dama dama*) were also found to be seropositive.

CvHV2

Serosurveys in semi-domesticated reindeer (*Rangifer tarandus tarandus*) in Finland, Sweden, Norway and Greenland have all revealed the presence of a BoHV1-related virus. Seroprevalences have ranged from 12% in Finland in 1977[50] to more than 48% in Norway in 2003–2006[52]. Although most reindeer in Scandinavia are semi-domesticated, meaning that although free-ranging they are owned and herded, serosurveys on wild reindeer populations in both Greenland and Norway have also shown

these animals to be infected[52,53]. Recent seroneutralization tests in Norway determined much higher titres against CvHV2 than BoHV1, a fact also confirmed by CvHV2 isolation in 2009. The same studies have shown that age and animal density represent risk factors for CvHV2 infections[51].

The reindeer subspecies *R. tarandus platyrhynchus* inhabiting the Svalbard archipelago in the high Arctic was screened in the early 1990s, with all tested animals being seronegative[54].

Like other alphaherpesviruses, CvHV1 and CvHV2 are transmitted directly by close contact between infected animals, e.g. licking, nuzzling, sneezing or venereal contact. Both viruses have the potential to infect the genital and respiratory tracts as well as the ocular mucosa, as demonstrated either by disease outbreaks or experimental studies of CvHV1 in England and France, and CvHV2 in Norway and Finland[45,47,55–58]. In experimental infections with either CvHV1 or CvHV2, animals usually display a transient hyperaemia in the mucosae during the initial period of viral excretion, with increased nasal and genital discharges in some cases[55,56,59].

For both agents, viral excretion titres reach their maximum between 4 and 7 days post-infection at the point of entry (respiratory or genital tract), decreasing quickly after that as the humoral response commences at around 10 days post-infection.

Although little is known about the viraemic potential of CvHV1, in the case of CvHV2 experimental studies show that after genital or respiratory infection the virus spreads throughout the body, reaching different organs, such as liver, lung, spleen and lymph nodes[44,56,57]. Vertical transmission was observed in several animals.

Both viruses have been shown experimentally to become latent in their hosts and reactivation, excretion and re-isolation has been observed experimentally[34,55,57].

Reindeer infected with CvHV2 can exhibit lesions in the skin of the eyelids, lips and gingiva, as described in Norway[48]. In the same experimental study in Norway, one abortion occurred, with CvHV2 being recovered from both maternal and fetal tissues. This strengthened the hypothesis that CvHV2 contributes to, or causes, abortion. CvHV2, like BoHV1, seems to have the potential to be involved in respiratory disease complex, where reactivation of the virus can lead to it reaching the respiratory pathways, with immunohistological findings showing it to be associated with epithelial hyperplasia and destruction[56].

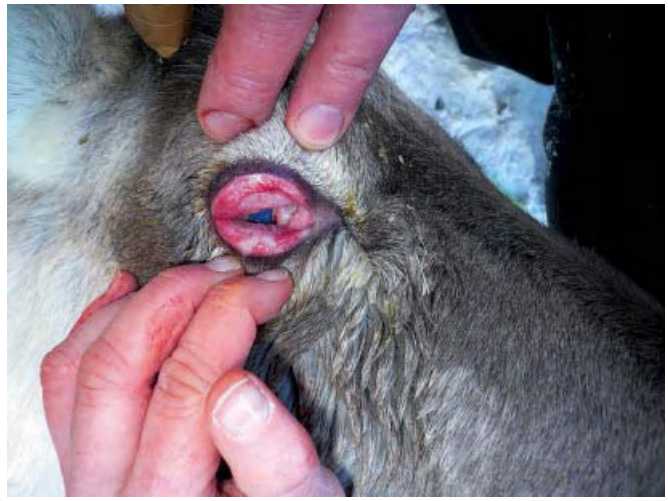

FIGURE 1.4 Infectious keratoconjunctivitis caused by *Cervid herpesvirus 2* infection in semi-domesticated reindeer during an outbreak in Norway in 2009. Severe degree of periorbital oedema. With permission from Das Neves, C.G. et al., 2010[48].

Outbreaks of keratoconjunctivitis have been reported to be associated with both CvHV1 and CvHV2 in red deer and reindeer in Scotland (1982) and Norway (2009), respectively[45,58]. In Scandinavia, similar outbreaks of ocular disease in reindeer have been recorded for more than 100 years.

Ocular disease is characterized, both in deer and reindeer, by purulent ocular discharge, hypopyon, uniform corneal opacity without ulceration, mucopurulent nasal discharge and photophobia. Moderate swelling of the periorbital tissues and marked oedema of the eyelids are also observed (Figure 1.4). In more severe cases, there is a secondary bacterial infection and haemorrhagic and purulent exudates appear, leading, in extreme cases, to blindness and destruction of the eye. *Moraxella bovis* has been reported in this ocular syndrome in reindeer in Finland, but in an outbreak in Norway in 2009 *Moraxella boviculi* was reported for the first time in reindeer. Because of the potential of many ruminant alphaherpesviruses to cross-react serologically, only tests based on virus detection such as isolation, viral DNA detection or restriction endonuclease analysis can accurately determine which virus is present in a given animal or population. The presence of viral particles in lesions can also be demonstrated by immunohistochemistry or electron microscopy.

There is no known treatment for cervid herpesvirus infections. When clinical signs are present, infected animals

should be isolated, as the virus spreads easily horizontally.

There are no known public health concerns associated with *Cervid herpesvirus 1* or *2*.

Cervid herpesviruses seem to pose little risk for domestic ruminant populations. Experimental studies indicate that bovines seem to be refractory to CvHV1 infection but can be infected with CvHV2, which can cause a mild rhinitis without latency or reactivation[59]. Both cross-infections have never been reported outside experimental conditions. In southern Scandinavia, reindeer and deer share common grazing areas; however, cross infections between CvHV1 and CvHV2 in these two species has not been studied.

HERPESVIRUS INFECTIONS IN AQUATIC MAMMALS

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All herpesviruses identified so far in marine mammals belong to the order *Herpesvirales*, family *Herpesviridae* and subfamilies *Alphaherpesvirinae* or *Gammaherpesvirinae*. Infection with *Phocid herpesvirus 1* (PhHV1; synonyms *Phocine herpesvirus 1*, harbour seal herpesvirus), an alphaherpesvirus, may cause systemic disease in seals, whereas infection with *Phocid herpesvirus 2* (PhHV2), a gammaherpesvirus, has not been definitively associated with disease in seals. Alphaherpesvirus infection in cetaceans may cause systemic, CNS or cutaneous disease. Gammaherpesvirus infection in cetaceans may cause genital disease (Table 1.3).

Infections with PhHV1 and PhHV2 occur in harbour seals (*Phoca vitulina*)[60,76] and grey seals (*Halichoerus grypus*)[77,78]. PhHV1 is mainly transmitted horizontally among juvenile harbour seals[83]. Systemic disease from alphaherpesvirus infection has been recorded in bottlenose dolphins (*Tursiops truncatus*)[64], a Cuvier's beaked whale (*Ziphius cavirostris*)[65] and a striped dolphin (*Stenella coeruleoalba*)[66]. CNS disease from an alphaherpesvirus infection has been recorded in a harbour porpoise (*Phocoena phocoena*)[67]. Cutaneous disease from alphaherpesvirus or unspecified herpesvirus infection has been recorded in an orca (*Orcinus orca*)[84], a beluga whale (*Delphinapterus leucas*)[68], a striped dolphin[69], a harbour porpoise[70], and a bottlenose dolphin[71]. Genital disease from gammaherpesvirus infection has been recorded in a Blainville's beaked whale (*Mesoplodon densirostris*)[74] and in bottlenose dolphins[72]. Transmission of genital herpesvirus in bottlenose dolphins, and probably other cetaceans, is likely to be sexual[72]. Herpesvirus infection has been identified in several other marine mammal species whose range includes European waters, but associated disease has not been confirmed by light or electron microscopy (Table 1.3).

The pathogenesis of PhHV1 infection in harbour seals is probably initiated by virus entry into mucosal tissues or blood. A mononuclear leucocyte-associated viraemia favours spread to lymphoid tissues, followed by dissemination to parenchymal organs. Virus replication in parenchymal organs leads to tissue necrosis and inflammation, and possibly death. Seroconversion may be associated with clinical recovery but not necessarily virus clearance[61]. Little is known about the pathogenesis of other herpesvirus infections in marine mammals.

Clinical signs reported for PhHV1 infection in harbour seals are nasal discharge and coughing, inflammation of oral mucosa, vomiting, diarrhoea, fever, anorexia, lethargy, lymphopenia and seizures[62,85]. Clinical signs are milder in older animals and are milder in grey seals than in harbour seals[77]. Cetaceans with cutaneous[86] or genital herpesvirus infection[72] appeared to be active and in good health.

Pathological changes in fatally PhHV1-infected juvenile harbour seals include necrosis in adrenal cortex, liver, brain, crypts of the small intestine, and tonsils. Intranuclear inclusion bodies (INIB) may be present at foci of acute necrosis, particularly in adrenal cortex and liver[61,63]. Fatal systemic herpesvirus infection occurred in two bottlenose dolphins[64] and in a Cuvier's beaked whale[65] without concurrent morbillivirus infection and in a striped dolphin with concurrent morbillivirus infection[66] was associated with foci of acute necrosis in multiple organs, with INIB in both parenchymal cells and syncitial cells. Cutaneous herpesvirus infection in cetaceans is associated with variably shaped skin lesions, which may be ulcerated[64,71,86]. They are characterized by both necrosis and hyperplasia of epidermis, with INIB in keratinocytes. Encephalitis in a harbour porpoise was associated with INIB in many neurons[67]. Genital herpesvirus infection in cetaceans is associated with plaques in mucosa of penis or

TABLE 1.3 Association between herpesvirus infection and disease in marine mammal species occurring in Europe.

Localization of disease	Host species	Pathologic diagnosis[a] by			Virologic diagnosis by		Subfamily	References
		Histo	IHC	EM	PCR	Culture		
Systemic	Harbour seal (*Phoca vitulina*)	yes	no	yes	yes	yes	alpha[b]	(60–63)
	Bottlenose dolphin (*Tursiops truncatus*)	yes	no	yes	yes	no	alpha	(64)
	Cuvier's beaked whale (*Ziphius cavirostris*)	yes	no	yes	yes	no	alpha	(65)
	Striped dolphin (*Stenella coeruleoalba*)	yes	yes	yes	yes	no	alpha	(66)
Central nervous system	Harbour porpoise (*Phocoena phocoena*)	yes	yes	yes	no	no	alpha	(67)
Cutaneous	Beluga whale (*Delphinapterus leucas*)	yes	no	yes	no	no	not determined	(68)
	Striped dolphin (*Stenella coeruleoalba*)	yes	no	no	no	no	not determined	(69)
	Harbour porpoise (*Phocoena phocoena*)	yes	no	no	no	no	not determined	(70)
	Bottlenose dolphin (*Tursiops truncatus*)	yes	no	yes	yes	no	alpha	(71)
Genital	Bottlenose dolphin (*Tursiops truncatus*)	yes	no	yes	yes	yes	gamma	(72,73)
	Blainville's beaked whale (*Mesoplodon densirostris*)	yes	no	no	yes	no	gamma	(74)
Cardiac	Harp seal (*Phoca groenlandica*)	yes	no	yes	no	no	not determined	(75)
Unconfirmed	Harbour seal (*Phoca vitulina*)	no	no	no	no	yes	gamma[c]	(76)
	Grey seal (*Halichoerus grypus*)	no	no	no	yes	yes	alpha[b]	(77)
	Grey seal (*Halichoerus grypus*)	no	no	no	yes	yes	gamma[c]	(78)
	Bottlenose dolphin (*Tursiops truncatus*)	no	no	no	yes	no	gamma	(73)
	Dwarf sperm whale (*Kogia sima*)	no	no	no	yes	no	gamma	(73)
	Risso's dolphin (*Grampus griseus*)	no	no	no	yes	no	gamma	(73)
	Bottlenose dolphin (*Tursiops truncatus*)	no	no	no	yes	no	alpha	(79)
	Striped dolphin (*Stenella coeruleoalba*)	no	no	no	yes	no	alpha and gamma	(80)
	Orca (*Orcinus orca*)	no	no	no	yes	no	alpha	(81)
	Sperm whale (*Physeter macrocephalus*)	no	no	no	yes	no	gamma	(82)
	False killer whale (*Pseudorca crassidens*)	no	no	no	yes	no	alpha	(82)
	Melon-headed whale (*Pepenocephala electra*)	no	no	no	yes	no	gamma	(82)

[a]Histo = histology; IHC = immunohistochemistry; EM = electron microscopy
[b]*Phocid herpesvirus 1*
[c]*Phocid herpesvirus 2*

vulva, characterized histologically by epithelial hyperplasia and dysplasia, with INIB in epithelial cells[72].

Diagnosis of herpesvirus infection can be done by PCR and confirmed by sequencing of the PCR product. Samples of choice for clinical diagnosis are nasal swabs for PhHV1 infection, peripheral blood mononuclear cells for PhHV2 infection and swabs, scrapings or biopsies for cutaneous and genital herpesvirus infections. PhHV1 infection also can be diagnosed clinically by demonstration of at least a four-fold rise in virus neutralizing antibody in paired sera. Culture has been successful for PhHV1 and PhHV2 on primary seal cells and Crandell feline kidney cells[60,76,78], and for a gammaherpesvirus from bottlenose dolphins (TTHV) on primary cetacean cells and on Crandell feline kidney cells[72]. Histological detection of INIB in postmortem tissue samples is suggestive of herpesvirus infection. Diagnosis becomes highly likely if suspect cells are shown to contain herpesvirus-like particles by electron microscopy or to express herpesvirus antigen by immunohistochemistry using a primary antibody against a related herpesvirus. Population screening for herpesvirus infection can be done on tissues and secretions by PCR for all herpesviruses, and on sera by ELISA or virus neutralization test for PhHV1, PhHV2 and TTHV.

The management and control of outbreaks of PhHV1 infection in juvenile harbour seals at rehabilitation centres is important because such outbreaks may cause severe mortality. The severity of such outbreaks may be mitigated by appropriate quarantine measures, veterinary care and nursing of seal pups[83]. In addition, a recombinant vaccine has been developed that is expected to be safe and effective in protecting harbour seals against PhHV1-related disease[87].

Public health and domestic health concerns for marine mammal herpesviruses are low, because there is no evidence for infection of humans or domestic animals with these viruses. Given that several herpesviruses may cause severe disease and death in affected animals, these pathogens may be significant for the health of marine mammal populations.

OTHER HERPESVIRUS INFECTIONS

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Infection of European hedgehogs (*Erinaceus europaeus*) with a herpesvirus classified as *Erinaceid herpesvirus 1* (ErHV-1) has been described in Europe[88–90]. Herpesvirus-like particles were demonstrated in the liver of a hedgehog with hepatitis in the UK[88], fatal herpesvirus infection was detected in a 3-month-old hedgehog in Sweden[89], and a severe viral meningoencephalitis caused by herpesvirus in an orphan hedgehog brought to a wildlife rehabilitation centre was described in Switzerland[90]. Herpesvirus was isolated on primary bovine fetal skin cells from the liver of the hedgehog in Sweden (Figure 1.5), and a cytopathic effect characteristic of alphaherpesvirus was observed within 48 hours[89]. Herpesvirus particles were observed by electron microscopy in the case in the UK[1]. Abundant viral antigen was detected by immunohistochemistry in the nucleus and cytoplasm of neurons and glial cells using antibodies for human herpes simplex virus type 1 and 2[90]. Little is known about the epidemiology. The case in Britain affected an adult female. In Sweden, a litter of four 2-week-old orphan hedgehogs had been hand reared up to the age of 3 months and placed with adult hedgehogs. Three of the hedgehogs in the litter died within 2 days of mixing the groups, and the remaining one on the fifth day. Only one was submitted for postmortem examination. The case in Switzerland affected a young female. In the cases in the UK and Sweden, the liver was the most severely affected organ, showing coagulative necrosis, fatty degeneration and mild inflammatory infiltrate (Figure 1.6). Intranuclear

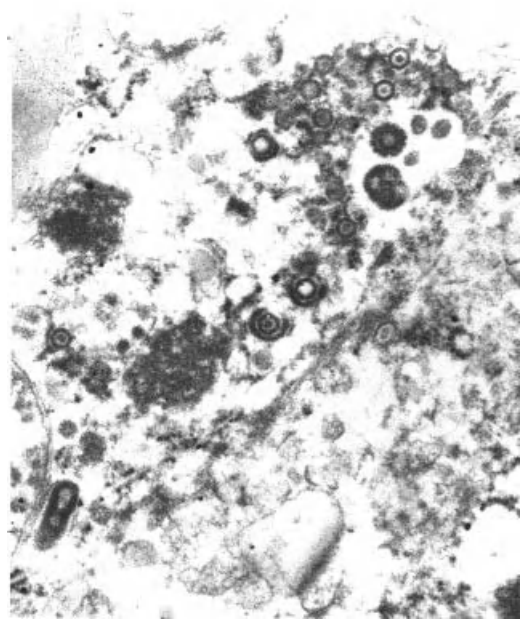

FIGURE 1.5 Electron microscopy of herpesvirus from a hedgehog with hepatitis cultured on primary bovine foetal skin cells.

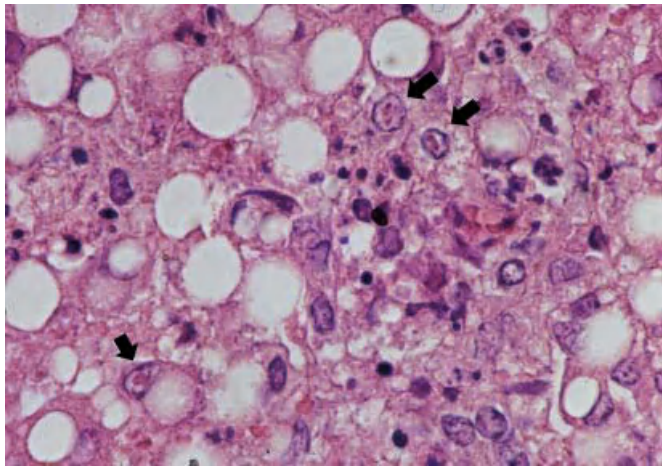

FIGURE 1.6 Histological section of liver of a European hedgehog with fatal herpesviral hepatitis, showing necrosis of hepatocytes and fatty degeneration. Intranuclear acidophilic inclusion bodies are observed in hepatocytes (arrows). Photo: D. Gavier-Widén, SVA.

acidophilic inclusion bodies were observed in hepatocytes (Figure 1.6) and Kupffer cells. Similar lesions were observed in the adrenal glands[89]. In the case in Switzerland, multifocal perivascular cuffing, diffuse meningeal infiltration with lymphocytes and plasma cells, numerous eosinophilic intranuclear inclusion bodies in neurons and glial cells of the cortex and to a lesser content of the thalamus and the brainstem and neuronal necroses were observed[90]. The clinical course in all the cases appeared acute and the hedgehogs were in good bodily condition. The case of meningoencephalitis clinically showed progressive incoordination, circling, and finally loss of appetite. It can be concluded that a potentially fatal alphaherpesvirus infection of hedgehogs occurs in Europe but that the virus is poorly characterized and information regarding the epidemiology and zoonotic aspects is not available. Whether hepatitis and meningoencephalitis is caused by the same herpesvirus genus remains a speculation.

Applying molecular methods, seven gamma- and one betaherpesvirus, belonging to seven different European bat species, were identified in 15 individual bats in Germany[91]. As the bats had been found at different locations and on different dates, it was considered unlikely that they originated from the same roost populations. None of the bats showed histological lesions that could be attributed to herpesviral infection. However, it is known from other species that infection with gamma- and betaherpesviruses often has a subclinical course, and the pathological significance/potential of the herpesviruses in these bats could not be elucidated. A herpesvirus (genus *Rhadinovirus*, subfamily *Gammaherpesvirinae)* was detected by nested PCR in an adult female serotine bat (*Eptesicus serotinus*) submitted to a rescue centre in Hungary. The bat had died with signs of icterus and anorexia within a day, in spite of supportive therapy. The causative role of the herpesvirus could not be proven[92].

Mustelid herpesvirus 1 (MusHV1; genus *Rhadinovirus*, subfamily *Gammaherpesvirinae*) has been reported to be frequent in badgers (*Meles meles*) in the British Isles. A high percentage of free-ranging badgers sampled at two geographically distinct locations (southwest of England and the Republic of Ireland) were found positive by PCR. The seroprevalence investigated by an in-house indirect ELISA, revealed antibodies in 36 out of 110 badgers tested. The antibody levels were higher in adults than in young badgers. MusHV1 has not yet been associated with lesions or clinical disease. When *Mycobacterium bovis*-infected and non-infected badgers were tested for MusHV1 antibodies, no significant difference between the two groups could be seen[93].

A serological survey of 65 sylvatic house mice (*Mus domesticus*) from three populations in northwest England revealed a high proportion (75%) of mice with antibodies to murine cytomegalovirus (MCMV). No information on the pathology or epidemiology was given[94]. High seroprevalence to MCMV has been reported in grey squirrels (*Sciurus carolinensis*) in North Wales, but the specificity of the serological assay is unknown and it cannot be ruled out that antibodies to betaherpesvirus from the two species are cross-reacting. It is not surprising that the seroprevalence to betaherpesvirus is high in several wild animal species, as similar observations have been made in domestic animals and humans.

Murine gammaherpesvirus 4 (MuHV4) was originally isolated from a bank vole (*Clethrionomys glareolus*) in Slovakia and further related herpesvirus strains were thereafter obtained from bank voles, wood mice (*Apodemus sylvaticus*) and a European shrew (*Crocidura russula*). The viruses are probably geographically widespread in the mouse and vole subfamilies. Wood mice are major hosts of MuHV4, with a seroprevalence of 13% and 24% in England and Northern Ireland, respectively[95], whereas bank voles show low seroprevalence. The virus resides initially in the respiratory system, causing bronchiolitis. MuHV4 has tropism for B-lymphocytes, which become latently infected. Lymphoproliferative disorders, including splenomegaly and B-cell lymphoma are characteristic for MuHV4 infection.

Felid herpesvirus (FHV) mainly causes upper respiratory tract diseases and conjunctivitis in domestic cats. In kittens it may cause ulcerative, dendritic keratitis. A study on 51 wildcats (*Felis silvestris silvestris*) from populations in France, Switzerland and Germany, revealed a seroprevalence of 4%[96], and a study in 50 wild cats in Scotland found that 16% had neutralizing antibodies to FHV[97].

HERPESVIRUS INFECTIONS IN WILD BIRDS

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Avian herpesviruses (AHV) are widespread in domestic poultry (chickens, turkeys, Pekin ducks, geese and Muscovy ducks) and cause a variety of conditions in domestic and free-ranging wild bird species (Table 1.4). Frequently, these viruses cause subclinical or latent infections, but under certain environmental conditions various forms of disease associated with high rates of mortality may occur. Clinical signs vary with host species, virus strain and environment. Almost none of the clinical signs are specific for AHV, but gross and microscopic lesions are suggestive of herpesvirus aetiology.

TABLE 1.4 Avian herpesviruses, their natural hosts and predominant types of macroscopic lesions.

Name of disease	Natural hosts	Predominant lesions
Marek's disease	Chicken, turkey, quail	Nervous system lesions, tumours
Duck plague	Waterfowl	Haemorrhages, necrosis
Infectious laryngotracheitis	Chicken, pheasant, quail	Haemorrhages, necrosis
Smadel's disease of pigeons	Pigeons and doves	Necrosis in intestine, organs
Inclusion body disease of owls	Owls	Necrosis in intestine, organs
Inclusion body disease of falcons	Falcons, eagles	Necrosis in intestine, organs
Inclusion body disease of cranes	Cranes	Necrosis in intestine, organs
AHV infection of storks	Black and white storks	Necrosis in intestine, organs
AHV infection of passerine birds	Passerines	Necrosis in intestine, organs

Lesions caused by AHV can be grouped into three categories. Only Marek's disease viruses cause visible lesions in peripheral nerves and lymphoid-cell tumours in visceral organs and skin. Diseases due to viruses causing duck plague and infectious laryngotracheitis are initially associated with haemorrhages and subsequent necrosis. All other AHV infections induce necrotic lesions in the digestive tract and in internal organs. All these viruses, or their genes, are frequently detected in subclinical infections and they may persist in latent forms during the entire life span of the infected host bird. Vertical transmission via embryonated eggs has not been proven for any AHV. All AHVs can be isolated from infected organs or swabs in embryonated chicken eggs and cell cultures derived from embryonic avian tissues or chick kidney cell cultures.

The taxonomic position of all currently known avian herpesviruses, order *Herpesvirales*, family *Herpesviridae*, subfamily *Alphaherpesvirinae*, has been reviewed[98]. A total of nine herpesviruses are still unassigned to any genus. Recent publications contain descriptions of a further nine avian herpesviruses that are as yet incompletely characterized and are not included in the list of assigned herpesviruses (Table 1.5).

DUCK PLAGUE/DUCK VIRAL ENTERITIS

In Europe duck plague is an important herpesvirus disease of domestic Pekin and Muscovy ducks, many species of free-living ducks, geese and swans, and is occasionally found in other aquatic birds. Duck plague is also called duck viral enteritis (DVE). Both names are misleading, because not only ducks can be infected and enteritis is not the only lesion in clinical cases. Other names for duck plague are fowl plague and *eendenpest* (in Dutch).

AETIOLOGY

Duck plague virus is currently considered to belong to the family *Herpesviridae* as *Anatid herpesvirus 1* (AnHV1) but is not yet assigned to any subfamily or species[98]. However, placing it in the subfamily *Alphaherpesvirinae* has been proposed[101]. AnHV1 has a linear, very large double-stranded DNA genome of 125–290 kbp that is contained within a T = 16 icosahedral capsid. The nucleocapsids are surrounded by a proteinaceous matrix, the integument and a lipid-containing envelope[98]. Nucleocapsids with a diameter of 91 to 93 nm are detectable in the nucleus of infected cells. After envelopment by budding through the

TABLE 1.5 Avian herpesviruses in the family *Herpesviridae*, subfamily *Alphaherpesvirinae*.

Genus	Name of species	Acronym	Common name
Mardivirus	*Columbid herpesvirus 1*	CoHV1	Pigeon herpesvirus
	Gallid herpesvirus 2	GaHV2	Marek's disease virus type 1
	Gallid herpesvirus 3	GaHV3	Marek's disease virus type 2
	Meleagrid herpesvirus 1	MeHV1	Turkey herpesvirus
Iltovirus	*Gallid herpesvirus 1*	GaHV1	Infectious laryngotracheitis virus
	Psittacid herpesvirus 1	PsHV1	Pacheco's parrot disease virus
Unassigned viruses in the family *Herpesviridae*	*Acciptrid herpesvirus 1*	AcHV1	Bald eagle herpesvirus
	Anatid herpesvirus 1	AnHV1	Duck plague herpesvirus
	Ciconiid herpesvirus 1	CiHV1	Black stork herpesvirus
	Falconid herpesvirus 1	FaHV1	Falcon inclusion body disease virus
	Gruid herpesvirus 1	GrHV1	Crane herpesvirus
	Perdicid herpesvirus 1	PdHV1	Bobwhite quail herpesvirus
	Phalacrocoracid herpesvirus 1	PhHV1	Lake Victoria cormorant herpesvirus
	Sphenicid herpesvirus 1	SpHV1	Black footed penguin herpesvirus
	Strigid herpesvirus 1	StHV1	Owl hepatosplenitis virus
Not placed in any taxonomic unit[a]	*Andigenid herpesvirus 1*	AnHV1	Toucan herpesvirus
	Estrildid herpesvirus 1	EsHV1	Exotic finch herpesvirus
	Estrildid herpesvirus 2	EsHV2	Exotic finch herpesvirus
	Estrildid herpesvirus 3	EsHV3	Exotic finch herpesvirus
	Fregata[b] *herpesvirus 1*	FrHV1	Magnificent frigate herpesvirus
	Lampropornid herpesvirus 1	LaHV1	Superb starling herpesvirus
	Serinid herpesvirus 1	SeHV1	Canary herpesvirus
	Tragopanid herpesvirus 1	TrHV1	Tragopan herpesvirus
	Weaver finch herpesvirus	PlHV1	Ploceid herpesvirus

[a] See Kaleta, 2008[99]
[b] See de Thoisy et al., 2009[100]

nuclear membrane, particles enter the perinuclear spaces and the endoplasmatic reticulum of the cytoplasm. Enveloped, spherical viral particles are 120 to 130 nm. Nucleocapsids and enveloped particles are detectable in infected cells of liver, small intestine, spleen, thymus and bursa of Fabricius. Only one serotype is known. Differences in virulence exist among isolates. Duck plague herpesvirus is sensitive to lipid solvents such as ethanol, isopropanol, dimethylether, chloroform, phenol and its derivates, glutaraldehyde, quaternary ammonium compounds, sodium hypochlorite (bleach), and organic acids such as formic and peracetic acid. These compounds destroy the infectivity of the virus within 30 minutes at concentrations of 0.5 to 2.0% at room temperature.

EPIDEMIOLOGY

GEOGRAPHICAL DISTRIBUTION IN EUROPE

The majority of outbreaks have been described in Northern and Central Europe, and North America. Clinical disease is predominantly seen in domesticated waterfowl. Both migrant and resident species of waterbirds can be affected. The virus may be distributed from circumpolar regions of Eurasia and North America to the Southern regions of these continents by migrating birds.

HOST FACTORS

Subclinical and latent infections occur frequently in many species and are independent of age and sex. Many species are susceptible to infection (see Table 1.6), although European teal (*Anas crecca*) and pintail (*Anas acuta*) appear resistant to experimental infection but still produce antibodies. During outbreaks a marked variation in species susceptibility is frequently observed. In a recent outbreak in domestic ducks and geese, many species that had not been considered susceptible before were affected[102]. Likewise, AnHV1 was isolated in Spain from common coots (*Fulica atra*) and crested coots (*Fulica cristata*), species that were previously considered resistant[103].

Natural infections have been described in ducklings as young as 7 days of age and in adult birds. Both sexes are equally susceptible. Stress due to physiological moulting,

TABLE 1.6 Hosts of duck plague virus of Eurasian anseriforms.

Order and species	Natural (N) or experimental (E) infection	Degree of susceptibility
Anseriformes		
Anas platyrhynchos, mallard duck	N/E	M
Anas querquedula, garganey	N/E	S
Anas strepera, gadwell	N/E	M
Anas penelope, European wigeon	N/E	S
Anas crecca, European teal	E	R
Anas acuta, pintail	E	R
Anas superciliosa, grey call duck	N	R
Anas discors, blue-winged teal	N/E	S
Anas rubripes, black duck	N	S
Aythya americana, redhead	N	S
Aythya valisinera, canvasback	N	S
Aythya affinis, lesser scaup	N	S
Aythya collaris, ring-necked duck	N	S
Aythya ferina, common porchard	N/E	S
Aythyia fuligula, tufted duck	N	S
Aix sponsa, wood duck	N/EN	SS
Bucephala clangula, goldeneye	NN	S
Bucephala albeola, bufflehead	N/E	S
Mareca americana, American wigeon	N/E	S
Somateria mollisima, common eider	N	S
Spatula clypeata, common shoveler	N/E	S
Mergus merganser, common merganser	N/EN/EN/E	S
Anser anser, greylag goose	N/E	SS
Anser caerulescens, snow goose	N	S
Anser albifrons, white-fronted goose	N/E	S
Anser fabilis, bean goose		S
Cygnus olor, mute swan	N	S
Branta canadensis, Canada goose	N/EN/E	
Branta leucopsis, barnacle goose	N	S
Dendrocygna autumnalis, red-billed whistling duck	N/E	S
Tadorna tadorna, shelduck		SS
Tadorna ferruginea, ruddy shelduck		M
Alopochen aegyptiacus, Egyptian goose		
Cairina moschata, Muscovy duck		
Gruiformes		
Fulica atra, common coot	N	R
Fulica cristata, crested coot	N	R
Charadriiformes		
Larus argentatus, herring gull	E	R
Larus ridibundus, black-headed gull	E	R

Degree of susceptibility: S = susceptible; M = moderately susceptible; R = resistant to infection

Some species of birds are of American or non-European origin but are kept in captivity in Europe

courtship, egg laying and incubation aggravates the clinical course of the disease.

ENVIRONMENTAL FACTORS

Most cases of duck plague in Europe occur during the winter, from January onwards, to early spring. The change from latency to clinically overt disease is regularly associated with environmental stressors such as aquatic pollution and prolonged periods of freezing temperatures that result in the gathering of large flocks of susceptible birds on small areas of unfrozen water. Such conditions create environmental stress factors and facilitate virus transmission. The effect of stress has been studied experimentally; oral administration of cyclophosphamide, an immunosuppressant agent, resulted in decreased resistance following challenge with a duck plague virus isolate, which did not cause mortality in immunocompetent mallards[104]. The practice of keeping large numbers of ducks and geese of different species in restricted and confined captivity to prevent exposure to avian influenza A virus, resulted in an outbreak of duck plague in 2007 in Germany[102].

EPIDEMIOLOGICAL ROLE OF AFFECTED SPECIES

Free-living, diseased and subclinically infected (with entire, infective virus) or latently infected (with viral genome that is not necessarily infective) waterfowl are considered to be the source of virus for susceptible free-living birds and domestic waterfowl. This is supported by the observation of seropositive subclinical virus carriers among free-living waterfowl. Infected wild birds may access farms with highly susceptible domestic Pekin ducks, Muscovy ducks and geese, infecting these domestic waterfowl and causing significant mortality among them.

TRANSMISSION

Infected birds excrete large quantities of duck plague virus in faeces and saliva, which results in contamination of water and grazing grounds. Oral and nasal infection is the most likely route for acquiring natural infection. Egg (vertical) transmission of the virus has never been confirmed. Living vectors are not required for virus transmission.

AnHV-1 can persist throughout life in a latent form in the trigeminal ganglion (TG), lymphoid tissues and in peripheral blood lymphocytes. Conversion may then occur, promoting latency to subclinical and productive

infection of infectious virus shed from the oropharynx and cloaca. The excreted virus remains infectious in contaminated fresh water for several days. The contamination of feeding grounds and roosting sites provides opportunities for lateral transmission. The infective dose is unknown.

PATHOGENESIS, PATHOLOGY AND IMMUNITY

The first steps of virus multiplication occur following oronasal infection of the upper respiratory and digestive tracts. After the initial infection, the virus is phagocytosed by macrophages and transported to the bloodstream, resulting in viraemia and colonization of internal organs, including the intestines. Parenchymatous organs develop focal haemorrhages followed by necrosis. The small intestinal lesions frequently comprise one or more ring-like areas of haemorrhages that can be seen from the serosal surface. These lesions are considered pathognomonic for duck plague. The oesophagal and cloacal mucosa contain haemorrhages, which develop into large necrotic areas. Haemorrhages and necrosis in the bursa of Fabricius are of diagnostic importance.

Owing to the rapid course of the disease, dead birds are generally in good body condition. Birds that have suffered for prolonged periods from detrimental environmental conditions may be in poor condition. As a result of extensive haemorrhages and loss of blood into the intestine, the body appears pale during necropsy. Prominent lesions are present in the proximal to distal parts of the intestine. The mucosa of the oesophagus, proventriculus, intestine, cloaca and bursa of Fabricius contain multiple haemorrhages that develop into extensive layers of necrosis of the mucosal surface and submucosa. The surface of the heart and the myocardium may show petechiae, ecchymotic or extended haemorrhages. The thymus shows initially a haemorrhagic inflammation, which changes during the course of the disease to necrosis, distinct atrophy and almost complete loss of thymocytes. The surface of the enlarged liver has a copper-like colour with intermingled small haemorrhages and pinpoint foci of necrosis. At later stages the liver appears dark bronze in colour and some areas are stained by bile. In more protracted cases these haemorrhages are replaced by large areas of necrosis. The spleen is enlarged and contains pale foci. The kidneys are swollen. The respiratory tract is not altered.

Microscopically, during the acute phase, multiple haemorrhages are prominent in almost all organs. These are subsequently replaced by necrosis in organs and ulcerations in the intestinal mucosa. INIB in the intestines and internal organs can be seen in the vicinity of the necrotic lesions.

The necrosis of lymphoid cells in the bursa of Fabricius and in the gut-associated lymphoid tissues (GALT) alters the immune responsiveness so that serum antibodies are either completely absent or only detectable in low titres in virus neutralization tests.

Death occurs as a result of anorexia and extended haemorrhages in intestines and parenchymatous organs. Necrosis of the intestinal mucosa results in invasion of bacteria with subsequent bacteriaemia. Infected ducks usually die after a few days of illness.

CLINICAL SIGNS AND TREATMENT

Clinical signs vary widely among species. The time interval between infection and the appearance of the first signs of disease is estimated to be between 3 and 7 days. Death usually follows 1 to 3 days later. Duck plague is clinically characterized by sudden onset of mortality without specific premonitory signs. Some susceptible birds appear listless, are reluctant to move and to fly, and the intake of food and water is reduced. Species-specific vocalization is absent in sick birds, even during handling. Occasionally, abnormal movements of the head and neck (torticollis) can be observed. Intestinal discharge may be watery, greenish and intermingled with fibrinous material. In severe cases, blood clots can be seen in the faeces. Other signs such as swollen eyelids, drooping of wings and incoordination are only rarely seen. Infection during egg laying results in smaller than normal clutch sizes but egg size and shell structure are not affected.

There is no known effective treatment of clinical duck plague. Palliative measures such as fluid therapy to compensate dehydration, provision of appropriate food enriched with vitamins and treatment of bacterial infections and internal parasites can be attempted to aid recovery.

DIAGNOSIS

Clinical signs are not specific. Gross pathology is of major diagnostic value.

Virus isolation is necessary to confirm a diagnosis of duck plague. Two to three days post-inoculation, a

round-cell type cytopathic effect appears in susceptible cell cultures. Electron microscopy on purified and concentrated gut content or faeces and ultrathin tissue sections can be useful for the detection of herpesviral particles. The application of PCR for accurate and rapid diagnosis is currently the method of choice[105]. PCR is performed on tissues (liver, kidney, spleen, intestines, cloaca) or on swabs from the cloaca or pharynx of live birds. The knowledge of the genome greatly supports the differentiation of AnHV-1 from other closely related viruses and is particularly useful for large-scale epidemiological studies.

Convalescent and immunized birds develop antibodies that can be detected in serum and egg yolk by a virus neutralization test. This test is useful for sero-epidemiological studies of all birds that are susceptible to duck plague virus. ELISA for detection of antibodies were successfully applied in commercial Pekin duck farms but have not been evaluated for testing of free-living Anseriformes.

MANAGEMENT, CONTROL AND REGULATIONS

Local outbreaks of mortality are not considered to pose a threat to any European waterfowl species, and no intervention is required. Birds that recover from natural infection appear resistant to reinfection.

An inactivated vaccine could potentially be applied to protect susceptible birds without the risk of introducing a modified live virus in free-living populations. However, formalin-inactivated adjuvanted vaccines had only a limited effect on subsequent experimental challenge. An inactivated vaccine is not commercially available in Europe. A live chicken embryo-adapted vaccine was developed to protect exposed juvenile and adult ducks[106]. Revaccination at yearly intervals is necessary if breeding birds are kept. Unfortunately, this attenuated live-virus vaccine is considered suitable only for specific situations applicable to a small number of birds, and it is therefore currently difficult to obtain from European vaccine manufacturers.

Duck plague is a reportable disease in the USA but not in European countries. There are no specific regulations from the European Union or other countries in Europe for monitoring and control of duck plague.

PUBLIC HEALTH CONCERNS

Duck plague herpesvirus is not transmissible to mammals and is of no public health concern. However, humans who are involved in the health monitoring of free-living birds may act as important mechanical vectors of the virus.

SIGNIFICANCE AND IMPLICATIONS FOR ANIMAL HEALTH

In the USA, a die-off in the neighbourhood of the Lake Andes National Wildlife Refuge, South Dakota in 1923 resulted in the death of approximately half of the 100 000 wintering waterfowl [107]. However, duck plague outbreaks of similar dimensions have not been reported in Europe. Nevertheless, the Lake Andes disaster clearly demonstrates that duck plague can assume devastating proportions among wild birds. The source of duck plague virus in domestic ducks and geese is unknown in almost all outbreaks. Free-living European waterfowl, especially the mallard, are frequently implicated as the source of virus, but without definitive proof. Carnivorous free-ranging mammals are not susceptible to duck plague virus but may act as mechanical vectors.

Recovered birds should be tested and only released to the wild if AnHV-1 and antibodies against it are not detected. Mixing of domestic waterfowl of unknown herpesvirus disease status, and wild waterfowl at farms or rehabilitation centres should be avoided.

MAREK'S DISEASE

Marek's disease (MD) (synonyms: polyneuritis gallinarum, fowl paralysis) is highly contagious and widespread in Europe in commercial and ornamental breeds of chickens, turkeys and quails. It is caused by *Gallid herpesvirus 2* (Marek's disease virus type 1) and *Gallid herpesvirus 3* (Marek's disease virus type 2). An additional member of the genus *Mardivirus* is the *Meleagrid herpesvirus 1* (turkey herpesvirus 1), which is commonly isolated from turkeys and chickens. As turkey herpesvirus is avirulent for all gallinaceous birds, it is widely used as a live virus vaccine for chickens to prevent losses due to MD.

Virtually all countries and regions in Europe with domestic chicken and turkey populations are infected with MD viruses (MDV) of different virulence.

Chickens (*Gallus gallus*) are susceptible to MDV. Genetic background (blood group alleles) influences the severity of the disease. Domestic Japanese quail (*Coturnix japonica*) and probably free-living European Common quail (*Coturnix coturnix*) can be infected under natural and experimental conditions and develop viraemia and tumor-

ous lesions. The domestic turkey (*Meleagris gallopavo*) may have visceral tumours but rarely neural lesions. Wild turkeys and other gallinacious species and birds of other orders resist infection. Very young chicks are more susceptible than juvenile or adult chickens. Gross pathological and histopathological lesions consistent with MD, but without demonstration of the virus, have been described in a large number of different avian species. In Europe these include the common buzzard (*Buteo buteo*), sparrowhawk (*Accipiter nisus*), mallard (*Anas platyrhynchos*), eagle owl (*Bubo bubo*), little owl (*Athene noctua*), domestic goose (*Anser anser*), mute swan (*Cynus olor*) and others[108].

MDV-infected chickens, quails and turkeys are the natural reservoirs, and may shed MDV throughout life. MDV matures only in cells of the feather follicle epithelium. Large numbers of MDV are found in epithelial cells and feather dander, from where they may be adsorbed onto dust particles. Transmission is facilitated by inhalation of virus containing dust. MDV is not vertically transmitted. Inhalation and conjunctival infection are the dominant routes of infection.

No obvious gross lesions are present in subclinically infected chickens. Neural lesions are associated with macroscopically visible thickening and discolouration of peripheral nerves, especially the vagus nerve, brachial plexus and ischiadic plexus. The ocular form consists of a unilateral iridocyclitis and panophthalmy. Large tumours are most frequently present in the ovary or testes and in the proventriculus. Histopathologically, nerves are oedematous, have focal accumulations of small lymphocytes ('Marek's cells') and a proliferation of Schwann's cells. Tumours are composed of small lymphoid cells of the T-cell type.

Live virus vaccination of newly hatched commercial chicks is common practice in hatcheries. In Germany, but not in other European countries, only the tumorous and neural forms of MD in chickens must be reported. Eradication of the virus has never been attempted. Prevention of early exposure to MDV, improved hygiene and early vaccination (at 1 day old) are commonly practised in Europe to control this disease in poultry.

INFECTIOUS LARYNGOTRACHEITIS

Infectious laryngotracheitis (ILT) is a respiratory disease in chickens, peafowl and captive and released pheasants caused by a herpesvirus, subfamily *Alphaherpesvirinae*, genus *Iltovirus*, type species *Gallid herpesvirus 1*[98]. ILT is endemic in some European countries and occasionally causes substantial losses.

Outbreaks of ILT may occur in all European countries with an intensive chicken industry. ILT is mainly seen in adult chickens and peafowl (*Pavo cristatus*) of both sexes. Some species of pheasants are highly susceptible. Guinea fowl (*Numida meleagris*), turkeys, quails, pigeons, ducks, geese, swans and passerines are resistant. Climate, season and temperature do not appear to affect the course of ILT. Subclinically infected chickens and farm-raised common pheasants (*Phasianus colchicus*) released for hunting are the source for pheasants in the wild.

Birds of some species develop mild signs such as bilateral serous conjunctivitis and rhinitis. Other species display bilateral serous conjunctivitis, respiratory rales and lethargy. Severe signs of disease are noted in other species that consist of serous-purulent conjunctivitis and rhinitis, swelling of eyelids, abnormal movements of the head (torticollis) and lethargy that ends in high levels of mortality. It is noteworthy to recognize that the degree of clinical signs does not correlate with the genus of these birds.

Birds suffering from ILT excrete large amounts of blood-stained tracheal mucus containing ILT virus, thus facilitating transmission to other susceptible species. Subclinically infected birds may excrete ILT virus from conjunctiva and oral secretions and can also be a source of infection by direct and indirect contact. Vectors play no role. No evidence exists for vertical virus transmission.

Following nasal and conjunctival infection and infection of the respiratory epithelium, mainly the trachea, virus multiplication occurs in mucosal cells of the respiratory tract. If diseased birds do not die of suffocation by mucus in the respiratory tract, recovery is likely. Virus transport to the TG occurs early during infection. ILT virus remains in a latent stage in the TG. Convalescent and immunized birds produce antibodies.

Most birds that die of ILT are in good body condition. The main lesions are restricted to the upper respiratory tract. Extended haemorrhages occur in the nasal cavity, conjunctival sac, periorbital sinuses, trachea and primary bronchi. The epithelium is oedematous and frequently detached from the submucosa. Histopathologically, oedema of the respiratory mucosa, haemorrhages and mucoid exudates are present, and epithelial cells frequently, but not in all cases, contain INIB.

The incubation period following natural exposure to virulent strains in chickens is 6–12 days. The disease in chickens, peafowl and some species of pheasants has a rapid course. Initial clinical signs consist of general depression, a

reduction in egg laying, reduced food and water intake and difficulties in breathing. These non-specific signs are followed by severe expiratory rales, nasal discharge of blood-tainted mucus, swollen infraorbital sinus and haemorrhagic tracheitis and increased or significant mortality. Milder strains of ILT virus cause respiratory depression, gasping and expectoration of bloody mucus[109]. The clinical signs in pheasants differ markedly between genera and species. There is considerable variation in clinical severity and mortality among the different species of pheasant found captive and free living (feral) in Europe.

The affected host species, the clinical signs and macroscopic lesions are suggestive of ILT. Confirmation is obtained by histopathological detection of lesions in the respiratory tract and the presence of INIB. Virus isolation is performed in embryonated chicken eggs or in chicken kidney cell cultures from samples of the respiratory mucosa. Inoculated embryos display pox-like foci on the chorioallantoic membrane. Large syncytia are present in cell cultures. Several PCR are applied to identify field and vaccine viruses[110].

Local outbreaks of ILT in domestic chickens are eliminated by culling. Total eradication of ILT appears possible owing to its narrow host range, the detection of latently infected birds by PCR and the sensitivity of the virus to chemical disinfectants, ultraviolet light, dryness and elevated temperatures.

Vaccination of adolescent and adult chickens with live attenuated vaccines is conducted in endemically infected areas by conjunctival installation (eye-drop method). Owing to the residual virulence of attenuated live vaccine viruses, care must be taken to avoid mixing vaccinated and unvaccinated chickens. Circumstantial evidence suggests that attenuated live ILT vaccines may regain their original virulence by serial passages in chickens. Also, the duration and protective capacity of ILT vaccines is relatively limited.

Great caution is required to prevent spread of vaccine-derived virus from chickens to pheasants and peafowl. Vaccines should never be used for any species of pheasant. Severe post-vaccinal reactions, including mortality, are likely in pheasants and peafowl.

Formal reporting of ILT to governmental authorities is not required. Legal regulations do not exist.

Exact data on the prevalence of ILT in domestic bird populations are not available. Domestic and free-living waterfowl, pigeons and passeriform birds are not susceptible. Mammals, including humans, are completely resistant.

SMADEL'S DISEASE OF PIGEONS

Smadel's disease, pigeon herpesvirus infection or ingluvitis of pigeons, is a contagious disease of predominantly young pigeons of all breeds (racing and fancy) of worldwide distribution. Single cases are also diagnosed in feral pigeons (*Columbia livia*) and of other birds of the family Columbidae. Generally, the pigeon herpesvirus can affect all species of the family Columbidae. Infection without subsequent development of clinical signs are frequently observed.

The aetiologic agent of Smadel's disease is a member of the order *Herpesvirales*, family *Herpesviridae*, subfamily *Alphaherpesvirinae*, genus *Mardivirus*, species pigeon herpesvirus, *Columbid herpesvirus 1*, CoHV1[98].

Exact data on the prevalence of Smadel's disease is not available. However, numerous reports provide evidence for the presence of the pigeon herpesvirus in all European countries and many pigeon lofts. Pigeon herpesvirus has been detected in all breeds of domestic pigeons (*Columba livia* f. *domestica*), feral pigeons, and other members of the family Columbidae. Young pigeons (squabs) are more susceptible to disease than adults. Free-living but also domestic pigeons of various breeds are frequently co-infected with a large variety of other infectious agents, which increases the severity of the clinical course of Smadel's disease. These agents include the pigeon circovirus, reovirus, adenovirus, *Salmonella typhimurium* var. copenhagen, *Chlamydia psittaci*, *Trichomonas gallinae*, yeasts and intestinal parasites. The disease in domestic pigeons occurs more frequently in the presence of environmental stressors. Feral pigeons in urban areas suffer frequently from chronic, immunosuppressive lead intoxication, which promotes the frequency and severity of the disease. Since the complete ban in Europe of the gasoline additive tetraethyl lead in 1997, the numbers of clinically overt forms of Smadel's disease in pigeons has fallen. Infected domestic and feral pigeons provided as food for captive birds of prey may result in lethal infections of these birds, so this practice is not recommended, or, if done, falconers should remove the head and neck of pigeons before feeding. Chronically infected female and male breeding pigeons transmit the herpesvirus to their squabs by feeding regurgitated crop milk during the first weeks of life of the squabs. Contact during courtship, preening and mutual feeding of adult pairs during mating does not result in virus transmission. Egg transmission of the virus has not been recorded.

Ingested virus replicates in the oropharynx region, followed by short-term viraemia and virus multiplication in

all internal organs. Squabs succumb as a result of severe epithelial lesions in the pharynx, oesophagus and crop and as a result of co-infections. The body development of clinically infected squabs is poor. Diphtheroid pharyngitis, oesophagitis and ingluvitis are prominent. The enlarged liver and spleen contain numerous pin-point white foci. Additional lesions caused by secondary infections are frequent. INIB are frequently present in epithelial cells of the upper digestive tract, liver and spleen. Surviving squabs develop antibodies.

Infection by pigeon herpesvirus only rarely results in clinically overt forms of disease. Important co-factors such as poor hygiene, overcrowding in lofts, and concurrent infections increase the likelihood and severity of clinical disease. Adult pigeons usually do not show obvious signs of disease, whereas young squabs are depressed and anaemic with poor growth and poor plumage. Mortality can reach 30 to 50%.

A presumptive diagnosis is based on young age, clinical signs, gross and microscopic pathology. Virus isolation confirms the aetiologic diagnosis. Antibody assays (neutralization tests) have no value for the diagnosis of individual cases, because most healthy pigeons possess circulating antibodies[99].

Squabs can be raised free of the infection if crop milk from virus-free parents is used as the only source of food. Chemotherapy using thymidine-kinase inhibitors (aciclovir or ganciclovir) has been tried with limited success. Vaccines are not available. The treatment of the prevailing secondary pathogens ameliorates the clinical course of the disease. Improved hygiene, including repeated cleansing and disinfection of the lofts, reduces the risk of exposure.

Pigeon herpesvirus 1 is not transmissible to mammals, including humans. As herpesvirus-infected pigeons are frequently concurrently infected by *Chlamydia psittaci*, special care is needed to prevent transmission of this zoonotic pathogen to people. There is also a potential risk of spill over of pigeon herpesvirus and also of chlamydia from domestic pigeons, to free-living columbiforms during racing competitions. However, definite proof for this assumption is not available.

INCLUSION BODY HEPATITIS OF OWLS, EAGLES AND FALCONS

Inclusion body hepatitis of owls (synonyms *Hepatosplenitis infectiosa strigum* (HSiS), inclusion body disease of owls), eagles and falcons (synonyms Inclusion body disease of falcons and eagles) are caused by unassigned viruses in the family *Herpesviridae*[98].

The herpesviruses of owls, eagles and falcons are *Owl herpesvirus 1* (hepatosplenitis virus or strigid herpesvirus 1), inclusion body disease virus of eagles (*Eagle herpesvirus 1*, acciptrid herpesvirus 1), and Inclusion body disease virus of falcons (*Falconid herpesvirus 1*), respectively.

Biological and virological properties of herpesvirus isolates from eagle owls, falcons and eagles are very similar, if not identical, to the *Pigeon herpesvirus 1*[111]. These viruses cause an almost identical gross and microscopic pathology[112], possess cross-reacting neutralizing antibodies[113], form similar bands in restriction endonuclease patterns[114] and yield similar sequence data of a fragment of the highly conserved herpesviral DNA polymerase gene using degenerate PCR primers[111]. These herpesviruses are readily inactivated by chemical disinfectants and by exposure to ultraviolet light.

The domestic pigeon (*Columba livia* f. *domestica*) and the ubiquitous feral pigeon (*Columba livia*) are considered the natural reservoirs[115]. Detailed, contemporary data on the prevalence of herpesvirus in Strigi-, Falconi- and Accipitriformes in Europe is not available.

Owl herpesvirus 1 induces a highly lethal disease or subclinical infection in free-living and captive birds of: the order Strigiformes, family Strigidae, subfamilies Asioninae, genus *Asio*; subfamily Striginae, genera *Strix*, *Megascops*, *Otus*, *Bubo*, *Nyctea*; subfamily Surniinae, genus *Athene*. Owls of all these genera present very similar gross, histopathological and ultrastructural lesions in liver, spleen, bone marrow and oesophagus. Most of these viruses were isolated from dead birds that lived free or were maintained in breeding and rehabilitation centres in Europe[116].

There is evidence of different susceptibility of owl species to herpesvirus isolated from an eagle owl (*Bubo bubo*). Experimentally, the European barn owl (*Tyto alba*) resisted infection, whereas nine identically infected owls of other species (five *Asio otus*, three *Athene noctua*, one *Aegolius funereus*) died with typical lesions in the liver, spleen and bone marrow[117]. Unfortunately, isolates from European barn owls are not available for molecular characterization. A herpesvirus was isolated from a barn owl but detailed characterization of this virus was not reported. Serologic studies in Germany showed neutralizing antibodies against an isolate from an eagle owl in 24 of 111 eagle owls but not in 61 barn owls[118]. Falcon herpesvirus induces lesions similar to the owl herpesvirus in falcons,

Falconiformes, genera *Hierofalco*, *Chiquera*, *Aesalon*, *Tinnunculus*[119]. Eagle owl herpesvirus was isolated from eagles in Germany (Accipitriformes, genera *Haliaeetus*, *Accipiter*, *Buteo*).

The viruses have been isolated from dead captive and wild birds and antibodies have been detected in live, apparently healthy birds, indicating infection in both young and adult hosts[120]. Infected European birds include eagle owl, long-eared owl (*Asio otus*), snowy owl (*Nyctea scandiaca*), little owl (*Athene noctua*), Tengmalm's owl (*Aegoluius funereus*) and great horned owl (*Bubo virginianus*). Tawny owl (*Strix aluco*) and barn owl proved resistant to a high dose of virus during experimental infections[117]. It has been noted that the susceptible species have yellow and orange irises, whereas the resistant ones have brown irises, although the relevance of this observation is not clear.

Circumstantial evidence suggests that raptors may become infected following consumption of infected pigeons and infected birds of prey. A German study yielded 12 strigid herpesviruses from 95 dead eagle owls and identified virus neutralizing antibodies in 116 of 695 serum samples derived from apparently healthy adult eagle owls. Consequently, breeders of eagle owls were advised to use only virus- and antibody-negative birds to produce young for release.

Oral transmission via consumption of herpesvirus-infected pigeons is the most likely mode of infection. Horizontal transmission from bird to bird by sharing prey or by mutual aggression may also be possible. Evidence for vertical or egg transmission has not been published.

Experimental studies showed spread of HSiS virus in the body of infected birds to palate, the choana, oesophagus, liver, spleen, bone marrow, thymus, trachea, lung, intestines. Virus was not detected in brain, heart, proventriculus or gizzard[117]. Virus-positive cells included hepatocytes and Kupffer cells, cells of connective tissues, lymphatic cells and epithelial and mesenchymal cells[121]. The progression of the disease is associated with rapid virus multiplication and organ dysfunction[116]. Dead owls were in relatively good body condition, suggesting a short course of the disease. The mortality rate is not known. Serologic data suggested that infected birds could recover.

Lesions in owls, falcons and eagles are uniform and consist of liver enlargement and small foci of necrosis in the liver, spleen and bone marrow. Intranuclear, eosinophilic inclusions in cells of these organs are characteristic.

Natural infection in owls was followed by an incubation period of approximately 1–2 weeks. However, after experimental infection general malaise and lethargy was noted after 3–4 days. The most frequent finding was sudden death[121]. Some owls had millet-seed-sized yellow nodules in the buccal palate and oesophagus. Similar signs were noted in falcons and eagles[112].

The gross and microscopic lesions are characteristic. Virus isolation in cell cultures of avian origin form the basis for the aetiologic diagnosis. Virus neutralization tests are used to demonstrate antibodies that indicate previous infection[113,122]. PCR can be applied to detect the DNA polymerase gene of herpesviruses[111].

Breeding of birds for subsequent release must be carried out using individuals that are free of virus and antibodies. Inactivated vaccines to protect healthy owls and falcons are of limited success. Cell-culture-adapted falcon herpesvirus live vaccine[123] did not provide effective protection against challenge with a homologous virulent virus obtained from a kestrel (*Falco mexicanus*). Treatment has no effect against the viral infection.

Release of herpesvirus-infected or antibody-positive birds must be avoided.

INCLUSION BODY DISEASE OF CRANES

Inclusion body disease of cranes, or hepatitis of cranes, is caused by *Gruid herpesvirus 1* (GrHV-1), an unassigned virus in the family *Herpesviridae*[98]. The virus caused fatal hepatitis in 12 grey-crowned cranes (*Balearica pavonina*) and in seven demoiselle cranes (*Anthropoides virgo*) in a safari park in Austria and additional losses in a zoo in Morbihan, France, in the winter of 1973–1974. In March and April 1979 a die-off in several crane species occurred in the International Crane Foundation, Baraboo, Wisconsin, USA; 18 out of 51 birds died suddenly. This apparently new disease in three locations has so far only been seen in captive cranes. It is not known whether any relationship exists between outbreaks in Austria, France and the USA as the origins of the birds in the respective collections is not known. The currently known spectrum of susceptible cranes comprises the sandhill crane (*Grus canadensis*), red-crowned crane (*Grus japonensis*), hooded crane (*Grus monacchus*) and Stanley crane (*Anthropoides paradisea*). All diseased birds were mature and both sexes were involved. The maintenance of cranes in overcrowded enclosures may have facilitated virus spread. The disease is

frequently lethal, but seropositive convalescent birds have been observed. Experimental infections provide evidence for susceptibility of white Pekin ducklings and adult coots (*Fulica americana*), whereas white leghorn chicks (*Gallus gallus*) and Muscovy ducks (*Cairina moschata*) were resistant. Crane herpesviruses from the outbreaks in Austria and France cross-react with a herpesvirus isolated from a bobwhite quail (*Colinus virginianus*)[124] and yield identical bands in restriction enzyme analysis[114].

Crane herpesvirus is excreted with faeces. Transmission via eggs was ruled out. The infective viral dose is unknown. It is likely that infection occurs by the oral route, but it is not clear if it results in an initial virus replication in the upper digestive tract. Postmortem data provide evidence for viraemia with subsequent dissemination in internal organs. The most prominent lesions are seen in liver and spleen, which are enlarged and with numerous grey foci. Necrosis is seen in the gastrointestinal tract, thymus and bursa of Fabricius[125]. Enteric lesions are occasionally observed. Histologically, numerous intranuclear inclusions are present in hepatocytes.

The course of the disease is rapid: birds succumb within 2 days. Although most infected cranes succumb, recovery and seroconversion is possible. Initial signs consist of depression, anorexia, lack of preening, enteritis and sitting with eyes closed[126,127].

The aetiological diagnosis is obtained by virus isolation in the cell culture yielding cytopathic effects, followed by characterization of the virus. Monoclonal antibodies that enable specific detection of crane virus by immunofluorescence and antibody assays in a competitive ELISA have been produced[128].

Separation of newly acquired birds in quarantine and serological monitoring should reduce the risk of introduction and subsequent spread. There is no vaccine or effective treatment.

So far, spread of crane virus from infected premises to free-living birds or white Pekin ducklings has not been reported. The role of the quail virus as a possible source of infection for cranes has been proposed but the relationship, if there is one, is not clear.

HERPESVIRUS INFECTIONS IN PASSERIFORMES

Although the order Passeriformes contains approximately half of all avian species, the isolation and characterization of herpesviruses from these birds are rarely described and there are no published reports in free-living European passerine birds. The few publications refer to captive pet birds such as canaries (*Serinus canaria* f. *domestica*)[129]. In Austria[130], Switzerland[131], Canada[132] and Illinois, USA[133], lethal diseases that are associated with conjunctivitis and respiratory distress were seen in gouldian finches (*Chloebia gouldiae*). Herpesvirus isolations were obtained from healthy appearing sharp-tailed mannikin (*Lonchura striata*), bronze mannikin (*Spermestes cucullatus*), common cardinal (*Cardinalis cardinalis*) and zebra finch (*Taeniopygea guttata*)[129]. Also, a herpesvirus was isolated from a disease outbreak in newly imported superb starlings (*Lamprotornis superbus*)[134] that is genetically related to a psittacid herpesvirus of the genotype 1[135]. So far, there are no publications providing evidence for lateral spread of these exotic passerine herpesviruses found in captive passerines, to endemic wild European species.

HERPESVIRUS INFECTION OF STORKS

The white stork (*Ciconia ciconia*) is a common bird in many parts of Europe. The causes of decline and recovery of white and black storks (*Ciconia nigra*) are carefully documented, and dead birds are usually comprehensively examined. Herpesviruses (*Ciconiid herpesvirus 1*) have been isolated and tentatively assigned to the family *Herpesviridae*[98]. The herpesvirus causes necrotic lesions usually in the liver and spleen[136]. Additionally, haemorrhagic enteritis was described in Spain[137]. Follow-up studies in rehabilitation centres in Germany provide evidence for a long-lasting, possibly life-long, cell-associated viraemia in disabled but otherwise normal adult white storks. It appears that storks can live with such viraemia for prolonged times, frequently for years, and produce healthy offspring[138]. The stork herpesvirus is antigenetically unrelated to any of the other avian herpesviruses.

PACHECO'S DISEASE

In 1931 Genesio Pacheco and Otto Bier[139] described, for the first time in great detail, a highly lethal disease in Brazilian large parrots and differentiated this apparently new disease from psittacosis.

The causative virus of Pacheco's disease (PD) is designated *Psittacid herpesvirus 1* (PsHV1), it is classified as a member of the family *Herpesviridae*, and stands as an unassigned virus in the subfamily *Alphaherpesvirinae*, genus

Iltovirus[8]. At least four, possibly more, major genotypes are known, with each genotype including two to four variants. Six serotypes are recognized that correspond well to genotypes[140].

PD affects many parrot species originating from several continents. The disease is seen mainly in Amazon parrots (*Amazona* spp., African grey parrots (*Psittacus erithacus*), macaws (*Ara* spp.) and cockatoos (*Cacatua* spp.). South American conures (*Aratinga* spp. and *Pyrrhura* spp.) are less frequently affected, but they often survive following infection and develop a carrier state associated with faecal virus excretion, which is important for lateral spread of virus.

In recent decades, free-living, sustainable populations of some parrot species, mainly parakeets of the Genus *Psittacula* spp., that have escaped from private collections have established in several Northern European and Mediterranean countries. Birds of this genus are not endemic in Europe but are susceptible to PD virus. So far, cases of PD in these free-living parrot populations have not been published, but PD does occur in captive psittacines in Europe. Natural transmission to endemic avian species in Europe has not been recorded.

Psittacine birds acquire the infection by oropharyngeal uptake of virus from contaminated food and water but also by coprophagia. Initial virus multiplication occurs in the upper respiratory and digestive tracts, followed by viraemia and spread of the virus to almost all the internal organs. In chronic cases, death follows as a result of emaciation, dehydration and dysfunction of multiple organs.

Postmortem findings in acute cases consist of good body condition (owing to the short duration of illness) and enteritis, enlarged liver and spleen with focal necrosis, enlarged ureters filled with urates. No prominent gross lesions are detectable in peracute disease forms.

Clinical signs of PD develop after an incubation period of 1 week and consist initially of lethargy, anorexia, ruffled feathers, closed eyelids and occasionally respiratory signs. During further progression of the disease greenish-yellow liquid droppings with larger amounts of urates are seen. Rarely, CNS disorders develop. The clinical course of the disease before death is a matter of days.

The diagnosis is based on virus isolation in cell cultures or by PCR. Virus differentiation is done by geno- and serotyping. Recovered birds have antibodies that can be differentiated into serotypes by neutralization tests. In histopathology, INIB can aid in the diagnosis.

All psittacine herpesviruses are sensitive to chemical disinfectants and radiation by ultraviolet light. Improved hygiene is recommended to reduce the risks of spread within bird collections. Only PD-negative birds should attend exhibitions. As psittacine herpesviruses consist of several geno- and serotypes, autogenous vaccines are very effective to prevent spread and further losses in affected bird collections. These vaccines – specific for each bird collection – are produced from cell culture-grown virus that is purified and inactivated by formalin and supplemented with potent adjuvants. Vaccinated birds develop neutralizing antibodies.

All non-psittacine birds, mammals and humans are resistant to infection by psittacine herpesviruses. Legal restrictions do not exist.

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CHAPTER

2

INFLUENZA VIRUS INFECTIONS

LESLIE A. REPERANT, ALBERT D.M.E. OSTERHAUS AND THIJS KUIKEN

INTRODUCTION

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Influenza A, B and C viruses belong to the family *Orthomyxoviridae*. They are typically spherical viruses of 80–120 nm in diameter with an envelope derived from the lipid membrane of host cells. Neutralizing antibodies recognize viral glycoproteins projecting from this envelope: haemagglutinin (HA) and neuraminidase (NA) for influenza A and B viruses, and haemagglutinin-esterase-fusion for influenza C viruses. Influenza viruses have a single-stranded negative RNA genome, which is segmented and prone to mutation and reassortment during the replication process. In general, influenza viruses replicate in epithelial cells and cause an acute infection that ends when the host develops a specific immune response. The host range of influenza differs according to genus. In particular, influenza A viruses infect a wide range of avian and mammalian hosts[1,2] and influenza B viruses, long thought to be restricted to humans, have been found in pinnipeds[3].

Influenza A viruses belong to the most important genus for wildlife. They are divided into subtypes according to the antigenic variation in their HA and NA proteins. To date, 16 HA and 9 NA subtypes have been described. Wild waterbirds are the natural reservoirs of all influenza A virus subtypes[1]. Influenza A viruses may also spread from wild birds or poultry to mammalian species, in which they may become established. In this way, influenza A viruses have become endemic in human, horse and pig populations. In addition, equine influenza A viruses have infected and potentially established in dog populations[2].

In wild waterbirds, influenza A viruses primarily cause an intestinal tract infection in the absence of clinical disease. Such low pathogenic avian influenza viruses (LPAIV) similarly cause an intestinal tract infection in poultry. However, upon evolution, by selection, into highly pathogenic avian influenza viruses (HPAIV) in poultry, they cause systemic infection with high mortality. In mammals, influenza A viruses typically cause a respiratory tract infection of variable clinical severity and pneumonia as a primary complication. HPAIV H5N1 forms an exception, as it may cause severe systemic infection in mammals.

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AVIAN INFLUENZA

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AETIOLOGY

Avian influenza A viruses can be classified based on their pathogenicity in poultry into low pathogenic and highly pathogenic forms. Nearly all isolates from wild waterbirds are low pathogenic avian influenza viruses (LPAIV). All known subtypes of LPAIV have been isolated from wild waterbirds, which are considered their natural reservoirs. All influenza A virus lineages occurring in other species, including established lineages of influenza A viruses in humans and other mammals, are thought to originate from avian influenza viruses of wild birds. LPAIV may be transmitted to poultry, where infection is subclinical or causes mild clinical disease. However, LPAIV of the H5 and H7 subtypes can evolve by selection in poultry into highly pathogenic avian influenza viruses (HPAIV) and cause systemic infection and severe, often fatal, disease in these species. HPAIV are rarely found in wild birds, and, if they are, typically they do not cause clinical disease. Only HPAIV H5N1 has caused disease in wild birds in Europe, sometimes in association with outbreaks with high mortality.

Low pathogenic avian influenza has no synonyms. Highly pathogenic avian influenza is also called fowl plague or fowl pest.

EPIDEMIOLOGY

GEOGRAPHICAL DISTRIBUTION

LPAIV are widely distributed across Europe. Partly as a result of up-scaling of avian influenza surveillance as a result of the threat of HPAIV H5N1, LPAIV have now been isolated from wild birds in most European countries (Figure 2.1A). Some eastern and southern European countries have not yet reported LPAIV infection in wild birds, although recent studies have provided serological evidence of LPAIV infection.

Most LPAIV haemagglutinin (HA) and neuraminidase (NA) proteins described to date have been isolated from wild birds in Europe (Table 2.1)[(4)]. Phylogenetic analysis of each of the eight genes of LPAIV isolated from wild

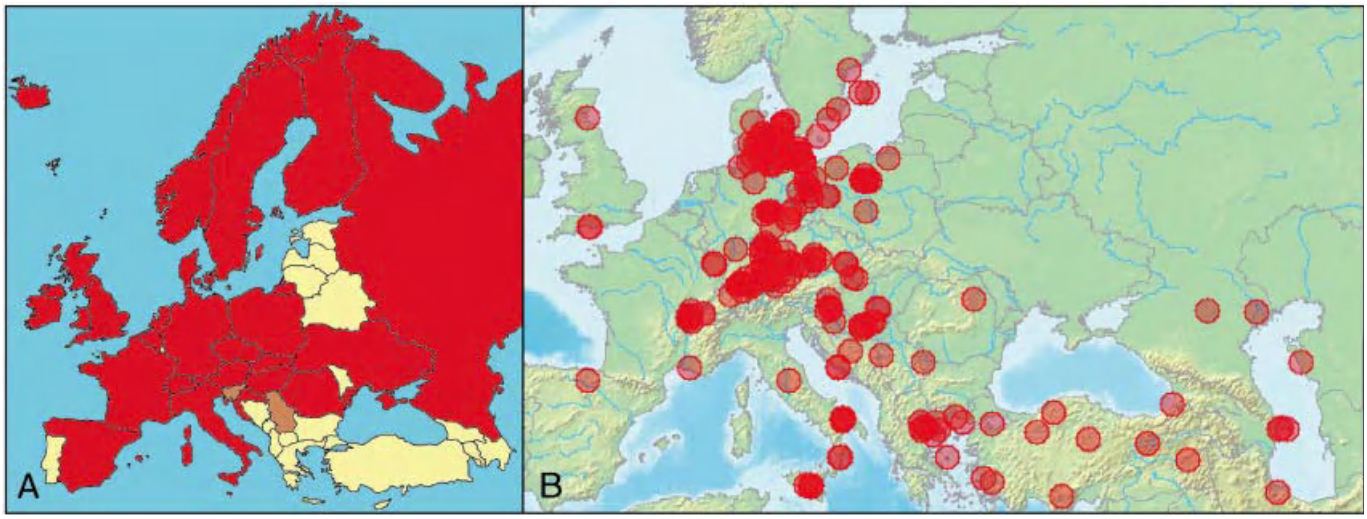

FIGURE 2.1 Geographical distribution of avian influenza viruses in wild birds in Europe. **A**. Geographical distribution of LPAIV. Red: countries where LPAIV have been isolated from wild birds and viral sequences deposited in a public database; brown: countries with serological evidence of LPAIV circulation in wild birds; yellow: countries with no published report of LPAIV infection in wild birds. **B**. Locations of wild birds found infected with highly pathogenic avian influenza virus H5N1 in Europe and the Near East between 2005 and 2009 (marked as transparent circles).

TABLE 2.1 Combinations of HA and NA proteins from LPAIV recovered in wild birds.

	H1	H2	H3	H4	H5	H6	H7	H8	H9	H10	H11	H12	H13	H14	H15	H16
N1	X	X			X	X					X					
N2	X	X	X	X	X	X			X	X	X					
N3		X	X	X	X		X				X					X
N4							X	X		X						
N5		X	X			X				X		X				
N6	X		X	X	X	X				X	X		X			
N7	X	X					X			X	X					
N8	X		X			X				X	X	X	X			
N9		X		X	X		X			X	X					

Green boxes: combinations of HA and NA proteins from LPAIV recovered in wild birds globally
X: combinations of HA and NA proteins from LPAIV recovered in wild birds in Europe

birds in Europe places them in a Eurasian clade. By contrast, LPAIV from wild birds in North America cluster in a separate, North American clade[5], and there may be a distinct phylogenetic clade for South American isolates[6]. Geographical separation of host species has probably shaped the gene pool of LPAIV into largely independently evolving lineages. Intercontinental dispersal of LPAIV of either clade has not been described to date, although there is growing evidence of reassortment of genes from LPAIV of different clades[7]. For instance in Europe, genes of the North American clade have been recovered from LPAIV isolated in guillemots (*Uria aalge*) in the northern Baltic Sea[8]. However, such intercontinental reassortment events remain rare. Therefore, despite shared migratory flyways used by LPAIV host species, e.g. the East Atlantic flyway, LPAIVs mix infrequently between the Eurasian and North American clades[5].

Since the autumn of 2005, HPAIV H5N1 originating from poultry in South-East Asia have infected wild bird populations across Eurasia (Figure 2.1B). HPAIV H5N1 has affected multiple wild bird species on several continents during the past 5 years. HPAIV H5N1 first reached Russia and south-eastern European countries in autumn 2005 and then spread to more western and northern countries during the following winter. Most outbreaks of fatal infection occurred during the winter and early spring of 2006 in multiple species of wild birds. HPAIV H5N1 reoccurred in wild birds in summer 2007, mostly in Germany, and were occasionally isolated in winter 2007–2008 and winter 2008–2009, in the UK and central Europe.

HOST FACTORS

LPAIV have been recovered worldwide in at least 110 wild bird species from 26 families belonging to 13 orders. In Europe, LPAIV have been recovered in at least 35 wild bird species, from 10 families belonging to 8 orders (Table 2.2). Despite their wide host range, LPAIV typically infect bird species that live in wetland and aquatic habitats, in particular species of the orders Anseriformes (geese, ducks and swans) and Charadriiformes (waders and gulls), as well as Eurasian coots (*Fulica atra*) of the order Gruiformes in Europe (Figure 2.2A).

The host range of avian influenza viruses (AIV) is partly determined by the specificity of the HA protein for the receptor by which the virus attaches to the host cell. AIV typically bind to sialic acid moieties with a α_{2-3} linkage to galactose. These receptors are present on the surface of avian host cells, notably epithelial cells of the intestinal tract. However, the conformation of these receptors on intestinal epithelial cells of ducks, gulls and chickens differ[9]. Such differences may contribute to the observed differences in susceptibility of different avian hosts to LPAIV infection.

Studies on prevalence of LPAIV infection in wild birds in Europe are based on isolation of LPAIV or detection of LPAIV nucleic acids in samples. The highest prevalence is

TABLE 2.2 List of wild bird species found infected with LPAIV in Europe. Prevalences are reported for species with more than 150 sampled birds.

Order, family	Species common name	Species scientific name	Country	N	%
Anseriformes, Anatidae	Greater white-fronted goose	*Anser albifrons*	The Netherlands, Sweden	4325	2.2
	Greylag goose	*Anser anser*	Germany, the Netherlands, Sweden	1432	1.5
	Pink-footed goose	*Anser brachyrhynchus*	The Netherlands, Sweden	285	2.1
	Bean goose	*Anser fabalis*	The Netherlands	466	0.6
	Barnacle goose	*Branta leucopsis*	The Netherlands	1257	0.6
	Brent goose	*Brenta bernicla*	The Netherlands, Sweden	715	0.6
	Northern pintail	*Anas acuta*	France, the Netherlands, Sweden	920	3.3
	Common teal	*Anas crecca*	France, Germany, Iceland, the Netherlands, Sweden	2414	8.3
	Eurasian wigeon	*Anas penelope*	France, the Netherlands, Sweden	3596	2.5
	Mallard	*Anas platyrhynchos*	Czechoslovakia, France, Germany, Hungary, Italy, Sweden, the Netherlands, Sweden	15962	8.7
	Garganey	*Anas querquedula*	France, Romania		
	Northern shoveler	*Anas clypeata*	France, the Netherlands	284	3.2
	Common shelduck	*Tadorna tadorna*	France, Italy, Sweden	1244	4.7
	Tufted duck	*Aythya fuligula*	The Netherlands		
	Long-tailed duck	*Clangula hyemalis*	Germany	157	1.3
	White-winged scoter	*Melanitta fusca*	Germany		
	Common scoter	*Melanitta nigra*	Norway		
	Common eider	*Somateria mollissima*	The Netherlands, Sweden		
	Bewick's swan	*Cygnus columbianus bewickii*	The Netherlands, Sweden	153	2
	Mute swan	*Cygnus olor*	Germany, the Netherlands	1362	1.5
Charadriiformes, Caradriidae	Waders		Sweden, Italy	3000	1.2
Charadriiformes, Laridae	Black-headed gull	*Larus ridibundus*	Germany, the Netherlands, Sweden	2395	1.5
	Common gull	*Larus canus*	The Netherlands	226	0.9
	Herring gull	*Larus argentatus*	The Netherlands	862	0.6
	Greater black-backed gull	*Larus marinus*	The Netherlands, Sweden		
	Mediterranean gull	*Larus melanocephalus*	France		
	Common tern	*Sterna hirundo*	Germany	875	1.5
	Arctic tern	*Sterna paradisea*	Germany		
	Sandwich tern	*Sterna sandvicensis*	Germany	351	0.3
Ciconiiformes, Ardeidae	Grey heron	*Ardea cinerea*	Romania		
Columbiformes, Columbidae	Eurasian collared dove	*Streptopelia decaocto*	Hungary		
Galliformes, Phasianidae	Common pheasant	*Phasianus colchicus*	Hungary	399	0.5
Gaviiformes, Gavidae	Arctic loon	*Gavia arctica*	Romania		
Gruiformes, Alcidae	Common murre	*Uria aalge*	Sweden	843	0.7
Gruiformes, Rallidae	Eurasian coot	*Fulica atra*	Germany, Hungary, Italy, the Netherlands	2610	2.6
Pelicaniformes, Phalacrocoracidae	Great cormorant	*Phalacrocorax carbo*	Germany, Romania	4500	0.4

typically recorded in dabbling ducks (*Anas* spp.), and notably in mallards (*A. platyrhynchos*) and common teal (*A. crecca*) with a prevalence of 8% (Table 2.2). Interestingly, high prevalence was also reported in a small sample of estuary and marine ducks, such as shelduck (*Tadorna* spp.), scoters (*Melanitta* spp.) and eiders (*Somateria* spp.) (Figure 2.2A). In contrast to North America, where spring prevalence of LPAIV in waders is high, low prevalence is reported in a few species of waders in Europe, and no LPAIV has been isolated from waders in Europe to date.

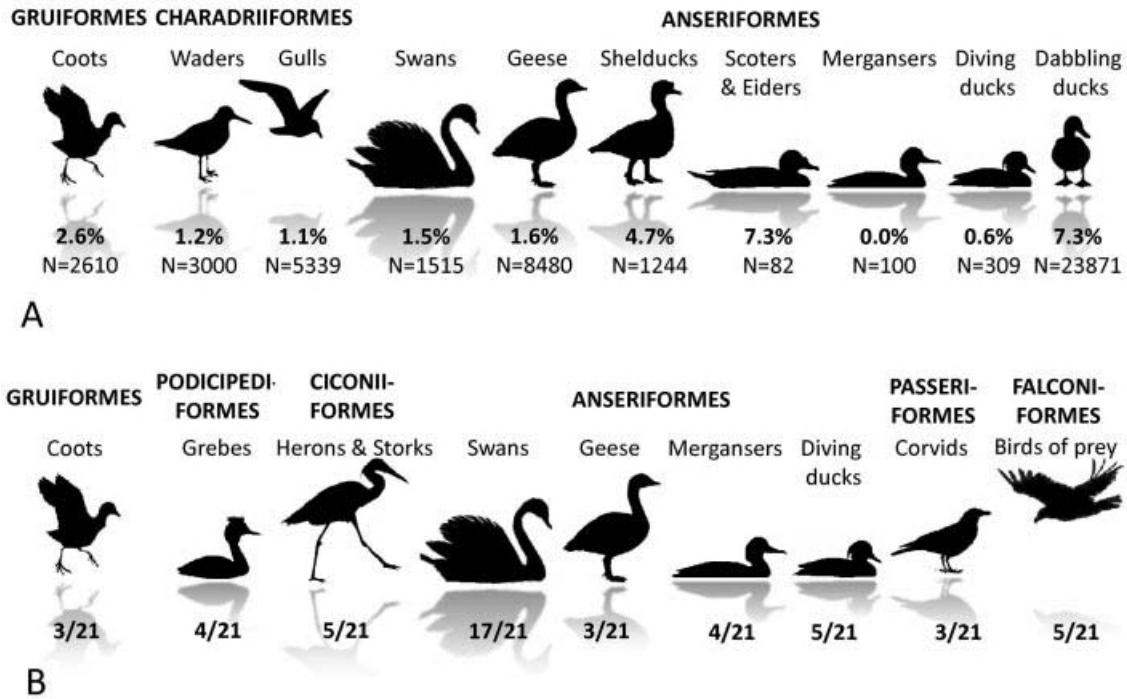

FIGURE 2.2 Avian host range of LPAIV and HPAIV H5N1 in Europe. **A**. Prevalence of LPAIV infection in the most frequently infected wild waterbirds in Europe (N is the number of birds sampled). **B**. Wild bird species most frequently found infected with HPAIV H5N1 in Europe (the number of affected European countries experiencing outbreaks of HPAIV H5N1 infection in each wild bird category is indicated).

Higher prevalence of LPAIV infection is reported in juvenile birds than in adults, in particular just before the autumn migration when many waterbirds congregate. LPAIV prevalence was 13% and 6% in 3982 juvenile and 1363 adult dabbling ducks, respectively, sampled during autumn in the Netherlands and Sweden between 1998 and 2006[4,10]. Juvenile birds are immunologically naïve to LPAIV, and this probably contributes to the observed age difference in LPAIV prevalence[4]. Gender does not appear to affect LPAIV prevalence in birds.

Waterbird ecology probably contributes to the observed higher prevalence of LPAIV infection in birds of the orders Anseriformes and Charadriiformes. Feeding behaviour, notably of dabbling ducks, and social behaviour and population sizes of most waterbird species will probably favour virus transmission[4]. Of particular interest is the difference in the prevalence of LPAIV infection in dabbling ducks and small diving ducks (*Aythya* spp.), possibly resulting from differences in diet and feeding behaviour. Dabbling ducks feed on the surface of the water whereas diving ducks feed below the water surface. Feeding on the surface of water may result in greater ingestion of contaminated water. Conversely, lower prevalence in swans and geese may reflect a lower risk of infection from grazing. In this light, high prevalence in shelducks and marine diving ducks is rather surprising, as these birds respectively graze and feed in deep saline waters that do not favour LPAIV environmental persistence. Therefore, additional factors probably contribute to the host range of LPAIV in waterbirds.

HPAIV H5N1 also have a wide avian host range, but infect some species that are rarely found infected with LPAIV (Figure 2.2B). In particular, birds not necessarily associated with aquatic habitats, such as corvids and birds of prey, were infected, this probably as a result of feeding on infected carcasses. More than 700 fatal cases of HPAIV H5N1 infection were confirmed in wild birds in Europe in 2006, mostly in mute swans (*Cygnus olor*), whooper swans (*C. cygnus*), common pochards (*Aythya ferina*) and tufted ducks (*A. fuligula*). In summer 2007, a massive outbreak reoccurred in wild birds in Germany and affected mostly black-necked grebes (*Podiceps nigricollis*) and great-crested grebes (*P. cristatus*), killing more than 200 individuals[11]. There are few data on potential correlations of HPAIV H5N1 infection with bird age or sex.

ENVIRONMENTAL FACTORS

LPAIV prevalence displays important geographical and seasonal variations[1,12]. In Europe, there may be a north–south gradient of LPAIV prevalence in wild birds, being typically higher in Scandinavian countries than in the more southerly located Netherlands[4]. However, prevalence data remain limited, and it is not known whether seasonal variations in wild bird demography and distribution play a role.

LPAIV infection in wild birds peaks in early autumn, when waterbird populations are composed of a high proportion of juvenile birds that congregate before migration, with a prevalence reaching up to 25%, e.g. in Sweden[10]. As migration proceeds, LPAIV prevalence declines and reaches low to undetectable levels during late winter. LPAIV prevalence in spring is typically lower than in autumn, yet can reach relatively high levels in Sweden (up to 9.5%)[10]. LPAIV prevalence in waterbirds at breeding grounds has rarely been reported. It was 3.5% in breeding dabbling ducks in northern Siberia[13].

HPAIV H5N1 emerged in wild birds in Europe at the time of autumn migration in 2005 and resulted in massive outbreaks during winter 2005–2006 and summer 2007. HPAIV H5N1 was isolated in wild birds during winter 2007–2008 and winter 2008–2009, displaying an unusual seasonality pattern. During winter 2005–2006, the temporal and geographical distribution of wild birds infected with HPAIV H5N1 coincided with the 0°C isotherm. This suggests that cold weather may have favoured HPAIV H5N1 transmission in Europe, either owing to the aggregation of waterbirds in ice-free areas, or to the persistence of the virus in the environment along the 0°C isotherm, or both[2,14].

EPIDEMIOLOGICAL ROLE OF THE AFFECTED SPECIES

Because of the observed high prevalence of LPAIV infection in birds of the orders Anseriformes and Charadriiformes, these species (and possibly coots of the order Gruiformes) are typically referred to as the natural reservoirs of LPAIV[12,1]. Many species within these orders migrate over long distances and have the potential to spread LPAIV over large geographical areas. Mallards and common teal have a higher prevalence rate than other waterbird species, and probably play a significant role as reservoirs. Annual turnover of mallards in Europe is estimated to be one-third, thus resulting in populations with one-third of juvenile naïve individuals, which will favour the maintenance and spread of LPAIV[4,10]. However, it is difficult to assess the role of other waterbird species: part or all of the waterbird community may play a role as the reservoir of LPAIV.

LPAIV of the H13 and H16 subtypes form a separate category. These viruses are phylogenetically distinguishable from other LPAIV, and are typically isolated from gulls, belonging to the order Charadriiformes[15]. Interestingly, the receptor-binding site of the HA of subtypes H13 and H16 differs from those of other LPAIV subtypes, possibly in adaptation to slightly different cell receptors in gulls[9]. Collectively, these findings strongly indicate that gulls are reservoir hosts for these subtypes.

Waders, of the order Charadriiformes, do not appear to play a significant role as reservoir hosts of LPAIV in Europe, contrary to what is observed in North America. Birds from orders other than Anseriformes and Charadriiformes are typically considered accidental hosts. However, more comprehensive surveillance studies extending to a larger number of wild bird species are necessary to determine the relative role of the different avian hosts of LPAIV.

Wild birds potentially play different roles in the epidemiology of HPAIV H5N1: sentinel, spreader and reservoir species. Some wild bird species, e.g. mute swans, whooper swans and tufted ducks, proved highly susceptible to developing fatal disease, and thus were found to be sentinels. Other wild bird species, e.g. mallards, became subclinically infected and shed virus for several days upon experimental infection, and may thus play a role in spreading the virus. There also is field evidence for the role of wild birds as spreaders of HPAIV H5N1. First, a healthy pochard and a healthy mallard have been found infected in Switzerland and Germany. Second, outbreaks of HPAIV H5N1 in Western Europe during winter 2005–2006 occurred mainly in wild birds. Third, the temporal and geographical association between the locations of these outbreaks and the 0°C isotherm strongly suggest that wild waterbirds were spreading HPAIV H5N1, following its introduction[2,14]. Whether wild birds can act as a reservoir of HPAIV H5N1 is an important but unanswered question, especially if eradication of HPAIV H5N1 in poultry succeeds.

TRANSMISSION

LPAIV are excreted mainly from the digestive tract of infected birds into the environment. This may involve

large quantities of virus: cloacal shedding was estimated in one study at more than 10^8 EID_{50} (median egg infectious dose) per gram of duck faeces, for a total of 10^{10} EID_{50} per day[16]. The viruses are thought to be mainly transmitted via the faecal-oral route, via ingestion of contaminated water (Figure 2.3A). Faecal-oral transmission is facilitated in aquatic habitats. LPAIV have been shown to persist for several months in environmental reservoirs, such as lake water[17]. Birds feeding on surface water may be more likely to be infected. Taken together, these factors favour waterbirds over terrestrial birds as the main hosts of avian influenza viruses.

Alternative routes of transmission are via the cloacal and respiratory routes. 'Cloacal drinking', i.e. uptake of water via the cloaca, has been suggested as a possible route of infection in waterbirds. The respiratory tract can become transiently infected by LPAIV, and excretion of LPAIV from the respiratory tract cannot be excluded.

The mechanisms of year-to-year persistence of LPAIV remain unclear, and several hypotheses have been proposed. On the one hand, year-round infection of wild birds may contribute to LPAIV persistence; LPAIV are usually undetectable during late winter in Europe, but this may be the result of low sampling efforts. On the other hand, environmental persistence of LPAIV may also contribute to their year-round persistence. Surprisingly, few studies have investigated LPAIV persistence in environmental samples, and none in Europe (for a review see Stallknecht and Brown[17]). LPAIV can persist for several months in faeces and water under adequate conditions. The duration of infectivity is highly dependent on LPAIV strains, yet is always higher in water at lower temperatures, with neutral to slightly basic pH and low salinity levels. LPAIV have been isolated from the surface water of lakes in North America and LPAIV RNA has been recovered from lake sediment in North America and from ice of frozen lakes in Siberia. Although infectious virus could not be isolated in the latter two, sediment and ice may form long-term environmental reservoirs of LPAIV. LPAIV may survive in lake water during winter, and infect birds heading towards their breeding grounds during spring migration.

Transmission from the virus reservoir in the environment may be independent of the presence of infectious birds, i.e. density-independent, because infectious virus persists in the water after the infectious bird has left; or it may be density-dependent, necessitating the presence of infectious birds. Studies of LPAIV dynamics in wild birds are rare, and density-dependent transmission is typically used as the main mode of transmission in mathematical models of the SIR (susceptible-infected-recovered) type. However, a few recent mathematical models of LPAIV transmission have incorporated both a density-dependent and density-independent form of transmission. The results of these models increasingly support an important role for environmental transmission of persisting LPAIV in the absence of infectious birds in maintaining LPAIV locally and from year to year[18,19].

The transmission mode of HPAIV H5N1 may be different from that of LPAIV. In contrast to LPAIV, HPAIV H5N1 are mainly excreted from the respiratory tract of infected birds. This may favour direct bird-to-bird transmission via respiratory secretions (Figure 2.3B). However, contamination of the environment by respiratory secretions and infected carcasses probably results in indirect oral transmission of the virus. The presence of HPAIV H5N1 in one mallard in January 2009 in Germany, without any report of additional outbreaks in Europe, may indicate environmental persistence of HPAIV H5N1, which have been shown to remain infective for 158 days in fresh water at 17°C, and for 26 days at 28°C[20]. In addition, bird species not necessarily associated with aquatic habitats were also found infected with HPAIV H5N1, notably corvids and birds of prey. This strongly suggests that predation and ingestion of infected bird carcasses is another mode of transmission of HPAIV H5N1 in wild birds.

PATHOGENESIS, PATHOLOGY AND IMMUNITY

The pathogenicity of avian influenza viruses in poultry is at least partly determined by the cleavability of the HA protein. The HA glycoprotein contributes to attachment and fusion of the lipid membranes of the virus and host cell. The HA protein of AIV must be cleaved by host proteases to become infectious and undergo a conformational change at low pH, leading to the fusion of the virus and host cell membranes. The HA protein of LPAIV is cleaved by extracellular trypsin-like host proteases present in the digestive and respiratory tracts of birds. By contrast, the HA protein of HPAIV has a polybasic cleavage site that can be cleaved by ubiquitous intracellular subtilisin-like host proteases such as furin, which are present in many cell types, contributing to high virulence.

Ingestion of water contaminated with LPAIV is considered the main route of infection in waterbirds[21], although 'cloacal drinking' and inhalation cannot be excluded. Thus, the first sites of LPAIV replication are the intestinal epithelium and to a lesser extent the respiratory epithelium (Figure 2.3A). If ingested, LPAIV must pass through and survive the acidic environment of the proventriculus of ducks; uncleaved HA of LPAIV appears more resistant to acidic pH than that of mammalian influenza viruses, and ingestion of a large amount of water may partly neutralize the acidic pH of duck stomach, which together probably allow infectious doses of LPAIV to reach their target site of replication in the intestine.

Little is known about the infectious dose of LPAIV in wild birds. Doses ranging between $10^{1.2}$ and $10^{3.3}$ EID_{50} have proven to be infectious[22], but higher doses were used in the past for experimental infections. Large quantities of LPAIV are shed in infected bird faeces[16], and LPAIV have been isolated from unconcentrated samples of lake water; thus ingestion of relatively high infectious doses may not be infrequent in nature.

The route of infection of HPAIV H5N1 may be inhalation of respiratory secretions or ingestion of water contaminated by respiratory secretions and carcasses. Ingestion of infected carcasses is another route of infection, based on the occurrence of disease in corvids and birds of prey (Figure 2.3B). Ingestion of meat infected with HPAIV H5N1 resulted in infection and disease in experimentally infected herring gulls (*Larus argentatus*)[23]. The first site of HPAIV H5N1 replication appears to be the respiratory epithelium. In contrast to LPAIV infection, cells along the intestinal epithelium are not, or are rarely reported to be, infected by HPAIV H5N1. A possible explanation is that HPAIV H5N1 is unable to survive the acid barrier of the proventriculus: HPAIV H5N1 is released from the host cell with cleaved HA protein, which is known to be sensitive to, and lose infectivity at, low pH[21].

Relatively low infectious doses of HPAIV H5N1 (around 10^4 median tissue-culture infectious dose ($TCID_{50}$)) result in productive infection of various wild bird species[23–25]. Inoculation of various doses of HPAIV H5N1 into mute swans[26], house sparrows (*Passer domesticus*) and rock pigeons (*Columbia livia*)[27] demonstrated a correlation between infectious dose, clinical outcome and duration of viral shedding. Lower doses typically resulted in less severe disease and longer viral shedding, although the total amount of excreted virus did not differ substantially in birds inoculated with different infectious doses.

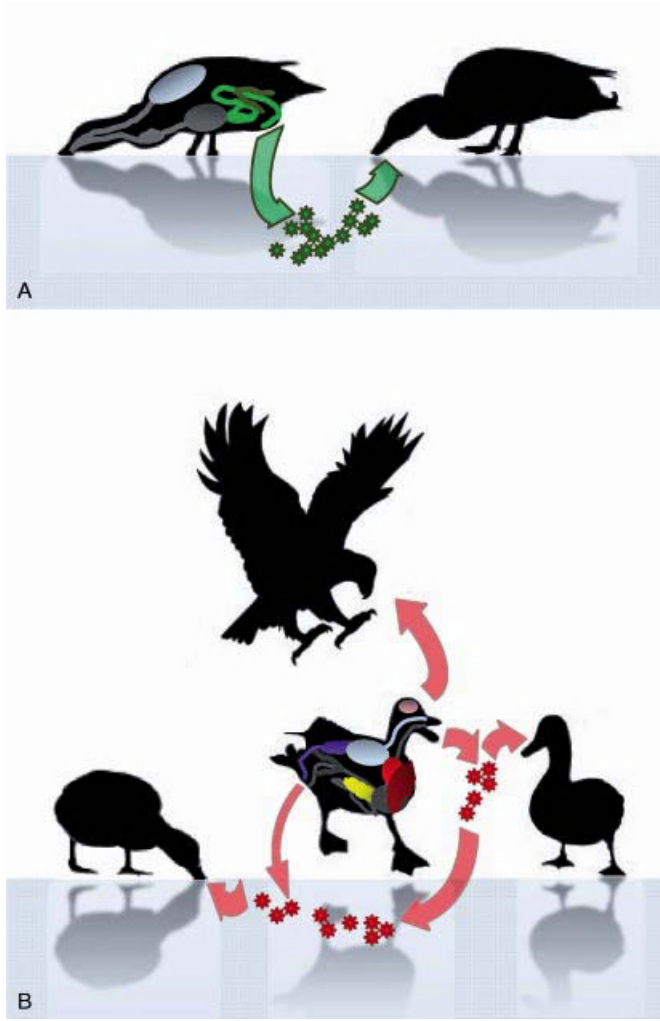

FIGURE 2.3 Routes of transmission and tissue tropism of LPAIV and HPAIV H5N1 in wild birds. **A**. LPAIV (green stars) are thought to be mainly transmitted via a faecal-oral route, and ingestion of contaminated water is considered the main route of infection in wild birds. LPAIV are recovered from the respiratory tract (blue) and digestive tract (green), infecting mostly intestinal epithelial cells. Infected birds do not present clinical signs. **B**. HPAIV H5N1 (red stars) are mostly shed via the respiratory tract, yet the exact route of infection in wild birds is not exactly known, and probably includes inhalation, and ingestion of contaminated water or infected carcasses. HPAIV H5N1 replicate in many organs in highly susceptible species, including the central nervous system (pink), the respiratory tract (blue), the heart (red), the liver (brown), the pancreas (yellow) and the kidneys (purple). In susceptible species, clinical signs include laboured breathing and neurological signs, and disease is often fatal. The intestinal tract (grey) is not infected.

The target cells for LPAIV infection in domestic ducks are epithelial cells of the intestinal tract and of the bursa of Fabricius (Figure 2.4), and to a lesser extent epithelial cells of the respiratory tract[16]. LPAIV are first isolated from the respiratory tract on 2 days post-inoculation (dpi),

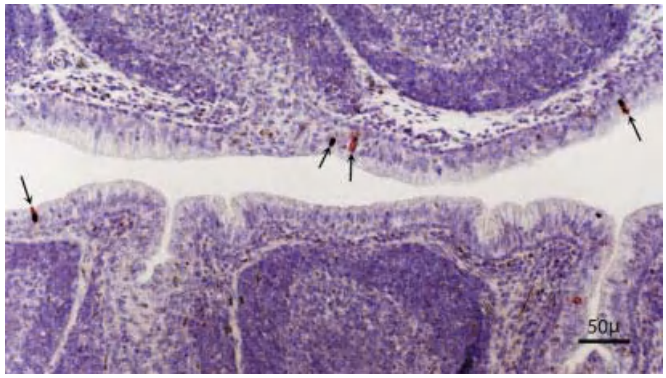

FIGURE 2.4 Cloacal bursa of a wild mallard duck naturally infected with LPAIV, subtype H2N3. Positive cells are scattered along the surface epithelium (arrows). Note the lack of histologic changes associated with the expression of influenza virus antigen in Cloacal bursa (bursa of Fabricius) of a wild mallard. Immunoperoxidase method for nucleoprotein of AI virus counterstained with hematoxylin. Reprinted with permission from Daoust P.-Y. et al., 2001.

and from the intestinal tract and bursa of Fabricius on 2–3 dpi and until 5–7 dpi[16,28–31].

LPAIV are shed via the cloaca of domestic ducks as early as 1 dpi, and cloacal shedding lasts typically 6–7 days. Cloacal shedding of 3–4 weeks has also been occasionally reported. A capture-recapture study of mallards naturally infected with LPAIV reported maximum cloacal shedding times of around 8 days, with a mean of 3 days[32]. The pathogenesis of LPAIV in waterbird species other than the domestic duck has not been described.

In contrast to LPAIV, which are typically restricted to the digestive tract and, to a lesser extent, the respiratory tract, HPAIV H5N1 have a wide tissue tropism in wild birds (Figure 2.3). The respiratory epithelium appears to be the initial site of HPAIV H5N1 replication. Subsequent viraemia may lead to secondary infection of other organs. However, the degree of dissemination differs greatly among species, from localized respiratory tract infection to dissemination to multiple organs. Once beyond the respiratory tract, HPAIV appears to have the strongest tropism for the central nervous system in many bird species, e.g. common teal, common pochards, tufted ducks, mute swans, whooper swans, herring gulls, house sparrows (*Passer domesticus*) and rock pigeons (*Columba livia*). The virus also may spread to other organs: replicating virus and high viral titres are typically recorded in pancreas, liver, adrenal gland, kidney, heart and skeletal muscle of experimentally infected wild birds (Table 2.3). In most naturally and experimentally infected wild bird species, parenchymal cells are the main target for HPAIV H5N1 replication, e.g. respiratory epithelial cells, pancreatic parenchymal cells, hepatocytes, adrenocortical cells, renal epithelial cells, myocytes and neurons. In contrast to HPAIV H5N1 infection in chickens, HPAIV H5N1 rarely, if ever, infects endothelial cells in most wild bird species. Exceptions are mute and whooper swans and tufted ducks[26,33,34], in which HPAIV H5N1 shows a clear endotheliotropism.

In contrast to LPAIV, HPAIV H5N1 is shed more from the respiratory tract than from the digestive tract, based on virus titres in pharyngeal and cloacal swabs, respectively. Pharyngeal shedding of HPAIV H5N1 starts at least 1 dpi and can last 5–7 days in experimental infection of most European species of wild ducks and swans[24,25]. In mute swans infected with low doses, HPAIV H5N1 were shed for up to 11 days[26]. In contrast, herring gulls shed HPAIV H5N1 for up to 3 days only[23]. Virus shedding from the cloaca is typically shorter and at titres of one to two logs lower than from the pharynx[23–25,27].

AIV infection in wild birds is typically acute and resolves with the development of a specific immune response. Regeneration of intestinal epithelial cells of wild birds infected with LPAIV is rapid, with histological evidence of regeneration as soon as 72 hours post-infection[30]. Damage caused by HPAIV H5N1 infection varies greatly among species.

Disease following infection with AIV may result from direct cytopathogenic damage caused by the virus replicating in host cells, from damage resulting from the host immune response, or from a combination of these. LPAIV infection in domestic ducks and wild birds causes no, or at most very mild, clinical disease, and the only recorded lesions are very mild degenerative changes of intestinal and bursal epithelium (see below).

By contrast, the effect of HPAIV H5N1 infection in wild birds ranges from subclinical disease to acute death, largely dependent on host species affected. Replication of HPAIV H5N1 in parenchymal cells of an organ results in marked necrosis and inflammation. Clinical disease is highly variable in timing and presentation. It may start between 4 and 9 dpi, and involve respiratory, nervous or general signs. Development of disease differs in those species, such as whooper swans, in which HPAIV H5N1 is endotheliotropic. Infection of endothelial cells causes blood vessel damage and results in haemorrhages and oedema, leading to clinical disease as early as 24 hours post-infection.

LPAIV infection in wild birds generally does not cause gross or microscopic lesions (Figure 2.4). However, very

TABLE 2.3 Infectious period, incubation time and clinical signs following natural and experimental infection with LPAIV and HPAIV H5N1 of the sublineage that spread to bird populations in Europe, in wild bird species.

Wild bird species	AIV	Type of infection	Maximum pharyngeal shedding (duration; N/Total)*	Maximum cloacal shedding (duration; N/Total)*	Clinical signs (incubation time; N/Total)	Mean time to death (N/Total)	Ref
Mallard	LPAIV	Natural	ND	ND (3–8 days; 59)	None	None	32
Mallard	LPAIV	Experimental	5.9 (7 days; NA)	5.8 (7 days; NA)	None	None	28,16,29,30,31,32
Mallard	HPAIV H5N1	Experimental	3.2 (4 days; 8/8)	0	None	None	25
Common teal	HPAIV H5N1	Experimental	1 (5 days; 3/8)	0.5 (1 day; 1/8)	None	None	25
Eurasian wigeon	HPAIV H5N1	Experimental	1.2 (2 days; 4/8)	0	None	None	25
Gadwall	HPAIV H5N1	Experimental	2.2 (6 days; 7/8)	0	None	None	25
Tufted duck	HPAIV H5N1	Experimental	3.2 (5 days; 6/8)	0	Laboured breathing, increased recumbency Neurologic signs: torticollis, circling, loss of balance, head tremors (3–4 days; 7/8)	4 days (3/8)	25
Tufted duck	HPAIV H5N1	Natural	ND	ND	Neurological signs: head tilt, circling, loss of balance, drooping wings	ND	34
Common pochard	HPAIV H5N1	Experimental	2.8 (4 days; 7/8)	0.7 (2 days; 2/8)	Laboured breathing, increased recumbency Neurologic signs: torticollis, circling, loss of balance, head tremors (3–4 days; 4/8)	4 days (1/8)	25
Mute swan	HPAIV H5N1	Experimental	5.6 (7 days; 5/5)	4.5 (4 days; 5/5)	Listlessness Neurological signs: seizures, tremors, marked inco-ordination (5–7 days; 5/5)	5–8 days (5/5)	24
Whooper swan	HPAIV H5N1	Experimental	6.3 (6 days; 4/4)	4.2 (5 days; 4/4)	Listlessness Neurological signs: seizures, tremors, marked inco-ordination (2–4 days; 4/4)	4 days (4/4)	24
Whooper swan	HPAIV H5N1	Experimental	4.2 (based on Ct values) (6–11 days; 12/12)	3.3 (based on Ct values) (6–10 days; 12/12)	Neurological signs: opisthotonus, torticollis, ataxia (4–7 days; 3/12) Sudden death (4–7 days; 3/12)	5–9 days (5/5) 8–14 days (6/7)	26

TABLE 2.3 (*Continued*)

Wild bird species	AIV	Type of infection	Maximum pharyngeal shedding (duration; N/Total)*	Maximum cloacal shedding (duration; N/Total)*	Clinical signs (incubation time; N/Total)	Mean time to death (N/Total)	Ref
Herring gull	HPAIV H5N1	Experimental	3.8–4.3 (2.5–3 days; 5/5)	<1.96 (1.5–3 days; 5/5)	Lethargy Neurological signs: seizures, head-tilt, head tremors, torticollis, severe imbalance (1–5 days; 5/5)	5 days (3/5)	23
House sparrow	HPAIV H5N1	Experimental	5 (4–9 days; 13/15)	4.3 (4–9 days; 13/15)	Lethargy, pulled-up feathers Neurological signs: loss of balance, head tremors, seizures Sudden death (4–7 days; rare)	5–8 days (13/15)	27
Rock pigeon	HPAIV H5N1	Experimental	<1.9 (1–2 days; 3/15)	<1.9 (1–2 days; 3/15)	Lethargy Neurological signs: imbalance, head tremors, seizures Sudden death (5–6 days; 3/15)	7–8 days (2/15)	27

ND – not determined; N/Total – number of infected birds shedding virus, presenting clinical signs or dying of infection over the total number of infected birds Ct-cycle threshold.

*maximum pharyngeal and cloacal shedding titres are expressed as $\log_{10}$ of median tissue-culture infectious dose ($TCID_{50}$) or median egg infectious dose (EID_{50}).

mild epithelial degeneration of the intestinal and bursal mucosa was observed microscopically in experimentally infected domestic ducks[16,28,29,31]. Also, aerosol inoculation of LPAIV in domestic ducks caused multifocal pulmonary consolidation and pleural oedema grossly; this corresponded microscopically with mild broncho-interstitial pneumonia[30].

HPAIV H5N1 infection in wild birds may cause severe necrosis and inflammation in multiple organs of susceptible species (Table 2.4)[23–27,33,34]. Surprisingly, this is often not reflected in the gross lesions. Gross lesions may be completely absent, or there may be multiple foci of necrosis in the pancreas (Figure 2.5, left panel), mild diffuse thickening of the air sacs and multifocal pulmonary consolidation. In addition, whooper swans and mute swans may have haemorrhages in multiple organs, including heart (Figure 2.5, right panel) and pancreas; this is in accordance with the endotheliotropism of HPAIV H5N1 in these species[24,26,33]. Microscopically, there are multiple foci of acute necrosis and inflammation in multiple organs, including pancreas, lung, air sac, brain (Figure 2.6), liver, heart and adrenal gland[25].

The immune response to LPAIV infection in wild birds probably involves both humoral and cellular elements[35], but the former has been studied most. In domestic ducks experimentally infected with LPAIV, serum IgM was detectable between 5 and 28 dpi; serum IgY of the 7.8S form (equivalent to IgG in mammals) became detectable at 9 dpi and increased in concentration until the end of the experiment at 33 dpi; and secretory IgX (comparable to IgA in mammals) became detectable at 1 dpi and peaked at 7 dpi in serum and at 12 dpi in bile[36]. Such secretory antibodies are probably present on other mucosal surfaces, such as the intestinal mucosa, based on expression patterns of IgA genes in domestic ducks[37]. As expected, domestic ducks reinfected with LPAIV have a secondary immune response, characterized by a faster and stronger increase in serum antibodies[29].

Humoral immunity is thought to protect mainly against LPAIV of the same HA subtype, but not against other subtypes[35]. This may explain in part the existence of the large number of HA subtypes in the wild waterbird community. Furthermore, mucosal antibodies on the intestinal mucosa are short-lived[36], and may not protect against

TABLE 2.4 Gross pathology, distribution of histological lesions and of influenza virus antigen in species of wild birds experimentally and naturally infected with LPAIV and HPAIV H5N1 of the sublineage that spread to bird populations in Europe.

Wild bird species	AIV	Type of infection	Gross pathology	Histological lesions	Distribution of AIV antigen	Ref
Mallard	LPAIV	Experimental	None Multifocal pulmonary consolidation, oedematous and cloudy pleura	None Broncho-interstitial pneumonia	Intestinal epithelial cells Respiratory epithelial cells along airways	28,16, 29–31
Mallard	HPAIV H5N1	Experimental	Mild air sacculitis	Lymphocytic air sacculitis Broncho-interstitial pneumonia	None	25
Common teal	HPAIV H5N1	Experimental	None	Lymphocytic air sacculitis Multifocal pancreatic necrosis	Respiratory epithelial cells Pancreatic cells Hepatocytes Neurons Myocytes (colon) Adrenocortical cells	25
Eurasian wigeon	HPAIV H5N1	Experimental	Mild air sacculitis	Lymphocytic air sacculitis	None	25
Gadwall	HPAIV H5N1	Experimental	Mild air sacculitis Mild multifocal pulmonary consolidation	Lymphocytic air sacculitis Multifocal pancreatic necrosis. Necrotizing hepatitis Multifocal encephalitis Necrotizing adrenalitis	None	25
Tufted duck	HPAIV H5N1	Experimental	Mild air sacculitis Mild multifocal pulmonary consolidation Moderate multifocal pancreatic necrosis Severe diffuse hepatic necrosis	Lymphocytic air sacculitis Broncho-interstitial pneumonia. Multifocal pancreatic necrosis Necrotizing hepatitis Multifocal encephalitis Necrotizing ganglioneuritis Necrotizing adrenalitis Myocardial necrosis	Respiratory epithelial cells Pancreatic cells Hepatocytes Neurons and glial cells Ganglion cells (intestinal plexi) Adrenocortical cells Cardiomyocytes	25
Tufted duck	HPAIV H5N1	Natural	ND	Mild rhinitis Haemorrhagic broncho-interstitial pneumonia Multifocal hepatic necrosis Multifocal-to-coalescing coagulative necrosis of pancreatic acini Splenic lymphoid depletion Multifocal myocardial fibre necrosis Multifocal adrenalitis Non suppurative encephalitis or meningoencephalitis	Respiratory epithelial cells Endothelial cells Pancreatic cells Hepatocytes Neurons Ganglion cells Adrenocortical cells Renal tubular cells Ovarian follicular cells	34
Common pochard	HPAIV H5N1	Experimental	Mild air sacculitis Mild to moderate multifocal pancreatic necrosis	Lymphocytic air sacculitis Broncho-interstitial pneumonia. Multifocal pancreatic necrosis Necrotizing hepatitis Multifocal encephalitis Necrotizing ganglioneuritis Necrotizing adrenalitis Myocardial necrosis	Respiratory epithelial cells Pancreatic cells Hepatocytes Neurons and glial cells Ganglion cells (intestinal plexi) Adrenocortical cells Cardiomyocytes	25

Mute swan	HPAIV H5N1	Experimental	ND	Multifocal to coalescing necrosis with mild to moderate heterophilic inflammation in brain and most parenchyma	Neurons, astrocytes, and glial cells Parenchymal cells of other organs	24
Whooper swan	HPAIV H5N1	Experimental	ND	Multifocal to coalescing necrosis with mild to moderate heterophilic inflammation in brain and most parenchyma	Neurons, astrocytes, and glial cells Parenchymal cells of other organs	24
Whooper swan	HPAIV H5N1	Experimental	Widespread haemorrhages (myocardium, brain, lungs) and petechiae in pancreas, liver Pancreatic necrosis	Necrotizing rhinitis Multifocal pancreatic necrosis Multifocal hepatic necrosis Multifocal encephalitis Necrotizing dermatitis	Respiratory epithelial cells Endothelial cells Pancreatic cells Hepatocytes Neurons, glial cells and ependymal cells Adrenocortical cells	26
Mute and whooper swan	HPAIV H5N1	Experimental	Pulmonary oedema and congestion Multifocal hemorrhagic pancreatic necrosis Subepicardial haemorrhages	Hemorrhagic broncho-interstitial pneumonia Multifocal pancreatic necrosis Multifocal to coalescing coagulative hepatic necrosis Multifocal lymphoplasmacytic encephalitis Spleen Multifocal lymphocyte necrosis in spleen and Peyer's patches Multifocal adrenalitis	Respiratory epithelial cells Endothelial cells Pancreatic cells Hepatocytes Neurons, glial cells and ependymal cells Intestinal epithelial cells (proventriculus) Mononuclear cells (Peyer's patches) Adrenocortical cells Kidney tubular epithelial cells	33
Herring gull	HPAIV H5N1	Experimental	ND	Multifocal pancreatic necrosis Multifocal encephalitis Heterophilic and necrotizing myocarditis Multifocal necrotizing adrenalitis	Pancreatic cells Neurons and glial cells Cardiomyocytes Adrenocortical cells	23
House sparrow	HPAIV H5N1	Experimental	Pulmonary oedema and congestion Pancreatic necrosis Splenomegaly	Multifocal to coalescing pancreatic necrosis Multifocal to coalescing necrotizing adrenalitis Lymphocytic air sacculitis Lymphoplasmacytic and necrotizing encephalitis Lymphocytic ganglioneuritis Myocardial necrosis and lymphoplasmacytic myocarditis Lymphocytic and heterophilic nephritis Lymphocytic orchitis Heterophilic oophoritis Necrotizing myositis	Pancreatic cells Adrenocortical cells Neurons and glial cells Cardiomyocytes Hepatocytes and Kupffer cells Kidney tubular epithelial cells Thecal epithelial cells, Sertoli cells, testis tubular epithelial cells Myocytes	27
Rock pigeon			Pancreatic necrosis	Multifocal lymphocytic encephalitis and meningoencephalitis Multifocal hepatic necrosis	Neurons and glial cells Hepatocytes Thecal epithelial cells	27

ND – not determined

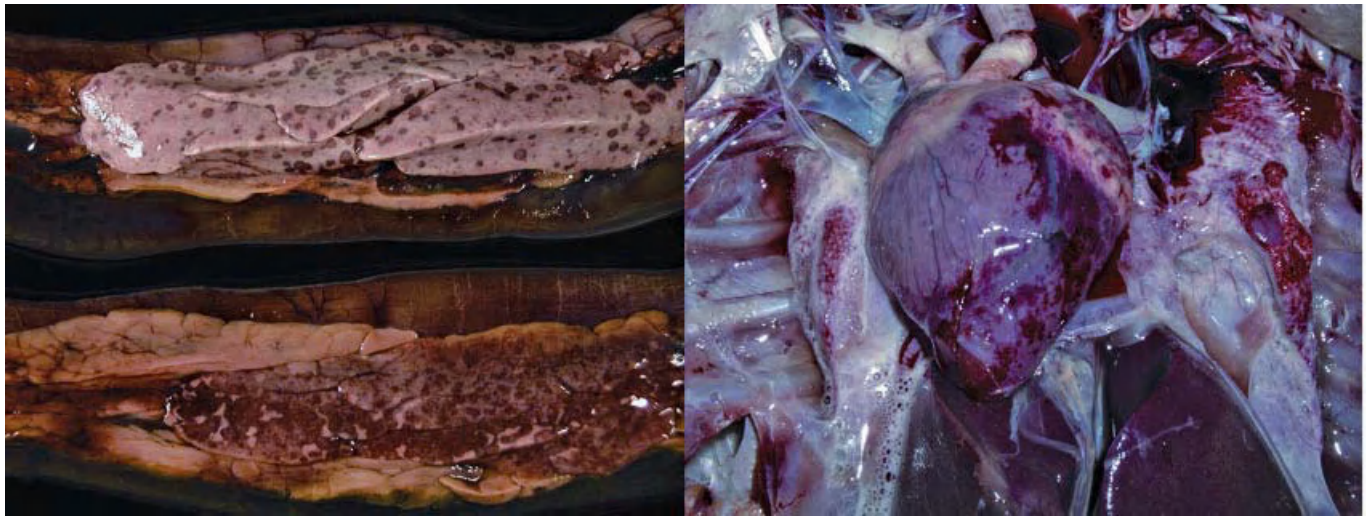

FIGURE 2.5 Gross lesions of HPAIV H5N1 infection. Left panel: Pancreas showing multiple foci of necrosis and haemorrhage in a whooper swan naturally infected with HPAIV H5N1. Right panel: Heart showing subepicardial haemorrhages in a whooper swan naturally infected with HPAIV H5N1. Reprinted with permission from Teifke, J.P. et al., 2007.

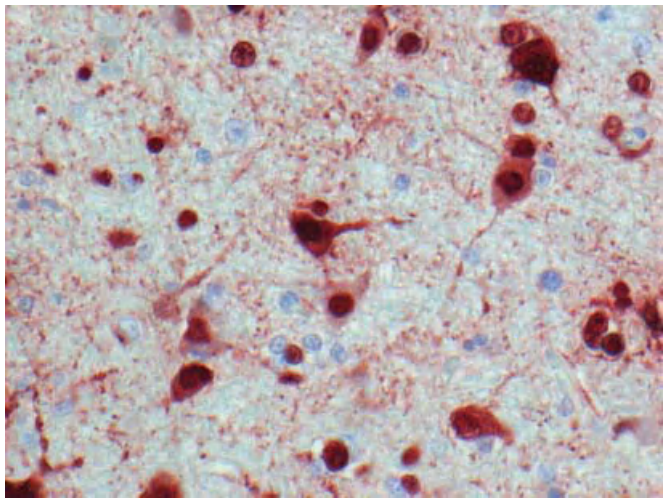

FIGURE 2.6 Histological lesions of HPAIV H5N1 infection. Severe encephalitis in a tufted duck experimentally infected with HPAIV H5N1, characterized by abundant influenza virus expression in neurons and glial cells (stained by immunohistochemistry that used a monoclonal antibody against the nucleoprotein of influenza A virus as primary antibody). Reprinted with permission from Keawcharoen, J. et al., 2008.

annual reinfection. However, lower prevalence of LPAIV infection in adult waterbirds strongly suggests that immunity, in particular heterosubtypic immunity, may reduce the susceptibility of older birds to reinfection.

Immunity to LPAIV protected domestic ducks and mallards against disease and greatly reduced virus shedding upon challenge with the same subtype[29,38]. Immunity to another subtype of AIV also reduced disease and virus shedding from challenge with HPAIV H5N1, but to a lesser degree[39].

CLINICAL SIGNS

In general, neither natural nor experimental LPAIV infection causes clinical signs in wild birds[40]. Likewise, experimental infection of domestic ducks and mallards with LPAIV typically results in subclinical infection. Despite the general absence of clinical disease, it has recently been suggested that LPAIV infection may cause physiological costs to wild birds[41]. Natural LPAIV infection of two Bewick's swans (*Cygnus columbianus bewickii*) was associated with reduced feeding and migrating performances, resulting in shorter and delayed spring migration[42]. Mallards, naturally infected with LPAIV, were significantly lighter than uninfected mallards, and virus titres negatively correlated with body mass of juvenile birds[32]. It remains unknown whether LPAIV infections are the cause or consequence in these observed correlations.

In contrast to LPAIV, the clinical effects of HPAIV H5N1 infection of wild birds range from subclinical infection to sudden death, both in natural and experimental infections. In natural infections, clinical signs are rarely reported, because most infected wild birds are found dead.

In Sweden, seven naturally infected tufted ducks were found alive and presented with neurologic signs, including head tilt, circling, loss of balance and drooping wings[34].

In experimental infections with HPAIV H5N1, clinical signs depend largely on host species but also on virus strain and inoculation dose[23–27]. Mute swans, whooper swans and house sparrows showed severe clinical signs and a high mortality rate, whereas dabbling duck species – mallard, common teal, Eurasian wigeon (*Anas penelope*), gadwall (*A. strepera*) – showed no clinical signs and no deaths. Rock pigeons, diving ducks – tufted duck, common pochard – and herring gulls were intermediate in severity of clinical signs and death rate. Mean time to death ranged from 4 to 14 dpi (Table 2.3). Clinical signs were first seen between 2 and 7 dpi. Clinical signs were lethargy, weakness, listlessness, dyspnoea and neurological signs, such as loss of balance, incoordination, circling, ataxia, opisthotonos, head tilt, head tremors, torticollis and seizures[23–27], and sudden deaths with no clinical signs were also reported [26,43]. Neurological signs were particularly conspicuous and were associated with cerebellar infection in experimentally infected birds.

DIAGNOSIS

LPAIV infection is generally subclinical, and the diagnosis in a live wild bird can be made by virus culture or by reverse transcription polymerase chain reaction (RT-PCR)[12,44]. Samples of choice are cloacal swabs and, to a lesser extent, pharyngeal swabs, which are placed in virus transport medium and either analysed directly or stored at −70°C until analysis. For virus isolation, specific pathogen-free embryonated chicken eggs are more sensitive than cell culture. Virus culture allows for full characterization of the virus isolate. For RT-PCR, primer pairs based on a conserved gene, such as the matrix gene, are used for initial detection. This may be followed by RT-PCR developed to detect specific HA subtypes, such as H5 and H7, or by virus culture.

The diagnosis of LPAIV infection in a dead wild bird can be made by virus culture, RT-PCR or immunohistochemistry[12,44]. Besides cloacal and pharyngeal swabs, samples of choice for virus culture and RT-PCR are tissue samples from intestine, bursa of Fabricius and lung. For immunohistochemistry, a primary antibody against one or more AIV proteins is used to detect AIV antigen in infected cells[45]. Immunohistochemistry makes it possible to co-localize microscopic lesions with the presence of AIV antigen, but only allows a diagnosis to the genus level. Samples of choice for immunohistochemistry are tissue samples from the same organs as above, but fixed in 10% neutral-buffered formalin. Only fresh tissues should be used, because AIV antigen expression is limited to epithelial cells, which are the first to desquamate during autolysis of the carcass.

HPAIV H5N1 infection should be included in the differential diagnosis of any unexplained mortality of wild birds, particularly of the species known to be susceptible to disease from these viruses, and also when birds show neurological signs. Other diseases to consider in such cases include Newcastle disease, botulism, avian cholera and blue-green algal poisoning[46]. The gross and microscopic lesions are too non-specific to make a presumptive diagnosis, but suspicion is increased with the gross observations of multiple necrotic foci in the pancreas and – in swans – multi-organ haemorrhage; and for the microscopic observation of acute necrosis and inflammation in multiple organs, including brain, pancreas, liver and adrenal gland. The differential diagnosis for such microscopic lesions should include Newcastle disease.

The diagnosis of HPAIV H5N1 infection in a live wild bird can be made by virus culture or RT-PCR, as for LPAIV, except that pharyngeal swabs are preferred to cloacal swabs. The diagnosis of HPAIV H5N1 in a dead wild bird can be made by virus culture, RT-PCR or immunohistochemistry, as for LPAIV. Again, pharyngeal swabs are preferred to cloacal swabs. Tissue samples of choice also are different: they should include brain, lung, air sac, pancreas, liver and kidney.

Screening of wild bird populations for both LPAIV and HPAIV is most commonly done by Taqman PCR of pharyngeal and cloacal swabs, by use of a probe for the matrix gene. Positive samples are subsequently analysed by virus culture to obtain the virus, to identify HA and NA subtype and to determine pathogenicity[12,47].

Other diagnostic tests available for AIV diagnosis include: nucleic acid sequence-based amplification (NASBA), loop-mediated isothermal polymerase chain reaction (LAMP-PCR), pyrosequencing and *in situ* hybridization for RNA detection; antigen-capture enzyme-linked immunosorbent assay (ELISA) for antigen detection; and agarose gel immunodiffusion (AGID), ELISA, hemagglutinin inhibition (HI) and neuraminidase inhibition (NI) tests for antibody detection[48].

MANAGEMENT, CONTROL, AND REGULATIONS

LPAIV infection is endemic in wild birds and management or control is not required for the purpose of wild bird health. However, wild birds are the source of LPAIV infecting poultry and potentially evolving into HPAIV (in the case of LPAIV of the H5 and H7 subtypes), which causes extensive mortality and production loss. Management and control measures thus are applied to reduce the risk of transmission of LPAIV from wild bird to poultry populations by improving biosecurity.

Surveillance of wild birds for AIV infection consists of sampling live birds for detection of AIV or AIV RNA, and necropsy of dead birds for detection of AIV, AIV RNA or AIV antigen.

Three main objectives of such a surveillance programme are: i) to identify areas considered at higher risk for introduction and occurrence of AIV in poultry owing to proximity of poultry holdings to mixing and resting areas of migratory birds; ii) to prevent the disease from entering poultry holdings by limiting contact between wild birds and poultry and by enhancing biosecurity measures; and iii) to detect and report AIV of the H5 and H7 subtypes in wildlife, and to adapt disease-prevention measures in areas where they are detected. Prevention measures, such as limiting contact between farmed animals and wild birds, and enhancing biosecurity measures, such as ensuring that the source of water for farmed animals is not used or contaminated by wild birds, are also applied for susceptible species of farmed mammals that can acquire AIV infection from wild birds, such as pigs and mink.

Wild birds historically played no epidemiological role in HPAIV epidemiology. However, they probably contributed to the geographical spread of HPAIV H5N1, and early detection of HPAIV H5N1 currently is one of the functions of AIV surveillance in wild birds. In the event of wild bird die-offs, necropsy of highly susceptible species, such as swans, diving ducks and grebes, should be reinforced. Detection of HPAI H5N1 in wild birds requires European member states to establish a 3-km control area and a 10-km monitoring area, where restrictions on movements of live poultry and poultry products apply, and where hunting of wild birds is banned. Poultry must be kept indoors to prevent contact with infected wild birds. Clinical inspections and laboratory investigations are required in poultry holdings in order to detect possible virus introduction. Biosecurity and disease awareness are raised. The European Commission also approved preventive vaccination programmes for birds kept in zoos in 17 member states to avoid stamping-out measures for captive wild bird species[49].

Domestic cats and other carnivores may be at risk of HPAIV H5N1 infection in areas where outbreaks in wild and domestic birds occur and should be monitored for infection. Measures to prevent contact between domestic carnivores and infected birds, and surveillance of unusual morbidity and mortality in domestic and wild carnivores, should be implemented[50].

PUBLIC HEALTH CONCERN

Transmission of AIV from wild birds to humans is rare. There is serological evidence that people in close contact with wild birds, such as duck hunters, can become infected with LPAIV[51]. However, the only reported transmission of AIV from wild birds to humans concerns two clusters of human infection with HPAIV H5N1 and six human deaths in Azerbaijan, where close contact with and de-feathering of infected wild swans was considered to be the most probable source of exposure[52]. Other sources of AIV infection in humans are most commonly from poultry, but also laboratory accident and pre- and *post mortem* examination of infected seals and possibly wild birds[53].

AIV infection in humans typically causes mild clinical disease, characterized by conjunctivitis or influenza-like illness, which resolves within 2 weeks However, HPAIV H5N1 infection in humans, although rare, causes severe pneumonia that often progresses to acute respiratory distress syndrome, with a case fatality rate of about 60%[53,54].

The protective measures when handling apparently healthy wild birds in areas where HPAIV H5N1 infection is not suspected are routine hygienic measures such as using gloves and protective eyewear when possible, washing hands with soap and water and disinfecting equipment and work surfaces[55]. In areas where HPAIV H5N1 infection has been identified in wild birds, wearing protective suits and respiratory masks and disinfecting tools and vehicles must be implemented to limit contamination and chains of transmission[49].

SIGNIFICANCE AND IMPLICATIONS FOR ANIMAL HEALTH

Both LPAIV and HPAIV have had implications for domestic and wild animal health. LPAIV may cross from wild birds to poultry. Poultry may then present with clinical disease upon infection or show reduction in egg or meat production[56]. Furthermore, LPAIV of the H5 and H7 subtypes may become highly pathogenic once introduced into poultry populations, potentially bearing high economical costs and a zoonotic risk. Poultry infected with LPAIV may become bridge species, transmitting LPAIV to other species, including domestic animals and humans. LPAIV have also resulted in outbreaks of severe respiratory disease in farmed mink and outbreaks of influenza-like illness in domestic pigs[2], potentially producing high economic costs. LPAIV have been transmitted from wild birds to free-ranging wild mammals and have resulted in outbreaks of severe respiratory disease in harbour seals. LPAIV outbreaks in farmed mink also call for awareness in the event of cross-species transmission of LPAIV to endangered European mink (*Mustela lutreola*).

Poultry is the major source of HPAIV in other domestic animals and in wild animals. In particular, HPAIV H5N1, which displays an unusually broad host range, have infected and caused severe systemic disease and death in domestic cats and dogs. In addition, HPAIV H5N1 causes death and severe disease in a wide range of wild birds, and in free-ranging mammals, i.e. a stone marten (*Martes foina*) and an American mink (*Neovison vison*) in Europe. The potential of AIV to cross the species barrier and to cause disease in novel host species thus calls for continued and extended surveillance of AIV in wild bird populations[57].

INFLUENZA IN AQUATIC MAMMALS

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Although there is evidence of both influenza A and influenza B virus infections in several species of marine mammals, only influenza A virus infections are known to have caused disease.

EPIDEMIOLOGY

In Europe, serologic evidence of exposure to influenza A viruses has been reported in one seal (species not specified) from the North Sea in 1988 (LPAIV of the H4 subtype), and in harp seals (*Pagophilus groenlandicus*) and hooded seals (*Cystophora cristata*) from the Barents Sea in 1991 and 1992 (subtype not determined)[58,59]. Influenza B viruses were isolated from harbour seals (*Phoca vitulina*) from the North Sea in 1999, and antibodies against the virus were detected in North Sea harbour seals and grey seals (*Halichoerus grypus*) between 1995 and 1999[3].

Outbreaks of disease caused by influenza A viruses in marine mammals occurred along the New England coast of the USA. Influenza A viruses of different subtypes (H7N7, H4N5, H4N6 and H3N3) were isolated from stranded harbour seals in 1979, 1982, 1991 and 1992, respectively[60–62]. In addition, LPAIV H13N2 and H13N9 viruses were isolated from a pilot whale (*Globicephala melas*) in 1984 near Maine, when major strandings were reported[63].

Only harbour seals and a pilot whale (*Globicephela* spp.) are known to have developed disease following infection with influenza A virus. LPAIV H1N3 were isolated from lung and liver samples of minke whales (*Balaenoptera acutorostrata*) collected in 1975 and 1976 in the South Pacific[64], but no details were provided on clinical signs or lesions in infected animals. Influenza B virus, which infects mostly humans, has been isolated from harbour seals, but it is unclear whether the virus causes clinical disease in this species.

In addition to harp seals, hooded seals, harbour seals and grey seals from European waters (see above), antibodies against avian and human influenza A viruses and influenza B viruses were also reported in Kuril harbour seals (*Phoca vitulina stejnegeri*) near Japan, in ringed seals (*Pusa hispida*) from Alaska and the Kara Sea, in Caspian seals (*Pusa caspica*), in Baikal seals (*Pusa sibirica*) and in sea lions (species not specified) from the Bering Sea[2,65]. Antibodies against influenza A viruses were detected in belugas (*Delphinapterus leucas*) from Arctic Canada, and common minke whales and Dall's porpoises (*Phocoenoides dalli*)

from the western North Pacific and Antarctic Oceans[66,67]. Further determination of the subtypes was either not possible or unsuccessful.

Little is known on the epidemiology of influenza A viruses in marine mammals. In North America, influenza A outbreaks in harbour seals typically occurred during late winter.

Marine mammals are considered accidental hosts for avian and human influenza viruses. However, influenza viruses can remain in seal populations for several months to several years. The outbreak of LPAIV H7N7 infection in North American harbour seals in 1979 lasted 10 months[60]. Likewise, LPAIV H4N5 isolated in North American harbour seals in winter 1983 was virtually identical to a virus isolated from the lungs of an emaciated seal found dead in June 1982, indicating that this virus may have been sustained for more than a year in the seal population[61]. In spring 1999, harbour seals from the North Sea were found infected with an influenza B virus closely related to strains that had circulated in humans several years earlier. Antibodies against this virus were found in archived sera of stranded harbour seals and grey seals (*Halichoerus grypus*), collected between 1995 and 1999. Together, these results suggest that the virus had circulated in seal populations for several years[3].

All influenza A viruses isolated from marine mammals are of avian origin, demonstrating cross-species transmission of LPAIV from birds to marine mammals[2]. Seabirds and pinnipeds frequently share habitats at roosting or haul-out sites, probably favouring cross-species transmission of LPAIV. Likewise, cetaceans may have close contact with seabirds at sea. They both feed concurrently on the same fish species, which may facilitate cross-species transmission. The detection of antibodies against LPAIV in different seal species further supports frequent cross-species transmission from wild birds to pinnipeds. Furthermore, the detection of antibodies against human influenza A and B viruses and isolation of influenza B viruses in seals indicates cross-species transmission of influenza viruses from humans to pinnipeds[3].

Once introduced into marine mammals, only influenza A viruses caused outbreaks of disease in harbour seals, which suggests seal-to-seal transmission, probably via the respiratory route. Some LPAIV isolated from infected seals behaved more like mammalian influenza A viruses, as they replicated better in mammalian than in avian species, suggesting that LPAIV may have adapted to mammalian hosts once introduced into seals[68].

PATHOGENESIS, PATHOLOGY AND IMMUNITY

Little is known about the pathogenesis of influenza virus infection in marine mammals. In harbour seals, LPAIV typically have a restricted tissue tropism for the respiratory tract, and are most frequently isolated from the lungs of infected animals. However, LPAIV were occasionally isolated from the brain of naturally infected seals, indicating that LPAIV can spread beyond the respiratory tract in this species[61]. LPAIV H7N7 were also isolated from the bronchial lymph node and from the third eyelid of experimentally infected harbour seals[68], and LPAIV H4N5 from bronchial, mesenteric and mandibular lymph nodes as well as corneal swabs of harbour seals, ringed seals and harp seals[61]. In the pilot whale infected with LPAIV near Maine, USA, viruses were isolated from the lungs and hilar lymph node[63], also demonstrating a preferred tropism for the respiratory tract in this species.

Necrotizing bronchitis and bronchiolitis, and haemorrhagic interstitial pneumonia were reported on *post mortem* examination of harbour seals that died of natural influenza A virus infection[60–62]. Lesions were absent or similar in experimentally infected seals, and co-infection, e.g. with *Mycoplasma*, is thought to have contributed to more severe disease in naturally infected seals[61,68].

Harbour seals, ringed seals and harp seals experimentally infected with a seal isolate of LPAIV H4N5 had detectable antibodies 2 weeks post-infection, declining by day 30 post-infection[61]. Sera from harbour seals caught along the New England coast of the USA collected at the end of the seal epidemic of 1979–1980 had detectable levels of antibodies against the H7N7 virus[68].

CLINICAL SIGNS

In North America, influenza A virus infection in harbour seals resulted in massive outbreaks of fatal respiratory disease[2]. Approximately 600 seals, representing about 25% of the local population, died of LPAIV H7N7 infection between December 1979 and October 1980[60], and another outbreak of fatal respiratory disease caused by LPAIV H4N5 resulted in a three- to four-fold increase in seal mortality between January and March 1983[61]. Strandings of harbour seals increased in winter 1991 and winter 1992, when LPAIV H4N6 and H3N3, respectively, were isolated from dead animals[62].

Clinical signs of influenza A virus infection in harbour seals included prostration, respiratory distress, mucopurulent to haemorrhagic oculo-nasal discharge and subcutaneous emphysema, resulting in a swollen neck and quivering of the muscles[60–62]. The disease developed within 2 to 3 days following infection, and some animals died within hours after feeding normally[60,68]. Experimental infection of harbour seals, ringed seals and harp seals with viruses isolated from fatal cases typically resulted in subclinical infection or milder but similar disease. Whereas harbour seals, ringed seals and harp seals experimentally infected with a seal isolate of LPAIV H4N5 did not develop disease[61], mild cough and mucopurulent oculo-nasal discharge were observed in harbour seals experimentally infected with the seal isolate of LPAIV H7N7[68]. In the pilot whale infected with LPAIV near Maine, USA, clinical signs were non-specific and included skin sloughing and extreme emaciation. The animal had difficulties swimming, diving and surfacing[63].

To date, influenza A viruses have not been associated with disease outbreaks in European seals. Influenza B virus was isolated from the throat swab of a juvenile harbour seal with respiratory problems in spring of 1999[3]. However, the seal was probably also infected with lungworms, and it remains unknown whether the virus was the aetiological cause of the respiratory signs.

DIAGNOSIS

The methods used for the diagnosis of avian influenza in birds can be applied to marine mammals.

MANAGEMENT AND CONTROL

No management and control measures are required.

PUBLIC HEALTH CONCERN

Five humans who handled seals infected with LPAIV H7N7 developed conjunctivitis, associated with high titres in their conjunctival swabs, demonstrating the zoonotic potential of the virus[69]. Precautions, such as wearing protective clothing and glasses, should be applied during necropsy of infected marine mammals.

SIGNIFICANCE AND IMPLICATIONS FOR ANIMAL HEALTH

LPAIV outbreaks may cause significant morbidity and mortality in harbour seals and potentially other marine mammals. In addition, there is evidence of cross-species transmission of influenza viruses of human origin to pinnipeds, although their pathogenicity for pinnipeds is unknown.

INFLUENZA IN OTHER SPECIES

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Wild species in Europe other than birds and marine mammals are likely to be accidental hosts for influenza viruses. Outbreaks of HPAIV H5N1 infection in wild birds in Europe have been occasionally accompanied by cases of fatal infection of wild carnivores, i.e. a stone marten in Germany, and an American mink in Sweden, both in 2006. Both animals were found in areas where wild birds were dying of HPAIV H5N1 infection and were likely to have fed on infected carcasses.

There is serologic evidence of infection of wild boar (*Sus scrofa*) with swine influenza viruses of H1N1, H3N2 and H1N2 subtypes in Germany, Poland and Spain. These subtypes frequently infect domestic pigs[2]. Seroprevalence varies geographically and by subtype, and ranges from less than 1% to 8%. Domestic pigs are descendants of wild boars, so wild boar are likely to be infected with swine influenza viruses via the respiratory route and may potentially act as a reservoir for swine influenza viruses.

In principle, mammals, reptiles and amphibians that are predators of – or scavenge on –wild waterbirds, or share habitat with them, can become infected with LPAIV. However, there is no virological or serological evidence for this in Europe.

The pathogenesis of HPAIV H5N1 infection in carnivores has been studied in experimentally infected domestic cats, red foxes and ferrets[70–72]. Feeding on contaminated

bird carcasses or meat may cause infection by two routes. The first route is inhalation of virus, causing infection of the respiratory tract. The second route – confirmed by direct virus inoculation into the stomach (Reperant et al., unpublished data)[72] – is ingestion of virus, resulting in infection of the intestine. Therefore, both the respiratory tract and the digestive tract may act as initial sites of HPAIV H5N1 replication.

From the initial site of replication, HPAIV H5N1 may spread to other organs via blood or lymph (Reperant et al., unpublished data). In mice, spread via neurons also has been demonstrated. Most wild carnivores found infected to date have HPAIV H5N1 in multiple organs, including the brain, lungs, liver, heart and kidneys. HPAIV H5N1 replication in epithelial or parenchymal cells results in necrosis and inflammation, which may be severe[2]. Little is known about the immune response of wild carnivores to HPAIV H5N1.

HPAIV H5N1 infection in carnivores causes systemic lesions with variable involvement of different organs, most consistently brain, lung and liver. Gross lesions may include consolidation or haemorrhage in the lungs, and foci of necrosis or haemorrhage in the liver. Microscopic lesions consist of foci of acute necrosis and inflammation in multiple organs. This includes non-suppurative encephalitis, broncho-interstitial pneumonia and necrotizing hepatitis, associated with viral replication in neurons and glial cells, bronchiolar epithelial cells, pneumocytes and alveolar macrophages, and hepatocytes, respectively. Other organs with microscopic lesions associated with virus replication include heart, pancreas, intestine, spleen, kidneys and adrenal glands[2].

Pathology of swine influenza infection in wild boars is not reported but in domestic pigs consists of necrotizing broncho-interstitial pneumonia.

Infection with HPAIV H5N1 may cause severe clinical disease and death in infected felids, mustelids and viverrids. Based on experimental infections in domestic dogs and red foxes, clinical disease in canids is less severe. Clinical signs in infected carnivores are high fever, respiratory distress, serosanguineous nasal discharge and neurological signs, such as loss of balance, hind limb paralysis, ataxia and circling. Clinical signs of swine influenza virus infection in wild boars are not reported. In domestic pigs, clinical signs are fever, coughing and stiffness lasting 1 to 2 weeks, and the mortality rate is low.

The laboratory methods used for diagnosis in birds can be applied to other species. Tissues sampled in carnivores suspected of HPAIV H5N1 infection should include lung, liver and brain. Tissues sampled in cases suspected of other influenza virus infections should include upper and lower respiratory tract.

For HPAIV H5N1, management and control measures in other animal species should be directed at preventing transmission from poultry or wild birds. HPAIV H5N1 vaccines have been developed for domestic cats, but their efficacy in other carnivore species is unknown. For swine influenza viruses, control measures consist of preventing contact between domestic pigs and wild boar.

HPAIV H5N1 infection is of public health concern. However, the risk of transmission to humans from infected carnivores is small compared with the risk of transmission from infected poultry. Appropriate protective measures should be taken during necropsy of carnivores suspected of HPAIV H5N1 infection. Hunters can be exposed to low pathogenic avian or swine influenza viruses and should also take appropriate protective measures during preparation of game or waterfowl.

HPAIV H5N1 can cause severe disease and death in many species of carnivores. This has implications for endangered carnivore species, either captive or free ranging, in areas where HPAIV H5N1 occurs in poultry or wild birds.

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CHAPTER

3

AVIAN PARAMYXOVIRUS INFECTIONS

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Avian paramyxoviruses (APMV) are members of the Order *Mononegavirales*, Family *Paramyxoviridae*, Genus *Avulavirus*. This genus contains currently nine species that are named *Avian paramyxovirus 1* (APMV1, type species *Newcastle disease virus*) and *Avian paramyxovirus 2* to *9*[1]. These APMV1 have a world-wide prevalence, a very wide host range and are of great ecologic and economic importance. APMV1 infection causes Newcastle disease (ND). Older publications used the name atypical fowlpest. Other names for ND are pseudo-fowlpest, pseudovogelpest, pseudopoultry plague, avian pest and avian distemper. ND viruses are also found in transcontinentally migrating birds. In Asia, ND is known as Ranikhet disease (India), Tetelo disease (Japan) or Korean fowl plague. In the USA, a neurotropic respiratory form of ND is known as avian pneumoencephalitis. Another clinical entity of ND is termed exotic Newcastle disease (END) in the USA, to differentiate it from endemic Newcastle disease.

Following natural exposure, highly virulent ND viruses (NDV) cause severe epidemics with high rates of morbidity and mortality in terrestrial, mainly gallinaceous and passerine, birds, ratites and storks. Less severe forms occur usually in shore birds (gulls, cormorants, shags, gannets, egrets)[2]. Relatively little disease expression is seen among the majority of aquatic birds.[3]. Raptors rarely acquire NDV[4].

AETIOLOGY

APMV1 comprise a large number of viruses that differ markedly from each other in their genome organization (at least seven genotypes), pathogenicity (pathotypes velogenic, neurotropic, mesogenic, lentogenic and avirulent-enteric), serological reactions (at least nine serotypes) and in their ability to induce immunity (immunotypes or protectotypes).

Each APMV1 virion consists of linear negative sense ssRNA genome that is not infectious[1]. The genome of 15–16 kb codes for six proteins. Four genes code for internal proteins. These are the RNA-directed RNA polymerase (L), the matrix protein (M), phosphoprotein (P) and nucleoprotein (N). Two additional genes code for proteins that are expressed on the surface of virus particles. These are the haemagglutinin-neuraminidase protein (HN) and the fusion protein (F).

Morphological studies reveal variable forms of particles that differ in size and form. Most particles are round to ovoid with a diameter of 120–170 nm, and they represent the majority of virions. Filamentous forms are much longer. Protrusions (spikes) on the virion surface are prominent, with an average length of 8–10 nm (Figure 3.1). Disrupted viral particles displaying herringbone-like

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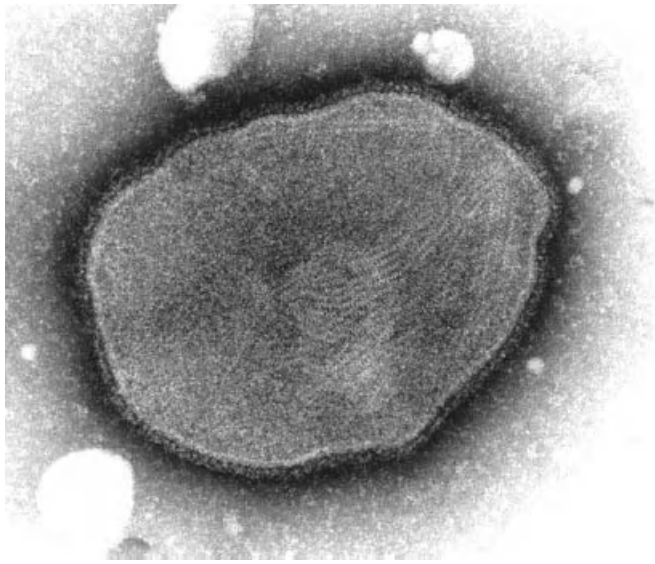

FIGURE 3.1 Morphology of an avian paramyxovirus. Note projections (spikes) on the surface of the particle and the internal herringbone-like structure of the viral RNA. Courtesy Dr Werner Herbst, University Giessen.

fragments of RNA are frequently seen and are of some help in differentiating APMV from other enveloped viruses of similar size.

All APMV are sensitive to lipid solvents such as ethanol, isopropanol, dimethyl ether, chloroform, phenol and its derivates, glutaraldehyde, quaternary ammonium compounds, bleach and other chemical disinfectants such as formic and peracetic acid. These compounds destroy the infectivity within 30 minutes at concentrations of 0.5 to 2.0% at room temperature.

EPIDEMIOLOGY

GEOGRAPHIC DISTRIBUTION AND HOSTS

Several huge epizootics have affected domestic chickens and turkeys since 1927. The geographic distribution of ND in free-living birds is markedly affected by three virus sources: i) free-living birds, which acquire NDV as an overspill from infected poultry; ii) the pigeon variant of APMV1 (also named pigeon PMV1, PPMV1), which was introduced to Europe from Middle Eastern countries in 1981–1982 and has since occurred not only in free-living feral pigeons (*Columba livia*) but also in other columbiformes such as the Eurasian collared dove (*Streptopelia decaocto*), wood pigeon (*Columba palumbus*) and other species that live in urban environments[5] (after the introduction of the pigeon variant of APMV1 to European countries, large proportions of wild birds succumbed during the following years[2]); iii) migratory birds, which are a less important source, that fly from Africa to Europe within the incubation or latent period or are traded within European countries.

Exact data on the geographic distribution of APMV1 for all European countries are not available. In Germany, examination of 3,672 cloacal samples from 78 wild migrating and resident bird species collected during 2003 to 2005 resulted in detection of 52 APMV1-positive samples[3]. APMV1 were obtained from mallards (*Anas platyrhynchos*), Eurasian coot (*Fulica atra*), a black-headed gull (*Larus ridibundus*) and a mute swan (*Cygnus olor*). APMV4 was obtained from mallards, teal (*Anas crecca*), European wigeon (*Anas penelope*), a black-headed gull and a magpie (*Pica pica*). APMV6 was detected in mallards, a mute swan and a greylag goose (*Anser anser*). The results indicated that predominantly apathogenic APMV1 circulates among waterfowl and waders (Anseriformes and Charadriiformes) that migrate from their Nordic breeding grounds to central Europe[3].

In a separate study, in Germany, a mallard yielded an APMV9[6]. APMV1 was not isolated from 658 organ samples of 110 greylag geese. However, haemagglutination inhibition (HI) antibodies against APMV4 were detected in one of 13 serum samples, against APMV6 in 10 of 13, and against APMV8 in two of 13 serum samples from this species[6].

ND has been described in 241 avian species from 27 of 50 orders[7] (Table 3.1). It appears reasonable to conclude that all species of European birds are susceptible to infection by NDV. However, the signs of disease and its outcome vary considerably with the affected host species. Many reports describe virus detection in birds that were found dead and none describe major die-offs among free-living birds in Europe. Young birds that acquire infection by virulent NDV before the development of immunocompetence seem to be more susceptible to new infection and disease than mature, immunocompetent birds. Convalescent birds with serum antibodies are resistant to disease but not to infection. The sex of birds does not affect infection and outcome of disease.

Virulent APMV1 as defined below in the diagnosis section, are of major importance as notifiable epizootics in

TABLE 3.1 Host range of NDV in free-living European birds (abridged; for details see Kaleta and Baldauf, 1988[7]).

Order Family	Clinical signs	Lesions
Gruiformes		
Gruidae	CNS, respiratory, enteric signs	Haemorrhages, enteritis
Ralliformes		
Rallidae	None	None
Charadriiformes		
Scolopacidae	None	None
Haematopodidae	None	None
Lariformes		
Laridae	None	None
Sternidae	None	None
Pelicaniformes		
Phalacrocoracidae	CNS, lethargy, enteritis	Encephalitis, haemorrhages
Columbiformes		
Columbidae	CNS, respiratory, enteric signs	Haemorrhages, nephritis
Strigiformes		
Strigidae	CNS, lethargy, respiratory signs	Enteritis, pneumonia
Falconiformes		
Falconidae	CNS, respiratory signs	Haemorrhages
Accipitriformes		
Accipitridae	Lethargy, CNS	None
Pandionidae	Lethargy, respiratory, enteritis	None
Ciconiiformes		
Ciconiidae	CNS, enteritis	Haemorrhages
Ardeidae	CNS, respiratory signs	None
Anatiformes		
Anatidae	None	None
Anserinae	None	None
Phasianiformes		
Phasianidae	CNS, respiratory signs, enteritis	Haemorrhages, encephalitis
Passeriformes		
Pittidae	None	None
Corvidae	None	None
Estrildidae	Lethargy, CNS	None
Passeridae	CNS, enteritis	None
Carduelidae	CNS, respiratory signs	None
Motacillidae	None	None
Sturnidae	CNS	None

CNS – nervous signs including torticollis, opisthotonus
None – either not reported or not observed

chickens and turkeys. Such strains have been frequently isolated from free-living gallinaceous, passerine and other terrestrial birds.

Mesogenic APMV1 have been detected so far only in domestic chickens. Lentogenic strains are frequently isolated from aquatic birds such as ducks, geese, swans and many shore birds. Avirulent enteric APMV1 has not been associated with any disease expression. Numerous viruses of this pathotype have been, in most cases, isolated from subclinically affected aquatic birds. As compared to APMV1, APMVs of the types 2 to 9 are of minor importance as causes of disease and losses. Such viruses are occasionally isolated from a variety of birds, mainly passerine and captive psittacine birds[2]. Only strains of the APMV3 cause respiratory signs and pancreatitis in turkeys and, in addition, disorders of the central nervous system in some captive psittacine birds, namely Australian parrots of the genus *Neophema*[8].

ENVIRONMENTAL FACTORS

Climate *per se* has no reported effect on the prevalence of disease. However, cold winters with frozen surface waters force aquatic birds to aggregate in large numbers on a few remaining open waters, which facilitates frequent and effective virus transmission. As NDVs are readily transmitted by the faecal-oral route, virus transmission is more likely. However, NDV in excreted faeces or saliva is readily inactivated by ultraviolet light from sunshine.

Moist soil and decomposing vegetation preserve the infectivity of the virus, whereas dry materials and higher temperatures inactivate virus rapidly. Several studies provide evidence for survival of NDV for days to months, but the data cannot be applied to the highly variable environmental conditions in nature, such as decomposition of organic matter, pH, organic acids found in the environment, microbial colonization (bacteria, yeasts, moulds), survival in arthropods and following ingestion by earthworms. Viral infectivity in carcasses, for example of hunted birds, can be retained for several months at freezing temperatures. Animate and inanimate vectors are not necessary for virus spread, and arthropod vector transmission does not occur.

TRANSMISSION

Saliva and faeces of ND-affected domestic poultry contain high amounts of NDV. Birds with access to poultry farms (house sparrows, swifts, swallows, crows) acquire infection by direct contact with virus-containing faeces or consumption of contaminated feed and water for poultry. Thus, horizontal transmission is the most important route. Also, live-bird markets displaying domestic, hobby, pet

and captured wild birds facilitate spread among birds. Local spread among waterfowl is likely to be due to contaminated water. International and intercontinental transmission may occur via commercial trade in live poultry and poultry products or offal. Virus transmission may occur from migrating wild birds returning from Africa to Europe. Free-ranging chickens in many African countries suffer from endemic ND, which facilitates transmission to migrating birds.

An epidemiological link between ND outbreaks in poultry and virus isolations in birds of prey has been observed, with a causal relationship between ND-infected chickens and domestic pigeons, as the prey of Falconiformes and Strigiformes, in which birds virus is subsequently detected[4].

PATHOGENESIS, PATHOLOGY AND IMMUNITY

Susceptible birds contract NDV mainly from contaminated feed and water and to a lesser extent by inhalation of virus-containing dust or dander.

Little is known about the minimal infectious dose of NDV for wild birds. In domestic chickens, five to ten infectious particles of virulent strains can produce disease and mortality by intranasal instillation in adult immunologically naïve chickens. Lentogenic and avirulent strains induce subclinical infection and immunity at a minimum dose of 10^5 to 10^6 embryo-infective doses.

The portal of entry of NDV is the epithelium of the upper respiratory tract. Experimental instillation of NDV directly into the crop, thus circumventing the respiratory epithelium, does not initiate infection in the chicken. Following initial virus replication in the epithelial layers, NDV is released from infected cells and phagocytosed by macrophages and thrombocytes. Subsequent virus transport in the bloodstream results in ND virus being detectable in all blood components: i) blood plasma; ii) macrophages; iii) attached to erythrocytes in intravasal agglutination of red blood cells with the formation of micro-emboli in various organs and infection of cells and cell cytolysis within organs. Consequently, death follows as a result of intravasal coagulopathy and organ dysfunction.

The development of disease and outcome of the infection depends on the avian host species, its immunity and the virulence of the infecting virus strain. The incubation period for domestic poultry is 5 to 7 days, and it is likely to be similar for other susceptible birds. The mortality rate depends on the species of bird, being highest in terrestrial birds with case fatality rates close to 100% (in commercial poultry flocks), and low – if at all – in aquatic birds. The few survivors develop protective circulating antibodies that can be detected 5–7 days post-infection by HI tests or enzyme-linked immunosorbent assays (ELISA). These antibodies consist initially of the immunoglobulin (Ig) M and later of IgG (in birds also named IgY) and IgA type and persist for several months. Antibodies can be used as indicators of a previous infection. The presence of antibodies is correlated with the elimination of infectious virus. Convalescent birds do not excrete infectious ND virus.

Antibodies in serum are transferred into the yolk during the growth of egg follicles. The quantity of antibodies in serum equates with that in yolk. During the last third of embryonic development, yolk antibodies pass into the small intestine of embryos, are absorbed by the mucosal cells and transferred to the blood circulation. A few days after hatching the antibody content in the blood of chicks reaches a maximal concentration and then declines thereafter. The duration of persisting antibodies depends on the metabolic decay of IgY and the growth rate of the chicks. Calculations of the elimination of maternal antibodies from the circulation resulted in half-life times in the range of 4–5 days.

Owing to the relatively short period of illness, there is usually no loss of body condition. The gastrointestinal tract is almost empty or contains mucus, and the subcutis and muscles are dehydrated. The kidneys are enlarged and the ureters filled with whitish to yellow urates. Besides these non-specific lesions, haemorrhages in the epithelium of the nasal cavity, trachea and bronchi, on the serosal surfaces in the body cavity, and in the mucosa of the proventriculus, intestines and bursa of Fabricius can be observed, but may be mild or absent[9].

An infection during the moulting period results in malformation of feathers, which appear larger than normal, with thinner and fragile shafts and smaller vanes.

Virulent NDV strains cause rapid death, and histological lesions might be completely absent. In more protracted cases, perivascular cuffing by lymphocytes are seen mainly in the brainstem, the medulla oblongata and the spinal cord but also in the respiratory tract and the kidneys.

In columbiform birds the pigeon variant of APMV1 (PPMV1) regularly causes spongiform lesions in the brain-

stem along with gliosis and necrosis of neurons. Indirect immunofluorescence on thin sections using monoclonal antibodies directed against the F and HN proteins demonstrates the presence of viral antigens in many tissues[10] with high specificity and sensitivity.

CLINICAL SIGNS AND TREATMENT

NDV-infected sick wild birds are predominantly found during late stages of disease. Information on initial signs is frequently lacking[9]. However, occasionally wild birds with ND display lethargy, unilateral conjunctivitis and rhinitis, and birds succumb readily. The pigeon variant of APMV1 frequently causes torticollis, opisthotonus and/or unilateral lameness of a wing or leg.

All virulent NDV strains cause systemic infections, which are expressed clinically in the following time sequence: severe lethargy, depression, anorexia, ruffled feathers, respiratory rales, cuffing, excretion of watery, greenish faeces and production of misshapen eggshells in laying females. As late sequelae, abnormal movements of the head and neck (torticollis), opisthotonus and unilateral lameness of wings and legs for prolonged times (all these signs persist for weeks to months). Feather tips of incoordinated wings display mechanical trauma. The duration of the disease may be as short as 2–3 days. Nervous signs may persist for many weeks to months in these cases; recovery is unlikely.

Mesogenic strains induce only minor respiratory signs. Lentogenic and avirulent-enteric strains are not associated with any clinical signs but seroconversion occurs.

There is no effective treatment of birds suffering from ND. Drugs such as amantadine or oseltamivir (Tamiflu) have no effect on penetration and uncoating of ND viruses.

DIAGNOSIS

Clinical signs, gross and histopathological lesions lead to a presumptive diagnosis. Detection of viral antigens in tissues of the respiratory tract and central nervous system by indirect immunofluorescence is of supporting value. A definitive diagnosis requires isolation and characterization of the causative virus as an avian PMV1 and identification of markers for virulence. In recent years, molecular techniques such as reverse transcription polymerase chain reactions (RT-PCR) support or replace conventional virus isolation procedures. Targets for the RT-PCR are mainly portions of the matrixprotein-, nucleoprotein- and fusionprotein genes.

Virus isolation is commonly done in embryonated chicken eggs by inoculation of the allantoic cavity of at least four eggs at day 9–11 of incubation. The inoculum consists of 10% w/v suspensions each derived from brain (cerebrum, cerebellum and medulla oblongata), nasal conchae, trachea, lung, spleen, liver, kidney and intestine. Tissue suspensions can be pooled. Inoculated eggs are further incubated at 37.8°C in a humidified atmosphere without turning. The chorioallantoic membranes, yolk sac and the embryo are checked for macroscopic lesions (haemorrhages, oedema). The obtained allantoic fluids (AF) are individually tested for haemagglutination (HA). The AF are tested in a semiquantitative manner for HA using phosphate-buffered saline as a diluent and a 1% suspension of washed chicken red blood cells. Most but not all AF agglutinate. If embryo death is noted at 24 hours post-inoculation or later but AF failed to agglutinate, a passage of AF derived from several embryos is required. The HA test is followed by HI tests using polyclonal antisera (preferentially raised in chickens) against APMV1 and APMV2 to APMV9. The results of the HI tests allow the allocation of the new isolate to one of the nine APMVs.

As an alternative to the rather simple, rapid and inexpensive HI test, virus neutralization tests in embryonated eggs or in permissive cell cultures (e.g. chicken embryo fibroblasts) may be used. If the results of the HI tests document the presence of an APMV1, direct electron microscopy of negatively contrasted AF without purification/concentration can be applied as a second independent line of evidence for confirmation of APMV.

Haemagglutinating, embryo-lethal isolates must be immediately submitted to the National Reference Laboratories (NRL) of the EU member states for confirmation of the diagnosis and determination of virulence of the isolate.

Based on their virulence for chickens, the APMV1s are subdivided on the basis of three *in vivo* tests into five pathotypes (velogenic, neurotropic, mesogenic, lentogenic and avirulent-enteric). The commonly used three tests provide numerical index values for virulence and, therefore, ease differentiation between pathotypes of deviating virulence. The intracerebral pathogenicity index (ICPI) test, which is the standard test to determine the pathogenicity, is performed in ten 1-day-old chicks and yields

values between zero (avirulent) and 2.0 (highly virulent). The intravenous pathogenicity index (IVPI) test is performed in ten 6-week-old chickens and yields indices between zero (avirulent) and 3.0 (highly virulent). The third test is performed in embryonated chicken eggs and provides the time in hours that is required to cause embryonic death (mean death time, MDT). Highly virulent viruses cause embryo death in less than 50 hours, whereas avirulent viruses do not cause regular embryo mortality within 6 days. These traditional *in vivo* tests have now been replaced or supplemented by molecular tests that focus on the F protein or proteins of other genes. These include the determination of multiple basic amino acids on the cleavage side of the fusion gene protein and other genes.

Most important are virulent APMV1 that cause high morbidity and mortality in domestic chickens and turkeys and are referred to as Newcastle disease, which is a notifiable disease in most countries of the world.

The definition published in the *Terrestrial Manual* of the World Organisation for Animal Health (OIE)[11] for an outbreak of ND reflects the current understanding of the molecular basis of virulence:

> *Newcastle disease is defined as an **infection** of **birds** caused by a virus of avian paramyxovirus serotype 1 (APMV1) that meets one of the following criteria for virulence:*
>
> (a) *The virus has an intracerebral pathogenicity index (ICPI) in day-old chicks* (*Gallus gallus*) *of 0.7 or greater*.
>
> (b) *Multiple basic amino acids have been demonstrated in the virus* (*either directly or by deduction*) *at the C-terminus of the F2-protein and phenylalanine at residue 117, which is the N-terminus of the F1 protein. The term "multiple basic amino acids" refers to at least three arginine or lysine residues between 113 and 116. Failure to demonstrate the characteristic pattern of amino acid residues as described above would require characterisation of the virus by an ICPI test."*

The term 'infection' in this definition of ND means that the virus isolate must be derived from a bird that may or may not present signs of disease, including mortality, lesions or seroconversion. The term 'birds' includes all avian species and not only domestic poultry.

By contrast, the OIE definition of ND for the purpose of international trade as published in the *Terrestrial Animal Health Code* (2009)[12] defines only an infection of poultry. Poultry is defined as 'all domesticated poultry, including backyard poultry, used for the production of meat or eggs for consumption, for the production of other commercial products, for restocking supplies of game, or for breeding these categories of birds, as well as fighting cocks used for any other purpose.' In annex III of the amended (in 2004) Council Directive 92/66EEC[13] 'Newcastle disease means an infection of poultry caused by any *avian paramyxovirus 1* with an intracerebral pathogenicity index in day-old chicks greater than 0.7.'

ANTIBODY DETECTION

Virtually all species of birds produce antibodies that can be detected in serum but also in conjunctival fluid (tears), lavage from respiratory and digestive tracts and in yolk of laid eggs. Such antibodies are present following natural exposure in convalescent or vaccinated birds and persist for up to 1 year. The method of choice for antibody assays in sera of wild birds is still the HI test. This test is universal, because antibodies in all avian species can be measured, and it is also independent of the material used (sera or others). Virus neutralization tests can also be used for all avian species but are more labour-intensive, costly and time-consuming. For large-scale testing of chicken and turkey sera, various modifications of ELISA have been developed and are commercially available, but as the ELISA is species-specific, sera from wild birds other than poultry cannot be directly tested. For this purpose blocking ELISA in various modifications are adopted.

MANAGEMENT, CONTROL AND REGULATIONS

ND is a notifiable disease in Europe and elsewhere. The duties of the European NRL are: i) to confirm the initial results of diagnostic laboratories; ii) to perform PCR to differentiate APMV1 from other APMVs; iii) to determine the virulence of the isolate by demonstration of multiple basic amino acids at the cleavage site of the F protein; iv) to perform the intracerebral pathogenicity test and calculation of an index value (ICPI) to determine the neurovirulence *in vivo*. The detection of virulent APMV1 by these four criteria constitutes the presence of a virus that causes the notifiable ND in those cases where the isolate originated from poultry. The local veterinary authorities and the national governments must be immediately

informed. In addition, the national government must inform the OIE. It is the duty of the government in cooperation with the local authorities to initiate countermeasures to eradicate infected and contact birds, including hygiene measures and disinfection.

The first (index) case of confirmed ND in poultry has a severe impact on national and international trade in live poultry and poultry products, including eggs. Formal regulatory requirements for ND confirmation in free-living birds (wild) are not in force.

Procedures in place for the management of ND outbreaks in poultry include 'stamping out' infected and in-contact poultry, incinerating dead and culled birds, destroying all eggs laid during the outbreak, cleansing farms with subsequent disinfection and stand-still of all movements and transport for at least 3 weeks. Such measures are not applicable to wild birds kept in captivity or for free-living birds. Captive birds and domestic pigeons are usually maintained during outbreaks in closed houses, and enforced biosecurity is mandatory. Vaccination with inactivated vaccines is used with the agreement of local veterinary authorities. Lentogenic (mild) strains (e.g. Hitchner B1, LaSota, Clone 30) have been used as live vaccines for poultry and many avian species since about 1960.

If free-living birds were found to be infected with virulent NDV, surveillance might be applied to monitor enhanced mortality among these bird populations.

Bird collections in zoos or similar establishments can be vaccinated in accordance with EU regulations in emergency situations due to outbreaks of ND in poultry in the immediate neighbourhood. Vaccination of poultry with live or inactivated vaccines is mandatory in most EU countries and completely banned in others (e.g. UK, Ireland, Denmark).

PUBLIC HEALTH CONCERN

All APMV1 field strains (irrespective of the virulence of a ND virus for chickens) and all live vaccine ND viruses, following accidental instillation in humans, cause a painful conjunctivitis but not a keratitis. ND virus can be isolated from conjunctival swabs in the acute phase of the illness. The inflammatory response lasts for about 2 weeks and clinical resolution occurs without sequelae. The conjunctivitis is in almost all cases unilateral and the serum of affected patients is free of detectable antibodies. Lateral spread from human to human has not been reported to date.

SIGNIFICANCE AND IMPLICATIONS FOR ANIMAL HEALTH

Companion mammals such as dogs and cats occasionally acquire infection with APMV1. Cattle, sheep, goats, horses and other equids are susceptible following experimental infection and react with antibody formation. However, all these species do not develop signs of disease and play no role in the epidemiology of ND.

NDV-infected poultry and hobby birds that are kept outside excrete virus in large quantities and can infect free-living wild birds in the vicinity.

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CHAPTER

4

CIRCOVIRUS INFECTIONS

DANIEL TODD AND CHRISTIAN GORTÁZAR

INTRODUCTION

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Circoviruses are non-enveloped, spherical viruses (20–25 nm in diameter), that possess circular, single-stranded DNA genomes (1.7–2.3 kb). They are the smallest of the DNA viruses that affect animals and are highly resistant to physical and chemical inactivation. The family *Circoviridae* contains two genera. *Chicken anaemia virus* (CAV) is the only species and the type species of the genus *Gyrovirus*, whereas the genus *Circovirus* presently comprises, or is soon likely to comprise, *porcine circovirus* types 1 and 2 (PCV1, PCV2), *beak and feather disease virus* (BFDV), *pigeon circovirus* (PiCV), *goose circovirus* (GoCV), *canary circovirus* (CaCV), *duck circovirus* (DuCV), *gull circovirus* (GuCV), *finch circovirus* (FiCV), *starling circovirus* (StCV) and *swan circovirus* (SwCV). Viruses belonging to the two genera can be distinguished in terms of virion size and morphology and genome organization. Viruses from both genera use a single structural protein, and also produce one or two non-structural proteins. To date, only CAV, PCV1 and PCV2 can be grown in cell culture, which has greatly restricted the characterization of the antigenic interrelationships of avian circoviruses. However, the ability to use DNA amplification methods to isolate complete genomic fragments has allowed circoviruses to be characterized and classified using nucleotide sequence-based methods. A novel circovirus will be recognized as a new member of the genus *Circovirus* if it shares less than 70% genome nucleotide identity and less than 75% capsid protein amino acid identity with any of the previously assigned members[1]. The circovirus identified in mute swans (SwCV) is likely to be recognized as a new genus member, whereas that recognized in Australian ravens (RaCV) is not. Circovirus-like viruses have also been described in ostriches and pheasants, but these have yet to be definitively characterized.

CIRCOVIRUS INFECTIONS IN WILD BIRDS

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Avian circovirus diseases cause feather abnormalities in some avian hosts, most notably psittacine species, but primarily they are causally associated with immunosuppression, which can lead to growth retardation and ill thrift (wasting), and increased susceptibility to secondary pathogens[2].

Infectious Diseases of Wild Mammals and Birds in Europe, First Edition. Edited by Dolores Gavier-Widén, J. Paul Duff, and Anna Meredith.

EPIDEMIOLOGY

Information relating to the distribution of circovirus infections of free-living avian species within Europe is confined to single case reports concerning gulls in Sweden[3], starlings in Spain[4] and mute swans in Germany[5]. Based on information from racing pigeons and farmed geese and ducks, it is likely that circovirus infections are prevalent within flocks and widespread in avian species, including many wild species.

The recent characterizations of taxonomically distinct circoviruses in different avian species, including pigeons, geese, ducks, gulls, finches, starlings and swans, support the views that novel circoviruses will be recognized in additional avian hosts and that avian circoviruses display high levels of host specificity. Information gathered from studies of psittacine species, pigeons, geese and ducks indicates that most birds become infected as juveniles.

No information is available relating to the influence of climate on the prevalence of avian circovirus diseases. Circovirus disease in gulls occurred in summer-autumn in New Zealand[6], whereas deaths (due to salmonellosis associated with circovirus infection) of starlings occurred in winter in Spain[4]. There is no information as to whether circoviruses use insect vectors, but this is considered unlikely.

Cross-infection between closely related avian species remains a possibility; however, given the high degree of host-specificity, it is unlikely to occur between unrelated species. Cross-infections between free-living birds such as ducks or geese and their farmed counterparts are considered possible.

Investigations with psittacines and racing pigeons indicate that circoviruses can replicate in the intestinal tract and can be excreted for weeks to months after infection. Because circoviruses are highly resistant to inactivation, environmental contamination is high and transmission by ingestion of infected material is likely. The detection of circoviruses in the reproductive tissues (PiCV) of adult birds and in embryos (PiCV, BFDV) suggests that vertical transmission of avian circoviruses is possible.

PATHOGENESIS, PATHOLOGY AND IMMUNITY

Information relating to pathogenesis is mainly confined to psittacines, racing pigeons and farmed geese. It is probable that most birds become infected within the first few weeks with horizontally acquired virus, although evidence from pigeons indicates that infections may also be acquired vertically. There is no information on the infectious dose required, mainly because of the absence of cell culture propagation systems for estimating virus infectivity.

Ingestion of virus-contaminated material is likely to lead to infection of the intestinal tract, a tissue in which the circoviruses of psittacines, pigeons and geese have been detected. Circovirus viraemias have been demonstrated in psittacines, pigeons and geese, and this is the probable mechanism by which circoviruses spread to other organs and tissues. In clinically affected birds, circoviruses can be detected in a wide range of tissues. For example, PiCV has not only been detected in lymphoid tissues including the bursa of Fabricius and spleen, but also in a wide range of other tissues, including the intestine, respiratory organs (trachea, pharynx, lung), kidney, liver and reproductive tissues (ovary and testes). Owing to their very limited protein-coding capacities, circoviruses are heavily dependent on the host cell's replication machinery and probably replicate best in rapidly dividing tissues such as epithelial cells. In diseased birds, circovirus inclusions are commonly found in the cytoplasm of macrophages (Figure 4.1). Such inclusions probably result from phagocytosing activity.

Most avian circovirus infections are likely to be subclinical, probably involving low levels of virus replication in a limited tissue range. Clinical manifestations including ill

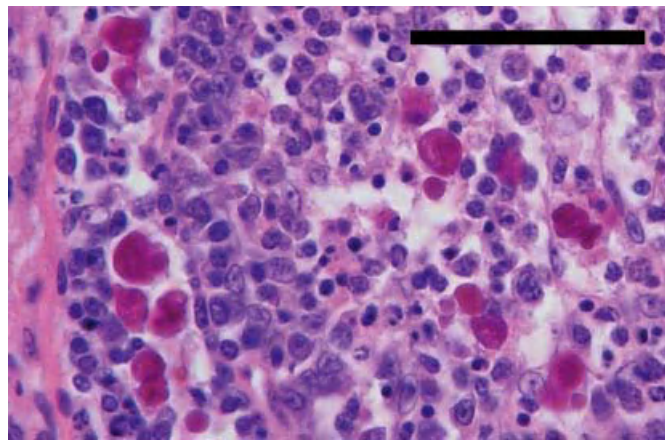

FIGURE 4.1 Bursa of Fabricius from a juvenile herring gull showing depletion of lymphocytes, eosinophilic inclusions in both cortex and medulla of the bursal follicle. Haematoxylin and eosin. Scale bar = 50 µm. (See Smyth et al., 2006)[3].

thrift, and feather abnormalities usually arise when high levels of virus are detectable within tissues. High virus levels may correlate with lymphocyte depletion and with the resulting immunosuppression. Studies with pigeons and geese suggest high levels of virus in tissues may take several weeks to develop. In chronically infected psittacine species, there is progressive feather loss and occasional beak deformity. Although cases of clinical remission have been observed, fatal outcomes, often resulting from secondary infections exacerbated by the circovirus-induced immunosuppression, are frequent. In non-psittacines the circovirus-induced immunosuppression causes less-specific clinical signs, including ill thrift and weight loss, with the clinical manifestations observed again being attributable to secondary opportunistic infections. For example, in the case of a southern black-backed gull (*Larus dominicanus*) the pathological findings were consistent with the possibility that a circovirus-induced immunosuppression allowed an overwhelming secondary infection with *Aspergillus* spp.[6]. A number of factors have been proposed to explain why some infections result in disease, whereas others remain subclinical. These include the route and age at which the birds are infected, the levels of maternal antibody, virus strain, infectious dose, and presence of co-infecting pathogens. Antibody responses to circovirus infections have been detected in a limited range of avian species, including psittacines, geese and pigeons. Experiments with psittacines have shown that maternally derived antibody is protective against a disease challenge.

Feather loss and beak deformities are the principal lesions associated with psittacine beak and feather disease, the most studied avian circovirus disease. Feather dystrophy and loss are usually symmetrical, with changes in the feathers including retention of sheaths, haemorrhages within the pulp, cavity fracture of the proximal rachis and failure to exsheath also being observed. Lymphoid depletion including bursal atrophy can be a feature of avian circovirus diseases, but these lesions can also be due to infections with other viruses. Many of the gross lesions observed in non-psittacines are attributable to secondary pathogens. At the microscopic level, affected birds show lymphocyte depletion and infiltration of monocytes. Basophilic globular or botryoid inclusions can also be observed in the cytoplasm of macrophages found within lymphoid tissues such as the bursa of Fabricius (Figure 4.1). Thin-section electron microscopy has shown that these inclusions comprise paracrystalline arrays of tightly packed circovirus particles (Figure 4.2).

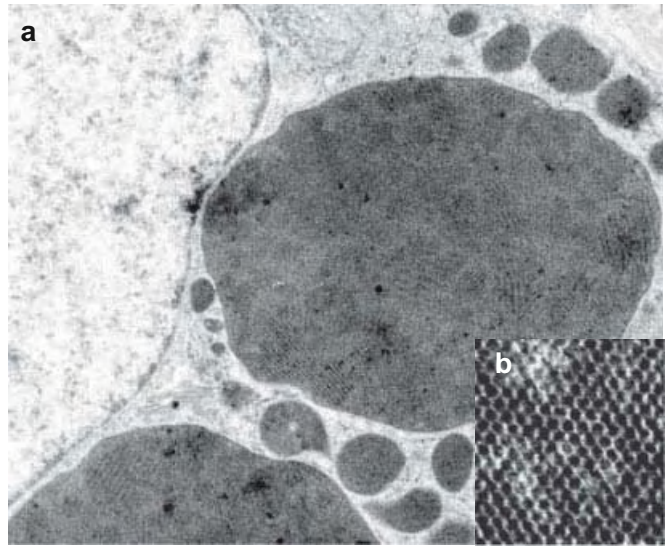

FIGURE 4.2 Thin-section electron microscopy performed with bursa of Fabricius tissue from a circovirus-infected psittacine species. Electron-dense inclusions containing paracrystalline arrays of tightly packed virus particles were observed in the cytoplasm of macrophage cells and are shown at low **(a)** and high **(b)** magnification. Image provided by Dr Joan Smyth, AFBI-Stormont, Belfast (See Smyth et al., 2006)[3].

CLINICAL SIGNS AND TREATMENT

Feather abnormalities are a common manifestation of BFDV infections in psittacines, and have also been observed in non-psittacine species, including Senegal doves (*Streptopelia senegalensis*) infected with a PiCV variant, pigeons, Australian ravens (*Corvus coronoides*)[7], finches, ducks and geese. Non-psittacine species frequently show non-specific clinical signs, including weight loss and ill thrift, with specific signs being attributable to secondary pathogens.

It may be possible to rehabilitate individual birds that are found sick by treating secondary infections. Clinical remissions have been observed with some psittacines and with Australian ravens, and infected psittacines have been successfully treated with avian gamma interferon[8].

DIAGNOSIS

Infections with circovirus-like viruses were originally diagnosed in pigeons, gulls and farmed geese based on the detection of characteristic, basophilic globular or botryoid

inclusions, which were first described in psittacines infected with BFDV. Electron microscopic techniques have also been used to detect paracrystalline arrays of small (14–17 nm) tightly packed, virus particles (Figure 4.2) within the inclusions and circovirus-like particles in tissue homogenates prepared from pigeons, geese, ducks and pheasants. However, owing to the absence of surface structure and variation in the accuracy with which small virus particles can be measured, definitive diagnoses should not be based on negative contrast electron microscopy alone.

So far, growth of most avian circoviruses in cell culture has not been described. This has meant that virus culture cannot be relied on for diagnosis, and it has also greatly restricted the ability to produce virus-specific antisera for use in antigen-detecting diagnostic procedures such as immunohistochemistry and cryosection/ impression smear fluorescent antibody tests. Molecular approaches are used for virus characterization and diagnosis. Degenerate primer-based polymerase chain reaction (PCR) methods and other novel DNA-amplifying methods have been used to amplify sub-genomic and full-length genome fragments specific to the majority of circoviruses recognized to date. Phylogenetic analyses based on sequence comparisons have then been used to establish whether the circovirus detected represents a new genus member. Knowledge of the circovirus sequences and the availability of cloned genome fragments have facilitated the development of diagnostic methods based on DNA detection, including *in situ* hybridization (ISH), dot blot hybridization (DBH) and PCR. PCR tests have been described for the specific detection of BFDV, PiCV, GoCV, DuCV, StCV and SwCV. Applications of these tests have shown that infections are prevalent and likely to be widespread. However, PCR detects subclinically infected birds as well as birds that are clinically affected by circovirus. Therefore, owing to its very high sensitivity, PCR may be of questionable use in disease diagnosis. Although ISH is less sensitive, its ability to demonstrate substantial amounts of virus DNA within lymphoid tissues may prove to be more valuable for diagnosing avian circovirus diseases (Figure 4.3). With birds infected with PiCV, GoCV and GuCV, virus DNA was detected by ISH in a range of tissues, many of which – for example liver – were not showing histological changes. A digoxigenin-labelled ISH probe prepared from a cloned PiCV genome fragment containing part of the rep gene has been shown to cross-react with GoCV, CaCV and GuCV. As such, this may prove to be a useful reagent for the detection of novel circoviruses in other avian species[3].

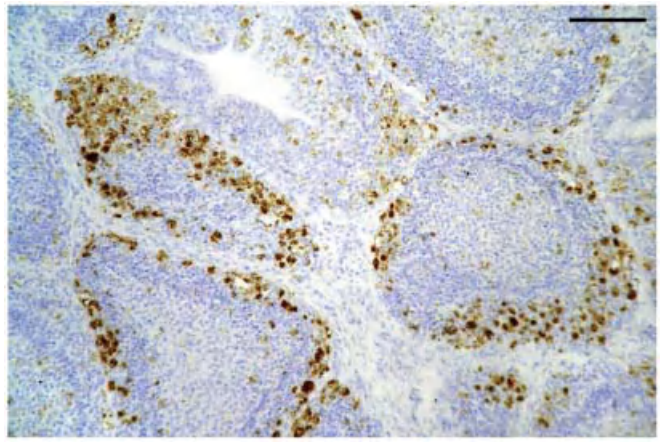

FIGURE 4.3 ISH showing positive labelling of circovirus DNA (stained brown) in the bursa of Fabricius from a juvenile herring gull. Infected cells are most numerous in the cortex of the follicles. ISH counterstained with haematoxylin and eosin. Scale bar = 100 µm. (See Smyth et al., 2006)[3].

PCR is likely to prove to be the most suitable test for screening populations for circovirus infections. Investigations with pigeons have shown that cloacal swabs taken many months post infection, are PCR-positive, and this may be the case for other avian species. Therefore, cloacal swab samples collected from captured juvenile wild birds or faecal samples collected from nesting sites will probably contain PCR-detectable virus resulting from subclinical and clinical infections. Serological testing is likely to be less useful, because antibody-detecting tests are available for very few avian circoviruses.

MANAGEMENT AND CONTROL

To date in Europe, outbreaks of circovirus diseases in free-living birds have been limited to those in occurring in gulls, starlings (*Sturnus vulgaris*) and swans, in which circovirus was detected in birds that were found dead. Management and control of circovirus disease in wild bird populations are impractical on the basis that it is difficult to administer vaccines or therapeutics to free-living birds.

PUBLIC HEALTH CONCERN

Avian circoviruses are highly host-specific, and infections of humans are considered highly improbable.

SIGNIFICANCE AND IMPLICATIONS FOR ANIMAL HEALTH

Although it is known that circovirus infections of farmed geese and ducks are common and widespread, at present there is no information about: i) the prevalence of GoCV and DuCV infections in free-living birds; and ii) whether the circoviruses that infect the free-living birds are identical to those that infect the farmed birds. On this basis, the possibility of cross-infections occurring between the farmed and free-living birds cannot be discounted. Cross-infections with BFDVs, which are known to infect a range of psittacine species, will occur between captive and free-living birds unless sufficient biosecurity is maintained.

CIRCOVIRUS INFECTION IN WILD BOAR

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Porcine circovirus type 2 (PCV2) is considered the aetiological agent of post-weaning multisystemic wasting syndrome (PMWS), a disease of pre-weaning and growing domestic pigs, which is also found in Eurasian wild boar. Infection with PCV2 does not always result in the development of PMWS.

PCV2 is a member of the genus *Circovirus* in the family *Circoviridae*. The genome contains three open reading frames (ORF): ORF1 encodes the replicase (rep and rep0) proteins involved in virus replication, ORF2 encodes the capsid (cap) protein and ORF3 encodes a protein that is not essential for PCV2 replication with potential apoptotic activities. Based on ORF2, the existence of two different genotypes within PCV2 (genotypes 1 and 2) has been suggested. Whereas pigs from PMWS-affected farms always harbour PCV2 genotype 1 (with or without sequences from genotype 2), pigs from non-PMWS affected farms had exclusively sequences from genotype 2[9].

PMWS has been described worldwide. In Europe, infection or exposure to PCV2 has been reported in wild boar from several countries, including Belgium, Czech republic, Germany, Italy and Spain[10,11]. Infection can occur at any age, and no clear sex effect on prevalence exists. PMWS, however, only occurs at early ages. Management conditions, age of the host, viral genotype and infection load with PCV2 are potential factors that influence the progress of the disease.

The high seroprevalence to PCV2, along with its widespread geographical distribution, suggest that PCV2 is endemic in the Eurasian wild boar. As this infection is widespread among domestic pigs, the reservoir role of wild boar is largely irrelevant. PCV2 is readily transmitted by direct and indirect contact. PCV2 primarily infects through close contact, such as nose-to-nose contact[12].

The respiratory system may be the route of entry of PCV2, as suggested by the capacity of the virus to infect bronchial and bronchiolar epithelial cells. Alternatively, PCV2 may infect the nasopharynx and tonsils and spread via blood or lymph. The incubation period for development of PMWS in pigs is approximately 2 weeks. The infection with PCV2 is systemic and may persist for a long period, causing prolonged viral shedding. Pigs with PMWS have higher viral load in serum and tissues than subclinically affected pigs. PCV2 has been detected in ocular, faecal and urinary swabs. Oronasal secretions, urine and faeces are potential routes of viral shedding[12]. Antibodies are detectable in wild boar of all age classes[10].

Marked lymphocyte depletion with moderate granulomatous histiocytic infiltration of lymphoid tissues was observed in 6- to 10-month-old wild boar piglets with PMWS[10]. Applying *in situ* PCR, viral genome was detected in the cytoplasm of macrophage-like cells in the lymph nodes, spleen, lung and liver. A small quantity of the PCV2 genome was also detected within the cytoplasm of epithelial tubular cells of the kidney[10,11].

In pigs, PMWS is clinically characterized by wasting, respiratory distress, pallor of the skin and, occasionally, jaundice[13]. Reproductive disturbance, including abortion, infected stillborn and non-viable neonate piglets has been described in domestic sows. PCV2 infection can cause similar pathology in wild boar as in domestic pigs, causing immunosuppression and facilitating the occurrence of secondary infections[11].

Serological methods for PCV2 include the immunoperoxidase monolayer assay (IPMA) and ELISA. The presence of PCV2 can be demonstrated by isolation in cell culture and by PCR. Owing to the lack of specificity of the clinical signs, the definitive diagnosis of PMWS is based on a combination of three different criteria: clinical signs, the presence of specific lesions in lymphoid tissues (consisting of lymphocyte depletion and granulomatous inflammation) and the presence of PCV2 in these tissues[13].

In the domestic pig industry, PCV2 infection is controlled through improved hygiene and by vaccination. Avoiding close contact between wild boar and domestic animals is important in general terms for disease control and eradication programs. Artificial management of wild boar populations, such as by the use of fencing and feeding, causes increased density and spatial aggregation and an increased risk of disease transmission. Natural management techniques and systems are therefore preferred.

PMWS might play a role in the population dynamics of intensively managed wild boar populations, as indicated by the following observations. Firstly, fewer piglets than expected were observed in intensively managed areas; secondly, juveniles from these sites had an extremely high seroprevalence suggesting recent PCV2 infection; and thirdly, a number of these farm-like areas reported abnormal piglet mortalities[10].

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CHAPTER

5

CALICIVIRUS INFECTIONS

J. PAUL DUFF AND DOLORES GAVIER-WIDÉN

INTRODUCTION

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Caliciviruses are usually about 40 nm in diameter, with cup-shaped surface depressions. *Calix* is Latin for 'cup' and refers to these depressions, seen in electron microscope preparations. This family of viruses is generally resistant to heat. The isolated virion RNA is infectious, and the viruses replicate in the cytoplasm of host cells. Currently four genera are recognized. The genus *Vesivirus* includes San Miguel sea lion virus (SMSLV), the vesicular exanthema of swine virus (VESV) and feline calicivirus (FCV), whereas the genus *Lagovirus* includes *rabbit haemorrhagic disease virus* (RHDV) and *European brown hare syndrome virus* (EBHSV). The two remaining genera contain Norwalk-like and Sapporo-like viruses, which are associated with human gastroenteritis. FCV probably infects all species of Felidae.[1]

RABBIT HAEMORRHAGIC DISEASE

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Rabbit haemorrhagic disease (RHD) (synonyms viral haemorrhagic disease (VHD), necrotic hepatitis, rabbit plague, rabbit X disease, rabbit calicivirus disease) is a highly contagious and infectious, acute but occasionally subacute, disease caused by the RHD virus (RHDV), a calicivirus. The only species affected is the European rabbit (*Oryctolagus cuniculus*).

AETIOLOGY

Initially RHD viral identity was unclear but a calicivirus aetiology is now accepted and supported by virus sequencing. RHDV belongs to the genus *Lagovirus*, which is used generically to refer to caliciviruses of lagomorphs. RHDV is antigenically related to, but distinct from, the calicivirus that causes European brown hare syndrome (EBHS), with which it shares approximately 71% nucleotide identity. RHDV is not closely related to the other known caliciviruses. RHDV is a small, round, 32–35 nm RNA virus with typical calicivirus structure and characteristic appearance in electron micrographs. The genome has a single positive-sense RNA strand. RHDV has a major capsid polypeptide (60 Kda). Various RHDV strains from around the world show 89–100% identity and with a few exceptions can be considered as one serotype. There has been no observed attenuation (reduction in viral virulence induced by passage through rabbits). The virus provides an opportunity to study viral evolution and viral acquisition of pathogenic properties. RHDV has *quasi-species* characteristics

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– that is, an array of slightly different RNA viruses, existing as many related but distinct 'strains' that are immunologically cross-protective to some degree. The complex inter-relationship between these is important in the understanding of the pathogenesis and epidemiology of the disease. A selection of strains distinct from the disease-causing strain require a brief mention.

- A smaller, smoother RHDV particle is detectable in some RHD livers and may arise from immunological degradation processes; it is a marker for chronic/subacute disease.
- A rabbit calicivirus related to RHDV was isolated from healthy farmed rabbits in Italy and named rabbit calici virus (RCV). Significantly RCV antibodies are cross-protective to RHDV infection. This virus is not virulent but is present at relatively high seroprevalence and replicates in the intestine rather than the liver. It is similar but not identical to the non-pathogenic RHD-like viruses identified in France and Australia.
- Another RHDV subtype, called RHDVa, causes disease in farmed rabbits but not in properly vaccinated animals. This variant varies from 'classical RHDV' in several amino acid substitutions, which alter the main neutralizing surface epitope.
- RHDV-like viruses; several countries including the UK, Czech Republic, Australia and New Zealand reported RHD antibodies from archived sera from animals sampled before 1984. These antibodies provide variable cross-protection to RHDV infection and in some of these populations may be the reason behind the variably low mortality seen following infection with classical RHDV.

Unlike some caliciviruses, RHD shows high species specificity. Experimental infection studies in a range of wild and domesticated bird species and 13 mammalian species[2], including lagomorphs such as the brown hare (*Lepus europeaus*) have not reproduced the disease. There are no accidental hosts, although foxes and some other natural predator species produce antibody without viral replication.

EPIDEMIOLOGY

Unusually, RHD first emerged as a point disease outbreak in place and time in China in 1984. Here it was seen initially in Angora rabbits imported from Germany a few days after they first arrived in China[3]. In less than 9 months it had spread to rabbitries within an area of 50,000 square kilometres, killing 500,000 farmed animals, and quickly spread to Korea. Independently, RHD was reported in domesticated rabbits in Italy in October 1986; whether it entered Italy from China is not known. By 1988, RHD had spread over large areas of the globe, including much of Europe, Russia, the Middle East and parts of Africa, Cuba, Mexico, USA, Uruguay and India, and in each was identified initially in farmed animals and subsequently in wild rabbits where these occurred. For instance, the disease occurred first in pet rabbits in southern England in 1992 (investigations could not find a likely source), and wild rabbits there in 1994, and by 1996 the disease was relatively widespread in both farmed and wild rabbits throughout Britain and Ireland.

Possible sources of RHDV include: mutation from the EBHS virus; mutation from a pre-existing non-pathogenic virus of rabbits; or transfer of a hitherto unknown virus from another animal species[2]. Complimentary serology results indicate that the second possibility is the more likely. Emergence of pathogenic virus may have occurred, or recurred, on several occasions. There may be a repeating cycle of evolutionary change whereby non-pathogenic, finely host-adapted strains change, probably by mutation, to highly pathogenic strains, and then subsequently change back by mutation to non-pathogenic forms.

The epidemiology of RHD is not well understood and the occurrence of outbreaks cannot be reliably predicted. Several factors are involved at the rabbit population level and include the interplay of the range of virus strains coupled with the presence or absence of respective neutralizing (protecting) antibody populations. Other important epidemiological factors include the presence of protective antibody in metapopulations before the arrival of clinical RHD; the lack of susceptibility of very young rabbits independent of their immunological status; the interplay with myxomatosis; the timing of breeding; and finally, seasonality.

RHD can be expected wherever there are wild rabbits in Europe. The disease has been found in isolated populations, particularly those on offshore islands. Maternal immunity is protective to about 6, or exceptionally 8, weeks of age. By some unknown mechanism not related to humoral immunity, young rabbits up to about 4–6 weeks of age are not susceptible to disease, and this allows a window of opportunity whereby exposure to the virus allows kits, in

the absence of maternal antibody, to develop a lasting immunity[4]. In effect, the innate protection ensures that rabbits in this age class will survive, and this period of juvenile protection therefore reduces the epidemic intensity and enhances subsequent population recovery.

Male and female rabbits are equally affected, and pyrexic females may abort prior to death. The disease in Europe shows variable seasonality and may occur in any month; however, in England it is more frequent in the autumn, whereas in most of France and Spain there is little seasonality. In Australia and parts of Spain rabbit populations in the drier areas appear to suffer long-term population reductions more than those in wetter regions. Vectors are important and wild carnivores and scavenger species, particularly gulls, are an important mechanical vector of infection. Mechanical transport of virus probably explains the stepwise progression of new epidemic fronts, for example with leaps of up to 280 km in England. Insect transmission exists and is the probable reason behind the escape of the virus in 1995 from the Wardang quarantine island, 5 km off the Australian coast, to the mainland and subsequent spread in Southern Australia. Windborne insect transmission from continental Europe may have accounted for an isolated outbreak of RHD on the east coast of England. There are no known reservoir animal species and it would be reasonable to assume that the virus would be removed from a population after it had killed all susceptible animals (fade-out). Viral RNA can be detected in populations after mortality ceases but does not necessarily equate to rabbit carrier status, as RNA may not be infective virus. Virus persistence in the environment occurs – for example, burrow systems may act as environmental reservoirs and rabbits themselves may be passive vectors through fur contamination. Clinical RHD may become almost endemic, persisting for years, owing to the continual circulation of RHDV in some large, heavily infected populations. Reintroduction of virus by the means listed above and by human actions regularly brings virus back into populations. Molecular evidence indicates that RHDV has been introduced to many countries on several occasions often by human activity, e.g. in moving subclinically infected live rabbits and rabbit meat. The rapid replacement of immune rabbits by susceptible animals is facilitated by the high natural mortality of rabbits (60% *per annum*). Mortality is difficult to assess and significant numbers of affected animals die undetected in warrens underground. In Australia RHD caused population reductions of 85% in arid areas, and 73% in coastal areas in year one, decreasing to a 12% reduction by the third year. However, the impact of RHD has declined in many populations over the years since first infection. High-intensity outbreaks in Spain suggested the existence of complete population immunological naïvety, whereas the variable intensity in other countries indicated pre-existing antibody protection.

In Spain RHD can cause two different pictures in adjacent rabbit colonies: one may crash, whereas the other has low mortality. In this country in particular, rabbit population recovery has been variable. The greatest losses in Spanish colonies occurred in areas where rabbits were already in low numbers[5]; when epidemic disease occurred it frequently recurred to the detriment of population recovery. A similar but less noticeable impact was seen in UK populations, where those experiencing heavy initial reductions due to RHD showed a poor recovery whereas populations with low initial mortality recovered well. Initial mortality on Ramsey, a UK marine island, was estimated at 5,000 deaths, with 40 deaths/ha. The outbreak was still active 4 months after onset. Outbreaks recurred on the island for three consecutive years and the population declined by 50% between 1994 and 1998. Further epidemiological postulations and questions that may be relevant, but are difficult to prove, include the following.

- RHDV has circulated in an avirulent form in Britain and possibly Europe for at least 50 years[6].
- Why avirulent RHDV changed to a pathogenic phenotype, what stimulated the change and whether the change was entirely due to viral mutation are not known.
- Serologically naïve rabbits may be infected with RHDV, or RHDV-like non-pathogenic virus, and not show overt disease.

RHD has not had a significant impact on the national wild rabbit population of several northern European countries, including the UK. Over recent years more RHD-like viruses have been identified. These have significance for understanding genome composition, molecular epizootiology and evolution of pathogenicity[7]. It remains possible that the virulence of RHDV for rabbits may not be defined simply in terms of unique genetic determinants of the viral genotype.

TRANSMISSION

Infection is by direct transmission, and indirect transmission facilitated by the stability of the virus in the

environment. Excretions and secretions are infective, as are carcasses and fomites such as bedding material. Infection can occur by oral, nasal, conjunctival and parenteral (insect bite) routes. Viral RNA is present in urine and faeces for up to 4 weeks post-infection in surviving animals. Virus persists well in the environment for up to 1 month at least; viral activity is not reduced by pH 3.0 conditions, and it persists experimentally in rabbit tissues for 18 months and for 100 days in dry cloth. It is stable with freezing (e.g. in frozen rabbit meat). Polymerase chain reaction (PCR) studies have shown RHDV nucleic acid in wild rabbit bones 7 years after an outbreak. Spread of virus in fomites and spread by people (on tools, equipment and vehicles) contaminated with virus is important in disease dissemination. Haematophagous insects, fleas and mosquitoes spread virus mechanically, while the oral and faecal excretions of flies (fly spots) are infectious. Transmission by flying insects has been considered as a reason for epidemics of the disease to 'jump' by large distances ahead of the disease front, disrupting any epidemic front. Spread by scavenger species, particularly birds, over long distances and onto islands also occurs and is important. Death is rapid, and consequently the period of clinical infectivity in the individual is short. Predators, including primary rabbit predators such as foxes and eagles, may excrete infective virus unchanged by digestive processes for short periods in their faeces, thereby potentially facilitating long-distance viral dissemination.

PATHOGENESIS, PATHOLOGY AND IMMUNITY

The incubation period is 1–3 days and death usually occurs 12–36 hours after onset of clinical signs. The clinical disease can be peracute, acute, subacute or, rarely, chronic. Infection only occurs in rabbits older than 40–50 days; the mechanism for this resistance is not known, but humoral immunity is not involved. Possibly implicated is a combination of lack of viral receptors in cells of the digestive and respiratory tracts and the innate immune physiology of the neonate.

Following entry of the virus into the rabbit body and infection through the mucosae of the respiratory and digestive tracts, there is limited local spread, primary replication and then haematogenous spread to the principal target organ, the liver. Replication of the virus in hepatocytes leads to cell death and apoptosis, presumably with local spread to neighbouring liver cells (Figure 5.1) and further hepatocyte necrosis (Figure 5.2), and ultimately to liver failure. The virus may also be isolated from the spleen (Figure 5.3), and occasionally from other organs. Most rabbits develop terminal disseminated intravascular coagulation (DIC) and shock. Occasional abortions are probably related to pyrexia, as viral infection of placental tissue and fetal tissue have not been demonstrated.

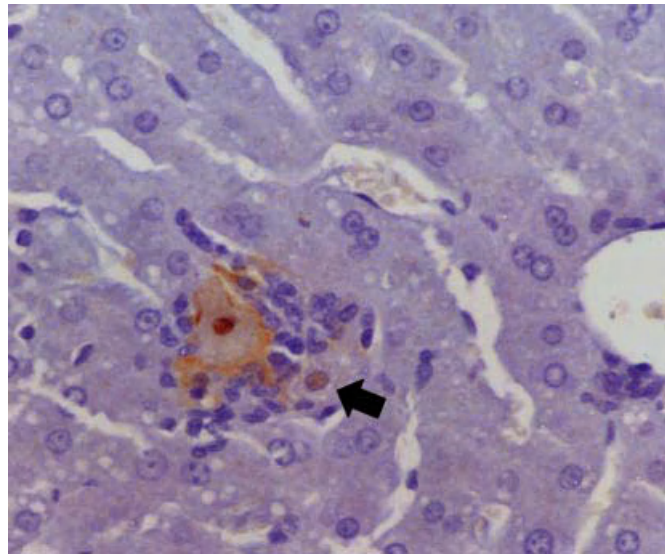

FIGURE 5.1 Histological section of liver of a rabbit 24 hours after oral inoculation with RHDV. A single hepatocyte is surrounded by inflammatory cells. Viral antigen (reddish brown coloration) is within the nucleus and on the cell membrane of this hepatocyte. Note the presence of antigen in the nucleus of an adjacent hepatocyte (arrow). Immunoperoxidase with primary monoclonal antibody to RVHD-virus. Photo: D. Gavier-Widén, SVA.

Humoral antibodies are frequently produced despite the rapid progress of clinical disease (antibodies are even detectable at 5–7 days post-infection, A. Lavazza, personal communication). Young rabbits with innate resistance to RHDV may develop immunity on exposure. It is assumed that surviving rabbits have a long, possibly life-long, immunity to RHDV. It is assumed that 'fitness', as assessed by other methods, offers no effective protection to RHD infection.

As the course of the disease is rapid, animals found dead are usually in good condition. RHD gross pathology is variable, lesions may be subtle and there are no consistent characteristic findings, belying the acute nature of the disease and extensive liver necrosis that causes death. In peracute disease no obvious gross lesions may be apparent.

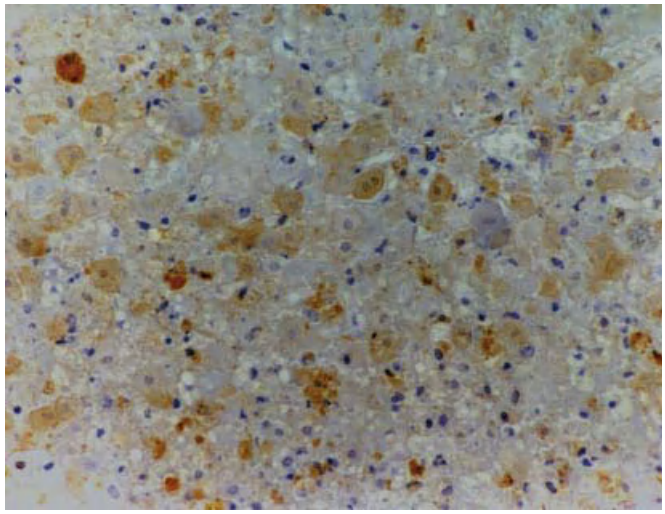

FIGURE 5.2 Histological section of liver of a rabbit that died 48 hours after oral inoculation with *rabbit haemorrhagic disease virus*. Viral antigen (brown coloration) is within the nucleus and cytoplasm of hepatocytes and along their cell membranes, in necrotic hepatocytes, debris and in a macrophage. Immunoperoxidase with primary monoclonal antibody to RHDV. Photo: D. Gavier-Widén, SVA.

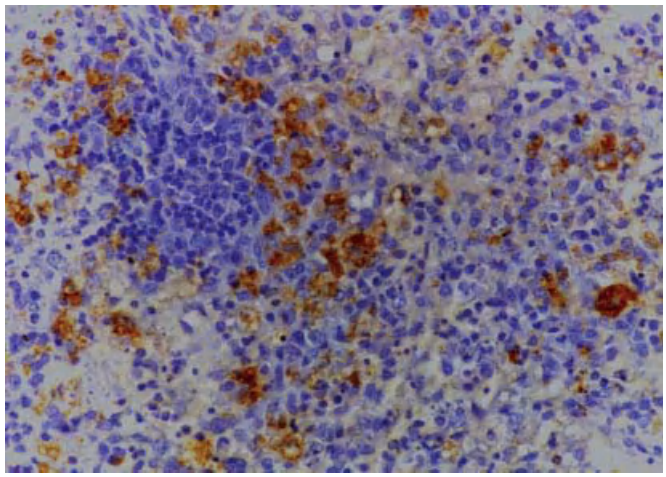

FIGURE 5.3 Histological section of spleen of a rabbit euthanized 4 days after oral inoculation with RHDV. Viral antigen is in the cytoplasm of macrophages. Immunoperoxidase with primary monoclonal antibody to 60 kD capsid protein of RHDV. Photo: D. Gavier-Widén, SVA.

The acute form is most frequent, with hepatosplenomegaly[7]. The liver is often a yellowish colour, and lobule necrosis may give rise to a reticular pattern. Haemorrhagic carcasses are relatively infrequent (the disease name is somewhat of a misnomer) and the variable presence of petechial and ecchymotic haemorrhage in various organs, pulmonary oedema and thoracic transudates and the presence of blood without clots have two causes: DIC and coagulopathy. However, haemorrhage, tissue congestion due to DIC and shock are not specific to RHD. Four lesions, although not specific for RHD, are reasonably consistent: i) liver changes as noted; ii) intense, sometimes violaceous, congestion of the tracheal mucosa often associated with copious tracheal froth; iii) marked congestion of the spleen; iv) variable lung changes, which may include haemorrhage, patchy congestion and thoracic transudates. There may also be blood-stained froth and blood clots around the nares and a mild catarrhal enteritis.

Histopathology reveals distinctive changes in the liver characterized by extensive (massive) liver necrosis, congestion and haemorrhages in animals that die peracutely to zonal hepatic necrosis, typically periportal and of variable extension in acute and subacute disease. Hepatocyte destruction may include nuclear pyknosis and karyorrhexis. Inflammatory infiltrates are moderate and consist of lymphocytes in portal spaces and sinusoids. There may be early calcification in survivors. There may be microthrombi and extensive haemorrhages in the lungs and kidney and lesions in the spleen vary from congestion to lymphoid necrosis associated with lymphopenia. Chronic disease pathology is putative and very infrequent if it does occur, and apart from non-specific mild icteric coloration of blood vessels the rare survivors do not have residual lesions.

CLINICAL SIGNS AND TREATMENT

Disease may be peracute with no clinical signs seen early in the epidemic and animals are found dead; acute (most frequent) and subacute disease show milder signs. Acute infection may produce clinical signs for 12–36 hours, with fever (>40°C), dyspnoea and epistaxis (Figure 5.4A) although bleeding from orifices is not always seen and not pathognomonic. Nervous signs, primarily as a result of hepatic encephalopathy, are frequent and include depression (Figure 5.4B), convulsions, incoordination, fitting, opishthotonos and vocalization (shrill screaming). In large outbreaks some rabbits are subclinically affected, and jaundice is reported in these before death[7].

There is no effective treatment for domesticated or wild animals. Hyperimmune sera may have a role in protection in the face of an outbreak but is probably impractical.

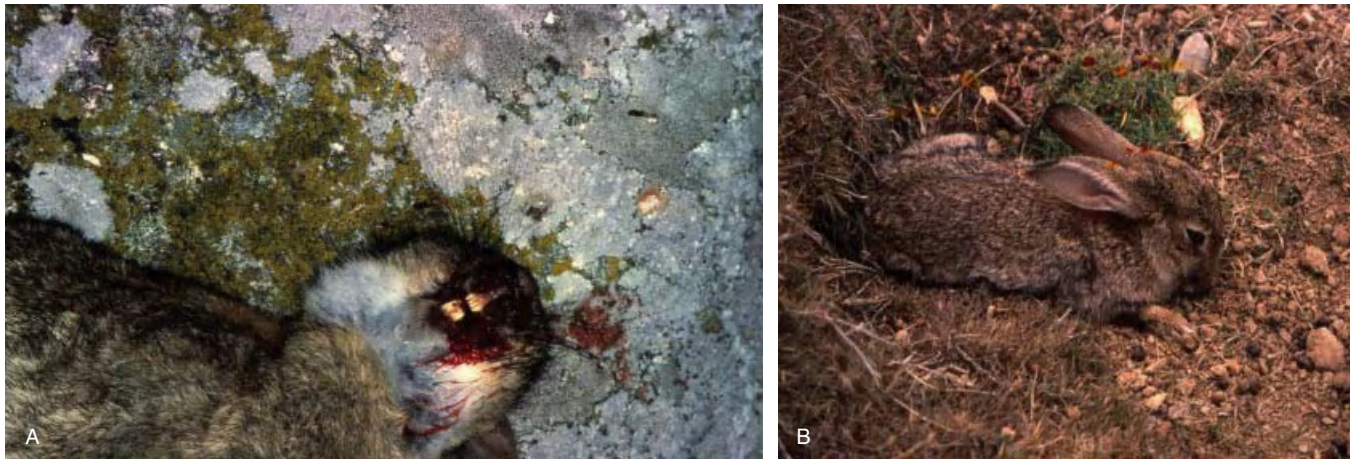

FIGURE 5.4 Wild European rabbits that died during a confirmed RHD outbreak on Ramsey Island, Wales. **A**. One dead animal shows bleeding from the nose, a clinical feature of some, but not all, RHD cases. **B**. The other rabbit shows profound depression, lying in abnormal recumbency in the open during daylight. Photo courtesy Roger Trout, Rabbit Wise, Hants, UK.

DIAGNOSIS

For detection of the virus the liver should be examined, as it contains the highest viral titre. *In vitro* isolation by virus culture has never been achieved. A haemagglutinin test utilizing human erythrocytes (type O) has been used; however, electron microscopy, enzyme-linked immunosorbent assay (ELISA), western blot, PCR and immunohistochemistry (to demonstrate RHD antigen in hepatocytes and splenic histiocytes) are more sensitive tests. Experimental infection of domestic (seronegative) rabbits is not usually necessary but can be employed in cases where diagnosis is equivocal

For serology, the tests most frequently used are haemagglutination inhibition or ELISA. Owing to the acute nature of the disease, early deaths may be caused by high titre viral infection in the absence of detectable antibody, and at the other extreme is the possibility of chronic low viral titre cases in the presence of antibodies (especially IgM). The diagnosis of RHD should be based on a range of observations from clinico-pathology and laboratory tests to histopathology. Clinical history of mass mortality in a rabbit warren affecting adult rabbits but not neonates over a period of up to 6 weeks should arouse suspicions of RHD. Myxomatosis in wild rabbits in Europe may occur concurrently. Histopathology will confirm the diagnosis on the basis of characteristic liver lesions in the majority of animals. Laboratory confirmation was based for years on electron microscopy, where massed ranks of calicivirus are seen in typical cases; however, ELISA and western blot methods are now more reliable. PCR should be considered the diagnostic method of choice, and this test can detect virus in tissues from rabbits that are severely decomposed or skeletal. Some caution with PCR results should be exercised, however, as positive livers may indicate viral contamination from other sources, or the animals could have a putative chronic form of disease in which lesions are in remission and death is caused by other causes. Population serology is important for epidemiological analysis[3]. Survivors of outbreaks also show seroprevalence, which is difficult to interpret, although the use of ELISA serology tests to differentiate serotypes is informative.

MANAGEMENT, CONTROL AND REGULATIONS

There is no effective treatment for RHD in domesticated or wild animals. Humane euthanasia should be considered for affected individuals.

There are no current EU regulations for RHD. The disease in domesticated and wild rabbits is reportable to the World Organisation for Animal Health (OIE). RHD was a notifiable disease in both wild and domesticated rabbits in some European countries, as in the UK between 1996 and 1998 but was denotified in the UK (and New Zealand) when it was no longer feasible to contain and eradicate the virus. The virus is generally resistant to many commonly used disinfectants however it is inactivated with 10% sodium hydroxide and 1.2% formaldehyde.

RHD is transmissible between wild and domesticated rabbits by direct and indirect methods, and it is therefore a trade-sensitive disease of importance in the many areas of the world where rabbit farming is commercially significant. RHD in domestic colonies and laboratory populations is controlled by very strict biosecurity and regular vaccination. These methods must be under regular review, as inadvertent introduction of the virus may easily occur.

Vaccination in wild colonies is impractical at present. A simple killed formalin-treated, adjuvanted vaccine is commercially available, providing protection for 6–12 months. Recently, a novel bivalent vaccine utilising a recombinent virus consisting of a laboratory-attenuated myxoma virus strain which has the capsid protein gene of RHDV inserted has become commercially available in Europe, providing 12 months protection against both RHD and myxomatosis[8]. In Spain, rabbits are a valuable species for hunters, and there have been initiatives to develop a recombinant vaccine attached to a live non-pathogenic virus (poxvirus) that would spread vaccine-derived non-pathogenic virus to a limited number of rabbits in contact with the vaccinated individuals. To date, however, this has not been licensed or used in the field. There is some evidence that RHDV strains may persist in regularly vaccinated domestic rabbit populations, although there is no obvious vaccine failure and several factors may be involved in this observation. RHD was eradicated in Mexico, where it was introduced in one shipment of rabbit meat from China; this was probably a result of the absence of wild European rabbits in that country. By contrast, eradication was not possible in Europe when RHD became firmly established in the natural reservoir population[3].

Management of RHD in wild populations globally can have opposing goals: in areas where RHD is used as a means of biological control of pest populations compared with areas in which control of RHD is sought to reverse population declines. Deliberate release of the virus as a form of biological control appears to vary in effect, depending on environmental conditions, time of release, bait quality, host factors, population size and climate. The results of biological control also depend on the presence of non-pathogenic or silent forms of the virus that may be circulating. Humans have had a significant direct and indirect role in transmission of RHDV in wild and domesticated populations in deliberate, identified ways, and covert, or accidental, ways.

PUBLIC HEALTH CONCERN

No other species, including humans, have been reported to be clinically affected by RHDV. Therefore the risk to public health from RHD viruses of currently known genomes is considered negligible. There is public concern when RHD mass mortality incidents occur, particularly if bodies are visible, and these frequently prompt requests for diagnosis and health assurance. No evidence has come to light to suggest that the virus crosses species barriers or that it infects people – for example, there was no serological response in 259 people occupationally exposed to the virus.

SIGNIFICANCE AND IMPLICATIONS FOR ANIMAL HEALTH

RHD is a major concern for rabbit breeders globally, and any colony of *O. cuniculus* no matter how geographically isolated could be infected. In the nearly 30 years since the first report of RHD the disease has caused significant animal losses throughout the world where rabbits are reared (China 140 million deaths, Italy 64 million deaths) and in wild populations. The losses in farmed animals have now been controlled by the regular use of commercial vaccine.

The European rabbit originated in the Mediterranean areas of Southwest Europe, where wild populations are currently in decline. In Spain the decline is considered to be multi-factorial in nature, related to habitat degradation, myxomatosis and RHD; however, since the arrival of RHD in 1988 populations have not recovered[5]. This led indirectly to increased hunting pressure on wild predators of rabbits. The combined effects of myxomatosis and RHD are significant in rabbit populations endemically infected with both viruses, but the complex synergy is not fully understood[4]. In Spain the rabbit is also obligatory prey for predators, including two critically endangered species, the Iberian lynx (*Felis pardina*) and the imperial eagle (*Aquila adalberti*). Conservation of rabbit populations here is the subject of renewed initiatives.

This is contrasted with the ecological picture in Australia, where the rabbit continues to thrive in suitable dry areas of the continent despite the introduction of RHD. Here the rabbit, introduced nearly 200 years ago, still has a deleterious effect on the environment and endemic fauna. By contrast, in Europe, hunters should be

advised to limit their control activities until after outbreak mortality ceases. Translocation of live rabbits and moving rabbit carcasses and viscera is a risk for dissemination of virus and should be avoided, as the effects of the disease introduction to new wild populations are entirely unpredictable. In the UK outbreaks are sporadic with highly variable impact on the rabbit populations, and in a few localities the decline in rabbit population has affected sensitive habitats, e.g. chalk grasslands and their threatened flora and fauna.

There is a potential untested threat from RHD to the world's rarer lagomorph species.

ACKNOWLEDGEMENT

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EUROPEAN BROWN HARE SYNDROME

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European brown hare syndrome (EBHS), also named leporid caliciviral hepatitis, is a calicivirus infection of free-living and farmed hares characterized by acute hepatitis.

AETIOLOGY

The *European brown hare syndrome* virus (EBHSV) belongs to the genus *Lagovirus*, family *Caliciviridae*. The virus is closely related antigenically to the rabbit haemorrhagic disease virus (RHDV), which causes similar hepatitis in domestic and wild rabbits (*Oryctolagus cuniculi*) and with which it shares epitopes of the major capsid protein gene (VP60). No cross-infection between the hare and rabbit disease occurs, probably because of the low homology between the respective EBHSV and RHDV. EBHSV has similar morphology and biochemical properties to RHDV; however, neither can be cultivated *in vitro*.

The EBHSV has been cloned and its genome has been fully sequenced. It was shown to be 7442 bases long, to have a nucleotide identity with RHDV of 71% and to have a similar genomic organization[9]. The EBHSV has evolved since the late 1980s, and new isolates have been identified in Europe.

EPIDEMIOLOGY

GEOGRAPHICAL DISTRIBUTION AND HOSTS

EBHSV appears to be highly host-specific, and infection is restricted to hares of the genus *Lepus*. It affects free-living and farmed European brown hares (*Lepus europaeus*) and mountain hares (*Lepus timidus*), and there is one report of EBHS in captive *Lepus capensis* in Belgium[10]. It is not known if other *Lepus* species are susceptible to EBHSV infection. EBHS has not been described in the published peer-reviewed literature in other species. Experimental infection of rabbits with EBHSV results in the development of low levels of antibodies, which do not protect against infection with RHDV. Similarly, infection of hares with RHDV results in a mild antibody response, which does not protect against EBHSV infection.

EBHS was first described as a new epidemic disease of unknown cause in the early 1980s in Sweden. Its viral aetiology was identified in 1988 by electron microscopy of liver homogenates of hares that had died from EBHS, which revealed viral particles that resembled those observed in the liver of rabbits with RHD[11]. EBHS occurred in Europe long before the emergence of RHD. Retrospective studies on archived material and old reports showed that European brown hares in England had antibodies to EBHSV as early as the 1960s[12], showed clinical disease and gross pathology consistent with EBHS (but unconfirmed by histopathology and virology) in 1982 and had first confirmation by histopathology in 1990[13]. EBHSV was identified by PCR on paraffin sections from the 1970s in Sweden. EBHS occurs today in many European countries, often endemically in free-living hares. EBHS has been reported in Belgium, Denmark, Finland, France, Greece, Slovakia, Sweden, Italy, Germany, UK, Croatia, Austria, Spain, Czech Republic, Poland and Switzerland. EBHS has not been detected outside Europe, except for a report in Argentina. Many seroprevalence studies on hare populations in various European countries have been conducted, showing a wide range in prevalence, from about 11% to up to 95%.

EBHSV is highly contagious and frequently causes short outbreaks of mortality. Mortality rates are high when the outbreaks occur for the first time in the area and the population of hares is immunologically naïve. In endemic

areas, where the majority of the hares have antibodies, cases are sporadic or mortality rates are low. Further exposure of immune hares to EBHSV results in boosting the antibody response. EBHSV may spread to new areas, often contiguous to endemic areas, causing new mortality events[14]. Clustering of cases in areas of more intensive agriculture, where the population density of European brown hares is higher, has been observed[15].

The time sequence of mortality in free-ranging hares was described in England: between 4 and 50 hares were found dead each week over a 3-week-period. The average number of deaths per incident was 28. In some cases mortality continued for 3–6 weeks[13]. In one event, approximately 100 hares died in a period of several weeks; this was estimated to be 60% of the population. More incidents were observed in areas with high population density[13].

Longitudinal studies in various regions of Europe have described seasonal clustering of EBHS mortality, but the seasons are different and cases of EBHS may occur all year round. For example, mortality was observed to be highest in the late autumn (October, November and December) in Sweden, coinciding with the period of highest hare population density in the year, and with a high proportion of hares' young-of-the-year, which have reached the age of susceptibility for infection[15] (see host factors below). Similar higher EBHS mortality during the autumn has been observed in England, France, Italy and Denmark. Highest mortality was observed in mountain hares in Finland in the spring and summer[14].

No wildlife reservoirs of EBHSV are known. It has been speculated that European brown hares that survive the infection act as reservoirs. However, maintenance of EBHSV endemically has also been recorded in populations of mountain hares in areas where brown hares are scarce[14]. In EBHS endemic areas, a small proportion of healthy hares are subclinical virus carriers, for example in one study in Italy, this was shown to be about 1%, in a population with a seroprevalence 67% and 78% in two consecutive years[16], and in a study in Poland it was 7.6% in a population with a seroprevalence of 38%[17].

EBHS is an important disease of farmed hares in Europe. The mortality varies from 10% to 100%. Mortality is high in the initial outbreak on farms, but often only about 10% of the hares succumb to EBHS in subsequent years. This is likely to be a result of the development of protective antibodies following the first exposure. Repopulation of hares in a farm 3–4 weeks after an outbreak, to replace the hares that had died, resulted in death of the new hares in 1 to 2 weeks after introduction. The source of the infection, whether carrier hares that had survived or the contaminated environment, was not known.

MOLECULAR EPIDEMIOLOGY

A French study on nucleotide sequencing of the partial capsid protein genes from EBHSV from different parts of the country between 1989 and 2003, and in comparison with a Greek strain, showed high level of conservation between EBHS viruses – the maximum nucleotide divergence was 11.7%[18]. The study revealed two major groups and several genogroups, and showed that old strains of EBHSV persist and evolve slowly in the locality of their origin. Several different genogroups co-exist and there is apparently an efficient dissemination of new genogroups[18].

The emergence of EBHS is not understood. It has been postulated that low or apathogenic EBHSV strains might have occurred in Europe before the disease was detected. A phylogenetic study indicated that EBHSV originated in Sweden and spread to western, eastern and southern parts of Europe[19]. Increasing numbers of EBHSV strains are being identified by molecular investigations; 24 strains are presently known[19].

HOST FACTORS

Early studies in Sweden diagnosed EBHS in 275 out of 2818 European brown hares and mountain hares examined by histopathology between 1980 and 1989. The majority (70.5%) of the EBHS cases occurred in adults (hares with fully developed gonads), and the rest in subadults (hares whose gonads were not fully developed); no cases were found in lactating hares. According to the anamnesis, 88.2% of the hares were found dead, 5.9% were moribund and euthanized and 5.9% showed abnormal behaviour and died after a short clinical course.

It was later confirmed in several countries that EBHS mortality occurs only in adult and subadult hares. No cases are described in hares younger than about 50 days, either naturally exposed (during outbreaks) or experimentally infected, indicating that they are resistant. The mechanisms of the age-related resistance are not known. Males and females appear to be equally susceptible to EBHS.

Genetic analysis of hares to detect possible familial susceptibility to EBHSV identified 15 different matrilinear

hare haplotypes, but they were not correlated to occurrence of EBHSV or antibodies[19].

TRANSMISSION

EBHSV is transmitted directly or indirectly. The virus is shed in faeces (probably transported from the liver, through bile into the intestine) and hares can be infected experimentally by the oral route. It is believed that the most important route of infection is the faecal-oral, and possibly the respiratory, but no specific comparative studies on susceptibility to infection by various routes and viral doses have been conducted. EBHSV is also considered to be transmitted via fomites.

PATHOGENESIS, PATHOLOGY AND IMMUNITY

Little is known about the pathogenesis of EBHS. Most experimentally infected hares die 2–3 days post-infection (dpi); some hares die later, up to about 7 dpi, and some hares survive. The liver is the main target organ, but the virus disseminates systemically. In terminal stages, hares develop disseminated intravascular coagulation and die with circulatory collapse, showing congestion in the lungs and other organs.

Characteristic gross lesions are fatty and friable liver, and signs of circulatory collapse, such as lung congestion and oedema, congestion of the mucosa of the trachea (Figure 5.5), and an enlarged and congested spleen. Stomachs well filled with ingesta and catarrhal enteritis in the small intestine, with thick mucus in colon and rectum, are often observed. There may be haemorrhages in any organ, but frequently on the mucosa of the stomach and in the lungs[15]. Signs of jaundice are observed in about one-third of the cases and are particularly frequent in subacute cases (Figure 5.5).

Histologically, in most cases, the acute development of liver lesions reflects the rapid course of the disease. The predominant, consistent and characteristic lesion is periportal hepatocellular coagulation necrosis (Figure 5.6), which coincides with the localization of EBHSV in hepatocytes in those areas. To the knowledge of the author, no other diseases cause this particular zonal liver necrosis in hares. The reason for the preferential periportal distribution of lesions and virus is not known. While liver necrosis occurs in all hares that die of EBHS, its extent varies, from a small periportal area (Figure 5.6), to the whole lobule (massive necrosis). There is also lytic necrosis with a loss of hepatocytes, leaving a few heterophils in an empty reticulin framework. In the peracute form there is massive liver necrosis, haemorraghes and scant or no inflammatory infiltrate. Viral antigen is located in the nucleus and cytoplasm of hepatocytes. In subacute cases there is periportal hepatocellular necrosis, fatty degeneration, infiltration of

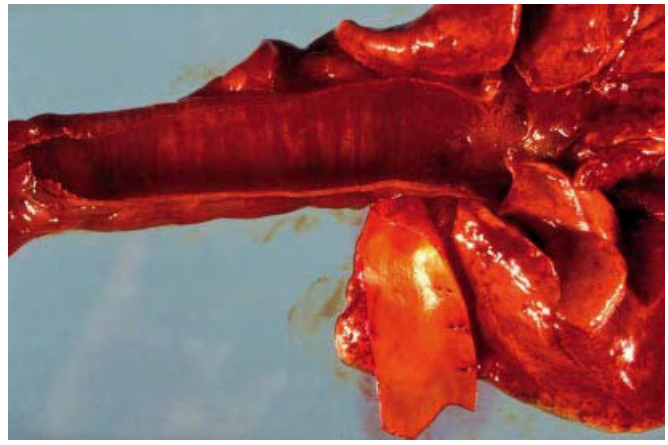

FIGURE 5.5 Lung, trachea and aorta of a hare with EBHS. Lung congestion and haemorrhages and congested tracheal mucosa. Froth in the opening of bronchi reflect lung oedema. The aorta has been opened to show yellow discoloration indicative of jaundice. Photo: Bengt Ekberg, SVA.

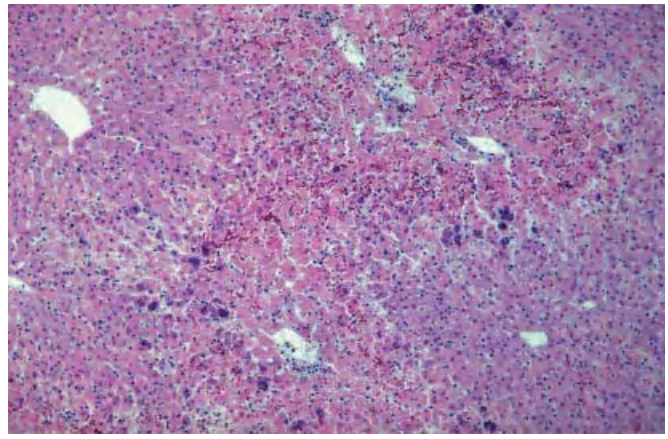

FIGURE 5.6 Histological section of liver of a hare with acute EBHS. Hepatocellular coagulation necrosis of periportal areas. Centrolobular (areas around the central veins) are spared and unaffected by the necrosis. Dystrophic calcification of hepatocytes is present. Inflammatory cell infiltrate is minimal. Photo: D. Gavier-Widén, SVA.

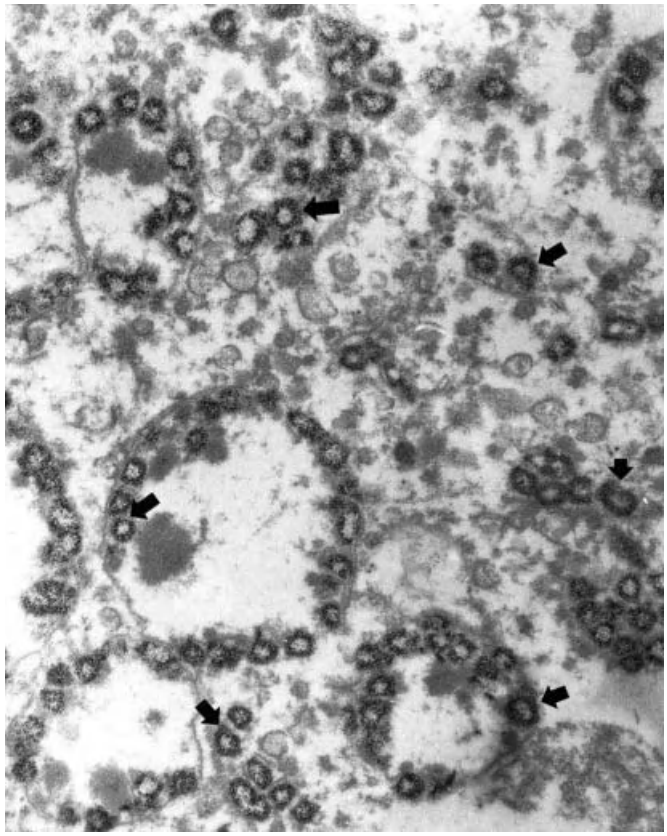

FIGURE 5.7 Electron micrograph of one hepatocyte liver of a hare with European brown hare syndrome. Mitochondria in the hepatocyte contain amorphous densities in the centre and large amounts of calcium as hollow spherules (arrows). Enlargement 9,000×. Photo: D. Gavier-Widén, SVA.

inflammatory cells and presence of virus in hepatocytes, which is more abundant in areas contiguous to the necrosis. Viral antigen is also present in the cytoplasm of macrophages (Kupffer cells); in cases with a longer clinical course of disease this is the predominant localization of virus. Acidophilic bodies, formed by condensation of degenerating hepatocytes, are often observed in sinusoids[15]. Granules of dystrophic calcification can be abundant in hepatocytes (Figure 5.6). They are formed by mitochondrial mineralization (Figure 5.7), and can be demonstrated with von Kossa staining in liver sections, even in autolysed cases. Chemical analysis of these livers reveals that the content of calcium is up to 20 times higher than that in normal hares[15]. With a longer clinical course, there is more marked inflammatory infiltration, bile duct proliferation and hepatocytic regeneration, manifested by the presence of binucleate hepatoctes. A small proportion of the hares develop chronic hepatitis and harbour virus in the liver, although at lower levels than in acute cases, for a period of unknown length.

About one-third of the hares develop kidney lesions, with tubular cell degeneration, necrosis and in some cases mineralization. There is lymphoid cell depletion or reduction in lymphoid organs, such as the spleen and lymph nodes[15].

An antibody response to EBHSV develops early after infection, at about 5 dpi. Hares that survive apparently develop life-long immunity, but the course of antibody levels during long periods of time are not known. Newborn hares may have antibodies to EBHSV, indicating passive transfer of immunity. Young hares (younger than about 50 days) with no maternal immunity that are exposed to the virus will develop antibodies but not clinical disease.

CLINICAL SIGNS AND TREATMENT

Hares may die peracutely without signs of disease (Figure 5.8). In cases with a longer clinical course of disease the hares may show nervous signs, which are probably the result of hepatic encephalopathy. The hares frequently lose fear of people, run in circles, are apparently blind, show loss of balance, opisthotonus, adopt abnormal postures, and terminally cramp and lie in prostration[15]. The hares die about 1–7 days after the first manifestations of clinical signs.

Experimentally, hares develop leucopenia by 24 hours post-infection, with values as low as 500 leucocytes per microlitre. Enzymes that indicate liver damage (aspartate aminotransferase and alanine aminotransferase) and bilirubin increase rapidly by day 4 post-infection, shortly before death. These changes are less marked in hares that survive the infection and reach normal values at day 10 post-infection[20]. Analysis of urine shows proteinuria, casts and haemoglobinuria[15].

Treatment is normally not feasible, but antiserum given early in the incubation period may prevent death.

DIAGNOSIS

Gross lesions are not always observed. The histological features of the liver (periportal necrosis) are characteristic of EBHS but are difficult to identify in cases with autolysis. To detect virus, the liver is the organ of choice, as it

FIGURE 5.8 European brown hare syndrome outbreak; dead adult brown hares collected in one day from an estate in southern England in October 1991. Photo: J. Paul Duff. AHVLA. Disease of Wildlife Scheme.

has the highest titres, but the spleen often contains EBHSV. The virus can be demonstrated by immunohistochemical or immunofluorescent staining of liver sections, and by haemagglutination (human type A or O red blood cells at pH 6.4 and at 4°C), negative staining electron microscopy of liver homogenate and western blot. Several antigen-detection ELISA methods have been developed and used widely. Immunocapture reverse transcriptase PCR (RT-PCR), based on genomic amplification of the virus captured on a plate coated with anti-EBHSV antibodies has been reported as a rapid method of high sensitivity. Several PCR techniques have been developed for the detection of EBHSV nucleic acids in fresh or formalin-fixed, paraffin-embedded tissues.

Serological tests are usually ELISA methods. Antibody detection ELISA can be conducted on blood collected on blotting paper, but with lower sensitivity than on serum. Haemagglutination inhibition can also be used for serology. Antibodies to EBHSV and RHDV show a low level of cross-reaction.

MANAGEMENT, CONTROL AND REGULATIONS

Eradication of EBHS in free-living hares is not feasible. Most hare restocking areas in Europe have endemic EBHSV, and translocation or import of hares from these areas into free areas should be avoided. In hare farms the safeguards for the prevention of virus entry include quarantine, serologic testing of new hares before introduction to the farm, and fencing to avoid contact with wild hares, using processed feeds rather than field-collected grass. Indirect infection through contaminated hay, or clothes and shoes should be avoided. Autogenous killed virus vaccines can be prepared at the beginning of an episode of EBHS mortality in farms to vaccinate the still-healthy hares.

PUBLIC HEALTH CONCERN

EBHSV does not affect humans.

SIGNIFICANCE AND IMPLICATIONS IN ANIMAL HEALTH

EBHS can have a significant impact during initial outbreaks or in sporadic outbreaks, and local declines of hare populations have been attributed to EBHS. Some reports describe recovery of populations within a few years because of the high reproductive rate of hares and the immunity acquired by the population resulting in lower EBHS mortality. EBHS was the most common disease of mountain hares in regions of Finland[14] and over much of Europe it remains a frequent disease of the European brown hare. EBHS causes significant losses in hare farms.

CALICIVIRUS INFECTIONS OF MARINE MAMMALS

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The San Miguel sea lion (SMSLV) virus, first isolated in 1972, is a calicivirus indistinguishable from the vesicular

exanthema virus of swine (VESV) and is also thought to infect marine fish such as the opal eye perch (*Girella nigricans*). SMSLV has not been detected in terrestrial European wild mammals.

Several caliciviruses have been demonstrated in cetaceans, including European cetaceans. Most are variants of the SMSLV. Cetacean calicivirus has been isolated from vesicular and ulcerative skin lesions in two Atlantic bottlenosed dolphins (*Tursiops truncatus*)[21].

Parapoxviruses and caliciviruses were detected by electron microscopy of skin lesions in two grey seal (*Halichoerus grypus*) pups from Cornwall, UK in 1991. The lesions were raised nodules 10–20 mm in diameter, mostly in the region of the sternum. The animals were taken into captive care, where the skin lesions increased in size and distribution. Both animals were eventually euthanized, and it was concluded that the caliciviruses were not playing a significant pathological role[22].

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CHAPTER

6

LYSSAVIRUS INFECTIONS

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The disease resulting from infection by a lyssavirus is called rabies in any animal species (*Rage* in French, *Tollwut* in German, *Rabia* in Italian and Spanish). The major clinical signs are related to an encephalomyeloradiculitis. The epidemiological situation of rabies in Europe is still a public health concern (from several EU neighbouring countries and a few new member countries of the EU). In Western Europe, the threat to public health was considerably mitigated during the 20th century owing to vaccination of domestic or wild animals. Nevertheless, rabies remains a significant health risk and a re-emerging disease. The disease still occurs sporadically as a result of introductions of animals incubating the virus, and some strains are prevalent among a small number of bat species. Moreover, within the EU borders, rabies remains enzootic in red fox (*Vulpes vulpes*), raccoon dog (*Nyctereutes procyonoides*) and dog (*Canis familiaris*).

AETIOLOGY

Rabies viruses (RABV) belong to the *Mononegavirales* order, *Rhabdoviridae* family and *Lyssavirus* genus. Lyssaviruses have a non-segmented RNA genome of negative polarity encoding five viral proteins (3′ to 5′): nucleoprotein N, phosphoprotein P, matrix protein M, glycoprotein G and polymerase L. The virion has a bullet-shaped form, 100–300 nm in length and 75 nm in diameter. Rhabdovirus infections have been detected in insects, mammals and plants. The lyssaviruses, which comprise rabies viruses, are adapted to replicate in the mammalian central nervous system. Historically, one viral species was believed to cause rabies; however, serological, antigenic and genetic methods have now demonstrated that there are at least 11 different viral species or genotypes. Bat lyssaviruses are probably the evolutionary ancestors of all the currently described lyssaviruses.

Only three genotypes are enzootic in Europe: the dog, fox and raccoon dog virus strains, which are closely related (genotype 1), and two groups of bat viruses, one isolated mainly from the serotine bat (*Eptesicus serotinus*) and identified as EBLV1 (European bat lyssavirus, genotype 5), and one from *Myotis* bats (*M. dasycneme* and *M. daubentonii*), named EBLV2 (genotype 6). Occasionally, other genotypes may be found in Europe following importation of animals from other regions of the world. Recently, a new lyssavirus (Bokeloh bat lyssavirus (BBLV)) was discovered from a Natterer's bat (*M. nattereri*) in Germany.

EPIDEMIOLOGY

Lyssaviruses are zoonotic infections that invariably spill over into non-maintenance hosts (such as humans, rumi-

Infectious Diseases of Wild Mammals and Birds in Europe, First Edition. Edited by Dolores Gavier-Widén, J. Paul Duff, and Anna Meredith.

nants and cats). Onward transmission within these dead-end hosts does not occur, so the successful transmission of RABV in new host species is likely to represent a major adaptive challenge. This is, in part, a reflection of the strong selective constraints that act on RABV, resulting in a high rate of deleterious mutation and hence in relatively low rates of non-synonymous substitution (nucleotide substitutions leading to amino acid changes), including at sites that might potentially enhance fitness. At a higher level, both the N and G gene phylogenies indicate that viruses sampled from other species of the family Canidae, such as foxes and raccoon dogs, as well as hosts belonging to other families within the Carnivora, e.g. the *Herpestidae* in southern Africa, are included in the phylogenetic diversity of dog RABV. Although no significant evidence for adaptive evolution has been found, recent studies strongly suggest that the dog has served as the main bridge for inter-species RABV liaison, generating viral lineages that then spread to other taxa[1]. Determining the genetic basis of the traits that govern cross-species transmission clearly represents a major goal for future research on RABV, although it is worth noting that patterns of cross-species transmission may also be influenced by the ecological factors that shape host contact rates.

GEOGRAPHIC DISTRIBUTION IN EUROPE

Rabies caused by RABV is widely distributed throughout the world and is present in all continents except Australia and Antarctica. In Europe, canine rabies has persisted for centuries. By the beginning of the 20th century, strict implementation of hygienic measures (dog supervision, feral dog control, mandatory carrying of muzzle when appropriate) resulted in the virtual disappearance of dog-mediated rabies, but the disease re-emerged in a focus south of Kaliningrad during World War II. The red fox was identified as the main reservoir for the virus, and this species, in subsequent decades, was responsible for the widespread dissemination of the disease. By the mid-1970s, fox rabies (also referred to as sylvatic or wildlife-mediated rabies), affected almost all countries within Western and Southeastern Europe. The incidence of fox rabies peaked during the early 1980s with around 16,000 to 25,000 annual cases and at this time the disease also reached its most widespread geographical distribution. Nevertheless, large regions in Europe have never experienced cases of fox rabies, or have had a long history of freedom from the disease. In addition, as a result of the oral rabies vaccination (ORV) of wildlife, since the late 1980s, the incidence of rabies in other parts of Europe has decreased dramatically. Within the last 25 years, fox rabies has been virtually eliminated in Western Europe and substantially decreased in Central European countries. At present (Figure 6.1), endemic areas are mainly restricted to the eastern and southern parts of Europe (the Baltic countries, Belarus, Russia, Ukraine and Moldavia) and the Balkan region. However, the recent emergence, or re-emergence, of fox rabies clearly demonstrates that this apparent steady decline in the number of cases is not inevitable: Italy, for instance, having been rabies-free, had cases of fox-associated rabies in 2008–2010, originating from the endemic regions of the Northwest Balkans.

Rabies in raccoon dogs predominantly occurs in north-eastern parts of Europe, e.g. the Baltic countries, Belarus, Russia and the Ukraine, and it is linked to a high population density of this host species, and to a specific variant of the fox rabies virus.

Dog-mediated rabies (synonym 'canine rabies') in Europe still persists in Turkey. At present, it is unclear whether a dog rabies strain still exists in dogs in regions of Eastern Europe such as Russia (where RABV infections are notified in dogs and other domestic animals). In addition, sporadic cases of dog rabies are periodically reported in rabies-free regions as a result of importation of dogs incubating the disease from rabies-endemic countries. In some of these introductions of RABV by a single infected dog, secondary cases result, which can create short 'chains' of transmission that, fortunately, finish in a dead-end host outbreak. These incidents legitimately result in the loss of rabies-free status of the country in which they occur (a problem encountered in France in 2008).

In addition to two specific lyssavirus genotypes (EBLV1 and EBLV2, mapped without distinction on Figure 6.2), a West Caucasian bat lyssavirus (WCBV), has been found in the European part of the Caucasus. The vast majority of bat rabies cases detected in Western Europe were identified as EBLV1. Further molecular characterization revealed the existence of phylogenetic sublineages 1a and 1b. While EBLV1a seems to occur in the lowlands and coastal plains along the North or Baltic Seas, and in Central Europe, sublineage 1b is restricted to the Iberian Peninsula, France and the Netherlands. Field surveys in Spain suggested that this virus is endemic in bat colonies. By contrast, EBLV2 has only been found sporadically in the Netherlands, UK, Switzerland, Germany and Finland[2]. Sero-surveys of bat populations performed in the UK suggest that EBLV2 is

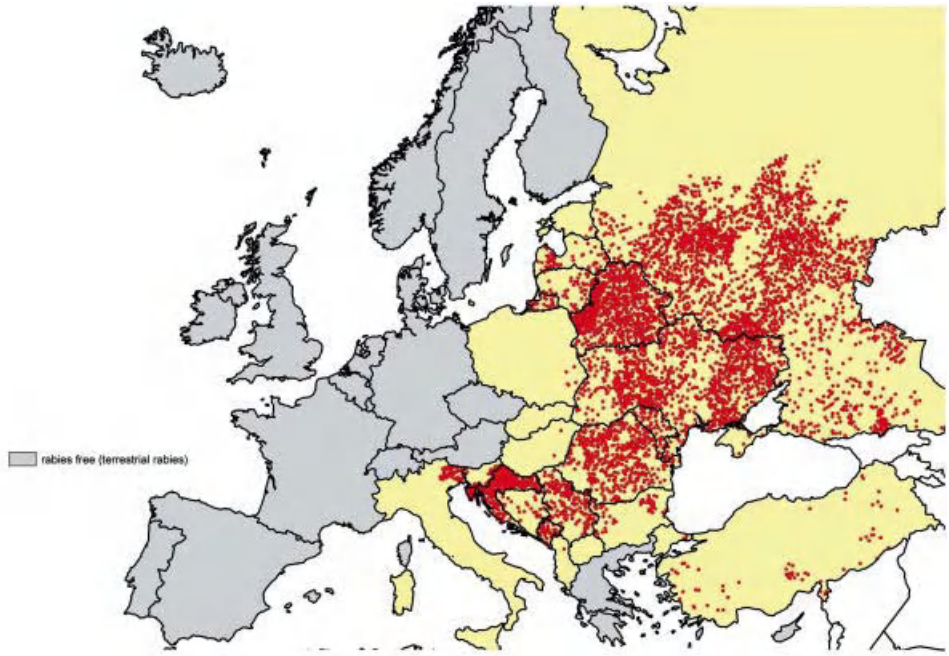

FIGURE 6.1 Rabies in terrestrial mammals; the situation in Europe in 2009.

FIGURE 6.2 Bat rabies cases in Europe from 1977 to 2009, as reported to the World Health Organization.

also endemic with a low prevalence. Considering the large distribution area of the suggested main reservoir species in Eurasia, ranging from Western Europe to Central Asia, there is reason to believe that infections of bats with those bat lyssaviruses could be far more widespread across Europe than observed by current surveillance.

SPECIES-RELATED SUSCEPTIBILITY

Only mammals are currently considered susceptible under natural conditions to rabies virus. However, species susceptibility is variable and, to a lesser extent, relates to individual factors such as immunological status. For rabies, the identification of susceptibility and resistance factors is difficult because of confusion with factors such as virus variants and ecological characteristics of the host that affect exposure risk and clinical outcome. Nevertheless, species affected by rabies can be classified into high (order Carnivora – canid, mephitid, procyonid, viverrid – or Chiroptera), moderate (felid, mustelid, ungulate (Artiodactyla) or primate) and low-receptivity/susceptibility (insectivore, lagomorph, monotreme, marsupial or rodent) species[3]. The susceptibility to RABV infection of marine mammals (cetaceans, pinnipeds) is unknown.

From an epidemiological point of view, each species either belongs to a reservoir or spillover host category, at least for a period of time. As a general rule, highly susceptible species are usually reservoir species; moderate- and low-receptive species are usually considered to be spillover hosts.

Towards the end of the 20th century, in Europe, the wildlife species most frequently affected with rabies has been the red fox, accounting for 75–80% of all recorded cases (90–94% of all wildlife cases). In addition, rabies cases were also reported in highly susceptible species, e.g. in raccoon dogs, jackals (*Canis latrans*) or wolves (*Canis lupus*), all other canids, and in the invasive raccoons (*Procyon lotor*, a procyonid translocated from North America). Infection in a wide range of other wildlife and domestic species has been reported, usually in a spatial and temporal association with fox rabies, e.g. stone martens (*Martes foina*) and pine martens (*Martes martes*), badgers (*Meles meles*) and other mustelids, and the roe deer (*Capreolus capreolus*), as well as in domesticated animals (dogs, cats and cattle, accounting for 89–95% of rabies cases in domesticated animals during recent decades); these are all considered to be spillover infections from foxes because all other forms of rabies disappeared in those areas where fox-mediated rabies was successfully eradicated. In recent decades, in the Baltic countries the epidemiological situation has changed, with the raccoon dog accounting for nearly half of the reported cases.

In Turkey, the domestic dog is the main reservoir species, and cases in other species – wildlife, domestic animals or humans – are the result of spillover events from dogs.

Bat rabies cases in Europe involve infectious cycles that are independent from sylvatic and canine rabies, and the epidemiology is significantly different. Out of 45 known indigenous bat species present in Europe, rabies infection has been detected in only 10 species. EBLV1 has been detected most frequently in serotine bats (*Eptesicus serotinus*, *Eptesicus isabellinus*), which are therefore considered to play a key role in the maintenance of this virus. Cases of EBLV1 have been found in bat species by using the standard fluorescent antibody test (FAT) for rabies diagnosis. Moreover, there is evidence of infection of other bat species (*Nyctalus noctula*, *Myotis myotis*, *Pipistrellus nathusii*, *Pipistrellus pipistrellus* and *Plecotus auritus*) by EBLV1, including some infected live individuals. Therefore, the role of the different bat species (as reservoirs, or perhaps victims of the active circulation of EBLV1 in other bat species) remains to be clarified.

By contrast, EBLV2 has been found only sporadically in *Myotis* bats (an abundant genus of bat species in Europe), e.g. *M. daubentonii* and *M. dascyneme*. The recently described WCBV has only been isolated once from a Schreiber's bat (*Miniopterus schreibersi*) in the European part of the Caucasus.

Infection of mammals other than bats with EBLV is rare. To date, occurrences of EBLV1 have been reported in a limited number of terrestrial mammals, including five domestic sheep, a stone marten and two domestic cats. Cases of EBLV transmission to humans were recently reported in Europe: since 1985, three and two human deaths from EBLV1 and EBLV2, respectively, have been suspected or confirmed. The suboptimal level of passive surveillance in wildlife species in many countries of Europe on the one hand and the limitation of investigation in species protected by law on the other hand, hinder the compilation of an accurate picture of affected bat species and associated host factors. This is in stark contrast to the situation in the Americas, where it was shown that almost all bat species are infected with bat-associated rabies viral strains.

ENVIRONMENTAL FACTORS

Sylvatic rabies in Europe is intrinsically linked to the behavioural ecology of its reservoir species, the red fox, and the interactions of parameters of two major biological associations, the virus–host and host–environment relationships[4]. The population density of reservoir species is mainly affected by the carrying capacity of an area, which in turn depends on its food resources, shelter availability and social regulation. A high population density of susceptible hosts increases the incidence but not the spread of rabies. In epizootic situations, the speed of colonization of new areas by RABV infection is narrowly dependent on the size of the home ranges of the host mammal species[5].

In the European Arctic tundra, red-fox-mediated rabies is absent. Here another reservoir species, e.g. the arctic fox (*Alopex lagopus*) fills the ecological niche, in particular in the tundra regions bordering Siberia. In the tundra, the rabies virus variant infecting the arctic fox is slightly different from that of the red fox and is closer to the dog virus from which it derived, probably in central Asia.

As the red fox is considered an opportunistic species, it can adapt and benefit from human activities. Anthropogenic changes, e.g. in the agricultural use of land, fragmentation of landscape, or urbanization, provide additional food resources and result in increased fox densities and colonization of new territories. Urban fox populations for instance, long considered a British phenomenon, have been increasing in Western and Central Europe for the past two decades. They posed a particular challenge for the final phase of rabies elimination in Germany when residual foci of the disease persisted in areas of high fox density in the southwest of the country[6]. The voluntary, or unintentional, moving of animals by people, sometimes when the animals are in a latent phase of infection, can influence the spread of rabies, and although in Europe translocation of incubating foxes was a rare occurrence, translocation of the raccoon dog from Asia to Europe, or, to a lesser extent, the raccoon from North America, effectively provided new susceptible host 'niches' for rabies virus transmission.

Natural barriers such as rivers or mountain ranges only temporarily block the spread of sylvatic rabies. In high mountainous areas such as the Alps, the spread of sylvatic rabies has been shown to follow the valleys. If the infection pressure is high enough, even large rivers or high mountains are crossed at their 'weakest points', e.g. at bridges, along lower mountain passes, or between end-to-end valleys. Nevertheless, natural barriers should be taken into account when implementing ORV campaigns in wildlife. Although regulated in Europe, translocation of wildlife may substantially contribute to the spread of the disease over large geographic distances, as was shown for the Americas.

Similar environmental pressures apply to dog-mediated rabies. However, canine rabies is more human-associated, as the dog is the principle reservoir and has a close relationship with humans. As a consequence of cultural, religious and social conditions, the incidence and spread of canine rabies in endemic areas is influenced by the social structure of the dog populations in those areas – that is, the owned, ownerless (community) or feral dog status.

European bat rabies is different from 'terrestrial' rabies, and the epidemiological traits relate to the abundance of different bat species in Europe, their different species behaviours and migration habits. As some bat species are migratory, the possibility of virus introduction and wider dispersal is possible, and consequently rapid spread might be expected. Furthermore, bat roosts constantly change location, depending on the seasons, and this may affect and enhance virus transmission opportunities between bats. Therefore, natural barriers do not appear at present to play a role in the spread of bat rabies.

Epidemiological Role of the Affected Species

Reservoir host species play a key role in the epidemiology of sylvatic, canine and bat rabies. The continuation of the infection in endemic areas in Europe relies on independent infectious cycles, in which the associated viral variants are constantly and periodically circulating among conspecifics (see the section on molecular epidemiology, below).

Regarding sylvatic rabies in Europe, there are several arguments indicating that the red fox is the main reservoir[4,5]. However, other potential reservoir hosts, such as the raccoon dog as well as the raccoon, need careful evaluation. Both species were introduced into European Russia and Germany, respectively, during the last century and progressively spread across Europe. They are important reservoir species for rabies in their natural habitats in Asia and America, respectively. Both species when infected pose a high zoonotic potential and must be considered potential reservoir species for classical rabies viruses in Europe too. They are prime examples of a human-made ecological problem resulting in establishment of additional reservoirs of high zoonotic potential with almost no observed inter-

species competition with the red fox. Although it is not yet proven, there is evidence that the raccoon dog could act as another rabies reservoir, as it is the second most reported species to be infected in Central and Baltic Europe, and it is mainly infected by one of the fox RABV variants, although its distribution is much larger in Europe than the distribution of this peculiar RABV variant. There are some areas with high populations of raccoons in Europe; however, fox rabies variants have not managed to adapt to this new host yet, and only sporadic spillover cases are reported.

Although spillover hosts, e.g. dogs and cats in the case of fox rabies, play an important role in the transmission of rabies to humans, this seems not to apply for bat-associated lyssavirus variants.

MOLECULAR EPIDEMIOLOGY

Both the N and G gene phylogenies indicate that viruses sampled from other species of the family *Canidae* (such as foxes and raccoon dogs), as well as hosts belonging to other Carnivora families, e.g. the *Herpestidae* in southern Africa, are included in the phylogenetic diversity of dog RABV. Although no significant evidence for adaptive evolution has been found, recent studies strongly suggest that the dog has served as the main link for inter-species RABV transmission, generating viral lineages that then spread to other taxa[1].

Dog-associated RABV form a distinct phylogenetic group among lyssaviruses of genotype 1, comprising six major clusters, identified as the Africa 2, Africa 3, Arctic-related, Asian, Cosmopolitan and Indian subcontinent clades[1]. The Cosmopolitan clade included dog, wolf and fox isolates from Europe, the Middle East, Iran and Kazakhstan and further east to the Republic of Tuva in Russia. It also included a number of dog RABV isolates from the Americas and from North, Central and South Africa, suggesting that they probably represent secondary migrations from Eurasia.

Further, the lack of mixing between clades supports the idea that, over longer timescales, the persistence of RABV in its enzootic stage does not depend upon regular immigration of infected individuals[7]. Rather, it is more likely that the dispersal of RABV reflects the gradual spatial spread of virus between animals that themselves move relatively small distances, as previously demonstrated in Europe with red foxes and raccoon dogs and in North America with raccoons.

In Europe, phylogenetic analysis of the gene sequence data revealed a number of distinct groups, each associated with a particular geographical area. This resulting pattern suggests that the rabies virus has spread westwards and southwards in Europe during the 20th century, but that physical barriers such as the Vistula River in Poland have enabled localized independent evolution[8]. During this dispersal process, two species jumps took place: one into red foxes and the second into raccoon dogs, although it is unclear whether virus strains are preferentially adapted to particular animal species or whether ecological factors explain the occurrence of the phylogenetic groups. In Europe, there are only three terrestrial non-flying mammal species that are capable of sustaining an epidemiological cycle on their own. These are the European red fox, the invasive raccoon dog, originating from Asia, and the domestic dog, which are infected by different specific variants of RABV.

TRANSMISSION OF TERRESTRIAL AND BAT RABIES

The abiotic environment is unlikely to be important in the transmission of the virus, as ultraviolet light, pH extremes, desiccation, organic solvents, excessive temperatures and putrefaction all result in rapid disintegration of the rabies virus, unless the virus is conserved in frozen carcasses. Transmission of lyssaviruses through intact skin does not occur, and therefore is not considered an exposure route for rabies. Oral and intranasal exposure, as well as aerosol transmission, theoretically may be possible under certain circumstances but do not play a role under field conditions. Therefore, based on the pathogenesis of rabies virus, the most reliable route of rabies transmission leading to an infection is via a bite or scratch from an infected animal, or less frequently, by licking the mucosa, or by virus deposition into breaks in the skin continuity, whereby the intact skin must be penetrated. For EBLVs repeated multiple low virus dose exposure is being discussed as a modified version of this bite inoculation.

Intra- and Inter-Species Transmission

Transmission of classical rabies and bat lyssaviruses predominantly occurs among conspecifics of the reservoir species and occasionally to spillover hosts. The transmission of lyssaviruses is promoted by the social ecology and

behaviour of the reservoir species. Annual population dynamics such as mating (sexual activity), birth, adolescence and dispersal lead to increased contact rates among conspecifics, enhancing the chance of virus transmission.

In addition, proximity to humans greatly influences the probability of contact with infected animals; consequently anthropophilic animal species such as the red fox or feral cat represent a higher public health risk.

European bats are solitary foragers but gregarious when roosting, forming colonies that may vary in size from a few individuals up to several thousand bats (e.g. *Miniopterus schreibersii* or *Tadarida teniotis*). Most species form transient groups during certain periods of the year, e.g. at hibernation roosts, maternity roosts, and mating roosts. Intra-species contact among members of these colonies are likely to be common[2]. A typical annual pattern of rabies peaks is seen with fewer cases during winter, as in terrestrial rabies. A better understanding of the social structure within and between bat colonies will improve our understanding of the EBLV transmission dynamics.

Several studies have investigated whether healthy animals could be potential carriers of rabies virus. So far this assumption remains highly controversial and should not lead to any modification of the control measures and quarantine periods of surveillance of suspect animals.

Rabid Behaviour and Virus Transmission

The incidence of rabies in a particular species is dependent upon its susceptibility and the probability of potentially infectious encounters. How the virus from the shedding animal comes in contact with the nerves of the receiving animal in natural conditions remains poorly documented and, for the most part, speculative.

Radio-collared animals have subsequently become rabid and been followed at a distance during the course of the disease[5]. Results were based on the follow-up of a small number of individuals and are difficult to generalize, but they suggest that a rabid fox loses its normal daily rhythm of activity, can express hyperactivity a few days before death, and finally expire within or just outside its original home range, unless it had started dispersal movements during the incubation period. These observations suggest that disease-induced paresis can induce antagonistic reactions from healthy congeners, favouring the transmission of the virus.

Virus transmission from diseased individuals to susceptible congeners drives the spatial expansion of rabies at a speed that relies on the latency period and activity range of animals, e.g. during the years 1960–1970, the disease wave front extended some 30–40 km per year.

In gregarious animals (e.g. fallow deer (*Dama dama*), greater kudu (*Tragelephus strepsiceros*)) or social mammals (e.g. hunting dog (*Lycaon pictus*), simian wolf (*Canis simensis*)), the virus can be transported through contact with infective saliva left on branches, or by mutual licking, and regurgitation. This 'natural' behaviour presents a route for passive virus passage from an infected shedder to a receptive congener.

Temporal variation of rabies incidence is mediated by the changing host behaviour of space use, circadian rhythms and social interactions. As a consequence, the incidence of fox rabies increases in March following an increase in the contact rate between foxes during the vulpine breeding season. By contrast, transmission of raccoon-dog- or raccoon-mediated rabies might be influenced by dormancy during the winter season.

Active aggression, the most spectacular clinical sign of rabies, is relatively rare and is not viewed as the most common way for virus transmission within species. However, it can be of major importance in lyssavirus evolution as an alternative method of transmission, allowing the virus to leap from one species to another (dog to fox, fox to raccoon dog).

Animals (or people) that inspect and investigate, or attack, a paralysed infected fox, or roe deer, cattle and other domestic ruminants, are more likely to be infected, and this is also the case with regard to people and bat rabies.

PATHOGENESIS, PATHOLOGY AND IMMUNITY

Animal rabies is characterized by encephalitis and myelitis, which cause a progressive alteration of the central nervous system (CNS) and a range of clinical features. Rabies virus was shown to be highly pathogenic in the red fox, with an incubation period varying from 11 days up to 15 months, depending on the dose and on the route of inoculation. During this period the virus can replicate locally in muscle cells or attach directly to nerve endings through several receptors. Having gained access to peripheral nerves, the virus travels in a retrograde direction within the axoplasm. When it reaches the CNS, there is massive viral replication. There, direct transmission of virus occurs from cell

to cell across synaptic junctions. At the onset of illness when evidence of neuronal dysfunction appears, there is little or no apparent histopathological change. The blood-brain barrier does not eliminate the virus. Centrifugal spread of virus from the CNS in somatic and autonomic nerves deposits virus in many tissues, including skeletal and cardiac muscle, adrenal glands, kidney, retina, cornea, pancreas and nerves around hair follicles. Productive viral replication takes place predominantly in the salivary glands, excreting virus that allows transmission to other mammals.

Rabies viruses have profound effects on the functions of infected and some uninfected neurons. Minor electroencephalographic changes during animal infection indicate neuronal dysfunction. However, a range of abnormalities are seen in magnetic resonance imaging (MRI) as human encephalitis progresses. Several hypotheses have been raised to explain the effect of rabies virus on neuronal function: changes in neurotransmitter functions could lead to failure of brain networking and regulation of responses, and disruption of neuronal metabolism, ending in the exhaustion of metabolic pools; infections of neurons by rabies virus may result in modifications of the expression of numerous genes involved in, for example, protein synthesis, neuron growth and interferon response.

The spread of the virus through the nerves does not promote the rapid onset of a strong immunological response. The serological response to rabies virus appears irregularly, and always late in the clinical course of the disease and is therefore of little value for diagnostic purposes or protection.

The neurotropism and clinical effects of bat lyssaviruses are known to be comparable to those of the RABV; when natural transmission to other mammals of these viruses occurs, clinical signs are similar to rabies. These 'terrestrial mammals' are dead-end hosts and unable to transmit an infectious, bat-derived virion to a new host. Nevertheless, RNA can be detected in salivary glands of infected animals[9]. Experiments have shown that terrestrial mammal susceptibility to EBLV is low by the intramuscular (IM) route; however, animals were sensitive to intracranial (IC) inoculation. Foxes inoculated IC exhibited clinical signs of rabies and succumbed from 14–24 days post–infection (dpi). All clinically ill foxes developed neutralizing antibodies, and viral antigen, infectious particles and viral RNA occurred in the brain. Salivary glands were infected in half of the animals, but none of the oral swabs collected from these animals were positive for viral RNA or infectious particles[10]. These data suggest that the chance of an EBLV spillover from bat to fox is low, but with a greater probability for EBLV1 than for EBLV2.

There is no specific gross pathology in rabies-infected animals. Some external skin damage can be linked with disturbances in behaviour induced by rabid encephalitis. Self-mutilation, injuries resulting from muscular spasm or paresis can be suggestive of the disease, mostly in small ruminants such as the roe deer, where abrasion of the forehead skin is a frequent finding in animals that have died from rabies infection.

Histologically, rabies shows mild changes. Apoptosis has been demonstrated in cell culture and in inoculated mice together with non-specific encephalomyelitis, with perivascular cuffing with lymphocytes or polymorphonuclear cells. Intracytoplasmic inclusion bodies – Negri bodies – in the neurons are typical of certain forms of brain rabies infection.

CLINICAL SIGNS

It is difficult to observe the progression of rabies in naturally infected free-living wild animals. There are several anecdotal descriptions of rabid animals that were observed while clinically active, or interacting with people in the countryside. They are probably biased towards the spectacular, with few observations of mute rabies. Most descriptions are usefully oriented towards clinical suspicion for surveillance purposes: alteration of normal behaviour (encephalitis), lack of muscular co-ordination or paralysis (myelitis) and swallowing difficulties due to pharyngeal paralysis are the most common findings reported in animal species. Clinical signs differ based on the route of administration; IM inoculation is deemed similar to the natural route (bite) for the administration of a lethal dose of virus.

The Rabid Fox (and Rabies in Other Carnivores)

Canine rabies is classically depicted as following three successive stages: prodromal, excitative and paralytic, the last of these being pre-terminal[5]. These stages can generally be transposed to describe the disease in other carnivores.

After a short incubation period (from 1 day up to 2 weeks, depending of the challenge dose and anatomical localization of virus inoculation, but believed to be about 4 weeks in the wild) a rabid fox is described as restless and

pacing; it loses its appetite and has a typical phonation/bark similar to that heard during the mating season; clonic and tonic convulsions are elicited by stimuli, but they rarely turn into the furious form; the third eyelid is frequently prolapsed. At the next stage of the disease progression, ataxia, paralysis, loss of locomotor function, and then coma, is followed by death.

In the natural environment, aggressive behaviour or spontaneous attacks are reported in about 10% of cases, leading to diagnostic submission. Rabid foxes are described as furious, inflicting injuries to themselves, or to animals and rarely humans. More frequently reported are constant prostration and complete apathy, with wild animals found in buildings, or at the edge of a street or path.

The Rabid Bat

Inoculated bats have altered reflexes, loss of appetite, tremors and paralysis. Aggressive behaviour is rarely observed in bats infected by the peripheral route. Furious behaviour occurs with IC inoculation of virus. Bats found alive and subsequently diagnosed as affected by EBLV are immobile, seen in daylight, occasionally squeaking, or instead lying on the ground with ataxia resulting in ineffective wing flapping. Such abnormal behaviour, if investigated, can result in a bite or scratch, exposing a human to infection. Injury by a predator, or intoxication, can lead to a similar behaviour and are differential diagnoses.

Rabies in Spillover and Other Hosts

Fox rabies has been frequently reported in wild cats and mustelids, with clinical signs similar to those described above. Rabies in roe deer is not a rare event, but usually animals are found dead, with clinical disease rarely being observed.

Laboratory mice are a common rabies animal model, with experimental rabies signs mostly involving ataxia, tremor and paresis[5]. However, rabies is not sustained in rodent populations, with the notable exception of sciurid species (such as marmots in America). Naturally acquired disease is reported as either lethargic (coma and death), or a furious (aggressive) stage preceding this.

The Dumb Form of Rabies

Rabies infection can frequently lead to death with no visible signs noted in animals that are not under constant human supervision. Silent excretion of virus has been described in bats and dogs[5]; so far these events are of a limited occurrence and do not impact on public health regulation.

For the rabies disease in wild animals, no sign is pathognomonic or species-specific, and it can be said that 'atypical is typical'[11]. In general, after a variable incubation period, a non-specific prodromal stage succeeds, which can only be observed in captivity; clinical signs of a progressive meningoencephalitis then will appear, characterized by abnormal behaviour (activity rhythm: diurnal movements for nocturnal species, loss of the usual fear of humans) plus disorders of locomotion (paresis and paralysis) and sometimes, excessive salivation. Death occurs after a few days. The severity of the signs appears to be related to the site of primary CNS lesions, as well as the viral strain, the dose and route of inoculation.

DIAGNOSIS

The identification of Negri bodies was the major diagnostic criterion until 1958, when the FAT was developed. The FAT has become the recommended procedure because it is fast, inexpensive and reliable when performed in a competent laboratory with high-quality reagents. Virus isolation is a routine back-up procedure, providing unambiguous identification of the aetiologic agent. The mouse inoculation test (MIT) is still in used in some countries. As the MIT yields delayed results (between 7 and 20 days), it was replaced in the 1980s by the rabies tissue culture infection test (RTCIT), employing murine neuroblastoma cells.

Laboratory diagnosis of rabies has been standardized through several expert committees under the supervision of the World Health Organization (WHO)[12,13], and the World Organisation for Animal Health (OIE)[14]. The main procedures for the routine diagnosis of rabies consist of FAT and RTCIT. These techniques are expensive and not suited for the analysis of large series of samples, for example from field epidemiological surveys. For large numbers of samples, an enzyme-linked immunosorbent assay (ELISA) (the rapid rabies enzyme immunodiagnosis, or RREID) was described. It was also more reliable than other tests when performed on autolysed specimens. However, the RREID is no longer commercially available. Another ELISA, named WELYSSA, which uses mouse monoclonal antibodies (MAbs) to capture the antigen (viral nucleoprotein), has been described recently[15]. In a

diagnostic setting, this test is intended for use in tandem with the FAT, which remains the reference technique. WELYSSA exhibits a good sensitivity and specificity for the lyssaviruses circulating in Europe.

Molecular techniques for the detection of rabies and rabies-related lyssaviruses usually use reverse transcription polymerase chain reaction (RT-PCR) methods targeting the viral nucleoprotein gene. However, another region of the lyssavirus viral genome, the polymerase gene, can be used for detection and phylogenetic analysis. This gene encompasses several highly conserved nucleotide blocks (as block III)[16]. A new reverse transcription hemi-nested polymerase chain reaction (RT-hnPCR) protocol employing this region was recently developed. Other techniques such as nucleic acid sequence-based amplification (NASBA) or loop-mediated isothermal amplification (LAMP) techniques have also been proposed but never tested in the European epidemiological context. These tests can be performed on necropsy specimens but also on filter (FTA) paper technology. This was shown to be useful for the storage, transport, collection and subsequent molecular analysis of viral rabies RNA, facilitating epidemiological investigations in the field[17].

Specific RT-PCR were also developed for field studies investigating the circulation in EBLV in Europe. More recently, a single, closed-tube, non-nested RT-PCR with TaqMan technology that distinguishes between classical rabies virus (genotype 1) and European bat lyssaviruses 1 and 2 (genotypes 5 and 6) in real time was developed. This TaqMan assay is done on only a single tube and for all reactions is rapid, sensitive and specific, and allows for the genotyping of unknown isolates concomitant with the RT-PCR[18].

Conventional seroneutralization methods were also widely used for surveys of EBLV circulation in the natural environment where bats roost[19], but this technique is expensive and time-consuming. Recently, glycoprotein (G-protein) cDNA from RABV (challenge virus standard-11) and EBLV1 and EBLV2 were cloned and co-expressed with human immunodeficiency virus (HIV) or murine leukemia virus (MLV) gag-pol and packageable green fluorescent protein (GFP) or luciferase reporter genes in human cells. The harvested lentiviral (HIV) vector provided high-titre pseudotype stocks, which were shown to be suitable for neutralization assays. These pseudotypes have two major advantages over live-virus neutralization tests: i) they can be handled in low-biohazard-level laboratories; and ii) the use of reporter genes such as GFP or beta-galactosidase will allow the assay to be undertaken at low cost in laboratories worldwide. This constitutes a robust microassay applicable to surveillance studies[20].

Seroneutralization (the rapid fluorescent focus inhibition test (RFFIT) or fluorescent antibody virus neutralization test (FAVN)) can be used to monitor rabies antibodies in a wild animal population, for example after vaccination campaigns. A virus neutralizing test using an indirect immunoperoxidase technique (VNT-IIP) for rabies has been developed for the titration of dog and cat serum samples in Japan. The VNT-IIP has the advantage that results obtained can be viewed by the naked eye. But this is still expensive and time-consuming. Recently, ELISA to titrate rabies antibodies in vaccinated wild and domestic carnivores have been described with varying sensitivity and specificity. Therefore, further evaluation organized by the Community Reference Institute for rabies serology (Anses Nancy, France) in the frame of international movements of pets[21] is needed before ELISA can be recommended for routine use and replace seroneutralization in Europe.

MANAGEMENT, CONTROL AND REGULATIONS

IN TERRESTRIAL CARNIVORES

The control of rabies has been among the greatest successes of wildlife disease management. Before the first quarter of the 20th century most recorded cases of rabies were in the domestic dog. As a result, many European countries adopted a number of measures for rabies management (quarantine, parenteral vaccination, registration of dogs, official notification of cases, muzzling and the elimination of stray and suspect dogs), which led to a marked reduction in the number of rabies cases throughout Europe and the progressive elimination of dog rabies and its associated virus. From the 1940s, a new expansion of rabies across Europe occurred, starting from Central Europe, with the primary host being the red fox. Since this is commonly considered a pest species, rabies management focused on population reduction through increased hunting, shooting, poisoning or gassing. This had little, if any, effect on the spread of the disease across Europe, even when population control was removing over 30% of the individuals each year. However, localized culling was claimed to have been successful at stopping the local spread of the disease.

From the 1970s, computer models have been used to simulate rabies spread, primarily in wildlife, and examine control options. After a preliminary field study[22], the critical threshold density, below which the epidemic would die out, was calculated at between 0.2 and 1.0 fox per km^2. As survival at this density will be very high, it does not appear possible for fox densities to be sustained below this level, by culling alone, as mortality is compensated by an increase in life expectancy.

In 1969 foxes were first immunized against rabies in the USA by the oral administration of a live attenuated strain of the rabies virus[23]. Field trials using similar strains (SAD strains) started soon after in Europe. Later, other approaches to rabies vaccination were developed, involving the delivery of the rabies virus surface antigen (glycoprotein) either in a genetically modified vaccinia (pox) virus (Vaccinia-rabies glycoprotein recombinant virus, or VRGv), or using the avirulent SAG-2 strain of live attenuated virus.

As oral immunization of wildlife is only possible using infectious (live) vaccine, all the above infectious vaccine strains can pose environmental safety problems, which have to be properly assessed before field delivery. Targeting the European red fox resulted in the near complete elimination of rabies from West- and Central Europe. Similar strategies have subsequently been used to control rabies in a range of other carnivores in the USA and Canada[24]. Unless there are reasons to change the distribution, the protocol for oral fox vaccination should follow the European Commission guidelines[25]. These state that following any re-emergence of disease, emergency vaccination of an area of 20–50 km width and a bait density of 18–20 and 20–30 baits per km^2 should be followed in areas of low and high fox density, respectively. In areas of particularly high fox density, there may be difficulties in eliminating the disease with vaccination on its own[26,27], and the above protocol has not been tested in such circumstances.

It is worth noting that there are two advantages of culling over vaccination. Firstly, culling may remove infected animals that do not then become rabid. Such individuals cannot be detected while incubating the disease. Secondly, culling reduces the overall productivity of susceptible animals. This latter effect can also be achieved with a fertility control agent, and modelling suggests this potentially has the greater effect. As fox culling can also result in increased movement[28] and thus may lead to an increase in disease spread, it is likely that future oral rabies developments will incorporate immunofertility agents.

In Eastern Europe, the spread of the raccoon dog has caused complications for rabies control, as rabies could spread more easily into areas of low fox density[29]. It appears to be a competent host, and contributes about half of the wildlife cases in some Baltic countries. The raccoon dog hibernates in very cold winters, but not in warmer ones, and this may affect the probability of disease spreading in a mixed community of foxes and raccoon dogs. Raccoon dogs continue to spread through Europe, and although the oral rabies vaccination appears to work well in this species, the effective increase in host density means that wildlife outbreaks in areas where both species are present need closer monitoring.

IN BATS

Currently, the bat lyssaviruses do not appear to affect the conservation status of the host bat species. In addition, human infection from these diseases in Europe is rare. However, monitoring infection and protecting human health when lyssavirus infection has been detected in a colony may require adaptive measures and informing the inhabitants of the house and neighbours. Sometimes it is practical to separate the bat roost from the rest of the habitation by filling cracks and blocking access of the bats to the rooms. In the case of caves, public access can be prevented by bars. Finally, if no solution can be found, the access of the bats to the roost could be closed at the time of the year when the colony has gone, to prevent re-colonization.

PREVENTION AND CURRENT EU REGULATION

Rabies is listed as a regulated disease in the EU for the purpose of eradication and monitoring programmes. Several decisions have been published following expert committee recommendations[13,14,25]. The objective of the programme in every member state is to eradicate the disease from wild animals (sylvatic rabies) or from domestic animals (urban rabies) if an outbreak occurs.

In addition, each member country is under an obligation to have a rabies surveillance scheme and a set of measures to manage the domestic pet and fur animal circulation inside the EU, or coming from third countries (individual identification, minimum age allowed for travelling, relevant vaccination, passport, etc.).

PUBLIC HEALTH CONCERN

In Europe, locally there can be an emergency or high alert status situation where an outbreak of wildlife rabies re-emerges or persists (e.g. Italy at the border of Slovenia since autumn 2008). Worldwide, 55,000 human deaths/year are recorded by the WHO, with an associated total of more than a million disability-adjusted life years (DALY) per year (a standardized comparative measure of extended disease impact). Studies carried out in affected continents indicate that the number of human rabies deaths may be up to 100 times higher than that officially reported[30]. Human mortality from endemic canine rabies can be estimated at between 24,500 and 90,800 deaths, with most occurring in Asia and Africa. Each year, there are about 12–13 million people receiving expensive post-exposure prophylaxis (PEP) in the world. Thus, reoccurrence of an epidemic form of rabies would be important, as the rabies virus is a significant pathogen and 100% lethal in infected individuals. In the EU, three different main threats can be anticipated:

- a former bat lyssavirus evolving to a new rabies variant communicable to terrestrial animals[31] ;
- sporadic reintroductions from dog rabies resulting in non-notified outbreaks in domestic carnivores, potentially spreading to wildlife and domestic animals;
- spread of fox or raccoon-dog rabies by local contagion across the borders of the EU or malicious/irresponsible translocation of infected wild animals (i.e. foxes, raccoon dogs or raccoons).

Other, less plausible risks, involve a classical rabies strain imported from the Americas by a bat hibernating in timber for instance, or emergence of a new bat lyssavirus strain. In both scenarios the result should be expected only as sporadic events.

Each reintroduction of dog rabies in Europe now is a challenge to public health infrastructure and represents a significant cost in resources, in terms of new preventative programmes, public concern abatement and post-exposure prophylaxis.

SIGNIFICANCE AND IMPLICATIONS FOR ANIMAL HEALTH

Little work has been done to accurately assess the impact of rabies on domestic and wild animal populations within Europe. Being weakly contagious, the infection does not need mass culling of stock animals to prevent its spread; nevertheless, in pet animals that are allowed to roam freely, non-vaccinated dogs or cats may have to be euthanized when their owner is living in close proximity to an identified case. Thus, direct or indirect losses due to rabies in domestic animals are usually limited to the vicinity of outbreaks, although indirect costs associated with preventive pet vaccination can be substantial. Movement of pets can be restricted within the areas of infected zones. In France, the cumulative cost of fox rabies control including oral vaccination during the period 1986–1995 was estimated to be US$261 million[32].

Among wildlife in Europe, rabies is expected to have a transient effect on maintenance host populations such as the red fox; but the impact of EBLV infection on insectivorous bats remains undocumented. Spillover host populations could be affected by rabies where populations of large predators are confined to a small areas (bear, lynx or wolf, for instance), a context that can still occur in Europe.

ACKNOWLEDGEMENT

The authors wish to dedicate this chapter to the memory of the late Jean Blancou, former general director of the OIE and leading scientist on rabies, who significantly contributed to the eradication of fox rabies in Europe.

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CHAPTER

7

MORBILLIVIRUS INFECTIONS

JOLIANNE M. RIJKS, ALBERT D.M.E. OSTERHAUS, THIJS KUIKEN AND KAI FRÖLICH

INTRODUCTION

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Viruses in the *Morbillivirus* genus, *Paramyxoviridae* family, are enveloped, single-stranded, negative-sense RNA viruses. They have a variable shape and size, ranging from spherical to filamentous and 150–1000 nm in diameter or length. They have a nucleocapsid core with a herringbone appearance, surrounded by a lipoprotein envelope (Figure 7.1). The genome consists of six genes (N, P/C/V, M, F, H and L), of which the haemagglutinin (H) gene is considered to be the most variable. Haemagglutinin forms part of the envelope and elicits the majority of neutralizing antibodies. Attachment of the virus to its host cell primarily occurs through binding of this haemagglutinin to signalling lymphocyte activation molecules (SLAM), which are restricted to cells of the immune system[(1)].

The stability of morbilliviruses in the environment is affected by sunlight, temperature, humidity and pH. They are rapidly inactivated by exposure to sunlight, but this inactivation is slowed by embedding in proteinaceous material. Morbilliviruses are stable at temperatures of around 0°C or lower, as long as freeze–thaw events do not occur. They are relatively labile at room temperature, but survive longer in the air at this temperature at low relative humidity. Morbilliviruses are rapidly inactivated at high temperatures. They are stable in a broad pH range. Being enveloped, morbilliviruses are readily inactivated by lipid solvents.

Eight species of the genus *Morbillivirus* have been identified to date: *Measles virus*, *Rinderpest virus*, *Peste-des-petits-ruminants virus*, *Canine distemper virus*, *Phocine distemper virus*, *Dolphin morbillivirus*, *Porpoise morbillivirus* and *Pilot whale morbillivirus*. The latter three are sometimes considered a single species, called *Cetacean morbillivirus*[(2)]. Species can be distinguished genetically, e.g. by divergence in gene sequences (Figure 7.2), and antigenically, e.g. by differential binding to monoclonal antibodies. Although each morbillivirus species is considered serologically monotypic, they cross-react in serological tests, and antibodies to one morbillivirus can protect to some extent against infection from another morbillivirus. Morbilliviruses are capable of infecting multiple species, sometimes from different orders (Figure 7.3). However, only one or two of these species may be crucial in maintaining the infection over time, such as cattle in rinderpest.

Infectious Diseases of Wild Mammals and Birds in Europe, First Edition. Edited by Dolores Gavier-Widén, J. Paul Duff, and Anna Meredith.

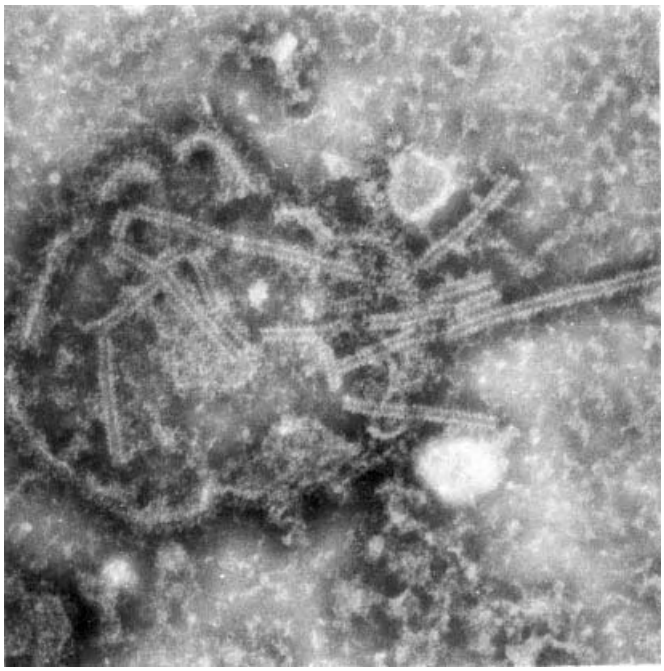

FIGURE 7.1 Negatively stained electron microscope photograph of phocine distemper virus, demonstrating herringbone-like structure of nucleocapsid. Fourth passage in VERO cells. 123,000×. Photo by Dr J.S. Teppema, courtesy of M. van de Bildt.

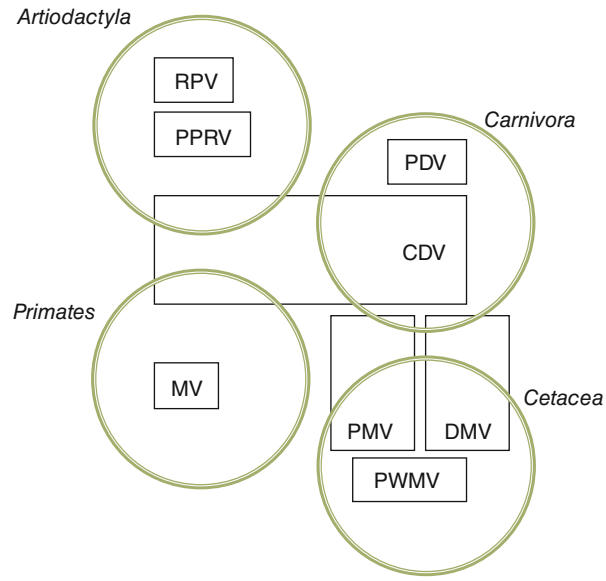

FIGURE 7.3 The orders to which host species infected by the eight known morbillivirus species belong.

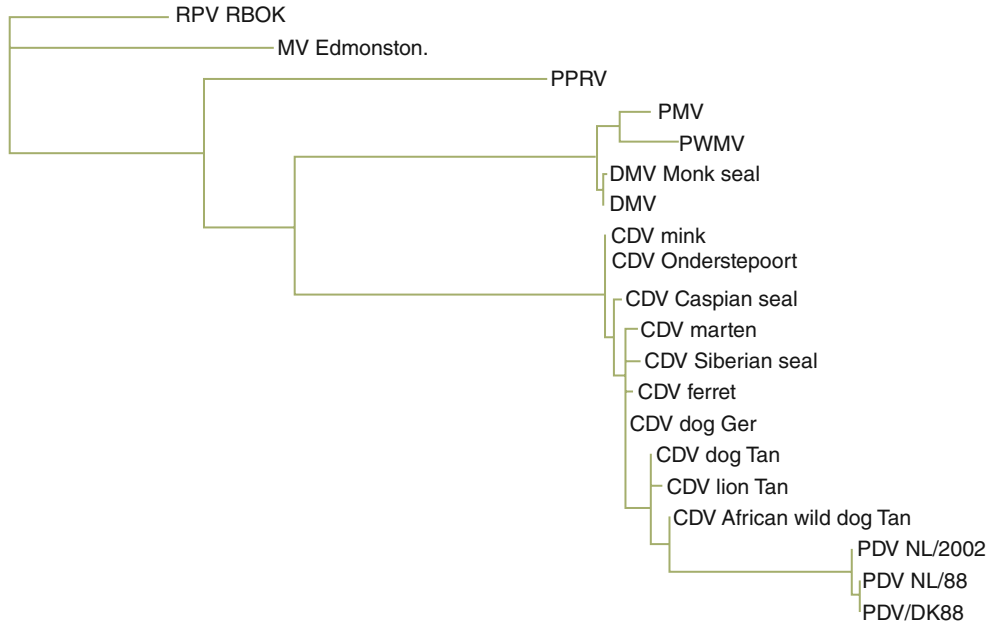

FIGURE 7.2 Phylogeny of morbillivirus species based on phosphoprotein gene fragments. The maximum likelihood tree was generated with the SEQBOOT and DNAML programs of the Phylip package with 500 bootstraps. GenBank accession numbers are given in parentheses. RPV RBOK = rinderpest virus RBOK strain (X68311), MV Edmonston = measles virus Edmonston strain (M89920), PPRV = peste des petites ruminants virus strain Nigeria 75/1 (AJ298897), PMV = porpoise morbillivirus (Barrett et al., 1993)[3], PWMV = pilot whale morbillivrus (AF200817), DMV Monk seal = DMV strain MSMV WA (Osterhaus et al., 1997)[4], DMV = dolphin morbillivirus (Z47758), CDV Mink = CDV European mink (AY130856), CDV Onderstepoort (AF305419), CDV Caspian Seal (Kennedy et al., 2000)[5], CDV Marten Ger = CDV marten Germany (AJ582389), CDV Siberian Seal (AF259551), CDV Ferret = CDV ferret Germany (AF259550), CDV Dog Ger = CDV dog Germany 1990 (AF259549), CDV Dog Tan = CDV dog Tanzania isolate A9411/15 (U53715), CDV Lion Tan = CDV lion Tanzania isolate 94-52.10 (U53712), CDV African wild dog = CDV African Wild dog Tanzania 2001 isolate LP/Tan/01 (FJ011000), PDV NL/2002 = phocine distemper virus, Netherlands 2002 (AF525288), PDV NL/88 = phocine distemper virus, Netherlands 1988 (AF525289), PDV/DK88 = phocine distemper virus, Denmark 1988 (X75960). Courtesy of M.W.G. van de Bildt.

In the individual host, morbillivirus initially is lymphotropic, resulting in lymphoid depletion and immunosuppression. This allows facultative pathogens to cause secondary disease, which is often the proximate cause of death. Later in the infection, morbillivirus can become neurotropic, causing primary neurologic disease. It also becomes epitheliotropic, resulting in the formation of aggregates of nucleocapsid-like structures in the nucleus and cytoplasm of infected epithelial cells. These aggregates are visible as intranuclear and intracytoplasmic inclusion bodies, which are an important diagnostic feature in histopathologic examination.

At the population level, morbilliviruses require large populations to remain endemic, because they are highly contagious, cause infections of short duration and induce long-lasting immunity in survivors. In populations where morbillivirus infection is not endemic and there is no herd immunity, infection typically leads to so-called 'virgin soil' epidemics with high mortality, and these are among the most severe die-offs known in wildlife.

CANINE DISTEMPER IN CARNIVORES

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Canine distemper (CD), also called *La maladie de Carre*, is an acute or subacute highly contagious disease that may manifest with signs of generalized infection, respiratory disease, hyperkeratosis, central nervous system (CNS) disease or a combination of these, and can be accompanied by severe immunosuppression. CD has been responsible for severe population declines in wild carnivores, and in domestic dogs CD continues to be a major disease, reoccurring despite vaccination.

AETIOLOGY

Canine distemper virus (CDV) is an enveloped, single-stranded, negative RNA virus of the family *Paramyxoviridae*, genus *Morbillivirus*. The virus is relatively fragile and quickly inactivated in the environment by ultraviolet light and by heat and drying. Temperatures greater then 50°C rapidly destroy the virus, and it is inactivated in several hours in tissues at 20–37°C. It may remain stable for weeks at 4°C and for years when frozen at −65°C. Common disinfectants readily inactivate CDV. The haemagglutinin (H) protein is a key component of the virus, responsible for the first virus–host cell interaction and initial virus entry. An adequate host immune response against the H protein may prevent CDV infection. The fusion (F) protein is an integral membrane protein-binding cell surface receptor of target cells. CDV is thought to have a single serotype, but is classified into up to nine lineages, e.g. America-1 and -2, Asia-1 and -2, European and Arctic. However, the distribution of the major lineages throughout the world is currently not clear. The sequence of pathogenic events depends on the strain. Certain isolates, such as Snyder Hill, A75/17 and R252 strain are highly virulent and neurotropic[6,7]. A small number of specific residues are important in the adaptive evolution of CDV through positive selection, owing to their interactions with cellular receptors or with the host immune system. Surveillance of sites 530 and 549 of the H protein in the molecular adaptation of CDV into novel hosts is an informative technique to assess the potential of CDV to spread into wildlife populations. Analyses show that pathogen evolution plays a crucial role in the establishment of CDV in novel carnivore host species. Taken together, molecular evolutionary analyses have revealed that the spread of CDV to novel host species may be associated with adaptation at signalling lymphocytic activation molecule (SLAM) receptor-binding sites 530 and 549 of the H gene[8].

EPIDEMIOLOGY

CDV has a broad host range. Members of nine families of the order Carnivora are susceptible to CDV infection, i.e. Ailuridae, Ailuropodidae, Canidae, Hyaenidae, Mustelidae, Procyonidae, Viveridae, Felidae and Phocidae. Additionally, in the order of even-toed ungulates CDV affects members of the family Tayassuidae, and in primates members of the family Cercopithecidae.

In European wildlife, infection and identification of CDV has been reported in several free-ranging carnivore species (Table 7.1).

CDV affects animals of all ages. Morbidity and mortality rates vary among species and age groups (usually higher mortality in young animals).

Observations on the epidemiology of CD in dogs may assist in understanding the disease in free-ranging species. In endemic areas where dog populations are high, clinical

TABLE 7.1 Evidence of CD infections in wildlife from Europe.

Species	Country	Antibody (AB), antigen (AG), lesions (L), PCR (P)	References
Mustelidae			
European mink (*Mustela lutreola*)	France	AB	9
American mink (*Mustela vison*)	France Denmark Spain	AB, AG, L	9, 10, 11
Polecat (*Mustela putorius*)	France Germany Spain	AB, AG, L	9, 10, 12, 13
Stone marten (*Martes foina*)	France	AB, AG, L, P	14
	Germany Czech Republic Spain Austria Italy		7, 9, 10, 12, 13, 15, 16, 17, 18
Pine marten (*Martes martes*)	France	AB	9
Weasel (*Mustela* spp.)	Germany	AB, AG, L	12, 13
Eurasian badger[a] (*Meles meles*)	Germany Czech Republic Austria Italy Switzerland	AB, AG, L, P	13, 15, 16, 17, 18, 19, 20, 21
Genet (*Genetta genetta*)	Spain	AG, L	10
Ursidae			
Polar bear (*Ursus maritimus*)	Norway	AB	22
Procyonidae			
Raccoon (*Procyon lotor*)	Germany	L, P	23
Felidae			
Iberian lynx (*Lynx pardinus*)	Spain	AB, AG, P	7
Eurasian lynx (*Lynx pardinus*)	Switzerland	L	19
Canidae			
Red fox (*Vulpes vulpes*)	Italy Germany Spain Portugal Luxembourg Norway Italy Switzerland	AB, AG, L, P	15, 17, 19, 21, 24, 25, 26, 27, 28, 29, 30, 31,32, 33
Arctic fox (*Vulpes lagopus*)	Norway	AB	33
Wolf (*Canis lupus*)	Spain Portugal Norway Sweden	AB	24, 27, 33

[a]In the UK, all blood samples from an intensively studied population of wild badgers were negative for CDV antibodies[34]

disease is mostly seen in pups following loss of maternal antibody at 3–6 months. In isolated populations of dogs, CD is epidemic, and outbreaks may be severe and widespread, and affect all ages. Domestic dogs have been considered as reservoirs of infection for wild carnivores[24].

In Germany, a higher prevalence of CDV antibodies was detected in wild canids in urban and suburban areas and no positive samples were detected in animals from rural areas. Phylogenetic analyses revealed a distinct relatedness to domesticated canine CDV isolates. Thus, the data support the concept of transmission of CDV between domestic dogs and wild carnivores[25].

In Bavaria, a severe outbreak of canine distemper encephalitis was observed in several wildlife species (red fox (*Vulpes vulpes*), Eurasian badger (*Meles meles*) and stone marten (*Martes foina*). The H genes of several representative virus samples during this outbreak had a Y549H amino acid substitution in the H protein. The data support the hypothesis that pathogen evolution at residue 549 in the SLAM-binding region of the H protein of CDV is associated, directly or indirectly, with disease emergence in novel host species[15].

In France, it was postulated that the high prevalence of CDV antibody in five mustelid species was possibly related to the fact that mustelids live in close proximity to humans, and are therefore more likely to come into direct or indirect contact with CDV-infected domestic dogs[9].

Although CDV is thought to be maintained in domestic dog populations worldwide, the virus has a very broad, and apparently expanding, host range. Transmission to wolves could occur through predation or scavenging of a domestic carnivore (dog or cat) carcass[24].

In Spain, the genome of CDV was detected in a feral cat, showing evidence of interspecific transmission of CDV[26]. It has been proposed that CDV-infected foxes could be a source of infection to other less abundant species that live sympatrically[27].

Since 2006, the Alpine region of North-East Italy has been experiencing a severe and widespread outbreak of CDV, affecting the wild carnivore population. Captured red foxes and Eurasian badgers showed CD-like signs (e.g. prostration, altered behaviour and conjunctivitis). CDV infection was confirmed by quantitative reverse transcription polymerase chain reaction (RT-PCR) of pooled organs, and sequence analysis of the H gene indicated that the fox strains were highly related to each other and to the strains identified in foxes in Bavaria, as well as to a canine strain identified in Hungary[28]. In Austria, strains isolated from badger and stone marten were assigned to the European wildlife groups[16]. Also the emergence and spread of a novel genetic cluster of CDV in wildlife populations of the Alps has been described[17]. This novel genetic group, denoted as WE/06–09 cluster, consists almost exclusively of viruses identified from wild carnivores (red fox, European badger, stone marten).

Moreover, this outbreak has reached wildlife (red fox and European badger) in Switzerland and Liechtenstein. Interestingly, there was evidence for the presence of morbillivirus infection in Swiss wildlife before this outbreak. Seroprevalence of about 25% was recorded in red fox and Eurasian lynx (*Lynx lynx*), indicating that exposure to a morbillivirus was apparently not uncommon in wild carnivores[19].

Transmission of CDV is primarily by aerosol or contact with oral, respiratory and ocular fluids and exudates containing the virus. Close association between affected and susceptible animals is necessary owing to the relative fragility of CDV in the environment, because the virus is quickly inactivated by ultraviolet light, heat and drying. Viral shedding occurs even if animals are subclinically infected, and virus may be shed for up to 90 days after infection.

PATHOGENESIS, PATHOLOGY AND IMMUNITY

As we have seen, the natural route of distemper infection is by inhalation of airborne virus or contact with oral, respiratory and ocular fluids and exudates containing the virus. Other possible routes are transplacentral or neonatal infection.

Virus replication occurs first in cells of lymphatic tissues in the upper respiratory tract. Within a few days, CDV multiplies in macrophages and spreads to tonsils and regional lymph nodes. After about 1 week, the virus may be found in systemic lymphoid tissues, lamina propria of the digestive system, and Kupffer cells in the liver. The systemic spread of virus corresponds to the development of fever and leucopenia; the latter is due to viral-associated loss of T and B lymphocytes. Destruction of cells of the immune system results in immunosuppression.

Between 1 and 2 weeks after infection, the host's immune response determines the outcome of infection. If there is a strong antibody response, no clinical illness develops and the virus is cleared from the tissues. If the animal mounts a

weak antibody response, illness may ensue, but approximately 3 weeks after infection the virus is cleared from most of the body, with the possible exception of the lungs, skin and CNS. The virus may be shed by these animals for several months. If the animal recovers, it may shed virus for 2–3 months[35]. Pathological changes of CD have been described in a variety of carnivores and are, with some exceptions, similar to those described in domestic dogs. They include interstitial pneumonia or broncho-pneumonia, as well as conjunctivitis, rhinitis and inflammation of the tracheobronchial tree. Catarrhal to haemorrhagic enteritis, and hyperkeratosis of the nose, lips, eyelids, ears, anus and footpads may be present. Older or more immunocompetent animals tend to develop leucoencephalomyelitis, with a predominance of lesions in the caudal brainstem and spinal cord. Absolute lymphopenia is common. One major difference compared with domestic dogs is the jaundice associated with CDV in raccoons (*Procyon lotor*), ranch-raised foxes and ferrets (*Mustela putorius*).

On histologic examination, CDV inclusions are most commonly cytoplasmic and acidophilic-staining. Lymphoid depletion is another typical histologic finding in animals with systemic disease.

Immunity to CDV infection involves both humoral and cell-mediated mechanisms. Viral proteins are expressed on the cell surface; thus immune-mediated cytolysis plays a role in clearing the virus from infected animals[35]. Neutralizing antibodies in domestic dogs develop 10–14 days post-infection (pi) and titres peak after 4 weeks; they may persist for 2 years after exposure. The titre levels at which antibodies are protective in wild carnivore species is unknown; neutralizing antibody titres between 20 and 100 probably provide protection[36]. Wild animals that survive CD probably have lifelong immunity to subsequent infection by CDV.

CLINICAL SIGNS AND TREATMENT

The incubation period ranges from about 1 week to 1 month or longer. The classic clinical signs are depression and mucopurulent oculo-nasal exudates. Fever, anorexia, vomiting and diarrhoea frequently occur. CNS signs may be concurrent or follow systemic disease. Neurological signs depend on the area of brain affected and include abnormal behaviour, convulsions, cerebellar and vestibular signs, paresis or paralysis and incoordination. Animals that recover from clinical CD or that have a prolonged illness may be in poor body condition. Regardless of the ultimate disease outcome, the infection is characterized by a dramatic decrease in white blood cells and an inhibition of lymphocyte proliferation in the first weeks after infection.

In canids and mink, juveniles appear to be most susceptible. In mustelids, the clinical signs first recognized are serous oculo-nasal exudate, photophobia, and hyperemia and thickening of the eyelids, lips and anus. Mortality is variable in adult mink (20–90%) and is approximately 90% in mink kits.

CD in procyonids is clinically similar to that described in canids. Raccoons with CNS manifestations are frequently reported as behaving abnormally. Hyperkeratosis of the foot pads, with marked thickening and deep cracks, is relatively common in raccoons that have a prolonged clinical course. Mortality in experimentally infected raccoons varied from 50 to 100%[35].

Infected animals with upper respiratory signs should be kept in environments that are clean, warm and free of draughts. Pneumonia is frequently complicated by secondary bacterial infection, which usually requires a broad-spectrum antibiotic therapy. Parenteral therapy is essential when gastrointestinal signs are present. Food, water and oral medications or fluids should be discontinued if vomiting and diarrhoea are present. Therapy for neurologic disturbances is less rewarding. In general, animals should not be euthanized unless the neurologic disturbances are progressive or incompatible with life.

In severe clinical cases passive immunization over several days with a hyperimmune serum (e.g. Stagloba®) in is recommended and should be repeated after 14 days.

DIAGNOSIS

An important diagnostic feature of CD is the presence of intracytoplasmatic and intranuclear eosinophilic inclusion bodies in epithelia, neurons and astroglia. Inclusion bodies are also often present in gastric mucosa, enterocytes and pancreatic and biliary duct epithelium, as well as epithelium of the respiratory and urogenital tract.

Immunohistochemistry is useful for detection of CDV antigen in formalin-fixed, paraffin-embedded tissues. The use of direct and fast viral nucleic acid detection methods such as RT-PCR is an extremely sensitive assay to detect CDV in various tissues. Viral RNA can be demonstrated in brain, lung, spleen, liver, kidney, urinary bladder and

colon samples as well as in buffy coat cells. In addition, they can be applied to phylogenetic analyses, which are often based on partial sequences of the F-gene[37].

A virus neutralization test is the standard serological test for antibodies against CDV.

MANAGEMENT, CONTROL AND REGULATIONS

On more than one occasion, wild carnivores have been translocated or brought into captivity while incubating CD. Thus, CD must be considered in management plans and during conservation efforts.

Vaccination against CDV in non-domestic carnivores has been problematic. Currently there is no safe and efficacious commercially available CDV vaccine for use in non-domestic species. Live-virus-vaccine-induced CD has occurred in domestic ferrets (*Mustela putorius furo*), black-footed ferrets (*Mustela nigripes*), European mink and several other wild carnivores[38]. Most important is the interference between attenuated live vaccines and maternal antibodies, resulting in insufficient protection of offspring from vaccinated females[39]. Additionally, the attenuated live vaccines are associated with a risk of reversion to virulence, and various wildlife species have suffered from fatal infections caused by live vaccines. Hence, safer alternatives such as inactivated virus vaccines, subunit vaccines or recombinant vaccines are recommended. DNA vaccines are being investigated as an alternative vaccination strategy against canine distemper to overcome some of the limitations of attenuated live vaccines[40]. The efficacy and safety of three different CDV vaccines in the Eurasian otter (*Lutra lutra*), the Asian small-clawed otter (*Aonyx cinereus*) and the North American river otter (*Lutra canadensis*) have been evaluated[41]. Most of the animals vaccinated with inactivated CDV vaccine showed no seroconversion. Experimental CDV immunostimulating complexes (CDV-ISCOM) induced a variable immune response that depended on the otter species. High serum antibody titres were observed in almost all animals vaccinated with commercially available modified life vaccines (MLV). Taken together, the results indicate a poor immune response to inactivated distemper vaccine, especially in Eurasian otters.

Recently, a chimaeric virus combining the replication complex of a measles vaccine strain with the envelope of a recent CDV wild-type isolate was produced[42]. The resulting virus did not cause disease or immunosuppression in ferrets and conferred protection from challenge with a lethal wild-type strain, demonstrating its potential value for wildlife conservation efforts.

In conclusion, adequately controlling CDV infection in wildlife in Europe is difficult. It requires concerted transnational actions, including effective surveillance and prompt gathering and dissemination of information[28].

There are no established EU regulations for wildlife.

PUBLIC HEALTH CONCERN

A suggestion has been made that Paget's disease, an inflammatory bone disorder in people, might be related to CDV acquired from exposure to dogs[6]. The presence of CDV has been shown in Pagetic bone samples and CDV can infect and replicate in human osteoclast precursors in a dose-dependent manner[43]. This is the first evidence that CDV should be considered as a potential zoonosis.

SIGNIFICANCE AND IMPLICATIONS FOR ANIMAL HEALTH

CD is one of the most important infectious diseases in carnivores. It has shown significant impact on free-ranging populations of highly susceptible species such as black-footed ferrets (*Mustela nigripes*)[44] and gray foxes (*Urocyon cinereoargenteus*)[45] in North America. In Europe, CD may be a potential risk for e.g. the highly endangered Iberian lynx (*Lynx pardinus*)[46][26]. The impact on populations of other susceptible species within Europe is less clear.

MORBILLIVIRUS INFECTIONS IN AQUATIC MAMMALS

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AETIOLOGY

Five morbilliviruses have been reported to cause natural infections in aquatic mammals (Table 7.2):

TABLE 7.2 Method of diagnosis, time, location and impact of morbillivirus infections in aquatic mammals in Europe.

Morbillivirus	Host species	Diagnosed by	Year(s)	Geographical location	Estimated number affected	Reference
Canine distemper virus	Caspian seal (*Pusa caspica*)	RT-PCR	1997	Caspian Sea		47
	Caspian seal	RT-PCR	2000	Caspian Sea	>10 000	5, 48
Phocine distemper virus	Harbour seal (*Phoca vitulina*)	Virus isolation	1988	Kattegat, Skagerrak, Baltic Sea, Wadden Sea, North Sea, N.E. Atlantic	23 000	49 50, 51 52
	Harbour seal (Grey seal, *Halichoerus grypus*)	RT-PCR	2002	Kattegat, Skagerrak, Baltic Sea, Wadden Sea, North Sea, N.E. Atlantic	30 000	53, 54
Porpoise morbillivirus	Harbour porpoise (*Phocoena phocoena*)	Virus isolation	1988, 1990	North Sea, N.E. Atlantic	10	55, 56 57
	Mediterranean monk seal (*Monachus monachus*)[a]	Virus isolation	1996	Mediterranean Sea (Greece)	1	58
Dolphin morbillivirus	Striped dolphin (*Stenella coeruleoalba*)	Virus isolation	1990–1992	Mediterranean Sea	>400	59
	Long-finned pilot whale (*Globicephala melas*)	RT-PCR	2006–2007	Mediterranean Sea	>21	60
	Striped dolphin	RT-PCR	2007	Mediterranean Sea,	>100	61
	Striped dolphin, Bottlenose dolphin (*Tursiops truncatus*), Long-finned pilot whale	RT-PCR	2007–2008	Ligurian Sea Mediterranean Sea	143	62
	White-beaked dolphin (*Lagenorhynchus albirostris*)	Virus isolation	2007	North Sea (Germany)	1	63
	Common dolphin (*Delphinus delphis*)	RT-PCR	1994	Black Sea	?	64
Pilot whale morbillivirus	Fin whale (*Balaenoptera physalus*)	Histology and IHC	1997–1998	North Sea	2	65
	Short-finned pilot whale (*Globicephala macrorhynchus*)	Histology	1986	Atlantic, Canary Islands	1	66

[a]Porporoise-morbillivirus-like virus

- *Canine distemper virus* (CDV) infection (canine distemper; synonym PDV2 infection);
- *Phocine distemper virus* (PDV) infection (phocine distemper; synonyms seal distemper, seal plague, PDV1 infection);
- *Dolphin morbillivirus* (DMV) infection;
- *Porpoise morbillivirus* (PMV) infection;
- *Pilot whale morbillivirus* (PWMV) infection.

For consistency in this section of the chapter, the terms 'CDV disease' rather than 'canine distemper', and 'PDV disease' rather than 'phocine distemper', are used.

EPIDEMIOLOGY

In 1988, about 50% of the North-East Atlantic population of harbour seals (*Phoca vitulina*) died within a few months. The causative agent was identified as a new morbillivirus, PDV[49–51]. A PDV epidemic of similar scale occurred for a second time in 2002, after the harbour seal population had returned to pre-1988 levels[53]. In 1988 a second new morbillivirus, PMV, was identified in harbour porpoises (*Phocoena phocoena*) that stranded on the coast of Northern Ireland[55]. A third new morbillivirus, DMV, was identified as the cause of a large epidemic in striped

dolphins (*Stenella coeruleoalba*)[59]. This epidemic started in the western Mediterranean Sea in 1990 and spread eastwards in 1991 and 1992. Two years later, an epidemic of morbillivirus disease occurred in common dolphins (*Delphinus delphis*) in the adjacent Black Sea[64]. Phylogenetic analysis based on the sequence of a nucleoprotein gene fragment showed 97% identity with dolphin morbillivirus (M. van de Bildt, personal communication). A second smaller DMV epidemic in striped dolphins in the western Mediterranean Sea occurred in 2007, and was preceded in 2006–2007 by an epidemic from the same virus in long-finned pilot whales (*Globicephala melas)* found stranded in the Strait of Gibraltar and southern Spain[60,61]. The Caspian seal (*Pusa caspica*) population, which is restricted to the Caspian Sea, suffered a large CDV epidemic in 2000[5]. In addition, infections with different morbilliviruses, including PWMV, have been identified in individual strandings of marine mammals in Europe (Table 7.2).

GEOGRAPHICAL DISTRIBUTION IN EUROPE

Morbillivirus infections have been diagnosed in multiple species of aquatic mammals in all the major waters of Europe, including the (North-)East Atlantic Ocean and the North, Baltic, Mediterranean, Black and Caspian Seas (Table 7.2). Rather than being endemic, they occur as epidemics in naïve populations or as isolated cases.

HOST FACTORS

Marine mammal species affected by morbillivirus disease belong to the suborders Pinnipedia, Odontoceti and Mystacoceti. There also is evidence of morbillivirus infection in the order Sirenia and in polar bears (*Ursus maritimus*) by serology and in sea otters (*Enhydra lutris*) by RT-PCR[67,68]. Therefore, many species of aquatic mammals should be considered susceptible to morbillivirus infection.

Many of the aquatic mammal species occurring in Europe are susceptible to fatal disease from morbillivirus infection: harbour seal (PDV), Caspian seal (CDV), harbour porpoise (PMV), striped dolphin (DMV), bottlenose dolphin (*Tursiops truncatus;* DMV and PMV)[69], long-finned pilot whale (*Globicephala melas*; DMV and PWMV)[70], white-beaked dolphin (*Lagenorhynchus albirostris;* DMV), common dolphin (DMV), short-finned pilot whale (*Globicephala macrorhynchus;* PWMV) and fin whale (*Balaenoptera physalus;* unidentified morbillivirus) (Table 7.2). Some species are known to be susceptible to morbillivirus infection, but the pathogenicity of these infections is not known, e.g. Mediterranean monk seal (*Monachus monachus;* PMV-like virus, DMV)[58], harp seal (*Pagophilus groenlandicus*; PDV), hooded seal (*Cystophora cristata*)[71]. The grey seal is unusual, because it appears relatively resistant to disease by at least one morbillivirus, PDV, based both on field and experimental data[72,73].

Aquatic mammals of all ages and both sexes appear to be susceptible to morbillivirus infection and disease, judging from their representation during reported epidemics and post-epidemic serological studies. However, age and sex may affect risk of infection and the severity of disease. PDV appeared to spread through the subadult fraction of the harbour seal population before the juvenile and adult fraction in the Dutch 2002 epidemic[74], indicating different probabilities of disease transmission among different age cohorts[75]. DMV affected mainly juvenile striped dolphins during the 2007 epidemic. A likely explanation is that the older animals that survived the 1990 epidemic had developed protective immunity[62]. PDV appeared to spread faster among males than among females of all age categories during the 2002 epidemic; possible reasons were higher contact rates among males and higher contaminant levels in tissues of male seals[54,74].

ENVIRONMENTAL FACTORS

Pollution, climate and fisheries activities have been associated with triggering the occurrence of morbillivirus epidemics in aquatic mammals, with enhancing mortality, or both. The tissues of harbour seals that died during the 1988 epidemic had high levels of pollutants, including polychlorinated biphenyls (PCBs) and dichlorodiphenyltrichloroethane (DDT). Based on a semi-field study comparing the immunological parameters in harbour seals fed contaminated or clean fish, it was concluded that the pollutant levels found in 1988 were sufficiently high to have caused immune suppression[76]. The same may have occurred during the 2002 epidemic in harbour seals, because the levels of PCBs had not decreased significantly (Kajiwara et al., unpublished data). Pollution may also have exacerbated the 1990 epidemic in striped dolphins, because tissues of animals that died during the epidemic had higher pollutant levels than those that stranded before[77]. By contrast, the tissue levels of pollutants in

Caspian seals that died during the 2000 epidemic were similar to those found in apparently healthy animals from previous years[48], as was also the case for striped dolphins stranded during the 2007–2008 epidemic, and before and after it[77].

The winters preceding the 1988 PDV, the 1990 DMV, and the 2000 CDV epidemics were unusually mild. It is speculative, but possible, that such weather events may trigger morbillivirus epidemics in aquatic mammals. Hypotheses include increased hauling-out patterns of seals, resulting in higher contact rates, and changes in fish abundance and seal species distribution, the latter resulting in a higher risk of cross-species transmission of morbillivirus[48].

Fisheries activities were speculated to have triggered the 1988 PDV epidemic indirectly. An unusual southward migration in the preceding winter brought harp seals – considered, on the basis of serological studies, to have been exposed and therefore a possible source of PDV – into the range of the North-East Atlantic harbour seal population. This movement may have been triggered by overharvesting of the fish stocks around Greenland, where harp seals normally forage[54].

The marine mammal populations in Europe that have undergone morbillivirus epidemics are probably not the reservoirs for these viruses; these reservoirs are unknown. The North-East Atlantic harbour seal population of about 50,000 is probably not a reservoir for PDV, as sera from before 1988 lacked specific antibody, seroprevalence declined between the 1988 and 2002 epidemics, and no infections were reported in this inter-epidemic period. Instead, the reservoir is speculated to be in aquatic mammals, e.g. harp seals, from the Northern Atlantic or Arctic Oceans, based on high seroprevalence in those populations, and one probable clinical case in a juvenile harp seal from Canada[78]. In any case, the P- and H-gene sequences of the 1988 and 2002 PDV isolates were virtually identical and thus probably had the same source[53,54].

The striped dolphin population of the Mediterranean Sea is probably not the reservoir of DMV, based on declining seroprevalence and lack of reported DMV infections between the 1990–1994 and 2007 epidemics. The reservoir of DMV is speculated to be in aquatic mammal populations in the Atlantic Ocean, e.g. in long-finned pilot whales, in which the seroprevalence is high[79]. Long-finned pilot whales with DMV infection in which the virus is genetically nearly identical to that in striped dolphins were found stranded along the Strait of Gibraltar just before the 2007 epidemic, and may have introduced DMV into the Mediterranean Sea via this route[60,61].

The reservoir for CDV that affected Caspian seals in 2000 could be the Caspian seal population itself, which was estimated at 111,000 in 2005[80]. Antibody to CDV was detected in Caspian seal sera as far back as 1993, and P- and F-gene sequences of the CDV from 2000 were identical to those of a CDV found in a Caspian seal in 1997. Alternatively, CDV could have been reintroduced repeatedly into Caspian seals from adjacent populations of terrestrial carnivores, e.g. domestic dogs or wolves[5,48].

It is not clear whether the North-East Atlantic Ocean harbour porpoise population of about 385,000[80] is a reservoir for PMV. Specific antibody was detected in harbour porpoises collected between 1991 and 1999 from the North and Baltic Seas and from the North-East Atlantic Ocean[79,81]. However, PMV has not been detected in harbour porpoises since its report in 10 harbour porpoises from the North and Irish Seas between 1988 and 1992[56,57,67], despite examination of over 500 carcasses found stranded or by-caught in the North Sea and North-East Atlantic Ocean between 1990 and 2000[82–84].

The little information available about morbillivirus infections in aquatic mammals is skewed towards coastal species in temperate climates. Epidemics occurring in more pelagic species, such as white-beaked dolphins and fin whales, may go unnoticed because their carcasses are less likely to reach a coast. Epidemics occurring in polar species, such as harp seals, also may go unnoticed because of low human densities in their range. Therefore, it is even more difficult to make any conclusions on the role of these pelagic and polar species in the epidemiology of morbillivirus infections.

TRANSMISSION

The route of transmission of morbilliviruses in aquatic mammals is speculative and experimental data is scant, but it is assumed to be similar to that in terrestrial mammals. PDV was isolated from nasal fluid of a harbour seal on day 11 post-inoculation[72].

The transmission of PDV in harbour seals may occur when they are in the water, where they have more direct contact than when they are hauled out on land. On land, direct contact is less common, but transmission by indirect contact might take place via infectious aerosols, secreta and excreta. Wind gusts could favour horizontal and concentrated movement of aerosolized virus.

Transmission of DMV in dolphins may take place by direct contact, e.g. during coitus, or by indirect contact, e.g. by aerosol[67]. Transmission by inhalation of expired blowhole secreta might be important because of the combination of aggregating in large schools, forceful expiration and inspiration, and breathing in a synchronized fashion[85].

PATHOGENESIS, PATHOLOGY AND IMMUNITY

Little is known about the pathogenesis of morbillivirus disease in aquatic mammals. Current concepts come largely from pathogenesis studies of morbillivirus infections in terrestrial mammals, supplemented by observations from both natural and experimental infections in aquatic mammals. Although morbillivirus in terrestrial mammals may enter via other sites (e.g. conjunctiva, digestive tract, urogenital tract and skin wounds), the main site of entry is assumed to be the respiratory tract. Harbour seals were successfully infected experimentally by droplets on the nasal mucosa[72]. Even in the respiratory tract, the primary target cells are probably immune cells expressing signalling lymphocyte activation molecule (SLAM), the receptor for morbillivirus. Peripheral blood leucocytes of aquatic mammals also express SLAM[86]. The first tissues found positive after infection are the lymph nodes of the head and lower respiratory tract.

Morbillivirus has a high infectivity, demonstrated for PDV in harbour seals, where a low dose is sufficient to cause fatal infection[87]. After amplification in the regional lymph nodes of the head and lower respiratory tract, the virus spreads via the blood to other lymphoid tissues, where a second cycle of amplification takes place. In experimentally infected harbour seals, leucocyte-associated viraemia occurs from 5 to 12 days post-inoculation, and sometimes beyond[72]. The resulting infection of the lymphoid tissues weakens host immunity, demonstrated in aquatic mammals by the severe lymphoid depletion of lymphoid organs associated with virus infection. The third stage of virus spread is to the epithelia of multiple organ systems (respiratory, digestive, urogenital), to the central nervous system (for some morbillivirus species), and to a variety of miscellaneous cell types such as fibroblasts. This systemic spread of morbillivirus also occurs in aquatic mammals[72]. Because not all these cell types express SLAM, morbilliviruses presumably have an alternative mechanism to enter cells.

Morbillivirus-induced immune depression allows facultative pathogens to spread and cause severe secondary disease. An example in PDV-infected seals is *Bordetella bronchiseptica*, which can spread to the lower respiratory tract and cause bronchopneumonia[88,89]. Spread of morbillivirus to the central nervous system causes necrosis of neurons and glial cells and associated inflammation, resulting in severe nervous dysfunction. Spread of morbillivirus to epithelial cells in various organ systems may lead to the development of intranuclear inclusion bodies (INIB) and intracytoplasmic inclusion bodies (ICIB), which are not necessarily associated with severe disease.

The speed and level of the immune response may influence the extent of morbillivirus spread throughout the body. Despite lymphoid depletion, infected hosts can mount an immune response. In harbour seals experimentally infected with PDV, neutralizing antibodies were first detected at 7 days post-infection and reached a maximum 3 weeks later[72]. An experimentally infected grey seal also developed antibodies[87]. Harbour seal pups, of naturally infected mothers, had detectable maternal antibodies until 3 months after birth, at which time they were fully susceptible to PDV infection[72].

In host species susceptible to morbillivirus disease, the vast majority of morbillivirus infections end abruptly within days after the onset of clinical signs, either because the host dies, or because the host has mounted an immune response that clears the virus from tissues. The infectious period is therefore generally short. It is not always fully understood how death is caused. Some hosts may die from neurological complications resulting directly from virus infection of the central nervous system, and others from disease caused by a facultative pathogen secondary to virus-induced immune depression. Many seals and dolphins dying during morbillivirus epidemics had a morbillivirus-associated encephalitis, a secondary bacterial bronchopneumonia, or both[88,89]. In hosts that completely clear the infection, the immunity is long-term, often lifelong, which explains why virtually no harbour seals older than 14 years stranded during the 2002 PDV epidemic, and why most of the striped dolphins that stranded during the 2007 DMV epidemic were juveniles[61,90].

Occasionally, viral clearance is incomplete. In particular, this involves animals with virus infection in the brain, which usually die within a few months. They are not known to be infectious beyond 2 months after infection. Very rarely, neurological disease only develops years after infection. In such cases, virus is restricted to the brain and may

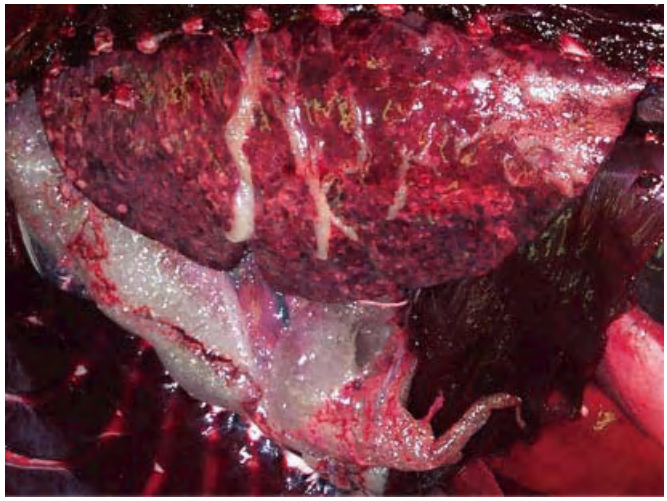

FIGURE 7.4 Pulmonary and mediastinal emphysema in a harbour seal with PDV infection. The ventral part of the lung is consolidated. Reprinted with permission from Rijks et al., 2008.[89]

be defective. Neurological cases after epidemics have been documented in marine mammals. Up to 4 years after the 1990 DMV epidemic, striped dolphins were found stranded dead with a DMV infection restricted to the central nervous system and associated with encephalitis[91]. Also, the authors have diagnosed encephalomyelitis in an approximately 14-year-old harbour seal in 2001, 13 years after the 1988 PDV epidemic (unpublished data). In this animal, infection was restricted to the central nervous system with a PDV variant that had a genetic signature that indicated it was defective, which suggests that it had survived for 13 years after becoming infected with PDV in 1988.

Aquatic mammals that die with morbillivirus infection have a nutritional state ranging from poor to good, and generally no food in the stomach[48,60,64,89,92–94]. The main gross lesions in several, but not all, aquatic mammal species with morbillivirus infection are in the lungs. Harbour seals with PDV infection, Caspian seals with CDV infection, and harbour porpoises with PMV infection have mutifocal pulmonary consolidation (Figure 7.4) characterized by irregular, red or grey, firm areas that sink in water. This is caused at least partly by secondary bacterial infections, of which *B. bronchiseptica* is most important in harbour and Caspian seals. Striped dolphins with DMV infection have multifocal atelectasis. A related lesion in harbour seals with PDV infection but not in other aquatic mammal species is marked emphysema, which is usually mediastinal, pericardial, retroperitoneal and subcutaneous (Figure 7.4). The occurrence of emphysema in harbour seals may be due to well-developed interlobular septa that prevent collateral ventilation in case of airway obstruction[89].

No gross pulmonary lesions were recorded in striped dolphins that stranded after the main part of the 1990 DMV epidemic, long-finned pilot whales and a white-beaked dolphin with DMV infection, and common dolphins with DMV infection.

Other gross lesions, which are often caused by secondary infections, include: enlargement, oedema and multifocal necrosis of the lymph nodes in long-finned pilot whales; multifocal erosive or ulcerative stomatitis of unknown aetiology in striped dolphins), pilot whales and common dolphins; similar lesions in the oesophagus, stomach, intestine or a combination of these in long-finned pilot whales, common dolphins and harbour porpoises; multifocal necrotizing encephalitis from *Aspergillus* sp. infection in striped dolphins; and suppurative bacterial balanoposthitis in Caspian seals and harbour porpoises.

The main organs in which microscopic lesions of morbillivirus infection are observed in aquatic mammals are lymphoid organs, brain and lungs, although lesions containing characteristic inclusion bodies may frequently be found throughout the body. Although there is an overall similarity in these lesions, characteristic changes may be more pronounced in one morbillivirus-aquatic mammal combination than in another (Table 7.3). Another factor to consider is that the morbillivirus-associated lesions in lungs and lymphoid organs often are confounded by co-infections from lungworms and secondary infections from bacteria and fungi.

Lymphoid organs (spleen, lymph nodes, Peyer's patches, thymus) have generally marked lymphoid depletion and lymphocytic necrosis. In long-finned pilot whales with DMV infection, however, the changes in the lymph nodes, considered to be the main microscopic lesion, have been described as multifocal necrotizing lymphadenitis[60]. In these pilot whales, as well as in striped dolphins, common dolphins and fin whales, syncytia have been observed in the remaining lymphoid tissue. Many histiocytes and some dendritic cells in lymphoid organs of Caspian seals have ICIB, and some syncytia in lymphoid organs of common dolphins and the fin whale have INIB. By immunohistochemistry for morbillivirus antigen, there is specific staining of lymphocytes, macrophages, dendritic cells, syncytia and (in common dolphins) fibroblasts.

Lungs have a multifocal broncho-interstitial pneumonia. Syncytia in alveolar and bronchiolar lumina are more

TABLE 7.3 Comparison of morbillivirus-specific microscopic lesions in aquatic mammals infected with morbillivirus.

Lesion	Tissue	Abundance of lesions per host–morbillivirus pair							
		Harbour seal PDV (88,89,95)	Caspian seal CDV (48)	Striped dolphin DMV early (59)	Striped dolphin DMV late (91)	Pilot whale DMV (60)	White-beaked dolphin DMV (63)	Common dolphin DMV (64)	Harbour porpoise PMV (56,94)
Inflammation	Lymphoid	–	–	–	–	++	n.d.	–	–
	Cerebrum	+	–	++	++	++	++	–	++
	Lung	++	+	+	–	+	+	+	++
	Other	–	–	–	–	–	–	–	+
Syncytia	Lymphoid	–	–	+	–	+	n.d.	+	–
	Cerebrum	+	–	+	–	+	–	–	–
	Lung	+	+	++	–	–	–	+	++
Inclusions	Lymphoid	–	+	–	–	–	n.d.	+	–
	Cerebrum	+	+	++	+	+	+	+	++
	Lung	+	++	++	–	–	–	+	++
	Other	+	++	+	–	–	n.d.	+	++

–, absent; +, present; ++, prominent; n.d., not done

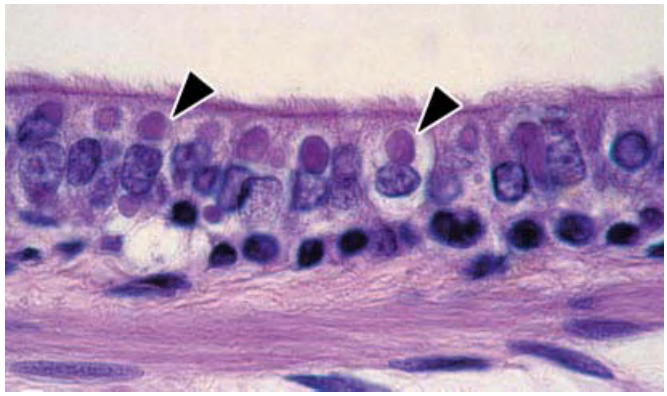

FIGURE 7.5 Acidophilic intracytoplasmic inclusions (arrowheads) in ciliated epithelial cells of a bronchiole from a Caspian seal with CDV infection. Haematoxylin and eosin. Reprinted with permission from Kuiken et al., 2006[48].

prominent in striped dolphins and harbour porpoises than in harbour seals, Caspian seals and common dolphins. Inclusion bodies, both intracytoplasmic and intranuclear, have been observed in macrophages and syncytia in alveolar and bronchiolar lumina, and in epithelial cells lining the alveoli, bronchioles, bronchi and trachea. These inclusion bodies are abundant and prominent in bronchiolar epithelium of Caspian seals (Figure 7.5) and in syncytia in striped dolphins and harbour porpoises, but rarer in common dolphins and harbour seals. By immunohistochemistry for morbillivirus antigen, there is specific staining of the same cell types as those in which inclusion bodies are observed.

The brain, mainly cerebrum, has a non-suppurative encephalitis in many striped dolphins[91,93] and a white-beaked dolphin with DMV infection[63], many harbour porpoises with PMV infection[56,94], some harbour seals with PDV infection[92,95], but not in Caspian seals with CDV infection[48]. Characteristic changes in the grey matter are neuronal degeneration and necrosis, gliosis, perivascular cuffing and oedema. In the white matter, demyelination is observed in many harbour seals with PDV infection, some striped dolphins with DMV infection, but not in harbour porpoises with PMV infection. Acidophilic or amphophilic INIB and ICIB have been observed in neurons, astrocytes, microglia and ependymal cells. By immunohistochemistry for morbillivirus antigen, there is specific staining of generally the same cell types as those in which inclusion bodies are observed, as well as perivascular lymphocytes.

Besides lymphoid organs, lungs and brain, microscopic lesions of morbillivirus infection may occur in many other tissues. These lesions are characterized by the presence of acidophilic or amphophilic ICIB and (less often) INIB. These inclusion bodies have been described in epithelial cells of the urinary tract, digestive tract, bile duct, pancreatic collecting duct, eye, lacrimal gland, mammary gland

and skin. The presence of inclusion bodies may be associated with degeneration and necrosis of affected epithelial cells, as well as with inflammation from secondary bacterial or fungal infections.

CLINICAL SIGNS

Clinical signs of morbillivirus disease are general malaise with or without involvement of the respiratory, digestive and reproductive tracts, central and peripheral nervous system including eyes, and skin. The clinical signs are partly caused by secondary disease from infection with facultative pathogens.

Most information about clinical signs of morbillivirus disease in pinnipeds comes from harbour seals, owing to detailed observation in rehabilitation centres. Harbour seals with PDV infection show clinical signs affecting the whole body (depression, lethargy, fever or hypothermia), central and peripheral nervous systems (abnormal posture, paresis, ataxia, seizures, head tremors and myoclonus of flipper or facial muscles), eyes (mucopurulent ocular discharge, redness of conjunctiva and sclera, corneal opacity, unilateral or bilateral blindness and nystagmus) (Figure 7.6), respiratory system (mucopurulent nasal discharge, coughing, tachypnea, dyspnea and subcutaneous emphysema), digestive system (diarrhoea), reproductive system (abortion) and skin (dermatitis)[67,96]. A subset of these clinical signs has also been seen in Caspian seals with CDV infection[48] and harp seals and hooded seals with PDV infection[71]. There is scant information about clinical signs of morbillivirus disease in cetaceans: most are found stranded dead or moribund. Striped dolphins with DMV infection can show clinical signs affecting the whole body (poor body condition leading to reduced flotation, craniodorsal subcutaneous oedema, tachycardia, weak sound emission), nervous systems (disorientation, striking against rocks, no interest in swimming, muscle tremors), respiratory system (abnormal respiratory rates), digestive system (ulcers in the oral mucosa) (Figure 7.7) and skin (increased burdens of external parasites)[61,67,93]. Common dolphins with DMV infection can exhibit seizures, uncontrolled trembling and dyspnea.

FIGURE 7.6 Mucopurulent discharge from eyes and nose of a harbour seal with PDV infection. Courtesy of J. Philippa.

FIGURE 7.7 Multifocal erosive and ulcerative stomatitis in a striped dolphin with DMV infection. There are multiple erosions and vesicles in the hard palate, and linear ulceration in the gingiva around the teeth. Reprinted with permission from Domingo et al., 1992[93].

There is no specific treatment for morbillivirus disease. Antibiotic treatment may be given against secondary bacterial infections.

DIAGNOSIS

The demonstration of acidophilic or amphophilic ICIB and INIB in typical tissues by microscopic examination is sufficiently specific to make the definitive diagnosis of morbillivirus disease.

The diagnosis of morbillivirus infection to genus may be confirmed by immunohistochemistry or serology. A

two-fold increase in virus-neutralizing antibody (mainly IgG) titre in serial serum samples is diagnostic[97], as well as a single positive IgM titre to morbillivirus by enzyme-linked immunosorbent assay (ELISA)[53]. Both the virus neutralization (VN) test and ELISA may give false negatives, because not all infected animals mount an adequate immune response[96].

The diagnosis of morbillivirus infection may be confirmed to species by PCR or virus isolation. Samples of choice are nasal and pharyngeal swabs from live animals and brain, lung, tracheo-bronchial lymph node, spleen, liver and kidney from carcasses. Brain samples should not be omitted because infection may be limited to this tissue[89]. Besides standard cell cultures, Vero cells expressing dog-SLAM may be used[98]. Morbilliviruses grow faster and to a higher titre on Vero-SLAM cells, and isolation of field viruses is more sensitive in these cells.

Population screening for morbillivirus infection is often performed on live animals by serology, and on dead stranded animals by PCR or immunohistochemistry on tissues. Blood samples from dead stranded animals also can be used for serologic screening, but because of haemolysis the more specific VN is then precluded and results are often not reproducible.

MANAGEMENT, CONTROL AND REGULATIONS

Management of morbillivirus infections in aquatic mammals depends on the susceptibility and conservation status of the host species, and on the virological and immunological status of the host population. In free-living aquatic mammals, population management needs to account for potential reduction of population size from recurrent epidemics and for risk of virus transmission between separate populations. Such virus transmission can occur directly through translocation of individual animals and indirectly through fishing or other human activities that influence the home range and migratory patterns of aquatic mammal populations. Vaccination by injection is impractical for free-living aquatic mammals. It is only considered when species are highly endangered, for example, the endangered Mediterranean monk seal[99].

In captive aquatic mammals, it is important to minimize the risk of virus introduction from both terrestrial and aquatic mammals by restricting access of other species and by following appropriate hygienic and quarantine procedures. Captive aquatic mammals can be vaccinated with an inactivated vaccine against the homologous or heterologous virus. Harbour seals brought into rehabilitation centres during PDV epidemics have been successfully vaccinated with inactivated CDV vaccines, including commercially available vaccines[100]. Recently, DNA vaccines have been developed[101]. The use of live attenuated vaccines is contra-indicated because of the risk of disease to the vaccinated animal and because of the risk of virus spread in the population.

CDV, PDV, DMV, PMV and PWMV infections are not notifiable, but the wildlife group of the World Organisation for Animal Health (OIE) does request their reporting. The morbillivirus OIE reference laboratory is the Institute for Animal Health, Pirbright Laboratory.

PUBLIC HEALTH CONCERN

The primary public health concern from morbillivirus infections in aquatic mammals is not from the virus itself but from secondary infections that are exacerbated because of the morbillivirus-induced immunosuppression. For example, morbillivirus-infected aquatic mammals have been diagnosed with toxoplasmosis[93], systemic *Streptococcus* spp., *Staphyloccus aureus* or *Escherichia coli* infections[88,89], which pose a risk to people handling such animals.

CDV, PDV, DMV, PMV and PWMV infections are currently not considered to be zoonoses. However, as for CDV, it cannot be ruled out that in the event of eradication of measles, humans could become a new ecological niche for a morbillivirus species from aquatic mammals[102].

SIGNIFICANCE AND IMPLICATIONS FOR ANIMAL HEALTH

Some morbilliviruses from aquatic mammals present a threat to domestic animals. Although DMV and PMV did not cause clinical disease in experimentally infected domestic dogs and ruminants[57], PDV did cause clinical disease in experimentally infected domestic dogs and experimentally or naturally infected mustelids[103,104].

Morbillivirus infections present a serious threat to endangered aquatic mammals, e.g. the Mediterranean monk seal. The high mortality they cause may reduce numbers to a level that results in extinction. Morbillivirus infections may also cause high mortality in less endangered aquatic mammals and need to be accounted for in their population management.

EXOTIC MORBILLIVIRUS INFECTIONS

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RINDERPEST

Rinderpest, also named *la peste bovine*, and *steppe murrain*, is the most devastating plague of cattle known in history. Rinderpest virus (RPV) is classified in the genus *Morbillivirus*, within the family *Paramyxoviridae*. Molecular studies indicate that it may be the oldest morbillivirus, from which the other members of the genus evolved.

Rinderpest caused massive losses in cattle and wildlife principally in sub-Saharan Africa during the end of the 19th century. Rinderpest also affected parts of East Africa, southern and central Asia and parts of the Middle East. Devastating epidemics of rinderpest occurred across Europe during the 18th and 19th centuries.

Outside Africa and Asia, Cervidae might be expected to be the main group of susceptible wild species; deer were reported to have been severely affected during the last British epidemic in 1865. Rinderpest may have caused high mortality in European bison (*Bison bosanus*) in 1890 and deer in 1865 in Europe[105]. In Western Europe, the last confirmed outbreak of rinderpest was in a zoo in Italy, and resulted from the importation of infected wildlife from Somalia[106].

The maintenance hosts of RPV are domestic cattle and African buffalo (*Syncerus caffer*). It is considered that wild animals do not act as reservoirs, and rinderpest was eradicated from areas with high populations of wildlife[107].

Transmission of RPV requires close contact between infected and susceptible animals. Animals that recover are immune for the rest of their life and do not excrete the virus.

The typical clinical signs in cattle are: depression, fever, ocular and nasal discharges, necrosis of the oral mucosa, diarrhoea, dehydration and death. Case-mortality rates with virulent strains of virus in highly susceptible cattle populations may exceed 90%. Clinical signs seen in susceptible wildlife are similar to those seen in domestic cattle.

Global efforts to eradicate rinderpest from livestock were successful. In 2010 the UN's Food and Agriculture Organisation announced the end of field operations and the successful conclusion of the Global Rinderpest Eradication Programme[108].

PESTE DES PETITS RUMINANTS

Peste-de-petits-ruminants (PPR), or goat plague, is a highly contagious, systemic disease of sheep and goats caused by a morbillivirus. The virus is distinct but similar to the rinderpest virus. There are four lineages of the PPR virus, which show some differences in virulence. PPR occurs endemically in sub-Saharan Africa, West Africa, the Middle East and Southern Asia, causing important economic losses. PPR occurred in Turkey, and serological evidence of PPR in small ruminants has been reported in Tunisia. Wild ungulates are also susceptible to PPR.

PPR was found in the United Arabian Emirates (UAE) in several wild ruminant species kept under semi-free-ranging conditions (bushbucks (*Tragelaphus scriptus*), impala (*Aepyceros melampus*), rheem gazelles (*Gazella subgutturosa marica*), Arabian mountain gazelles (*Gazella gazella cora*), springbuck (*Antidorcas marsupialis*), Arabian gazelles (*Gazella gazella*), Nubian ibex (*Capra nubiana*), barbary sheep (*Ammotragus lervia*) and Afghan markhor goat (*Capra falconeri*)). Diagnosis was confirmed by morphological, immunohistochemical, serological and molecular findings. Phylogenetic analysis revealed that the virus strain belongs to the lineage IV, which is different to some previously isolated PPR strains from the Arabian Peninsula[109].

In Cervidae, fatal and subclinical disease was reported in white-tailed deer (*Odocoileus virginianus*)[110]. PPRV specific antibodies were detected in free-roaming goitred gazelle (*Gazella subgutturosa subgutturosa*) on a farm in Anatolia (Turkey)[111]. To date, clinical PPR has not been reported in free-living European wildlife, but it has the potential to cause severe disease in some European wild species, in particular in wild caprines and ovines.

PPR is characterized by extensive necrosis of the mucosae of the oral cavity and gastrointestinal tract as well as pneumonitis. Clinical signs include serous or mucopurulent ocular and nasal discharges, with plaques of necrosis in the nasal and oral mucosae and diarrhoea.

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CHAPTER

8

ORBIVIRUS INFECTIONS

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INTRODUCTION

The genus *Orbivirus*, within the family *Reoviridae*, has a worldwide distribution and consists of 14 groups or species of virus, plus a number of unclassified viruses. Each of the virus groups includes a variable number of serotypes and antigenic complexes, and reassortment is reported to be able to occur between at least some members of a group but not between members of different groups[1].

Orbivirus virions have an indistinct outer capsid and a genome composed of 10 segments of dsRNA. The intact virions are about 80 nm in diameter and the core particles about 60 nm. Virus infectivity is stable at pH 8–9, but there is a marked loss of infectivity outside the pH range of 6.5–10.2. In sterile blood samples or when held in serum, viruses may remain infectious for decades at less than 15°C. They are rapidly inactivated on heating to 60°C but are resistant to treatment with solvents and detergents.

Depending on the virus, the vertebrate hosts of orbiviruses include ruminants (domestic and wild species), equids (domestic and wild species), rodents, bats, marsupials, sloths, birds and primates, including humans[1].

Orbiviruses replicate in, and are principally transmitted between, their vertebrate hosts by blood-sucking arthropods, which are biological vectors. Depending on the virus the vectors include mosquitoes, phlebotomines (sandflies), *Culicoides* biting midges and hard and soft ticks. Infection of vertebrate hosts *in uteri* may also occur with some viruses[1]. Orbivirus infection apparently has no ill effects on the arthropod vector, but in the vertebrate hosts the results of infection can range from subclinical to fatal depending on the virus and the host. Several orbiviruses cause significant diseases in domestic and/or wild livestock, and probably the most important of these are African horse sickness (AHS), bluetongue (BT) and epizootic haemorrhagic disease (EHD). BT has been attracting much international attention recently because of its massive and severe incursions into and through Europe.

BLUETONGUE

Bluetongue virus (BTV) causes a non-contagious, infectious, insect-borne disease of wild and domestic ruminants, known in English as bluetongue, in French as *fièvre catarrhale du mouton* and in Spanish as *lengua azul*. BTV is the type species of the genus *Orbivirus* and exists as 24 – soon to be 25 – serotypes.

BTV virions consist of a double-layered capsid surrounding a core particle. The diffuse outer layer comprises two structural proteins (VP2 and -5), which are responsible for the induction of serotype-specific neutralizing antibodies. The outer capsid encloses a core particle of 32

Infectious Diseases of Wild Mammals and Birds in Europe, First Edition. Edited by Dolores Gavier-Widén, J. Paul Duff, and Anna Meredith.

ring-like capsomeres arranged in icosahedral symmetry and consisting of five further structural proteins (VP1, -3, -4, -6 and -7). Within the core particle is the 10-segmented dsRNA genome.

EPIDEMIOLOGY

GEOGRAPHICAL DISTRIBUTION

Until recently, the global distribution of BTV was thought to lie approximately between 35°S and 40°N. Within these bounds it has a worldwide distribution occurring in the Americas, Africa, the Middle East, the Indian subcontinent, China, South-East Asia and northern Australia. Historically, it also occasionally invaded Iberia and a few Greek islands. Cyprus is the only area of Europe where BTV has occurred regularly.

Subsequent to the 1979–1980 outbreaks in Greece (Lesbos, Rhodes), BTV remained absent from Europe (excluding Cyprus) for almost 20 years. However, in late 1998 new incursions commenced, mainly affecting domestic ruminants. The outbreaks started in several Greek islands close to Turkey but spread to include European Turkey, mainland Greece, Bulgaria, Macedonia, Albania, Bosnia, Croatia, Montenegro and Serbia. There were several unique features to this incursion. First, it involved four serotypes of BTV (1, 4, 9 and 16) whereas all previous incursions into Europe had involved only a single serotype at a time. Second, serotypes 1, 9 and 16 had never previously entered Europe; and third, the incursion extended to the River Sava in Serbia, which at 44° 50′N is almost 500 km further north than BTV had ever been recorded in Europe[2].

In 2000 BTV was also recorded for the first time on Italian territory, in Sardinia. The origin of the outbreak was probably northern Tunisia, where the virus had been active since late 1999[3]. This incursion was due to BTV2 and was the first occasion this serotype had been recorded in Europe. It spread from Sardinia to Sicily and to several regions on mainland Italy. In the period 2002–2003 a new phase of the incursion commenced when, in addition to BTV2, BTV4, BTV9 and BTV16 were also identified and disease was detected in eight provinces in Italy[3]. BTV4, BTV9 and BTV16 had already been reported from the eastern Mediterranean Basin (see above) but the route of incursion into Italy is uncertain, although illegal movement of animals is suspected. Outbreaks due to one or more of these serotypes have continued in some Italian provinces until the time of writing (2010).

In late 2000, BTV invaded French territory for the first time, when BTV2 was identified in Corsica and the outbreak continued until mid-2001. In 2003, a further incursion into Corsica occurred when BTV4 was identified, and this was followed in 2004 by BTV16.

In September–October 2000 the first BTV incursions into Spanish territory since 1960 commenced. The initial outbreaks, on the Balearic islands, were due to BTV2, although in 2004 BTV4 was also isolated there[4]. Subsequent incursions of BTV4, from 2004, involved wide swaths of mainland Spain and Portugal. This latter incursion probably originated in Morocco and/or Algeria and after starting in southern mainland Spain spread northwards and westwards. In 2007 a fresh BTV incursion from North Africa occurred. This involved BTV1, and the virus spread northwards, reaching South West France in late 2007 and the north coast of France in late 2008.

Separate from the BTV incursions into southern and eastern Europe, and the incremental progression northwards of some serotypes, in 2006, an incursion of BTV8 directly into northern Europe occurred from a sub-Saharan source. BTV8 was first detected near Maastricht in The Netherlands in August 2006 and spread along a broadly east–west axis to involve most of the country, virtually the whole of Belgium, much of North West Germany, Luxembourg and northern areas of France[2]. After a pause during the 2006–2007 winter, the virus continued to spread with increased vigour across northern Europe, involving many additional areas, including England, Denmark, Sweden, Norway, the Czech Republic, Poland and Hungary. The occurrence of BTV8 in Norway in late 2008 at approximately 60°N is the most northerly record for BTV anywhere in the world (see Figure 8.1 for a map of the bluetongue serotypes in Europe in early 2009).

HOST FACTORS

BTV is probably able to infect all species of wild and domestic ruminant. Severe disease usually occurs only in high-yielding domestic breeds of sheep and some species of New World wild ruminant (e.g. white-tailed deer (*Odocoileus virginianus*), black-tailed deer (*O. hemionus*), pronghorn antelope (*Antilocapra americana*), bighorn sheep (*Ovis canadensis*)). This means that the vast majority of BTV infections are subclinical. This covert presence of the virus, alternating with outbreaks of severe disease, has had a considerable and adverse effect upon international trade in bovines and ovines. The effects of BTV on wild European ruminants have not been investigated in detail,

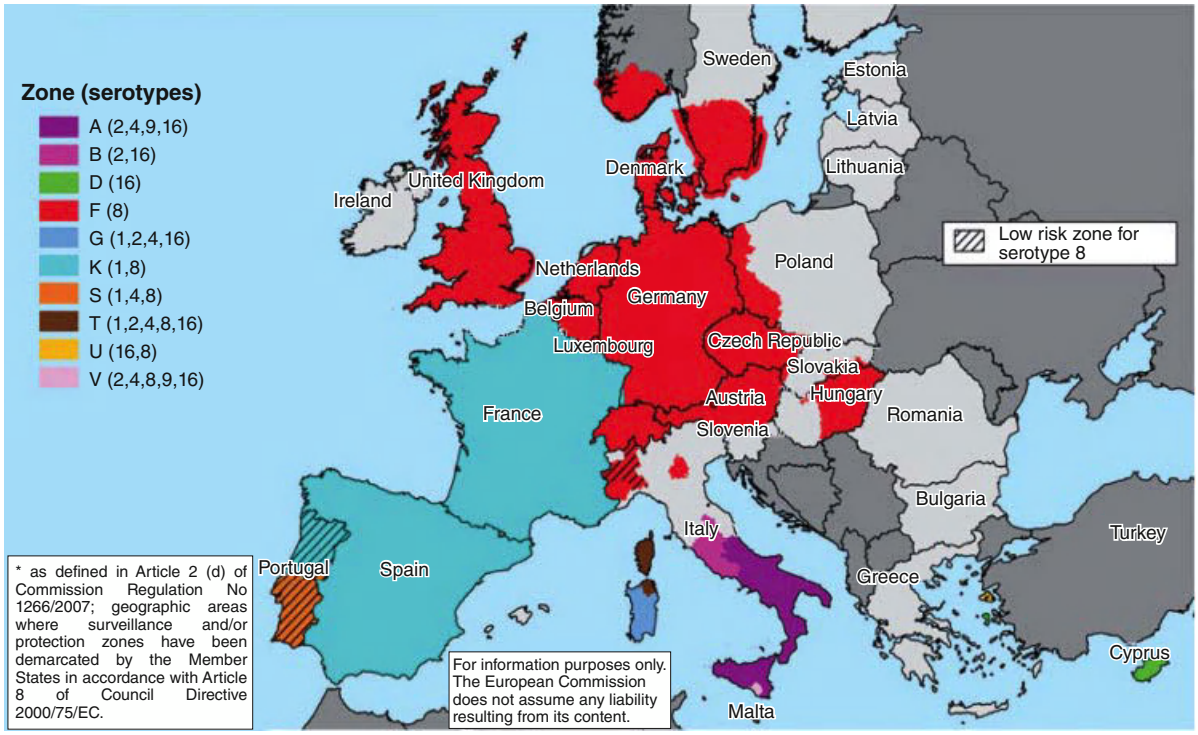

FIGURE 8.1 The European bluetongue virus restriction zones and the geographical locations of the different serotypes in early 2009.

although there are recent reports of BTV antibodies and/or viral RNA in red deer (*Cervus elaphus*), fallow deer (*Dama dama*), Ibex (*Capra pyrenaica hispanica*) and mouflon in Spain[5], red deer, mouflon and roe deer in Germany[6] and red deer in Belgium[7].

Until recently BTV seemed to be a cause of disease in wild ruminants only in New World species. However, during the recent incursion of BTV8 into Europe clinical disease was seen in 62 captive animals originating from Europe, Asia and the Americas, and belonging to three ruminant families; Bovidae, Cervidae and Camelidae. The Bovidae seemed to be most susceptible, with four species (American bison (*Bison bison*), European bison (*B. bonasus*), Siberian ibex (*Capra sibirica*) and musk ox (*Ovibos moschatus*)) showing high morbidity and a mortality of greater than 10%. None of over 200 African ruminants of 20 species were clinically affected[8].

Antibodies to BTV-8 were identified in 40.4% of free-ranging red deer in Belgium but with no evidence of disease or increased mortality[7].

In disease-susceptible ruminant species, there is no particular age-related difference in severity of clinical signs, but these do vary between species, breeds and individuals, and depending upon the serotype/strain of the infecting virus, and certain ill-defined interactions with the environment – for example, stress and strong sunlight exacerbate disease signs.

There is no sex-related difference in the extent and severity of clinical signs in domestic ruminant species, but transplacental transmission may occur, particularly with cell culture-adapted or live vaccine viruses. In such instances resorption, abortion, birth of weak or deformed offspring, or the birth of viraemic offspring may result. The northern European strain of BTV8 is unusual as transplacental transmission in cattle seems to be astonishingly common and rates in excess of 30% have been reported[9]. The reasons for this are uncertain, but this strain of BTV8 originated from sub-Saharan Africa, a region where live BTV vaccines have been used for decades. It is possible that this BTV8 strain has acquired one or more genome segments from a vaccine virus, by reassortment, enhancing its ability to cross the placental barrier. Such reassortants have been demonstrated in the field in Europe[10].

ENVIRONMENTAL FACTORS

Vectors and Transmission

BTV is an arbovirus and so is transmitted between its hosts almost entirely by the bites of vector species of arthropod,

which are certain species of *Culicoides* biting midge. The distribution of BTV is therefore limited to those regions where vector species occur and its transmission to those times of the year when climatic conditions are favourable for adult vector activity and for virus replication in, and transmission by, them.

In most epidemic zones BT is usually seen in late summer and autumn, as this is when high numbers of adult vectors occur and when the temperature is suitable for transmission. In such situations the annual bouts of disease may reflect new virus introductions or low-level persistence. Reintroduction is possible if endemic viral foci are close by, as infected *Culicoides* may be transported on the wind over distances in excess of 100 km[11]. As BTV has not been proven to be transmitted vertically through its vectors and is rarely transmitted directly from vertebrate to vertebrate, except in the case of transplacental transmission, long-term persistence usually occurs only in areas where adult vectors are present virtually throughout the year. If vector-free periods (VFP) do occur, persistence may still be possible, if they are shorter than the maximum period of viraemia in the local ruminant population (up to 54 days in sheep and 60 or more days in cattle), otherwise the last infected host will have died or recovered before new vectors emerge. The part played by wild ruminants in such overwintering is uncertain, as the maximum duration of viraemia has not been determined for any wild species.

BTV Vector Species of *Culicoides* in Europe and Climatic Effects on Vectors

Culicoides imicola is an Afro-Asiatic species and has long been known as a BTV vector in those parts of the Mediterranean Basin affected before 1998. The 1998–2005 BTV incursions into many parts of the Mediterranean Basin previously unaffected, including areas beyond the known range of *C. imicola*, was an unexpected turn of events. This suggested either that the range of *C. imicola* had expanded or that other species of *Culicoides* were, for the first time, transmitting the virus. In the event, the answer seems to include both these possibilities. Surveys in the BTV-affected areas from 2000 recorded *C. imicola* in many regions where it had been looked for in the 1970s and 80s but not found, i.e. mainland Greece, mainland Italy, Sicily, Sardinia, Corsica, the Balearics, eastern Spain and parts of southern mainland France[2]. The range of this species, therefore, seems to have expanded northwards, in recent times, to include much of the northern coast of the Mediterranean Sea and most of peninsular Italy. It has been suggested that this expansion is being driven by ongoing changes in European climate, which made the 1990s (i.e. the period when BTV incursions commenced) the warmest decade on record, and that extensions in the range of *C. imicola* have been into those areas that have warmed the most[12].

However, BTV has spread beyond the new range of this vector[2], and even within the overall distribution of *C. imicola*, transmission has occurred in locations where it is either rare or absent[12,13]. This means that in these areas indigenous European vector species of *Culicoides* must be transmitting the virus. Recent studies have shown that two widespread and abundant Palaearctic *Culicoides* species complexes (*C. obsoletus* plus *C. dewulfi* and *C. chiopterus*, and *C. pulicaris*) are the likely vectors[2]. Earlier studies suggested that these species had very low oral-susceptibility rates for BTV[14]. However, the recent climate warming in Europe is likely to have increased their importance as vectors, both by increasing their population sizes and by enhancing their susceptibility to BTV infection through temperature-controlled virus developmental effects[15].

The role of these indigenous vectors in the transmission of BTV was further confirmed in 2006, when an incursion of BTV8 occurred directly into northern Europe. Despite the absence of *C. imicola*, the virus was transmitted widely during 2006–2008[2].

EPIDEMIOLOGICAL ROLE OF THE VERTEBRATE HOST SPECIES

Historically, it is likely that the primary BTV cycle involved wild African ruminants and *Culicoides* midges. The virus now seems to be maintained in a midge–cattle cycle and, once a certain level of infection is attained, spills over to initiate a secondary cycle in sheep. This generally occurs in late summer or autumn when vector populations are maximal. Cattle are the preferred hosts of vector midges and consequently are more frequently infected than are sheep; however, they rarely develop clinical disease but do exhibit an extended viraemia and are therefore an ideal virus reservoir. In essence, sheep are the tip of the iceberg and are where disease is usually seen; cattle are the covert reservoir where the vast majority of the virus resides.

Little information exists on the role, in BT epidemiology, of free-living wild ruminants in Europe. However, such data that do exist suggest that during a BTV outbreak

the larger Cervidae are regularly infected and, in the absence of clinical disease, may act as covert reservoirs of infection for domestic livestock. In Spain, BTV mean seroprevalences in red deer of up 21.9% (309/1409), in fallow deer (*Dama dama*) of 35.4% (34/96), in roe deer (*Capreolus capreolus*) of 5.1% (2/39), in mouflon (*Ovis aries*) of 13.2% (9/68) and Barbary sheep (*Ammotragus lervia*) of 25% (1/4) were recorded[16]. Similarly, in Belgium seroprevalence rates of up to 40.4% were recorded in red deer[7]. Also in Spain, recent work has shown that BTV RNA is able to be detected by reverse transcription polymerase chain reaction (RT-PCR) in the blood of red deer experimentally infected with BTV1 and BTV8, for periods of up to 112 days post-infection (dpi)[17]. However, as has been demonstrated by many researchers, the mere presence of viral RNA has no relevance to transmission. It is therefore important that this Spanish work is followed up by elucidation of the duration and titre profile of 'live virus' viraemia in common European deer species so that the potential for these animals to act as BTV reservoirs can be accurately assessed.

Interestingly, limited data suggest that BTV may also be able to infect some carnivore species. Serological evidence from Africa suggests that large carnivores may be infected, although smaller species are not. The data indicate that the large carnivores are infected via an oral route, presumably through feeding on BTV-infected ruminants[18]. Also, in North America the use of a canine vaccine contaminated with BTV caused abortion and death in pregnant bitches. In neither of these cases is it known whether a viraemia develops subsequent to infection or whether vector *Culicoides* habitually feed upon large carnivores. Consequently, currently there is no evidence to suggest that dogs or other carnivores are important in the natural transmission of BTV.

PATHOGENESIS, PATHOLOGY AND IMMUNITY

The pathogenesis of BTV infection presented below is from domestic ruminant studies unless otherwise stated.

BTV is inoculated intradermally into its ruminant hosts by the vector *Culicoides*, but the minimum infectious dose is uncertain. However, a single bite from an infectious vector midge is sufficient to infect a ruminant[19] and individual vectors transmitted between 0.32 and 1.78 median tissue-culture infectious dose ($TCID_{50}$) of virus[20]; consequently, the minimum infectious dose presumably lies somewhere within this range.

In its ruminant host, BTV first multiplies in regional lymph nodes before spreading systemically. Virus replication occurs primarily in endothelial cells of capillaries and small blood vessels. In disease-susceptible species, cytopathic changes in these cells lead to vascular occlusion, stasis and exudation, which eventually give rise to hypoxia, oedema and haemorrhage, and to secondary lesions in the overlying epithelium. The severity of the lesions is influenced by the species of animal, stress and mechanical abrasion, and they develop mainly in tissues exposed to the environment, e.g. oral mucosa and coronary bands. Exposure of animals to strong sunlight exacerbates lesion severity, and the mortality rate can also escalate when infected animals are exposed to cold, wet conditions.

There is no published information on the development of BTV lesions in any wild European ruminant species. No evidence of increased mortality was observed in red deer in Belgium during an outbreak of BTV8[7], or in Spain during outbreaks of BTV1 and BTV4[21]. Neither were any BTV-induced lesions detected in red deer experimentally infected with BTV1 or BTV8 in Spain[17].

Following replication in lymphoid tissues and endothelial cells, BTV usually appears in the circulation 3–6 dpi. Viraemia reaches a peak of 5.0–6.0 logs ml 7–8 dpi and is accompanied by a febrile reaction lasting 4–8 days. In sheep the maximum duration of viraemia detected is 54 days, although usually it is only 14–15 days. Viraemia in cattle peaks at a similar level to sheep but tends to have a longer duration (7–50 days) and occasionally may extend to 100 days[22]. The maximum duration of viraemia in other ruminant species (domestic goats, bontebok (*Damaliscus pygargus*), white-tailed deer, elk (*Cervus elephus*), Arabian gazelle (*Gazella* spp.) and water buffalo (*Bubalus bubalis*)) has not been determined, but live virus has been detected in the circulation of these species for periods of between 10–50 days.

In blood most BTV is sequestered within membranes of erythrocytes and cells of the buffy coat, where it is protected from antibody. In consequence, virus and antibody may coexist in the circulatory system and virus is likely to persist until the 'infected' erythrocytes are cleared.

Abortion or malformation, particularly of the central nervous system of lambs and calves (e.g. cerebellar hypoplasia and hydranencephaly (absence of cerebral hemispheres with cerebrospinal filled sacs)) and of other areas

(e.g. the leg joints, causing arthrogryposis) may follow infection of pregnant ewes and cows with laboratory-modified strains of BTV or vaccination with live virus vaccines. There is little published information dealing with the infection of wild ruminant species with such strains of BTV.

In 2007, two Eurasian lynx (*Lynx lynx*) in a Belgian zoo that had eaten ruminant fetuses and stillbirths from areas where many cases of BT were occurring died. The first animal died 2 days after developing clinical signs and the second 5 months later. The animals showed petechial haemorrhages and lung congestion and oedema. Histologically, acute vasculitis and enlarged endothelial cells were observed in muscle, myocardium and lung. BTV8 was recovered from the lungs of the first animal, and BTV antibodies were identified in the second[23].

In disease-susceptible species, lesions relate to the severity of damage to the microvascular system. Lesions can include: congestion, hyperaemia, haemorrhage, erosion and ulceration of the mucosa of the upper gastrointestinal tract; oedema and haemorrhage of the lymph nodes; haemorrhages within the subcutis; subintimal haemorrhages in the pulmonary artery, pulmonary oedema, pleural and pericardial effusions; facial and submandibular oedema; oedema within the fascial planes of the muscles of the abdominal wall; and necrosis of the skeletal and cardiac muscles[24]. Microscopic lesions in skeletal and cardiac muscle range from acute myonecrosis to more chronic lesions with fibrosis and infiltration of mononuclear inflammatory cells. Pulmonary oedema is characteristic of many fatal BTV infections but is not pathognomonic. Changes within skin capillaries and capillaries adjacent to lesions are variable, but acutely affected vessels may exhibit endothelial hypertrophy with perivascular oedema and haemorrhage, and perivascular accumulation of lymphocytes and macrophages[24].

Cattle rarely exhibit significant pathological changes. However, generalized lymphadenopathy with petechial haemorrhages on the tongue, spleen and kidneys was observed in a bovine infected with BTV8.

White-tailed deer develop consumptive coagulopathy (disseminated intravascular coagulation) leading to haemorrhagic diathesis as a consequence of BTV-induced endothelial damage[25]. South American camelids showed severe, acute congestion and oedema of the lungs. The alpaca also had erosions in the oral cavity[26,27].

Type-specific neutralizing antibodies to BTV can be detected from 10 to 12 dpi. After field infection they persist for at least 6 months, and possibly for life. Subsequent to vaccination, they usually persist for a shorter period; accordingly, annual re-vaccination is recommended. The sequential inoculation of two or more BTV serotypes induces a heterotypic response that extends for several months. Consequently, in animals that are naturally infected by several serotypes, the range of neutralizing antibodies is much wider than the number of serotypes actually involved.

Although cellular immune responses play an important role in protection against BTV[28] information on these responses is limited and has been obtained from domestic sheep and cattle. BTV can induce interferon (IFN) *in vitro* and *in vivo*. However, its influence on virus clearance and the adaptive immune response is unknown. Indeed, viraemia continues beyond the detection of IFN, suggesting that BTV may be at least partially resistant to this antiviral mechanism[29]. Ruminants infected with BTV frequently develop lymphopenia, although the underlying mechanism is unknown. Cytotoxic T cells (CTL) are important for protection against intracellular pathogens, and anti-BTV CTL have been demonstrated in BTV-infected sheep[28].

CLINICAL SIGNS AND TREATMENT

Clinical signs are generally associated with sheep, and hence most descriptions of disease apply to this species. In sheep, disease can vary from subclinical to severe, including death. The first sign is fever, and the animal may appear depressed. This is followed by hyperaemia of the oral cavity and swelling of the mucous membranes, leading to oedema of the lips, tongue and muzzle. The animal may be anorexic. There is lachrymation, serous nasal discharge, becoming mucopurulent, excessive salivation and conjunctivitis. At a later stage there may be necrosis of the epithelium of the nose and mouth. Oedema may extend to the ears and brisket, and there may be wool-break. The feet often exhibit coronitis, leading to lameness. The animal may be reluctant to stand. Torticollis may also be seen. In pregnant ewes, abortion or mummification of the fetus may occur, and at term deformed or weak lambs may be born. Paradoxically, cyanosis of the tongue, from which coloration the disease derives its name (bluetongue) is less common.

In cattle, infection is usually inapparent. Where clinical disease does occur, BT is seen as a transient fever followed by hyperaemia and erosions of the nose, buccal and lingual mucosa and, rarely, the teats. Affected cattle salivate exces-

sively and may walk with a stiff gait. Usually, less than 1% of infected cattle show clinical signs, although in the recent epizootic of BTV8 in Europe anecdotal reporting suggests that 2–3% of cattle were clinically affected, and abortion, loss in milk yield and reductions in weight gain occurred. In addition, the European strain of BTV8 has been shown to cross the placental barrier in cows infected during pregnancy[30]. The outcome of these pregnancies ranged from healthy unaffected viraemic calves to weak dying neonatal calves, and 'dummy' calves. BT in goats is rarely seen and when it does occur is usually mild. Certain wild ruminants, such as the North American white-tailed deer, pronghorn antelope and bighorn sheep may develop severe BT, similar to that seen in improved sheep. Lethal infections have also been described in captive South American camelids (llamas and alpacas) in Europe that developed acute respiratory distress and died within 24 hours[26,27]. Similarly, two yaks (*Bos grunniens grunniens*) also kept in captivity in Europe developed clinical signs. Death in one animal occurred 7 days after examination[31].

Apart from supportive treatment there is no specific therapy for BT.

DIAGNOSIS

A presumptive diagnosis of BT in disease-susceptible species, based on clinical signs and lesions, can be made. However, in most ruminants BTV infection is subclinical, and laboratory confirmation by virus isolation/identification or serology is required.

Confirmation of BT or a BTV infection is via antigen detection and identification and/or serology.

ANTIGEN DETECTION AND IDENTIFICATION

- Identification of viral antigen directly from samples by group-specific conventional or real-time RT-PCR assays or the antigen detection enzyme-linked immunosorbent assay (ELISA)[32]. PCR assays are preferred over ELISA because of their increased sensitivity, and real-time over conventional PCR assays for the same reason.
- Isolation of infectious virus via intracerebral inoculation of suckling mice or intravenous inoculation of embryonating hens' eggs, followed by adaptation to cell culture. Virus identification is attempted, first using group-specific conventional or real time RT-PCR assays or an antigen detection ELISA, and then using type-specific RT-PCRs or virus neutralization tests (VNT)[33]. PCR assays are preferred over VNT, as they do not require virus adaptation to cell culture.

Despite the high sensitivity, specificity and speed of PCR-based assays, a major disadvantage is that they detect viral RNA, not infectious virus. Virus isolation should be used in addition to PCR to confirm the presence of live virus.

SEROLOGY

- Identification of BTV antibodies is undertaken using a group-specific, antibody detection ELISA followed by the serotype-specific serum neutralization test[34].

MANAGEMENT, CONTROL AND REGULATIONS

Movement of domestic ruminants and their germ plasms from infected to free zones should be restricted. Following an outbreak of BT in domestic animals or wildlife in a zone that has previously been free, attempts should be made to limit further transmission of the virus and to achieve eradication as quickly as possible. BT should be notified immediately to the responsible authority and the World Organisation for Animal Health (OIE). In endemic situations where BT occurs most years, annual vaccination of domestic ruminants with inactivated or live vaccines, effective against all known local serotypes of BTV, is recommended.

LEGISLATION

The way in which the above measures are applied, the tests to be used and the other responsibilities of the national veterinary authorities are described by the OIE[34,35]. EU legislation is in line with the OIE and sets out procedures for BT control within the Union[36]. The World Trade Organization[37], under the mandate of its Sanitary and Phytosanitary (SPS) Agreement, safeguards harmonization, equivalence and transparency of trade by publishing health standards for animals and animal products.

PUBLIC HEALTH CONCERN

No public health concerns have been identified.

SIGNIFICANCE AND IMPLICATIONS FOR ANIMAL HEALTH

BT is rightly considered one of the most feared ruminant diseases. During the current BTV incursions into Europe, well over 1.5 million sheep have died from the direct effects of the virus, and indirect costs in terms of sheep/cattle morbidity, reduced productivity, loss in trade and vaccination will be many times the direct figure.

For wild ruminant species, the financial and health costs of BTV are likely to be less significant. High levels of mortality and morbidity in small numbers of captive wild ruminant species from South America, Asia and Europe have been reported. It is not known what would happen should populations of such species be involved in an epizootic, but it is conceivable that BTV could pose a threat to some BT-susceptible wildlife species that are rare or have a restricted distribution.

OTHER ORBIVIRAL DISEASES

Like BTV, *epizootic haemorrhagic disease virus* (EHDV) is multi-typic, affects ruminants and is transmitted by *Culicoides*. EHDV has an almost worldwide distribution but has not yet been recorded in Europe. Nevertheless, it has recently expanded northwards – in 2006 EHDV9 was identified in Morocco, Tunisia and Algeria, and EHDV7 in Israel[38], and in 2007 EHDV6 was identified in western Turkey, all for the first time[39]. These range extensions and the disease caused in cattle persuaded OIE to re-classify EHD as notifiable from mid-2008[40]. European cervids (red, fallow, roe and muntjac deer) were experimentally infected with EHDV1 but did not develop clinical signs. However, cell culture passaged virus was used in all but three deer, a procedure that tends to attenuate orbiviruses. Otherwise there seem to be no published data on EHDV in European wild mammals. In view of this paucity of information and, as EHD is one of the most important diseases of deer in North America, its emergence on the threshold of Europe is a matter of concern.

African horse sickness (AHS) has a similar epidemiology to BT and EHD but affects equids. The horse is an indicator species, and in naïve populations mortality rates exceed 80%. Lower but significant mortality also occurs in mules and donkeys. Zebra support virus replication but rarely exhibit clinical disease. AHS virus (AHSV) has a track record of extension from Africa across the Middle East to India, and also into Iberia. Recently increased AHSV activity has been detected in Ethiopia and West Africa, from where it has previously invaded Europe[41].

In addition to EHDV and AHSV, other exotic orbiviruses are known or suspected of causing disease in ruminants (e.g. the Palyam viruses) or equids (e.g. *equine encephalosis virus* (EEV)), but there is no published information on their effects in European wild mammals. In common with BTV, EHDV and AHSV, EEV may be extending its range and has recently been identified in Israel. All of these recent *Orbivirus* range changes may be linked to high vector mobility and ongoing climate change.

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CHAPTER

9

FLAVIVIRUS INFECTIONS

HUGH W. REID, HERBERT WEISSENBÖCK AND KÁROLY ERDÉLYI

INTRODUCTION

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The arthropod-transmitted flaviviruses were originally classified according to epidemiological and antigenic criteria as Group B arboviruses and subsequently on an ultrastructural basis as Togaviruses. These taxonomic criteria have been replaced by highly analytical molecular technologies that recognize a distinct group of positive-stranded RNA viruses known as the *Flavivirus* genus, named after the first identified virus of the group, *Yellow fever virus*. The genus is classified in the family *Flaviviridae*, which includes two other principle genera: *Pestivirus* (*Bovine viral diarrhea 1* and others) and *Hepacivirus* (*Hepatitis C virus*).

According to their ecology, the *Flaviviridae* can be divided into three main clusters: tick-borne viruses, mosquito-borne viruses and viruses with no known arthropod vector. Within the tick-borne viruses, the mammalian tick-borne virus group (which harbours the *Tick-borne encephalitis virus*) and the seabird tick-borne virus group are distinguished.

Flaviviruses are small (approx 50 nm in diameter), consisting of a dense inner core surrounded by a lipid envelope, which is formed of two major viral proteins: the E (envelope) protein, which is the major viral antigenic determinant; and the M (membrane) protein. Within the envelope is a discrete nucleocapsid consisting of a capsid protein and the genomic RNA.

There are more than 50 identified *Flavivirus* species, the majority of which are recognized as human pathogens causing fever, encephalitis and haemorrhagic fever. The majority are transmitted by mosquitoes and are distributed globally, predominantly in the tropics. In Europe, only a comparatively small number of mosquito-borne flaviviruses (e.g. *West Nile virus*, *Usutu virus*) has been found. There is, however, an important group of tick-transmitted viruses present throughout the northern temperate zones known as the tick-borne encephalitides (TBE). Characteristically, flaviviruses are maintained in an occult vector/wildlife cycle in the absence of any disease. It is only when human activity interposes into or perturbs this cycle that disease occurs. Serious wildlife mortality can occur when these viruses are geographically translocated. *Yellow fever*

Infectious Diseases of Wild Mammals and Birds in Europe, First Edition. Edited by Dolores Gavier-Widén, J. Paul Duff, and Anna Meredith.

virus continues to cause periodic mortality in South American wildlife, centuries following its introduction from West Africa. In recent years, the *West Nile virus* has been introduced into North America, where it has been responsible for extensive mortality in the native avifauna and its subtler but distinct effect has also been recognized in Europe. Climate change and the predicted increase in human population will probably contribute to further similar introduction events and gradually modify the distribution of endemic areas.

WEST NILE VIRUS INFECTION

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West Nile virus (WNV) infection (also known as West Nile fever and West Nile disease) infects a wide range of vertebrate species, and its clinical manifestations range from asymptomatic infection, through febrile and neurological disease to acute fatal infection, depending largely on the affected species and the virulence of the virus strain.

AETIOLOGY

WNV belongs to the *Flavivirus* genus in the *Flaviviridae* family. From the 12 viral sero-complexes defined within the *Flavivirus*, WNV is assigned to the Japanese encephalitis virus complex. This group comprises genetically and antigenically closely related viruses such as the *Japanese encephalitis virus*, *Murray Valley encephalitis virus*, *St Louis encephalitis virus*, *Usutu virus*, WNV and others.

WNV was isolated for the first time in 1937 in Uganda from a febrile human patient. This virus has a wide distribution, extending from Africa and Madagascar over Southern and Eastern Europe to the Middle East, India, Central Asia and Australia. The virus was reported for the first time in the western hemisphere in 1999[1] and has subsequently extended its distribution there.

WNV strains can be classified into at least six separate lineages, which significantly differ from each other, exhibiting wide genomic diversity (76–77% nucleotide identity)[2]. Lineage 1 WNV strains can be divided into three clades, which are also related to the geographical distribution of these viruses. Clade 1a comprises European, African and, since 1999, North American strains, whereas Australian (Kunjin virus) and Indian isolates form clade 1b and 1c, respectively. Lineage 2 WNV strains had been mostly isolated in southern Africa and Madagascar, but their endemic presence has been recorded in Central-Eastern Europe since 2004[2]. Rabensburg virus strains establishing lineage 3 of WNV were isolated in the Czech Republic from *Culex pipiens* mosquitoes, whereas lineage 4 is represented by a single virus (LEIV-Krnd88-190) isolated in 1998 in Russia. Recently analyzed WNV strains isolated in India during the second half of the 20th century form an additional lineage 5 of WNV.

WNV is an enveloped virus with a small virion of about 40–50 nm in diameter. The round nucleocapsid shows icosahedral symmetry, and it is surrounded by a host-derived lipid envelope. The complex nucleocapsid structure consists of the viral RNA and multiple capsid protein molecules. The WNV genome is an 11,000-base-long, linear, positive-sense, single-stranded RNA strand, lacking the polyadenylated tail at the 3′ terminus but containing a methylated nucleotide cap at the 5′ end of the genome and several conserved secondary motifs regulating viral replication. The cross-neutralization analyses and monoclonal antibody binding assays reflect the genomic differences and antigenic variability among virus strains[3].

EPIDEMIOLOGY

GEOGRAPHICAL DISTRIBUTION IN EUROPE

Evidence of WNV infection in Europe was first detected in 1958, when WNV-seropositive human patients were found in Albania. In Europe the virus was simultaneously isolated for the first time in 1963 from humans and mosquitoes from the Rhône delta, France, and from humans and *Hyalomma marginatum* ticks from the Volga delta, Russia. Further WNV isolates were obtained soon after from humans, wild birds, horses, rodents, mosquitoes and ticks in Portugal, Slovakia, Moldavia, Ukraine, Hungary, Romania, Czechoslovakia, Italy and other countries[4,5]. Early epidemiology was based on the study of WNV epidemics in Egypt during the 1950s. Human West Nile fever cases were diagnosed from the 1960s in the South of France, Russia, Spain, Romania, Belarus, Ukraine and Czechoslovakia[4]. A new wave of outbreaks, generally associated with significant human morbidity and mortality, emerged in the 1990s in Algeria, Romania, Tunisia, Russia,

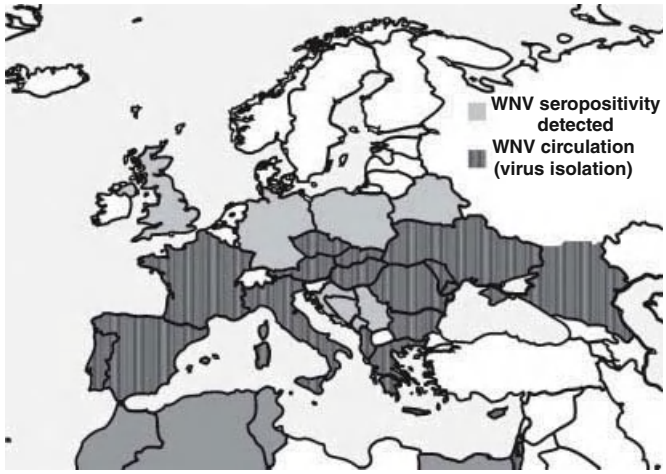

FIGURE 9.1 Map of Europe, indicating the areas of WNV presence and circulation (dark grey) and countries where seropositivity (pale grey) to WNV has been detected in birds or mammals.

Israel and Sudan. During the same period, WNV outbreaks in the Camargue region of France and in Northern Italy affected primarily horses, but human cases were also recorded. In 2003 an outbreak of lineage 1 WNV affected a flock of domestic geese in the South of Hungary. The first occurrence of a lineage 2 WNV strain on the European mainland was detected in infected birds of prey in the southeast of Hungary during the summer of 2004[6]. This viral strain has become enzootic in the Carpathian Basin, reaching Austria in 2008 on a westward range expansion and causing an unexpected human outbreak in Greece in 2010. Lineage 1 viruses continued to circulate in Northern Italy during 2008–2009, affecting primarily horses and humans. WNV lineage 1 activity was also detected on the Iberian Peninsula between 2001 and 2007 in both Spain and Portugal. Figure 9.1 shows areas of WNV presence and circulation and countries where seropositivity to WNV has been detected in birds or mammals in Europe.

HOST FACTORS

In contrast to the high rates of clinical disease and mortality reported in North American wild bird species following the 1999 introduction and subsequent expansion of WNV in the western hemisphere, the circulation of WNV in the Old World has not resulted in mass mortality or disease outbreaks in native European bird species. However, closer monitoring and targeted examinations recently revealed that some species regularly succumb to WNV infection in Europe. WNV-related clinical disease and mortality in both wild and captive birds of prey has been regularly demonstrated in Hungary since 2004. Wild and captive (falconry) goshawks (*Accipiter gentilis*) are most often diagnosed with the disease, frequently with high mortality (e.g. 27 WNV-related mortality cases in Hungary were confirmed by both pathology and virology between 2004 and 2009). Non-native gyr falcons (*Falco rusticolus*) and Harris hawks (*Parabuteo unicinctus*) and a single native peregrine falcon (*Falco peregrinus*) kept for falconry also suffered WNV-related mortality in the area. WNV infection with mortality was diagnosed in nestlings and fledglings of free ranging red footed falcons (*Falco vespertinus*) and sparrowhawks (*Accipiter nisus*) in Hungary. Goshawks and a captive bearded vulture (*Gypaetus barbatus*), snowy owl (*Bubo scandiacus*) and keas (*Nestor notabilis*) succumbed to WNV infection during the Austrian expansion of lineage 2 WNV. In Spain, WNV-associated disease and mortality was diagnosed in Spanish imperial eagles (*Aquila adalberti*) between 2001 and 2005[7] and a golden eagle (*Aquila chrysaetos*) in 2007[8]. Further cases of non-fatal clinical WNV infections were diagnosed in a golden eagle and a Bonelli's eagle (*Hieraaetus fasciatus*) in 2007. The virus strains isolated from these birds belonged to lineage 1 of WNV. Mortality of WNV-infected subadult white storks (*Ciconia ciconia*) was recorded in Israel during the autumn migration in 1999[9].

Clinically healthy birds were infected with lineage 1 WNV during the 2008 WNV epidemic in Northern Italy. WNV was demonstrated by reverse transcription polymerase chain reaction (RT-PCR) in samples collected from magpies (*Pica pica*), carrion crows (*Corvus corone*), rock pigeons (*Columba livia*), Eurasian jays (*Garrulus glandarius*), starlings (*Sturnus vulgaris*), one cormorant (*Phalacocorax carbo*) and a yellow-legged gull (*Larus michahellis*). WNV belonging to lineage 1 was also isolated in 2004 from a magpie and a house sparrow (*Passer domesticus*) found dead in the Camargue, France. A single magpie shot in the area of the 2010 Greek epidemic was found infected with the lineage 2 WNV strain also isolated from the human cases and mosquitoes. Additional WNV isolates were also obtained earlier from the following wild bird species in Europe: barred warbler (*Sylvia nisoria*), turtle dove (*Streptopelia turtur*), pied wagtail (*Motacilla alba*) on autumn migration, and green sandpiper (*Tringa ochropus*), lapwing (*Vanellus vanellus*), black-headed gull (*Larus rid-*

ibundus) and turtle dove returning on migration from their wintering grounds.

Serological studies detected prior exposure to WNV in: house sparrows, magpies, scops owls (*Otus scops*), willow warblers (*Phylloscopus trochilus*), whitethroats (*Sylvia communis*), woodchat shrikes (*Lanius senator*), hoopoes (*Upupa epops*), blackcaps (*Sylvia atricapilla*), common redstarts (*Phoenicurus phoenicurus*) and pied flycatchers (*Ficedula hypoleuca*) in the Camargue, France; in common coots (*Fulica atra*) in Spain; in pied flycatchers (*Ficedula hypoleuca*), common redstarts (*Phoenicurus phoenicurus*), red-backed shrikes (*Lanius collurio*), garden warblers (*Sylvia borin*), white storks (*Ciconia ciconia*), ospreys (*Pandion haliaetus*), red kites (*Milvus migrans*), a goshawk and mute swans (*Cygnus olor*) in Germany; in sparrows, white stork nestlings, mute swans and a hooded crow (*Corvus* corone) in Poland; in common coots, common kingfishers (*Alcedo atthis*), reed warblers (*Acrocephalus scirpaceus*), sedge warblers (*A. schoenobaenus*), marsh warblers (*A. palustris*), Savi's warblers (*Locustella luscinioides*), reed buntings (*Emberiza schoeniclus*), blackcaps, penduline tits (*Remiz pendulinus*), blue tits (*Parus caeruleus*) and starlings in the Czech republic; and other wild bird species in the UK, Sweden, Spain, Portugal, Belarus, Bulgaria and Romania.

Humans and horses are the only mammalian hosts in which clinical WNV is reported in European endemic areas. WNV has occasionally been isolated from small mammal species (e.g. bank voles (*Clethrionomys glareolus*), yellow-necked mice (*Apodemus flavicollis*), domestic mammals (e.g. sheep, cattle) and amphibians (lake frog (*Rana ridibunda*)). Seropositivity to WNV was also detected in a number of domestic and wild mammalian species[4,10].

ENVIRONMENTAL FACTORS

West Nile fever in Europe occurs primarily during the period of peak mosquito activity (July to September). Environmental determinants of mosquito activity and population dynamics are crucial for triggering WNV outbreaks. Mosquitoes are the primary vectors involved in the transmission of WNV, and the virus has been detected in at least eight mosquito species in Europe and more than 60 species in North America. In Europe, the virus has been isolated from *Culex modestus*, *C. pipiens*, *Mansonia richardii*, *Aedes cantans*, *A. caspius*, *A. excrucians*, *A. vexans* and *Anopheles maculipennis*. *Culex pipiens* is the most significant vector species among the above because of its preference for feeding on birds. The potential to serve as a true vector of WNV depends on several characteristics, such as biological competence, host preference and population density. It has been suggested that, depending also on the genotype of the virus, higher ambient temperatures may effectively shorten the WNV transmission cycle and thus also accelerate the geographic spread of the disease. WNV may spill over from its endemic bird–mosquito cycle through 'bridge vectors' that feed on both bird and mammal hosts. A seasonal shift towards preference for mammal hosts later in the transmission season has been observed in *C. pipiens* and *C. tarsalis*, which may enhance their importance as bridge vectors. Although WNV has been detected in several tick species (i.e. *Hyalomma marginatum*, *Dermacentor marginatus* in Europe) the competence of soft or hard ticks as true vectors of WNV is still uncertain. It has been indicated that overwintering infected vectors also play a role in the maintenance of endemic WNV cycles under different climatic conditions.

EPIDEMIOLOGICAL ROLE OF THE AFFECTED SPECIES

Birds are the principal hosts of WNV, and viraemic birds maintain the infectious cycle through ornithophilic mosquito vectors. It is not yet known which European bird species may serve as reservoirs, amplifying or transporting hosts of WNV. The repeated WNV outbreaks in Europe indicate that the virus is regularly (re)introduced from endemic areas in Africa by infected migratory birds. Migratory passerines have been identified as potential long-distance carriers of WNV in North America, but their potential to play a similar role in Europe has not been proven. Larger trans-Saharan migrant species (e.g. white storks) are probable candidates for this role. The monitoring of the local amplification of WNV and the short distance dispersal of WNV has been attempted in abundant, relatively sedentary species such as magpies, house sparrows and feral pigeons. Preliminary results indicate that these species could be suitable indicators of local WNV circulation, but their role in the epidemiology of the disease is, as yet, unknown.

The level of viraemia in mammals is generally considered to be too low to allow for a significant role of mammalian hosts in the maintenance of the natural cycle of WNV, and mammals are usually considered to be dead-end hosts. However, experimental evidence from several North American small mammals (e.g. fox squirrels (*Sciurus*

niger), eastern grey squirrels (*S. griseus*), western grey squirrels (*S. carolinensis*), eastern chipmunks (*Tamias striatus*) and eastern cottontail rabbits (*Sylvilagus floridanus*)) suggests that some mammalian species may play a role in the maintenance of the natural WNV cycle.

Studies during the WNV epidemic in North America revealed that a wide range of wild and domestic mammals as well as reptiles (over 30 species) acquire the infection and seroconvert; furthermore, some species may develop high levels of viraemia. The isolation of WNV strains from wild rodents in Hungary during the 1970s also points to the potential role of small mammals in the maintenance of endemic WNV foci.

TRANSMISSION

Although WNV is predominantly transmitted by mosquito bites in a bird–mosquito cycle, there are also alternative routes of WNV transmission. The spread of WNV by the oral route has been suggested by both experimental results and the circumstances of certain natural WNV infection cases of scavengers and birds of prey in both North America and Europe. Circumstantial evidence for this hypothesis is derived from the explosive geographic expansion of WNV (beyond the speed modelled for a pure mosquito-borne cycle) and the high incidence of WNV in scavenging corvids, and in diurnal and nocturnal birds of prey in North America, as well as in goshawks in Hungary and Austria. It has been found that infected birds may excrete significant quantities of the virus in their faeces ($>10^8$ pfu/g in American crows), so the potential importance of the horizontal faecal-oral transmission route has still to be fully assessed[3].

PATHOGENESIS, PATHOLOGY AND IMMUNITY

The primary route of WNV transmission is through the bite of infected mosquitoes. A viraemia in the bitten animal or bird, of varying length and intensity, ensues after an initial stage of localized viral replication at the inoculation site and in regional lymph nodes. Birds usually develop high levels of viraemia, which may last for 7–8 days. The viraemic stage of WNV infection in mammals is short and circulating virus levels are generally low. The virus is disseminated to its primary targets, the brain, meninges, spinal cord, heart, kidneys and spleen, as well as other organs. WNV replicates in a range of cell types but with a high preference for neurons, for example Purkinje cells, myocardiocytes, tissue macrophages, blood monocytes, renal cells and intestinal crypt epithelial cells[11]. Virus replication within the target cell results in cell degeneration and necrosis, accompanied by focal and perivascular or often extensive, diffuse inflammatory reactions.

In birds, antibodies to WNV appear 7–11 days post-infection (pi) and may persist for more than a year. Passive immunity transmitted vertically to offspring by antibody transfer into the yolk of bird eggs was found to last on average for 27 days in pigeon squabs. The clearance of WNV infection is achieved primarily through the effect of neutralizing antibodies, but both humoral and cellular mechanisms play a role in modulating immune reactions to WNV. Genetic susceptibility of the host species, host age and immune status, virus strain, route of infection and the infectious dose may all influence the pathogenesis of WNV infection[3].

Macroscopic lesions are non-specific. The usual findings are signs of circulatory collapse, general congestion of internal organs and cardiac enlargement. Affected birds and mammals may be in poor body condition. More sporadic findings, including a pale, mottled myocardium, splenomegaly and leptomeningeal haemorrhages, may also be associated with WNV infection.

Microscopic lesions associated with WNV infection are almost always present in the brain and the heart[6]. Severely affected birds usually have a multifocal, lymphocytic panencephalitis accompanied by marked gliosis, and neuronal degeneration comprising chromatolysis, necrosis and neuronophagia. Lesions in the brain are frequent in the outer layer of the cerebellum. Multifocal haemorrhage can be present in some cases and a predominantly lymphocytic meningitis may also be a prominent feature. Perivascular cuffing by lymphocytes and plasma cells may be irregular or incomplete. Lesions in the cerebellum and brainstem are often mild. Spinal cord lesions are usually found within the grey matter and focal mononuclear infiltrations can also be present in the peripheral nerves of the sciatic plexus. Degenerative changes detected in association with inflammatory lesions affect Purkinje cells in the cerebellum and neurons of the brainstem and cervical spinal cord together with demyelination in the cerebellar white matter.

Myocardial lesions were found to be present in a large proportion of WNV cases. A mild to severe multifocal lympho-histiocytic myocarditis often extends and involves the epicardium and the endocardium. Non-inflammatory

changes may also be present in the form of disseminated myocytolysis, multifocal haemorrhage and mineralization. Brain and heart lesions may have different or inverse severity in the same bird, e.g. cases with severe heart lesions may present mild or minimal brain involvement, and *vice versa*. This might reflect the acute or prolonged clinical course of the disease in affected individuals.

Lymphoid depletion is frequently found in the spleen, along with multifocal proliferation of reticulocytes and sometimes with signs of necrotizing splenitis. Some cases may exhibit multifocal interstitial lympho-histiocytic nephritis. Liver lesions range from multiple, focal haemorrhages and interstitial lympho-histiocytic inflammatory foci, sometimes containing heterophilic granulocytes, to acute multifocal coagulative necrosis of hepatocytes and moderate to severe congestion. Lungs are usually congested, with multifocal, acute haemorrhages and sometimes associated with a secondary bronchopneumonia. Mild lymphocytic enterocolitis and proventriculitis is a frequent feature accompanying WNV infection in birds.

The presence of WNV antigen in lesions can be demonstrated with immunohistochemistry (IHC) by using specific antibodies. The organs demonstrating the most pronounced immunolabelling are the brain and spleen, followed by the heart, kidneys, sciatic plexus and small intestine. In some cases viral antigen can also be visualized in other organs such as liver, lung, trachea, pancreas and thyroid gland. WNV antigen is most often observed in the cytoplasm of neurons, glial cells of the brain (Figure 9.2), macrophages, blood monocytes, myocardiocytes, fibrocytes, tubular epithelial cells of the kidney, endothelial cells and smooth muscle cells of arteries, the epithelial cells lining the air capillaries of the lungs, enterocytes, pancreatic exocrine cells, hepatocytes, smooth muscle cells of the *lamina muscularis* mucosae in the small intestine and follicular epithelial cells of the thyroid gland.

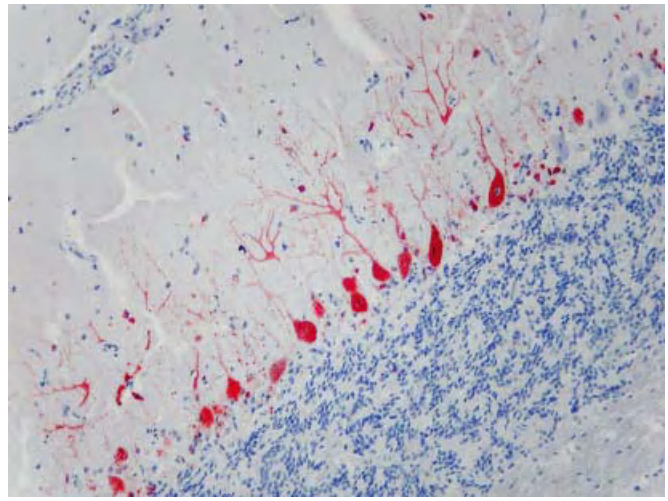

FIGURE 9.2 WNV antigen (red) in the cerebellum of a goshawk (positivity in neuronal axons and Purkinje cells). Immunohistochemistry (WNT 15R4 monoclonal antibodies, Horseradish-peroxidase labelled polymer (EnVision+ Kit), Mayer's haematoxylin counterstain, 200×).

CLINICAL SIGNS AND TREATMENT

Birds are the principal hosts of WNV, and clinical manifestations of WNV infection range from subclinical infection, through a wide range of clinical signs to death. European wild bird species appear to be predominantly clinically unaffected by WNV infection. This may be attributed to their long co-adaptation to infection with the virus. However, as certain bird species do exhibit increased susceptibility to WNV infection it seems likely that sporadic or small-scale disease events and mortality caused by WNV in Europe have not been recognized in the past.

Most bird infections are subclinical or produce mild signs of disease. However, these cases are also believed to result in high-level viraemias (as established for house sparrows and house finches (*Carpodacus mexicanus*) in North America) and so serve as important reservoir and amplifying hosts of WNV. Susceptible species, such as the goshawk and the sparrowhawk in Europe may be more seriously affected by the disease, initially showing depression, ataxia, anorexia, impaired vision and rapid loss of body condition, eventually developing more obvious neurological signs such as head tremors, paresis and seizures. The duration of clinical disease varies from an acute, fatal disease lasting for only one or two days, to a more prolonged illness that may lead to either death or recovery. Complete recovery may ensue in captive birds that are provided with supportive therapy and supplementary feeding as the neurological signs gradually subside.

In most mammals, including humans, clinical signs – if present – range from a mild, transient, flu-like, often febrile illness, to a neurological disease usually indicative of encephalomyelitis. The general symptoms may comprise headache, fatigue, body aches, swollen lymph nodes, fever and occasionally abdominal rash, and the neurological signs may include neck stiffness, stupor, disorientation,

coma, tremors, convulsions, muscle weakness and paralysis.

In the absence of wildlife cases, horses can be significant indicators of local WNV activity. About 10% of WNV-infected horses show signs of clinical illness – comprising primarily generalized clinical signs, ataxia and sometimes more severe neurological signs – which is fatal in 20–40% of clinical cases.

Treatment protocols for clinical WNV cases are limited. Supportive therapy and supplementary feeding have been found to be essential to maximize the chances of recovery of individual patients from WNV infection. Captive, usually older, goshawks that survive the acute phase of the infection and maintain good body condition are found to recover from the infection, although chronic lesions resulting in impaired vision or minor neurological disorders may remain in some cases.

DIAGNOSIS

Direct demonstration of WNV in an infected host can be performed by virus isolation or RT-PCR. In the first case, the viable virus is isolated on cell cultures (e.g. Vero, RK13 or mosquito cells) either directly from host tissue samples or indirectly following mouse inoculation. The identification of the isolated virus may be performed by monoclonal antibodies (MAB) in virus neutralization, enzyme immunoassay, immunofluorescence assay or by RT-PCR.

RT-PCR can also be used to demonstrate directly the presence of viral RNA in clinical or pathological samples. The virus can be directly demonstrated in clinical blood samples (serum) during viraemia, cerebrospinal fluid, pharyngeal and cloacal swabs of birds or in fresh samples of the brain, spleen, heart and other parenchymal organs. Further identification and characterization of virus isolates may require the sequencing of amplified PCR products or full viral genomes.

Aggregates of WNV-labelled virions can be visualized *in situ* in infected organs by IHC performed on formalin-fixed tissue samples using WNV specific monoclonal or polyclonal antibodies. IHC may help elucidate the pathogenesis of WNV infection in different host species and their role in the epidemiology of WNV, also providing evidence for the pathogenicity of WNV strains by demonstrating and quantifying the association of the virus with lesions.

Neutralizing IgM and IgG antibodies to WNV can be detected by several serological methods. Considering the potential need for the testing of a wide range of bird and mammal species, the suitability of the test used for surveys or diagnostic purposes must be assessed beforehand. One such convenient test is a commercially available capture enzyme-linked immunosorbent assay (ELISA), which can be used on multiple species. Because of the high possibility of cross-reactions between flaviviruses in ELISA tests the diagnosis of WNV infection should be confirmed by the state-of-the-art plaque reduction neutralization assay (PRNT) performed in Vero cells that is specific for WNV. However, since a live virus strain is used in PRNT, it can only be performed in biosafety level 3 high-contingency laboratory facilities. Other tests that can be used for WNV serology are the haemagglutination inhibition test (HI), indirect immunofluorescence antibody test (IFAT) and microsphere immunoassay (MIA).

MANAGEMENT, CONTROL AND REGULATIONS

As the transmission cycle of WNV depends primarily on the presence and abundance of competent mosquito vectors, one of the recommended measures to reduce the risk of infection in urban and suburban areas is to control and limit mosquito breeding sites such as ditch water, small receptacles containing stagnant water and small water bodies, and to prevent mosquito bites by the use of repellents, netting and other methods. To the author's knowledge, no attempts have been made so far to control WNV infections in wild animal populations. A passive surveillance scheme of human and horse encephalitis cases, complemented by passive surveillance of bird mortality and sentinel birds in endemic areas is recommended by the EU Scientific Committee on Veterinary Measures Relating to Public Health.

Extra-label vaccination – that is, the use of vaccine for species not covered by the manufacturer's licensed instruction, with inactivated or recombinant WNV vaccines designed to be used in horses has been applied in captive individuals belonging to susceptible bird species (e.g. birds of prey). The efficacy of vaccination seems to vary on a wide scale, and it is apparently influenced by the type and make of the vaccine as well as the specific host species.

Animal cases of WNV infection are reportable to the World Organisation for Animal Health (OIE), whereas the human cases are reported within the EU and an epidemiological assessment of the WNV situation is performed by the ECDC.

PUBLIC HEALTH CONCERN

WNV is a zoonotic agent causing mainly sub-clinical infections in humans, but the infection develops into a flu-like illness (West Nile fever) in about 20% of human cases, and fatal encephalitis is recorded in about 1% of infected individuals. Immunocompromised and elderly people are at a higher risk of clinical infection. Severity of the disease may depend on the pathogenicity of the virus strain, and in this regard both the lineage 1 strain expanding through North America and the lineage 2 strain circulating in Central Europe and Greece are considered highly neuroinvasive.

SIGNIFICANCE AND IMPLICATIONS FOR ANIMAL HEALTH

Apart from humans, horses are the mammalian species most significantly affected by WNV infection. The severity of WNV-induced neurological disease may vary greatly and can lead to mortality. WNV is therefore considered an increasingly important disease of horses, and equine cases are also important indicators of WNV presence.

The current knowledge about the effect of WNV on European wild bird species is fairly limited. As clinical disease and mortality have so far only been recorded in rare cases and in a few species, the situation seems to be in stark contrast with the North American experience following the introduction of the virus. Focal impacts on the breeding success and population sizes of susceptible species such as the goshawk and sparrowhawk, mainly through increased fledgling mortality, may be expected in areas with high seasonal viral activity. The exposure of hitherto unexposed European bird populations through the potential expansion of the range and intensity of WNV circulation may result in unforeseen mortality events in the above-mentioned or other highly susceptible northern species such as the gyr falcon.

USUTU VIRUS INFECTION

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Usutu virus (USUV) is a member of the Japanese encephalitis group among the mosquito-borne flaviviruses.

Prior to 2001 USUV had only been found in certain mosquito and bird species in Africa and it had not been associated with disease. In 2001 a more virulent strain of USUV emerged in Central Europe, causing considerable avian mortality and affecting wild birds of the order Passeriformes (especially blackbirds (*Turdus merula*)) and birds of the order Strigiformes (especially great grey owls (*Strix nebulosa*)) kept in captivity[12] (Table 9.1).

Currently, one USUV strain is endemic in several locations of Central Europe, where it has been involved in episodes of bird mortality since 2001. Emergence of this strain started in Eastern Austria, and spread to Hungary (Budapest, 2005), Northern Italy (Milan area, 2006) and Switzerland (Zurich, 2006)[13]. According to a serological survey in Austria a large number of bird species were infected with USUV, but only certain vulnerable species developed clinical disease and died[17]. The factors that predispose a host to develop severe disease are unknown. In owls, vulnerability seems to be limited to species native to Northern Europe. Single USUV seropositive birds have also been found in European countries that have not experienced USUV-associated bird mortality so far, such as the UK, Czech Republic, and Poland. As virus has not been recovered it is not known whether it is the previously mentioned strain or different, less-virulent strains of USUV that are present in these countries. A different USUV strain has been detected by RT-PCR from *Culex pipiens* mosquitoes in Spain (Catalunya)[18] that has not been associated with bird mortality.

USUV infection and associated disease is seasonal, occurring between July and September within the temperate climatic zone of Central Europe. This seasonality is related to vector abundance and activity. Although no thorough studies on vector preference and vector competence of USUV have been undertaken so far, preliminary results point towards the ornithophilic mosquito species

TABLE 9.1 Bird species affected by documented USUV infection-associated mortality in Europe.

Order	Common name	Scientific name	Number of reported cases	References
Passeriformes	Eurasian blackbird	*Turdus merula*	165	12, 13, 14, 15, 16
	House sparrow	*Passer domesticus*	31	12, 13
	European robin	*Erithacus rubecula*	3	13, 15
	Blue tit	*Parus caeruleus*	2	12, 13
	Great tit	*Parus major*	2	15
	Nuthatch	*Sitta europea*	1	15
	European greenfinch	*Carduelis chloris*	1	13
	Songthrush	*Turdus philomelos*	1	15
Strigiformes	Great grey owl	*Strix nebulosa*	11	12, 13, 16
	Tengmalm's owl	*Aegolius funereus*	6	13, 16
	Hawk owl	*Surnia ulula*	2	13
	Pygmy owl	*Glaucidium passerinum*	1	13

Culex pipiens as the principal vector[19]. *Culex pipiens* prefers to use human-made larval breeding sites, such as water-holding containers, ornamental pools or used tyres. The abundance of virus-positive *Culex pipiens* is proportional to certain environmental factors, in particular to prolonged periods of very hot and dry weather. Such conditions decrease the time span of mosquito development and allow more mosquito generations to be generated. The maintenance of viral activity within endemic areas is established by overwintering infected female mosquitoes. Birds do not seem to play a role in the maintenance of the virus over cold weather periods, as persistent infections have not been noticed so far. Virus transmission is most likely due to infected, competent mosquitoes that excrete the virus in their salivary glands while taking a blood meal. For the related WNV, however, there is also experimental proof of direct transmission between birds. It is currently unknown if direct transmission could also be relevant for USUV.

In vulnerable bird species USUV has a broad tissue tropism, and viral replication occurs in the central nervous system (CNS), striated and smooth muscle cells, fibroblasts, epithelial cells of intestinal, respiratory and urinary tracts, lymphatic tissue, cells of the ovary and testis and other tissues. The organ manifestation phase seems to be preceded or accompanied by viraemia, as USUV has been detected by RT-PCR in blood samples or peripheral blood mononuclear cells (PBMC) from clinically ill birds. The widespread viral replication in the body of the birds leads to multi-organ failure. The course of the disease is usually acute to peracute, and in these cases the immune response is not relevant. However, less vulnerable bird species, which seem to be infected subclinically, seroconvert and generate haemagglutinating or neutralizing antibodies. The seroconversion of a high percentage of susceptible birds may result in the development of flock immunity, which reduces the USUV-related mortality within wild bird populations.

Gross pathology is characterized by hepato- and splenomegaly (Figure 9.3), but other findings such as hyperaemic lungs or seromucous enteritis are inconsistent and probably not directly linked to USUV infection.

The most prominent histological lesions are present in the brain (neuronal necrosis, glial nodules and occasionally mild perivascular cuffs around cerebral vessels) (Figure 9.4), myocardium (myocytolysis and non-suppurative inflammation), spleen (necrotizing splenitis) and liver (multifocal necrosis). Using IHC, viral antigen can be found in many cells and tissues, such as neurons and glial cells in the brain, myofibres of the heart, smooth muscle cells, fibrocytes and endothelial cells of blood vessels, dendritic cells and macrophages of the spleen and smooth muscle cells and fibrocytes of the splenic capsule, glomeruli and tubular epithelial cells of the kidney, glandular epithelial cells and smooth muscle cells of the intestine, alveolar and endothelial cells of the lung, and intravascular monocytes. In the liver, only Kupffer cells are labelled, although this is infrequent.

Most of the birds that succumb to USUV infection are found dead, and thus clinical signs are reported infrequently. Observed clinical signs include apathy, ruffled plumage, inability to fly, incoordination and neurological disturbances.

Laboratory diagnosis is usually requested from dead birds. As avian mortality can be due to a number of

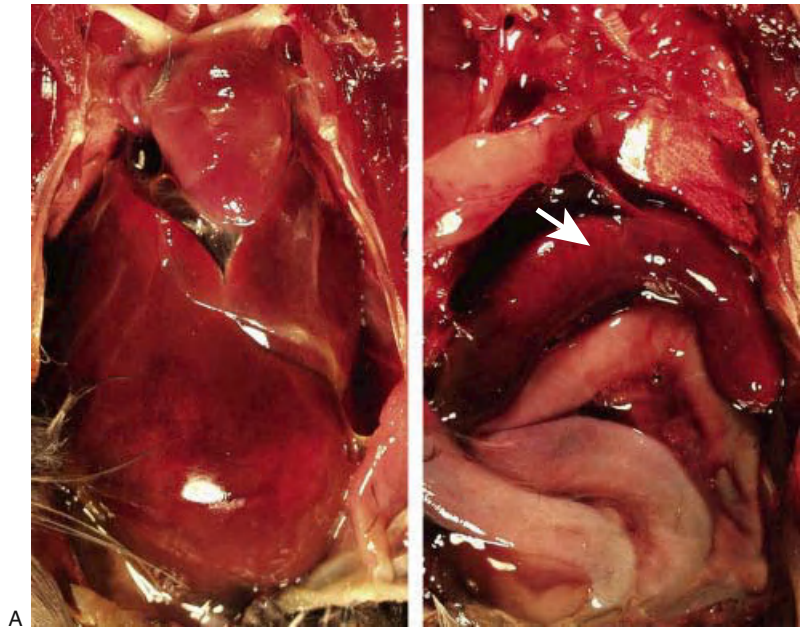

FIGURE 9.3 Blackbird with USUV infection: severe enlargement of (**A**) liver and (**B**) spleen.

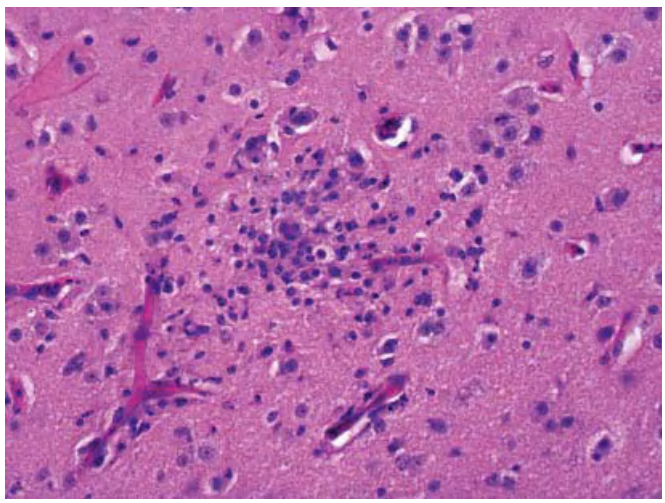

FIGURE 9.4 Non-suppurtaive encephalitis, predominantly characterized by glial nodules is present in some cases of lethal USUV infections. Cerebral cortex, blackbird, haematoxylin and eosin.

reasons, including infections with the related WNV, diagnostic assays for discrimination of these two flaviviruses are necessary. Lesions of USUV and WNV infections are similar, and even the use of IHC will not provide entirely clear results because of potential cross-reactivity of the antibodies used. The most frequently used and reliable method is RT-PCR, both conventional or in a real-time set-up. With this method, viral RNA can be reliably amplified from various tissues. PCR amplification has been complemented by nucleotide sequencing, in order to prove the identity of the virus and to gain information about the virus strain involved. PCR diagnosis using blood samples from sick birds has been proven to be successful[14]. As the course of the disease is short, serological methods are not useful for diagnosis. However, they have provided valuable data on the prevalence and distribution of the infection in various regions of Europe. When performing serological tests it has to be kept in mind that there is a high degree of serological cross-reactivity with other flaviviruses and that only tests based on virus neutralization are able to provide convincingly specific results.

No specific control measures of USUV activity are considered necessary, as even in areas with high wild bird mortality epidemics tend to be regionally restricted and to occur only seasonally.

So far there is no significant public health concern regarding USUV. Serological data confirm a relatively large percentage of human infections in endemic areas, but clinical cases presenting as transient rashes are exceptional findings[19]. However, there are two human case reports of immunocompromised individuals diagnosed with neuroinvasive disease. Experimental infections of chickens and

geese could not demonstrate the pathogenicity of USUV in these avian species.

LOUPING-ILL

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Louping-ill is a viral disease that has been recognized in domestic animals and, rarely, humans for many years but has also been established as a significant cause of mortality in wild red grouse (*Lagopus lagopus scoticus*).

The cause is a flavivirus belonging to the closely related TBE complex of viruses transmitted by ixodid ticks and distributed throughout the northern temperate regions.

Initially louping-ill was described as a condition of domestic sheep in the UK, but a similar disease of sheep has been found in Norway, Spain and Bulgaria, while cattle, horses, pigs, South American camelids, dogs and humans have been affected in the UK.

Infection of humans is only rarely diagnosed, but disease is relatively common in all categories of domestic livestock as well as wild red grouse exposed to the virus.

The pathogenicity of *Louping-ill virus* for red grouse resembles that of a virus exotic to the habitat of the red grouse. However, red grouse are one of the few vertebrate species almost unique to the UK, as is the louping-ill virus. Before the early 1400s, the upland rough pastures in northern Britain were largely used as summer pastures for cattle. The replacement of this traditional practice with large-scale sheep-raising probably enhanced the host potential for *I. ricinus* ticks, effectively introducing the species to this habitat. Furthermore, abundance of alternative hosts would have been meagre, and ticks would have predominantly relied on sheep for blood meals. It is speculated that these changes facilitated the adaptation of an ancestral TBE virus, which had either been previously maintained in a forest/woodland ecosystem in Britain or was introduced *de novo* from continental Europe. Over the next 400 years *Louping-ill virus* evolved to this specific habitat and was progressively dispersed across the British Isles.

The red grouse is a highly specialist feeder with a predilection for heather (*Calluna vulgaris*). Molecular analysis of the envelope gene of approximately 50 isolates confirms that *Louping-ill virus* has evolved from an ancestral TBE virus over approximately 400 years. There is thus excellent agreement between the historical appraisal of the probable evolution of *Louping-ill virus* and molecular analysis of contemporary isolates.

As transmission of *Louping-ill virus* in nature is dependent on the vector tick *Ixodes ricinus*, factors determining the activity and distribution of the tick dictate the epidemiology of louping-ill. Virus is transmitted trans-stadially and there is no evidence of transovarial transmission. Ticks become infected when they ingest viraemic blood, but relatively high titres of virus are required to establish infection. Thus only nymphs and adults that have derived their blood from a susceptible viraemic host as larvae and nymphs, respectively, are capable of transmitting virus.

To determine which vertebrates might contribute to amplifying the virus, a series of experimental infections in candidate domestic and wild species was undertaken to establish the titres of virus that developed in blood. The only mammalian species that consistently developed viraemias of a sufficient intensity to infect ticks were domestic sheep, in which viraemias exceeded threshold levels to infect ticks for 1 to 3 days. Similar experiments established that red grouse also developed sustained viraemias substantially above threshold levels to infect the tick. However, approximately 80% of infected birds died with a florid virus-induced encephalitis.

Subsequent experiments also established that mountain hares (*Lepus timidus*) could provide infected blood meals through a mechanism called co-feeding, i.e. ticks feeding on adjacent areas of skin to an infected tick could acquire infection through local transmission of virus in interstitial fluid in the absence of viraemia.

These studies suggested that the only essential maintenance vertebrate host of louping-ill viruses was domestic sheep, whereas infection of other species was tangential and only makes a minor contribution to virus maintenance. Field studies have supported this view and, where louping-ill virus becomes established, it is responsible for heavy mortality among red grouse, with populations declining and often disappearing[20].

Field studies of grouse suggested that the numbers of ticks parasitizing grouse was relatively low and could not explain the high incidence of louping-ill in red grouse. Examination of grouse droppings indicated that grouse consumed numerous ticks, and experiments confirmed that grouse could readily be infected by the oral route. It was concluded that the majority of grouse became infected through ingestion of infected ticks, a route of transmission that accounted for up to 95% of infections[21].

Following inoculation of virus, replication occurs in the lympho-reticular system over 4 to 5 days. This is accom-

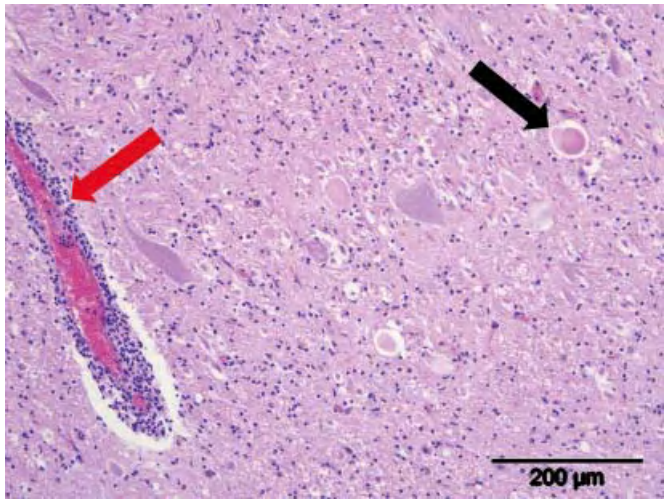

FIGURE 9.5 Histological section of the brain of a sheep with clinical louping-ill. Note the characteristic perivascular cuff comprised of lymphoid inflammatory cells (red arrow) and necrotic neurons (black arrow). Haematoxylin and eosin stain. Courtesy of Dr Mark Dagleish (Moredun Research Institute).

panied by minimal clinical manifestations, although a febrile response and some malaise may occur. It is during this phase of the disease that animals become viraemic and virus may invade the CNS. Decline and elimination of viraemia is associated with the appearance of neutralizing and haemagglutination-inhibiting antibody, which is maintained for life in survivors. Virus persists in the brain for a few days following elimination of infectivity from the periphery and lesions characterized by a non-suppurative encephalomyelitis and neuronophagia develop[22] (Figure 9.5). Depending on the species involved, a variable proportion recover.

Louping-ill is an RNA virus that can be isolated readily in tissue culture. However, virus is most usually detected today by specific RT-PCR[23]. IHC labelling of non-suppurative encephalitic lesions if combined with PCR detection of viral nucleic acid are diagnostic. Of great value in epidemiological investigation of this disease in wildlife is serology, and this has been used on red grouse and brown hare among other wild species in the UK to monitor the efficacy of the strategies mentioned below. Though there have been several attempts to apply ELISA tests for the detection of antibody, the HI test[24] has been found to be consistently reliable across a spectrum of domestic and free-living mammals and birds.

Sustained control strategies designed to suppress ticks and virus circulation through intensive acaracide treatment of sheep, vaccination of all sheep and reduction of alternate wild animal tick hosts, where required, have resulted in a substantial reduction in the prevalence of louping-ill virus and the recovery of grouse populations[25].

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TICK-BORNE ENCEPHALITIS

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Tick-borne encephalitis is a flaviviral nervous system disease of humans, which sporadically also affects several animal species and for which wild mammals serve as reservoir hosts.

AETIOLOGY

Tick-borne encephalitis virus (TBEV) belongs to the family *Flaviviridae*, genus *Flavivirus*. The species of TBEV is grouped into three subtypes: the European, the Far Eastern and the Siberian subtypes[26,27]. This assignment is based on their shared pathogenicity for humans rather than on genomic similarities. A different classification of TBEV has been proposed according to genome sequence data, which includes the assignment of tick-borne encephalitis and louping-ill viruses to the same species (TBEV), of which there are four viral types (*Louping-ill virus*, including Spanish, British and Irish subtypes; *Western tick-borne encephalitis virus* (W-TBEV); *Eastern tick-borne encephalitis virus* (E-TBEV) including far eastern and Siberian subtypes; and *Turkish sheep encephalitis virus*, including the Greek goat encephalitis virus subtype)[28]. Louping-ill has already been described in this chapter.

EPIDEMIOLOGY

The distribution range of W-TBEV is from the Urals in Russia to the Alsace region in France, Scandinavia in the north and parts of the Mediterranean areas along the

Adriatic coast to the south. The far eastern subtype is distributed from the Baltic countries to northeastern China and northern Japan, and the Siberian subtype is found in Siberia and the Baltic states. Thus, in parts of the Baltic states the three virus types occur simultaneously. TBEV is not a primary cause of clinical disease in wildlife, but it has a considerable pathogenic potential for humans. Wild mammals are the most important reservoir hosts and also useful sentinels for estimation of virus activity.

For efficient transmission, the virus must be capable of multiplication within the vector. The vector then carries the pathogen to a range of hosts that play different roles within natural foci. The principal vector of W-TBEV is the tick *Ixodes ricinus*, and there is an overlap in the distribution pattern of this tick species and W-TBEV. In the European part of Russia and in Asia, *I. persulcatus* is the most prevalent vector, which is correlated with the presence of the E-TBEV strains.

Reservoir hosts of TBEV are small mammals, such as yellow-necked mice (*Apodemus flavicollis)* and bank voles (*Clethrionomys glareolus*). Infected ticks, by the mechanism of trans-stadial transmission, remain virus carriers for their entire life and are the most important source of virus. The most relevant transmission event between ticks seems to be co-feeding of several ticks of different developmental stages on one reservoir host. By this mechanism infected tick nymphs are able to transmit the virus to non-infected larvae, which take their blood meal on the same host. Viraemia of the host is not a prerequisite, because the infection occurs by means of infected skin cells. The small mammal hosts have evolution-based adaptation to the virus and do not develop disease. Indicator hosts of TBEV are humans and wild animals larger than rodents, such as hares (*Lepus europaeus*), roe deer (*Capreolus capreolus*), foxes (*Vulpes vulpes*) and wild boar (*Sus scrofa*). These species become infected accidentally, and although they are not able to transmit the virus back to feeding ticks, they support virus circulation by enabling the ticks themselves to survive and reproduce. These species become infected with TBEV and show seroconversion; thus in endemic areas seroprevalence in large wild mammals can be used as indicators for TBEV transmission within a geographical region. Wild birds are accidental hosts of TBEV. Birds can become infected and develop viraemia, but generally neither participate in virus circulation nor form a significant nutrition source for ticks[29]. Wild birds, according to seroprevalence studies, are infrequently infected with TBEV and have not been shown to play an active role in maintenance of viral activity in endemic foci.

Transovarial transmission in ticks has been demonstrated experimentally. This, as well as the vertical transmission by small mammals to their offspring, does not seem to play a major epidemiological role in the transmission of TBEV. TBEV is mainly transmitted by infected ticks, but transmission by unpasteurized milk or milk products from clinically inapparently infected sheep and goats has been repeatedly described.

Seroprevalence between 14% and almost 50% in small mammals and of 10% in large mammals has been shown in different endemic areas in Austria and Germany. In an endemic area in Russia, viral nucleic acid was recovered by RT-PCR in 58% of investigated *Sorex araneus* shrews, 44% of *Clethrionomus rutilus* and 20% of *Apodemus agrarius* mice[30–32].

PATHOGENESIS, PATHOLOGY AND IMMUNITY

Experimentally, the pathogenesis of TBE is characterized by initial viral replication in endothelial cells and macrophages at the site of the tick bite, followed by transport of the virus to the lymphatic system and viraemia. At this stage, the virus attains access to the nervous system, where it replicates in neurons, leading to their necrosis, and initiates an inflammatory response.

Little information is available on the pathology of natural TBE in wild animals.

A euthanized mouflon (*Ovis ammon musimon*) found moribund in a hunting reserve in eastern Austria had a severe infestation with *I. ricinus* and neuropathological examination revealed a marked non-suppurative leptomeningoencephalitis, with the most severe inflammatory changes in the brainstem. Characteristic histological features were neuronal necroses with accompanying neuronophagic areas, diffuse microglial activation and numerous lympho-histiocytic perivascular cuffs (Figure 9.6). IHC demonstrated TBEV antigen in numerous neurons (Figure 9.7) and also in the cytoplasm of macrophages. Partial nucleotide sequencing showed a close relationship to the TBEV strain, Neudörfl, which had been isolated several years previously in the same area[33].

A Barbary macaque (*Macaca sylvanus*) from a group of about 200 animals in a large, outdoor enclosure of a monkey park in a TBE-endemic area in southern Germany

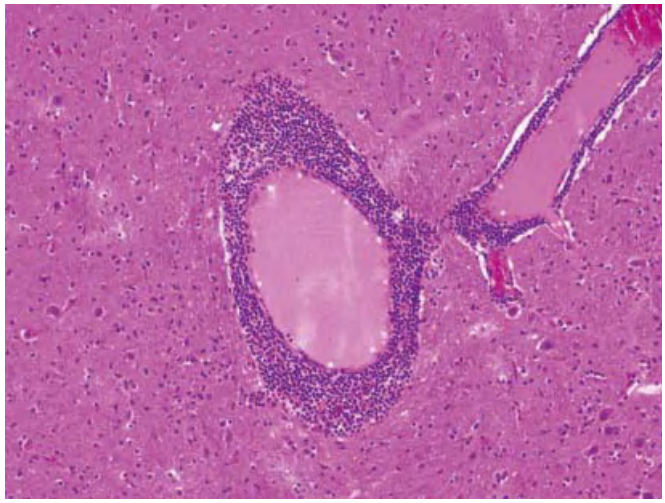

FIGURE 9.6 Severe non-suppurative encephalitis characterized by perivascular cuffs consisting of lymphocytes and monocytes. TBE, mouflon, haematoxylin and eosin.

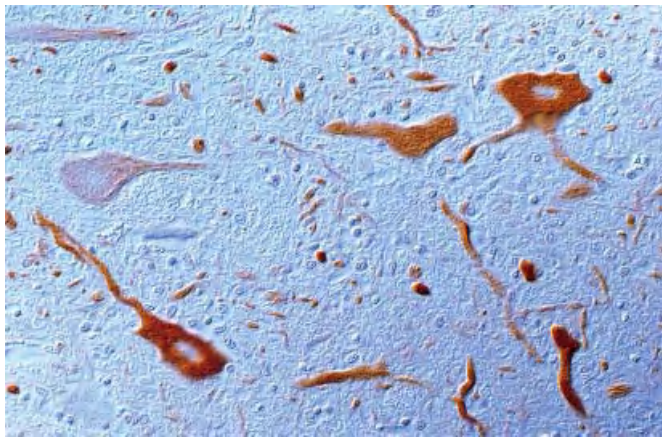

FIGURE 9.7 TBE viral antigen can be demonstrated as brown reaction products in the cytoplasm of neurons and in neuronal processes. Mouflon, IHC.

showed staggering paresis of the hind legs, incoordination and intermittent opisthotonos. Four days after the onset of clinical signs, the animal became comatose and was euthanized. Histologic examination of the brain showed moderate perivascular inflammatory cuffs and mild diffuse infiltration of brain parenchyma with mononuclear cells in almost all brain areas, including basal ganglia and cerebellum. In addition, mild mononuclear inflammatory infiltrates were present in the meninges. Single neuronophagias were also observed. Microglial nodules were not detected. IHC demonstrated viral antigen in several neurons and their processes, including Purkinje cells of the cerebellar cortex and pyramidal neurons of the temporal cortex. Genetic analysis of the entire genome of the isolated TBEV strain showed a typical member of the W-TBEV subtype[34].

There are no reports of clinical disease and pathological changes in wild rodents. Experimentally infected laboratory mice show extensive neuronal necroses and encephalitis.

CLINICAL SIGNS

Clinical disease and death due to TBE have been recorded infrequently in domestic animals (dogs, horses, goat, sheep). Especially in dogs, which next to humans are most susceptible to disease, the clinical signs and the neuropathology are well studied[35]. In fatal as well as surviving cases, there is fever and severe neurological signs, such as tremor and ataxia leading to convulsions and epileptiform seizures.

DIAGNOSIS

Diagnostic tests for TBE are comparatively straightforward in dead animals but more challenging in clinical samples from living animals. In dead animals, there are neuropathological findings consistent with an infection with a neuronotropic virus, accompanied by the presence of antigen in neurons. In addition, TBEV can be isolated or amplified by RT-PCR from samples of the nervous system. Confirmation of TBE in animals with transient or no clinical signs depends on the detection of viral genome and, more importantly, of specific antibodies. Viral isolation or RT-PCR from either blood or cerebrospinal fluid (CSF) plays a minor role in TBE diagnostics, because the virus is usually cleared from the blood before the onset of CNS clinical signs. Thus, diagnostic assays are mainly based on the detection of specific antibodies. As a rule, TBEV immunoglobulin M (IgM) and usually TBEV IgG antibodies are present in the first serum samples taken when CNS signs become manifest. Intrathecal IgM and IgG antibody response can be detected in CSF, but several days later than in serum. Enzyme immunoassays are usually used for specific serodiagnosis. Also haemagglutination inhibition is widely used, but it measures all antibody classes and requires a rise in antibody titre for definitive diagnosis. Because of high cross-reactivity of the antigenic

structure in the flavivirus, possible diagnostic difficulties could arise in areas where other flaviviruses co-circulate (e.g. *West Nile virus*). In such cases, neutralization tests for the confirmation of TBEV specificity of the antibodies are needed[36].

MANAGEMENT, CONTROL AND REGULATIONS

The vaccines against TBE are only licensed for use in humans and not for animals. As the virus is maintained in transmission cycles between small rodents and ticks, once infected a tick carries the virus for the rest of its life; therefore the elimination of the virus from natural endemic foci is not feasible.

PUBLIC HEALTH CONCERN

The risk of acquiring TBE is correlated with the likelihood of tick exposure; thus the infection rate in endemic areas is higher in those individuals who have more frequent outdoor activities. The infection rate within the population of a country in which TBE is endemic can be markedly reduced by vaccination programmes. This has been shown in Austria, where a large percentage of inhabitants are vaccinated against TBE.

Apart from *Japanese encephalitis virus*, TBEV is the encephalitogenic flavivirus with the most severe impact on human health in Europe and Asia. Each year it is responsible for several thousands cases of encephalitis in humans, and the fatality rate is significantly higher with the eastern subtypes (up to 20%), than with the western subtype (approximately 1%).

OTHER FLAVIVIRUSES

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Most of the tick-borne and mosquito-borne flaviviruses use wild birds or mammals as hosts. If these hosts have a co-evolutionary adaptive history with the virus, they are not affected by clinical disease. In addition to the flaviviruses of major importance, a few examples of flaviviruses of minor importance to wildlife populations are presented below. With one exception (*Bagaza virus*) none of these viruses has been detected in European wildlife. However, there is a potential for their introduction into Europe, particularly as some occur in countries bordering Europe.

TICK-BORNE FLAVIVIRUSES

OMSK HEMORRHAGIC FEVER VIRUS

Omsk hemorrhagic fever virus (OHFV) is a unique species among the tick-borne flaviviruses, although it is serologically related to tick-borne encephalitis virus. The natural foci of OHFV are in the Omsk and Novosibirsk regions and also in western Siberia, which comprise forested areas and open wetlands. There were major epidemics of Omsk haemorrhagic fever (OHF) after World War II that were associated with the neglect of arable land and the explosive multiplication of *Dermacentor reticulatus* ticks and the narrow-skulled vole (*Microtus gregalis*), which are considered efficient vectors and hosts of the virus. After re-establishment of agriculture the population density of these species declined, and outbreaks of OHF became rare. A second transmission cycle that has become increasingly relevant involves the muskrat (*Ondatra zibethica*) as amplifier host of OHFV. In contrast to small mammals the virus is highly pathogenic for the introduced muskrat, which develops encephalitis with high fatality rates. Muskrats are currently also the main infection source for humans, as most cases occur in hunters who acquire infection during skinning of these animals. After experimental infection, clinical disease and death has also been observed in birds of prey, such as the marsh harrier (*Circus aeruginosus*), kestrel (*Falco tinnunculus*), long-eared owl (*Asio otus*) and rook (*Corvus frugilegus*). Infection of humans is characterized by a haemorrhagic fever with a generally mild course and a fatality rate between 0.5 and 3%[37].

KYASANUR FOREST DISEASE VIRUS

Kyasanur Forest disease virus (KFDV) is the major tick-borne virus from southern Asia. It is geographically restricted to the Karnataka State of southern India. It causes a human febrile disease with haemorrhagic and encephalitic manifestations and a case fatality rate of up to 10%. Human cases of the disease are frequently associated with epizootics in monkeys, such as langurs (*Semno-*

pithecus entellus) or bonnet monkeys (*Macaca radiata*), which may exhibit mortality rates of up to 85%. The major tick vectors belong to the genus *Haemophysalis*, and wild mammals, such as the Blanford rat (*Rattus blanfordi*), the striped forest squirrel (*Funambulus tristriatus*) and the house shrew (*Suncus murinus*) are subclinical hosts that develop high viraemia titres. A novel but closely related virus emerged in 1992 in Saudi Arabia, called *Alkhurma virus* (or *Alkhumra virus*); over 60 human cases of Alkhurma haemorrhagic fever disease have been reported, mainly in sheep handlers and butchers, which indicates that blood or secretions from infected animals are infectious[37].

POWASSAN VIRUS

Powassan virus (POWV) is the only recognized North American member of the tick-borne flaviviruses. Owing to the low frequency of human infections (in total fewer than 50 cases), POWV is one of the less well studied of the human pathogenic flaviviruses. POWV constitutes a genetically and phenotypically diverse group of viral strains. The overall nucleotide sequence identity among all isolates of this virus species is 84% and the amino acid identity is 94%. Thus, several studies of nucleotide sequence data differentiate POWV into two distinct genetic lineages, the deer tick virus (DTV) lineage and the POWV lineage. POWV is transmitted mainly by *Ixodes* (*Pholeoixodes*) *cookei*, whereas DTV is maintained by *I. scapularis*. POWV is apparently widely distributed in the northern hemisphere, with isolates reported from Russia, Canada and the USA. Based on serological studies, a number of vertebrates are considered to act as maintenance hosts: for example, red squirrels (*Tamiasciurus hudsonicus*), chipmunks (*Tamias amoenus*), groundhogs (*Marmota monax*) or white-footed mice (*Peromyscus leucopus*). POWV infection of these vertebrate hosts results in a silent infection characterized by lack of clinical signs and low levels of viraemia. Clinical disease in animals is not known. Human disease is characterized by meningoencephalitis, with a case-fatality rate of approximately 10%[38].

MOSQUITO-BORNE FLAVIVIRUSES

BAGAZA VIRUS

Bagaza virus (BAGV) seems to be synonymous with *Israel turkey meningoencephalitis virus*, which causes non-suppurative meningoencephalitis and myocarditis in turkeys (*Meleagris gallopavo*) in Israel and South Africa. In 2010, a strain of BAGV emerged in southern Spain and caused mortality of wild Galliformes (pheasants (*Phasianus colchicus*); red-legged partridges (*Alectoris rufa*))[39]. This event recalls the permanent possibility of introduction of viruses into novel environments.

JAPANESE ENCEPHALITIS VIRUS

Japanese encephalitis virus (JEV) is the most important of the encephalitogenic flaviviruses, leading to approximately 50,000 human cases per year, with a fatality rate of up to 25%. The natural distribution range of this virus is South-east Asia and Australasia. The vectors are *Culex* spp., predominantly *C. tritaeniorhynchus*. Virus activity is naturally maintained through bird–mosquito cycles with ardeid birds (heron family), particularly black-crowned night herons (*Nycticorax nycticorax*), little egrets (*Egretta garzetta*), or plumed egrets (*E. intermedia*) as maintenance hosts. The virus does not cause disease in birds. Pigs (*Sus scrofa*, both domestic and feral) are important amplifying hosts and are recognized as frequent drivers of epidemic activity. In pigs, natural infections are generally inapparent, except for occasional stillbirths and abortions when pregnant sows are infected. Experimental infection of piglets with high virus titre innoculae resulted in non-suppurative encephalitis. JEV also causes encephalitis in horses, but as with humans, horses are dead-end hosts and not involved in onward transmission. Orang-utans (*Pongo pygmaeus*) have also been implicated as possible vertebrate hosts in sylvatic cycles of JEV transmission in Borneo[40].

ST. LOUIS ENCEPHALITIS VIRUS

St. Louis encephalitis virus (SLEV) is distributed over most of the American continent. Birds of the orders Passeriformes and Columbiformes (perching birds and pigeon family) seem to be the major vertebrate hosts and mosquitoes from the genus *Culex* the principal vectors. Between 1933 and 2000 a number of epidemics with a total of at least 10,000 severe human cases including more than 1,000 fatalities were reported from several southern and mid-western US states. The last major epidemic with 222 laboratory-confirmed human cases and 11 deaths was recorded in Florida in 1990. Endemic St. Louis encephalitis affects an average of 25 individuals per year in the

USA. Infected wild birds, as well as mammals, have never been shown to develop clinical illness[41].

YELLOW FEVER VIRUS

Yellow fever virus (YFV) is estimated to be responsible for approximately 200,000 human clinical cases per year, with a case-fatality rate of approximately 20%. YFV is native to Africa, where the vast majority of cases occur. In the early 1600s, the virus was introduced to South America during the slave trade. It is maintained in an enzootic cycle involving monkeys and the canopy-dwelling mosquitoes from the genera *Aedes* spp. (Africa) or *Haemagogus* spp. (South America) in tropical rain forests. Humans may be accidentally infected (jungle yellow fever). However, the virus is periodically introduced into urban areas, where it is able to establish epidemics with *Aedes aegypti* as vector and with humans as the only natural host. Although African primate species involved in the transmission cycle because of a stable host–parasite relationship show subclinical infection, many South American species, such as howler monkeys (*Alouatta* spp.), spider monkeys (*Ateles* spp.), squirrel monkeys (*Saimiri* spp.) or owl monkeys (*Aotus* spp.), may succumb to YFV infection, probably due to a comparatively short time span of co-adaptive evolution. The virus, both in humans and primates, is viscerotropic, resulting in liver damage[42].

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CHAPTER

10

PESTIVIRUS INFECTIONS

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INTRODUCTION

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Pestiviruses represent causative agents of diseases that have a significant health and financial impact for the livestock industry worldwide. Clinical signs of pestivirus infection include diarrhoea, respiratory problems and bleeding disorders.

Pestiviruses have a single strand of positive-sense RNA, which is around 12.5 kb long and have spherical structures of 50–60 nm in diameter. The genomic RNA contains one continuous long open reading frame flanked by 5′ and 3′ non-translated regions. The molecular biology of pestiviruses shares many similarities and peculiarities with the human hepaciviruses. Pestiviruses are sensitive to drying (desiccation) and are rapidly inactivated by a pH of less than 3 and greater than 11. Temperatures of 70°C for a minimum of 60 minutes inactivate the virus.

One remarkable biological property of pestiviruses is the existence of two biotypes that are recognized according to the morphological changes they cause during growth in tissue culture cells: non-cytopathogenic (ncp) pestiviruses replicate without obvious cytopathic effect, whereas cytopathogenic (cp) viruses lead to lysis of target cells.

All pestiviruses are able to cross the placenta and to infect the fetus. Such intrauterine infections may lead to abortion, stillbirth, fetal malformation, or weak or apparently healthy offspring. Of particular importance is the capability of ncp pestiviruses to establish persistent infections in fetuses not yet immunocompetent. Such animals acquire immunotolerance to the virus, and they are persistently infected for the rest of their lives. Various pestivirus vaccines exist and the correct vaccine strain should be given, depending on the herd's location and the endemic strain in that region. This vaccination must be given regularly to maintain immunity.

Based on the organization and expression of their genomes as well as their defined serological reactivity, *Bovine viral diarrhea virus*, *Border disease virus* and *Classical swine fever virus* are classified in the genus *Pestivirus* within the family *Flaviviridae*.

Pestiviruses have a broad range of hosts and primarily infect a wide variety of ungulates mainly belonging to the order Artiodactyla. Pestiviruses isolated from various species of wild ruminants and their phylogenetic relatedness are shown in Figure 10.1. In addition to various ruminant and pig species, camels (*Camelus dromedarius*), guanaco (*Lama guanicoe*), rabbits (*Oryctolagus cuniculus*) and wallaby (*Macropus rufogriseus*) appear to be susceptible to pestivirus infections.

Infectious Diseases of Wild Mammals and Birds in Europe, First Edition. Edited by Dolores Gavier-Widén, J. Paul Duff, and Anna Meredith.

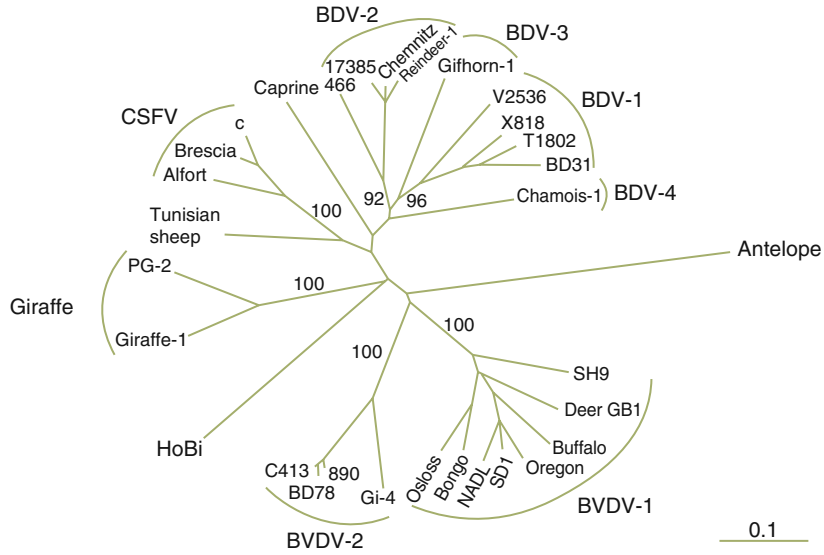

FIGURE 10.1 Phylogenetic tree indicating relatedness among pestivirus genotypes; after Vilcek & Nettleton (2006)[1].

Traditionally, pestiviruses are named after the host from which they were isolated and the diseases they cause. However, pestiviruses are not strictly host-specific. Thus, individual domestic species of artiodactyls can be infected by different pestiviruses.

PESTIVIRUS OF CHAMOIS AND BORDER DISEASE

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Border disease virus (BDV) is responsible for border disease (BD), first reported in sheep in the border region between Wales and England in 1959 and now distributed worldwide. It is a congenital disease characterized by barren ewes, abortions, malformations, stillbirths, birth of small weak lambs and persistent infections of the offspring.

AETIOLOGY

BDV is a member of the genus *Pestivirus* that mainly infects sheep, but also goats and occasionally cattle and pigs. Among pestiviruses, BDV has the greatest genetic diversity and six major genotypes have been reported, although new subgroups are being proposed.

EPIDEMIOLOGY

Evidence for BDV infection in wildlife is scarce. Antibodies against BDV have been demonstrated in roe deer (*Capreolus capreolus*), mouflon (*Ovis ammon*) and Alpine chamois (*Rupicapra rupicapra*)[2,3]. Isolation and characterization of BDV has been described in a reindeer (*Rangifer tarandus*) and a European bison (*Bison bonasus*) from a German zoo (BDV2 genotype)[4]. In free-ranging wildlife, there were no reports of infection with BDV until its detection in an outbreak of a previously undescribed disease in Pyrenean chamois (*R. pyrenaica*) in 2001[5]. Chamois BDV was characterized as BDV4 genotype, which is the same genotype as the BDV circulating in sheep in Spain[6].

The geographical distribution of the disease associated with BDV infection in Pyrenean chamois comprises an extensive area of the Central and Eastern Pyrenees in Spain, Andorra and France. Although the disease has been reported throughout this area, the prevalence varied in the different mountain massifs and valleys, with severe outbreaks reported (Figure 10.2). The Pyrenean chamois has been the only species affected by the disease, even though it shares the habitat with other wild and domestic

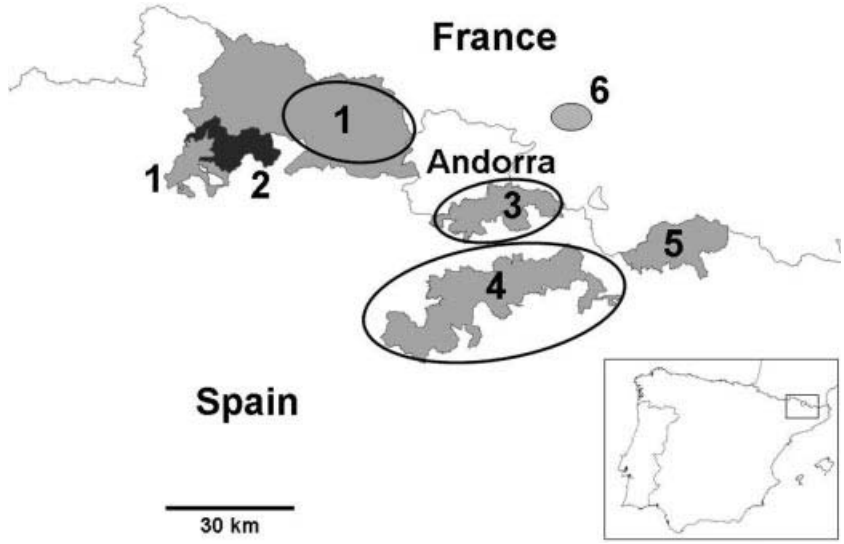

FIGURE 10.2 Map of the Central and Eastern Pyrenees showing the extent of the three main outbreaks of disease (circled areas) and the main protected areas: 1. Alt Pallars-Aran National Hunting Reserve; 2. Aigüestortes i Estany de Sant Maurici National Park (in black); 3. Cerdanya-Alt Urgell National Hunting Reserve; 4. Cadí National Hunting Reserve; 5. Freser-Setcases National Hunting Reserve; 6. Orlu National Game and Wildlife Reserve.

ungulates. No abnormal mortality has been recorded in any other species in areas where chamois were affected and serological and virological investigations indicated that chamois BDV infection among other species was unlikely[7]. Nevertheless, BD is widespread in sheep in Spain, although little is known about its prevalence and clinical significance. BDV strains from chamois and sheep may co-circulate in the Pyrenees, and it is possible that they are a source of infection for other wild and domestic species, as ruminant pestiviruses are not strictly host-species-specific.

The main outbreaks of disease in Pyrenean chamois have affected important protected areas: Alt Pallars-Aran, Cerdanya-Alt Urgell and Cadí National Hunting Reserves (NHR). In addition, isolated cases of diseased chamois have been found throughout the area, including Aigüestortes i Estany de Sant Maurici National Park (Figure 10.2). The high, and in some cases, extreme mortality that occurred during these outbreaks, leading to the death of thousands of chamois, has no precedent in pestivirus infections in wild ruminants. Mortality was difficult to assess accurately because most of the diseased chamois would not have been found due to the large size and remoteness of the affected areas. However, the annual censuses provided an indirect measure of mortality by showing a decrease in the population. In Alt Pallars-Aran NHR, the estimated population decrease was 31% for the whole reserve and 42% in the main affected area. In Cerdanya-Alt Urgell and Cadí NHR, the estimated decrease was 86% and 63%, respectively[8,9]. It has been estimated that for the whole Central and Eastern Pyrenees from Northeastern Spain, chamois population decreased from approximately 13,000 animals in 2000 to about 9,000 in 2006, a 30% reduction.

The epidemiology of the disease has followed different patterns in each of the different outbreaks. Research is being conducted to determine associated factors, such as pathogen, host and host population characteristics and ecological factors. Lack of immunity at the population level and the presence of secondary infections may have been important in determining the outcome of BD. Secondary infections have supposedly been magnified by the immunosuppressive effects of BDV infection and are most likely to be responsible for the mortality. The first outbreak of disease at Alt Pallars-Aran NHR showed a similar seasonal pattern for the 2 years it lasted (2001 and 2002): most of the affected chamois were found between late winter (February) and spring (June). Piroplasmosis was the most common secondary infection and severe lesions consistent with haemolytic anaemia were observed in spleen and kidney[8]. The second outbreak at Cerdanya-Alt Urgell NHR in 2005 produced the highest mortality. It lasted

almost 2 months, and bacterial pneumonia was the major contributing factor to the deaths. The third outbreak at Cadí NHR lasted for about 31 months and spread progressively to cover an extensive area of chamois habitat. Bacterial pneumonia was also the most common pathological finding in this outbreak[9]. *Pasteurella* spp. and *Mannheimia haemolytica* were frequently isolated from the lung lesions. The role of other concurrent infections that can influence the development of the disease has only partially been studied.

The absence of significant mortality in some parts of the Central and Eastern Pyrenees remains poorly understood. The chamois population is relatively continuous and abundant over the whole mountain chain. However, some areas with high density of chamois, such as Aiguestortes i Estany de Sant Maurici National Park, which is only 7 km away from a severely affected area, have not been affected. It was not until March 2009 that the virus was detected and isolated from the first reported clinical case. Factors such as natural movement of chamois, climatology and possibly other unknown factors, may have influenced this geographical distribution. Freser-Setcases NHR, in the Eastern Pyrenees, has probably the highest density of Pyrenean chamois from all the study areas (Figure 10.2). To date, no abnormal or mass mortality has been detected, despite this population being connected to the population of Cadí NHR. However, BDV similar to the previous chamois isolates were identified and isolated in 2006 from a healthy chamois and in 2007 from an isolated case of disease[9]. Therefore, the virus has reached or at least is present in this reserve, but to date it has not caused significant mortality. Prevalence of antibodies against pestivirus in this area was high (71% in 2003), thus, the immunological status at the population level may have been an important factor for the absence of the disease in this area. Nevertheless, the seroprevalence has been decreasing gradually since then, and was 38.5% in 2008. If this trend continues in the next few years, it could lead to an epidemic, if the virus is still circulating and if the population is losing antibody protection.

In a retrospective study performed to detect BDV in archived frozen spleen from chamois from the Central and Eastern Pyrenees between 1990 and 2000, two pestiviruses were identified by reverse transcription polymerase chain reaction (RT-PCR) and isolated from one healthy chamois and one diseased chamois with pneumonia, both sampled in 1996 in Freser-Setcases NHR. Sequence analysis revealed that the two viruses were grouped into the BDV4 genotype, closely related to the other BDV chamois isolates, indicating that BDV strains from chamois origin may have been present in the chamois population several years before the first cases of the disease in 2001[10]. Therefore, among host and ecological factors, virus mutation and transformation into a pathogenic strain should be strongly considered.

After the first severe die-off in 2001 and 2002 in Alt Pallars-Aran NHR, a high seroprevalence (71.9%) was observed in the chamois population. However, it decreased significantly after 2004, and since then frequent cases of BDV infection have been detected, suggesting that the infection became endemic, but could still be responsible for the continued slight decline in the chamois population over recent years[6]. In the French Pyrenees, at the Orlu National Game and Wildlife Reserve, periodic oscillations of the chamois population have not been explained, because no diseased chamois have been observed and mass mortality has never been reported. However, in a retrospective epidemiological study, the high prevalence of viraemic animals suggested that BDV infection in chamois may have had a clinical effect on reproduction and on juvenile mortality, which may explain these variations in the population size[11].

There are few data on host and environmental factors associated with BDV infection in Pyrenean chamois. No sex or age predisposition was observed in two of the main outbreaks reported. However, during the first outbreak, which had lower mortality, adult males were predominantly affected[8]. In the French Pyrenees, at the Orlu National Game and Wildlife Reserve (Figure 10.2), the same BDV genotype as in Spain was detected in chamois; however, no mass mortality or clinical signs have been observed. In this area, higher seroprevalence and low prevalence of infection have been reported in adult chamois than in individuals aged 2 years or less. This concurs with the dynamic of the infection in domestic ruminants, in which young animals are most susceptible to acute infections[11].

In sheep, the key for the transmission of BD is persistently infected (PI) animals that are infected during gestation and then become immunotolerant after birth, shedding high titres of virus throughout their lives. Thus, BDV transmission occurs mainly by direct contact by the oronasal route between PI animals and susceptible animals. Infection in non-pregnant animals is usually subclinical and leads to acutely infected animals, which are considered to be inefficient virus transmitters. However, by

contrast, in naturally and experimentally infected adult Pyrenean chamois, detection by RT-PCR and virus isolation of BDV from oral, nasal and rectal swabs, urine samples and sera indicated a high level of virus excretion and viraemia[12] (Cabezón, unpublished data). Thus, acute infection in chamois leads to a high viraemia and efficient virus elimination that would explain the high horizontal transmission rate of the virus in the severe epidemics reported.

The existence of PI animals in Pyrenean chamois has not been confirmed. In domestic ruminants, confirmation of PI status requires identification of virus in two separate samples with a minimum of 21 days. More than 100 free-ranging diseased chamois have been studied since 2001, and almost all were viraemic and antibody-negative, using an enzyme-linked immunosorbent assay (ELISA) test. However, they could not be re-tested to check their PI status because most of them died shortly after capture. The epidemiological pattern of the three mentioned outbreaks, with rapid development and very high mortality, indicate that it is unlikely that the diseased chamois were PI. The diseased chamois included adult and old animals, which is uncommon in BD of sheep, because PI animals have poor performance and survival. Another aspect is that the presence of PI animals in domestic ruminants is associated with moderate to high flock seroprevalence. Previous data from chamois populations before the outbreaks is only available from the Cadí NHR, where in 2005 a low antibody prevalence (5%) was reported, suggesting that it was unlikely that there were PI animals in the population[9]. A diseased BD Pyrenean chamois survived in captivity for more than 1 month, and blood samples were taken five times during this period. Serology using a blocking ELISA test and virological studies by RT-PCR to detect pestiviral RNA in this animal showed no antibodies against pestivirus but the presence of BDV at all five time points. Accordingly, this chamois can be considered a PI animal[5]. However, in an experimental infection of adult chamois, virus shedding was confirmed for more than 1 month until the animals were euthanized and antibodies were not detected using the same ELISA test, but high titres were detected with a virus neutralization test (VNT), using the homologous strain (Cabezón, unpublished data). Thus, BDV infection in non-pregnant Pyrenean chamois induces a long-lasting infection and an antibody response. In naturally infected chamois, the lack of antibody response should be further confirmed with a VNT using homologous strains.

In healthy seropositive Pyrenean chamois, high cross-reactivity was observed among different reference ovine BDV and *Bovine viral diarrhea virus* (BVDV) strains, which is frequent in pestivirus infection in domestic ruminants[6], and even the different BDV chamois isolates show different cross-reactivity. Therefore, cross-neutralization studies should be performed because antigenic differences may exist, not only between sheep and chamois BDV strains, but also between chamois isolates from the different locations of affected areas in the Pyrenees.

Vertical transmission was demonstrated in the fetus of a naturally infected female chamois that died before parturition[12]. In another study, three pregnant female Pyrenean chamois were experimentally infected with a BDV chamois strain. All animals became viraemic, one female aborted and all three females died before the end of gestation (Martin, unpublished data). Therefore, the existence of PI in chamois may be possible, but owing to the severity of the disease in this species, females may abort or die before giving birth.

PATHOGENESIS, PATHOLOGY AND IMMUNITY

The extreme severity and mortality of the disease associated with BDV in Pyrenean chamois is unprecedented in pestivirus infections in wild ruminants. The pathogenesis of the disease in chamois is different from BD in sheep. While healthy young and adult sheep exposed to BDV experience only mild or inapparent disease, chamois infected with BDV develop severe clinical signs and lesions. Embryonic and fetal death, abortion and the birth of dead or weak animals with poor growth, which are characteristic of BD in sheep, are very difficult to study in free-ranging animals, like chamois, because aborted fetuses, birth products and weak neonates and calves are quickly removed by predators and scavengers in the wild and are not readily found by disease investigators.

Experimental infection studies using a chamois BDV isolate have been performed in pig, sheep and Pyrenean chamois. Infected pigs and sheep developed a short viraemia (almost undetected in sheep) and an important antibody response and virus clearance, without presenting clinical signs, macroscopic or histological lesions. However, five Pyrenean chamois presented a persistent viraemia from day 2 post-infection through all the experimental period (34 days), with seroconversion starting at day 11

post-infection (Cabezón, unpublished data). In all experimentally infected chamois, the homologous BDV4 was isolated from sera, main organs and nasal, oral, rectal and urine swabs. Although no evident clinical signs were detected, significant pathological lesions were observed in the brain, similar to those observed from field cases of infected chamois. Therefore, these results suggest that this BDV is the primary aetiological agent of the disease.

At necropsy, the most consistent finding in naturally infected chamois is emaciation with serous atrophy of fat. Enlargement of lymph nodes, especially the superficial cervical ones, is frequently observed. Bacterial bronchopneumonia, ranging from small pneumonic foci to large areas of lung consolidation, is the most common internal lesion. Other lesions are associated with concurrent infections and include abscesses, verminous pneumonia and orchitis. The most severe microscopic lesions are found in the brain. They consist of oedema, diffuse moderate spongiosis and moderate to evident diffuse gliosis with astrocyte hypertrophy. Occasionally, glial nodules are detected. Neuronal degeneration and necrosis occur throughout the brain, with the hippocampus and the Purkinje cell layer in the cerebellar cortex most severely affected[8]. Occasionally discreet inflammatory infiltrates of mononuclear cells surrounding blood vessels have been observed. In lambs with BD, lesions in the central nervous system are different, as they are infected during gestation. Mainly, they consist of a deficiency of myelin and an increased density of interfascicular glia, which can persist for years.

In the skin, the alopecic areas show follicular atrophy and telogenization of the remaining hair follicles. In the epidermis, hyperplasia and melanosis with evident orthokeratotic hyperkeratosis is observed, and in the dermis, mild mononuclear interstitial inflammatory infiltrates can be seen occasionally. These lesions are different from those described for BD in lambs, which consist of abnormal and straight birthcoat in fine or medium fleeced breeds, with an increased size of primary wool follicles and decreased number of secondary follicles.

The immune response to infection with BDV in Pyrenean chamois is not well understood. The epidemiological data indicate that populations with moderate to high seroprevalence of antibodies may be protected against disease, but affected populations and diseased animals do not have such antibody protection. However, in experimentally infected animals, despite the production of neutralizing antibodies, virus clearance was not attained.

CLINICAL SIGNS

Clinical signs most commonly observed in naturally infected Pyrenean chamois are non-specific, and include emaciation, depression, weakness and difficulties in locomotion. Skin lesions are frequent and consist of different degrees of alopecia and skin hyperpigmentation. The alopecia ranges from small asymmetric areas on the head (mainly at the base of ears and periorbital regions), neck and trunk, to extensive areas, with hair persistence only on ears, distal parts of the limbs and tail (Figures 10.3). Affected animals frequently exhibit abnormal behaviour, with absence of flight reaction and tameness, allowing their easy capture. These alterations may be related to the microscopic brain lesions observed. Dyspnoea is a frequent clinical sign, always associated with secondary bacterial pneumonia.

Pale mucous membranes are associated with anaemia caused by cachexia and inflammation, but also by intense tick infestation and presence of piroplasms (*Theileria* sp. OT3) in some cases[13,14]. Other clinical signs occasionally observed in chamois with BD are diarrhoea and keratitis, both probably associated with secondary infections.

Haematological and serum biochemical studies in naturally infected chamois showed reduced red blood cell count, haemoglobin concentration, packed cell volume, mean corpuscular volume, lymphocyte count, glucose,

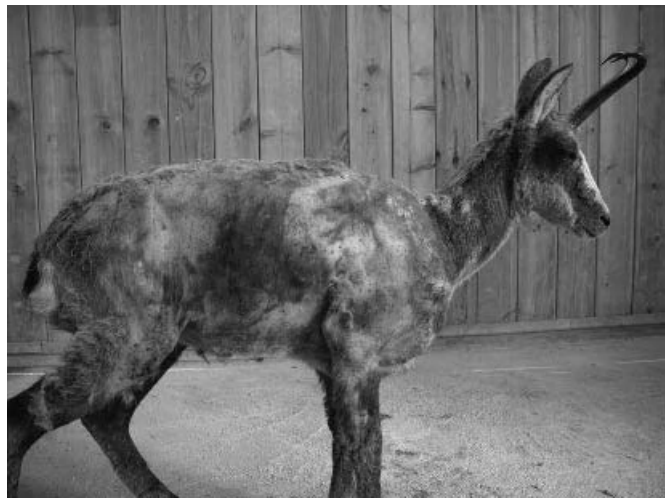

FIGURE 10.3 Extensive alopecia, emaciation and skin hyperpigmentation of a Pyrenean chamois (*Rupicapra pyrenaica*) with BDV infection.

lactate, triglycerides, creatinine, total protein concentrations and alkaline phosphatise. They also demonstrated higher neutrophil and platelet counts, total bilirubin, urea and aspartate aminotransferase. Most of these changes are non-specific and may be associated with the cachexia and inflammation in the diseased chamois, whereas the lymphopenia may be directly related to the BDV infection[14].

DIAGNOSIS

The diagnosis of BDV infection in Pyrenean chamois presents little difficulty in the field if animals are observed with emaciation, alopecia, depression, dyspnoea and abnormal behaviour. Nevertheless, laboratory confirmation is necessary, as other diseases described in chamois, such as sarcoptic mange, dermatophytosis, pneumonia, toxoplasmosis and cerebral coenurosis, should be considered in the differential diagnosis. RT-PCR and virus isolation have been used to detect pestivirus in chamois, as described for domestic ruminants[15]. Antigenic characterization of isolates of BDV has been performed by the immunoperoxidase monolayer assay (IPMA) test using monoclonal antibodies C-16 (pestivirus-specific), CA-3 and CT-6 (BVDV1-specific), WV433 (BVDV2 specific) and WS363 (BDV-specific)[8]. Viral antigen can be also demonstrated in most of the tissues of PI domestic animals by immunohistochemistry. Preliminary studies in chamois have also been performed on formalin-fixed, paraffin-embedded spleen and kidney samples, using the monoclonal anti-pestivirus antibody 15C5 directed against the 48 kDa glycoprotein of BVDV with positive results[5].

For population screening, there are several commercial ELISA antibody and antigen diagnostic tests for BDV, although they are not validated for wild ruminants. These have been used in several investigations in Pyrenean and Alpine chamois, and in some cases the results were confirmed by VNT. Six pestivirus strains have been used for these studies: namely BVDV1 strain NADL, BVDV2 strain atypical, BDV strain Spain 97, BDV strain Moredun, BDV strain 137/4 and BDV strain chamois, isolated from a diseased chamois in 2002. In healthy seropositive chamois, higher titres for sheep BDV strains than for the chamois strain have frequently been found. This could indicate an additional exposure in Pyrenean chamois to another BDV strain, probably of ovine origin, apparently without pathological consequences[6].

MANAGEMENT, CONTROL AND REGULATIONS

Control and eradication measures for pestivirus infection in domestic ruminants are mainly based on detection and removal of PI animals and vaccination. As it is not really known if PI animals are important for the spread and maintenance of the disease in Pyrenean chamois, and as these practices are not feasible in free-ranging populations of wild ruminants in remote mountain areas, it is unlikely that the infection can be controlled in the near future.

Control programmes for BD in sheep are not currently in place in the EU. BD is not included in the World Organisation for Animal Health (OIE) list and there is no designated reference laboratory.

PUBLIC HEALTH CONCERNS

There are no public health concerns, as humans are not susceptible to BDV infection.

SIGNIFICANCE AND IMPLICATIONS IN ANIMAL HEALTH

BDV infection in sheep has a worldwide distribution and causes economic losses through the impact on reproduction and health. In wildlife, no other information on mortality in species other than Pyrenean chamois has been reported.

ACKNOWLEDGEMENT

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BOVINE VIRAL DIARRHOEA

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Bovine viral diarrhoea (BVD) and mucosal disease (MD) are generalized viral infections caused by pestiviruses

affecting a broad range of hosts, mainly artiodactyls. Wildlife may be infected with more pestivirus species than have been identified at present.

AETIOLOGY

Bovine viral diarrhoea virus (BVDV) is a positive-sense, single-stranded RNA virus 12.5 kb in size with a single open reading frame encoding four structural proteins (the capsid protein and the envelop glycoproteins E^{rns}, E1 and E2) as well as six non-structural proteins. The virions are pleomorphic, spherical structures 50–60 nm in diameter, with a bilaminar envelope of cellular origin surrounding a semidense core of 20 to 25 mm diameter. Virions mature within intracytoplasmic membranes, and the virus is liberated by exocytosis of virus-containing membrane vesicles. Infectivity of BVDV is lost at elevated temperatures (e.g. mean half-life at 37°C and pH 7 is 7 hours) and by treatment with detergents and lipid solvents. The viruses are able to withstand a relatively broad pH range (pH 3–11).

Two antigenically distinct genotypes of BVDV exist (BVDV1 and 2) with further subdivisions discernable by genetic analysis. Isolates within these groups exhibit considerable biological and antigenic diversity (Figure 10.4). BVDV1 is clustered into as many as 11 distinct genotypes. Two main genotypes (2a and 2b) have been described for BVDV2[17,18]. BVDV2 represents around 50% of the BVDV isolates in North America. In Europe, more than 90% of the BVDV isolates are BVDV1. BVDV1 was isolated in 1954 and BVDV2 was first described in the early 1990s in North America as an emergent highly pathogenic viral genotype. Besides North America, BVDV2 has also been reported in Germany, Belgium, France, the Netherlands, Austria, Slovakia, Italy and the UK but not in Sweden, Norway, Spain, Slovenia or Denmark[19,20].

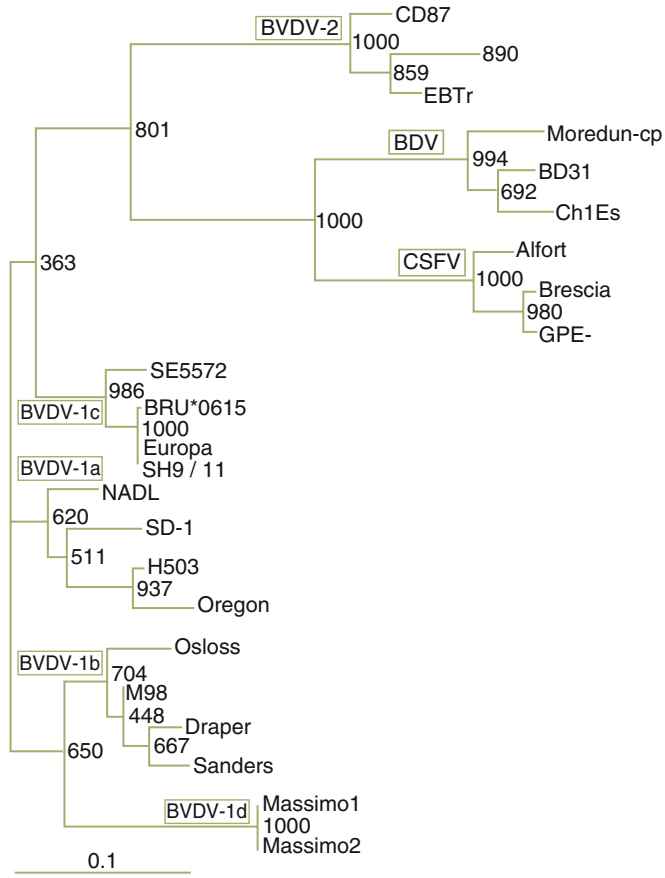

FIGURE 10.4 Phylogenetic tree based on 5′ – UTR sequence comparison from different pestivirus (mainly BVDV) strains; modified and used with permission. (See Giangaspero et al., 2001)[16]. Giangaspero et al. (2001)[16].

EPIDEMIOLOGY

GEOGRAPHICAL DISTRIBUTION AND HOSTS

Serologic surveys and virus isolation have provided evidence of infection with BVDV or related pestiviruses in more than 40 species of free-ranging wildlife worldwide. However, the extent of disease caused by BVDV in wild species is more difficult to assess, as many reports lack viral isolation and the clinical signs of various BVD viruses are insufficiently specific. The distribution of BVDV infections in wildlife from Europe is shown in Table 10.1.

In addition to serological evidence of BVDV in wild species in Europe, several characterizations of isolates have been conducted. Isolates from two seronegative free-ranging roe deer (*Capreolous capreolus*) from Northern Germany were analysed[33,34] and found to belong to the BVDV strain called SH 9/11. If the pathogenesis in deer is similar to that in cattle, the two isolates from roe deer probably were taken from PI individuals or those who had an acute transient form of BVD. The SH 9/11 strain is highly homologous (up to 93% identity) to 'classical' BVDV strains. However, SH 9/11 has characteristic variations in its 5′ UTR distinct from all other BVDV-strains and it is distinct from the other cattle isolates tested. Other isolates from roe deer were found in Hungary[35] and in Southern Germany[36], but phylogenetic analyses were not performed in these cases.

TABLE 10.1 Evidence of BVD infections in wildlife from Europe.

Species	Country	Antibody (AB), virus isolation (VI), lesions (L)	References
Cervidae			
Red deer (*Cervus elaphus*)	Denmark, Norway, Italy, UK, France, Germany, Austria	AB, VI	2, 21–32
Roe deer (*Capreolus capreolus*)	France, Norway, Germany, Hungary, Sweden, Spain	AB, VI, L	2, 23, 25, 26, 28, 30, 33–40
Fallow deer (*Dama dama*)	UK, Italy, Germany	AB, VI, L	2, 22, 27, 41–46
Sika deer (*Cervus nippon*)	UK	AB	22
Reindeer (*Rangifer tarandus*)	Norway	AB, L	30, 47, 48
Moose (*Alces alces*)	Sweden, Norway	AB, VI, L	30, 37, 49
Chinese water deer (*Hydropotes inermis*)	UK	AB	22
Bovidae			
Chamois (*Rupicapra rupicapra*)	France, Italy, Germany	AB, VI	25, 28, 32, 40, 50
Ibex (*Capra ibex*)	France	AB	25
European bison (*Bison bonasus*)	Poland	AB	51, 52
Suidae			
Wild boar (*Sus scrofa*)	Italy, Germany	AB	28, 32, 45, 53, 54
Lepridae			
Rabbit (*Oryctolagus cuniculus*)	Germany	AB, VI	55[a], 56[a], 57

[a]Experimentally infected individuals

Moreover, BVDV was isolated from captive fallow deer (*Dama dama*) from Germany and Sweden and from captive and free-living red deer (*Cervus elaphus*) from UK and France. Pestivirus was isolated and confirmed by genetic methods in captive reindeer (*Rangifer tarandus*) and European bison (*Bison bonasus*) (Germany)[58]. However, in the light of new results the reindeer isolate was typed as *Border disease virus 2*[4]. BVDV was also isolated from farmed alpacas (*Lama pacos*) in the UK. Phylogenetic analysis showed that this strain was of subgenotype 1b[59].

The principal source of BVDV within a population is PI individuals, which, by virtue of a high titre of virus shed in their secretions and excretions, infect healthy individuals. These PI carriers are especially important as virus excreters in maintaining the infection, because the virus is too unstable to survive long in the environment[60]. PI animals typically constitute 1–2% of the population[61]. Transmission of BVDV by acutely infected animals is not as efficient because the virus is only excreted for a relatively short time (2–15 days). The virus may also be present in aborted fetuses, fetal membranes and uterocervical fluids and semen. Transmission by mechanical and insect vectors (*Stomoxys calcitrans* or *Haematopota pluvialis*) has been reported[62,63]. In livestock in Europe BVDV has been endemic in all countries where no systematic control was applied. Approximately 50% of all cattle herds in Europe have PI animals, and 90% of all cattle become exposed during their lifetime[64].

The role of pestiviruses in wild ruminant populations and the interactions between wild ungulates and domestic livestock are not well understood. Suspected sources of the virus from wild animals include direct contact with infected livestock, shared feed and watering areas, or the presence of pestivirus-infected individuals within wildlife populations. Several investigators have speculated that an independent cycle occurs among wild ruminants in the absence of domestic animals. It has been hypothesized that distinct BVDV strains circulate in the free-ranging roe deer populations[33,34]. This hypothesis is supported by the results of serological surveys; on the one hand, no significant differences in antibody prevalence among deer in habitats with high, intermediate and very low density of cattle were found, suggesting that free-ranging deer can become infected with BVDV without having contact with cattle[2]. On the other hand, a causal relationship between the spread of BVDV in cattle and its occurrence in deer has been suggested, and it has been speculated that wild ruminants may serve as a reservoir of BVDV for cattle[62]. At present there is no evidence that BVDV spreads from free-living animals to domestic ruminants. However, theoretically it cannot be excluded that transmission of a highly virulent pestivirus strain from domestic to wild animals or

vice versa could occur and have a profound effect on both eradication programmes and wildlife populations[1].

PATHOGENESIS, PATHOLOGY AND IMMUNITY

Bovine fetuses infected with ncp BVDV during the first trimester of gestation may become PI with the virus. These fetuses are born persistently infected with the BVD virus and remain in this state for the rest of their lives until death; death is usually caused by the MD manifestation of BVDV infection. The PI fetuses usually develop as unthrifty calves that may be otherwise normal. PI bovines infected with ncp BVDV may succumb to fatal MD when superinfected with a cp strain. MD usually occurs in calves and young cattle between 6 and 24 months of age but also occasionally in older animals.

Two patterns of disease development can be observed. In one, cattle become moribund within 2–3 weeks after superinfection, developing an early-onset of MD. In the second pattern, animals recover after a transient phase of mild disease, but they may develop a late-onset MD after a time period varying from 4 weeks to several years. Both patterns of MD terminate in severe diarrhoea, erosions of the mucocutaneous membranes, anorexia, depression and ocular and nasal discharge, followed by generalized deterioration of the affected animal. Erosive and ulcerative lesions in the alimentary tract and depletion of lymphoid tissues are regarded as being characteristic for MD. Animals eventually show skin lesions and lameness due to interdigital ulceration and inflammation of the coronary bands.

Erosive to ulcerative lesions of oral mucosa and other mucocutaneous membranes of the digestive tract and catarrhal to fibrinous-diphteroid enteritis accompanied by general physical impairment are typically found in the MD form of BVDV infection in cattle.

Comparison of the course of BVDV infection in livestock to that in wild ruminant species is difficult, and at present there is insufficient data to make comparisons between the complex pathogenesis of BVDV in cattle and pathogenesis of BVDV infection in any wild species. Transplacental infections have not been reported in wild animals; however, mummified fetuses and stillborn and normal healthy fawns were observed in white-tailed deer (*Odocoileus virginianus*) experimentally inoculated with a BVDV originally isolated from a white-tailed deer in North America[65]. Inoculation of red deer, white-tailed deer and mule deer (*Odocoileus hemionus*) with ncp BVDV resulted in subclinical infections[21,66]. In reindeer, infection with cp BVDV resulted in transient mild diarrhoea, coronitis and laminitis[67]. The transmission of BVDV1f from a PI lesser Malayan mousedeer (*Tragulus javanicus*) to two bovine calves has been demonstrated[68]. In another study a lesser Malayan mousedeer, persistently infected with ncp BVDV type 1f, was experimentally superinfected with a cp BVDV type 1c, which antigenically partially matched the endogenous strain. Following superinfection, the lesser Malayan mousedeer did not develop clinical signs or lesions consistent with MD[69]. This may indicate a different course of BVDV infection in the lesser Malayan mousedeer than in domestic ruminant species.

Development of antibodies to BVDV has been reported in reindeer[67], and in mule deer and white-tailed deer[66]. In naive animals, antibodies are detected 8–15 days after infection; however, no systematic research has been performed regarding titre development in wild species after experimental infection. Therefore, it can be only assumed that, like in cattle[70], titres persist for life in wild ungulates.

CLINICAL SIGNS

The reason for the worldwide distribution of BVDV lies in its ability to cause two types of infection: i) transient (or 'acute') infections in the majority of cases; and ii) persistent infection.

The usual form of a BVDV infection in livestock (and probably in wildlife) is subclinical, but acute disease of high morbidity and low mortality or chronic disease of low morbidity and high mortality may occur. Initial clinical signs include pyrexia, anorexia, nasal discharge and lacrimation, leucopenia or general physical impairment followed by diarrhoea of varying intensity and duration.

The primary visible clinical signs in wild ungulates are haemorrhagic mucosal inflammation and general physical impairment (e.g. weight loss, apathy, immunosuppression). In several cases animals have erosive, ulcerative and nerotizing lesions of the digestive mucosa and catarrhal to haemorrhagic enteritis.

Some BVDV isolates in livestock may cause the so-called haemorrhagic syndrome, characterized by petechial haemorrhages in skin and mucous membranes, due to severe thrombocytopenia.

Transplacental infection with BVDV may, depending on the time of gestation, result in the development of a PI fetus, abortion, stillbirth, fetal malformation (cerebellar hypoplasia, microphthalmia, mandibular brachygnathism) or weak neonates. Early fetal losses are manifested as infertility and prolonged intervals between successive calvings as a result of these early embryonic deaths. Abortions can occur at any stage of gestation, depending on when the dam is infected. PI animals may appear either completely healthy or show abnormal signs such as growth retardation.

DIAGNOSIS

Various methods are available for diagnosing acute or persistent BVDV infections: immunohistochemistry (IHC), antigen and antibody ELISA, RT-PCR assays, and virus isolation. For detection of seropositive reactors by ELISA in wildlife, competitive ELISA is recommended, because specific anti-species antibodies are usually lacking. However, VNT is the 'gold standard' for the detection of specific antibodies in wild animals.

Acute infection with BVDV may be demonstrated by showing seroconversion using sequential paired serum samples (at an interval of at least 2 weeks) from several animals in the group.

Buffy coat cells, whole blood, washed leucocytes or serum are suitable for isolation of the virus from acutely infected or suspected PI live animals. At necropsy, samples of thymus, spleen, lung, liver, mesenteric lymph nodes, tonsils, intestine and kidneys should be collected for virus culture or RT-PCR. Viral antigen can be detected in acetone-fixed frozen tissue sections with immunofluorescent antibody techniques or in formalin-fixed paraffin-embedded tissues by IHC analysis. Confirmation of MD is possible by histopathology to demonstrate typical lesions coupled with IHC to show association of viral antigen with lesions, and if necessary isolation of the cp biotype as well as the ncp biotype.

PUBLIC HEALTH CONCERNS

Specific anti-BVDV antibodies have been found in up to 87% of animal handlers and veterinarians[71]. Among children under 2 years, pestivirus antigens were present in 24% of specimens from diarrhoea episodes that could not be explained by more common enteric pathogens[72]. Currently, genotypes of pestivirus RNA were detected in live virus vaccines for human use (e.g. mumps, measles)[16].

MANAGEMENT, CONTROL AND REGULATIONS

Control of BVDV infection of cattle is generally achieved by vaccination with attenuated live, modified live or killed BVDV vaccines, and by the identification and removal of persistently infected carriers of the virus.

Live vaccines contain cp BVDV. This vaccine is unable to produce PI animals. However, fetal infections after vaccination have been observed. Most probably they were attributable to non-cp contaminants of the vaccine. Furthermore, if PI animals are vaccinated with cp BVDV strains, MD may be triggered due to genetic recombination between the vaccine and the persisting virus. Killed vaccines are safer to use than live vaccines and are mostly used in wild animal species. However, they tend to be substantially less effective than live vaccines and require frequent boosters (twice a year). Various inactivated vaccines contain both cp and ncp strains of the virus.

In livestock, large-scale control efforts have been implemented in France, the Netherlands, Germany and Italy. Time-limited, project-type control efforts have also been implemented in Greece and Spain. In the UK, Ireland, the Netherlands and Slovenia, only killed BVDV vaccines are licensed. The Scandinavian countries and Austria do not permit the use of BVDV vaccines; instead, large-scale eradication schemes are in place. To build upon the growing interest in BVDV control a Thematic Network on BVDV Control, funded by the European Union Commission's 5th Framework Programme was formed in 2002 (www.bvdv-control.org)[73].

Wild ruminants share range (pasture), feed and water sources with cattle; therefore development of adequate control programmes is dependent on knowing whether there are reservoirs of BVDV on the ranges other than domestic cattle[74]. It is assumed that wildlife reservoirs can seriously undermine the effectiveness of any domestic BVDV control strategy by acting as potential sources of virus that can infect cattle, especially at the wildlife–livestock interface[75]. Also, it has been suggested that the reintroduction of BVDV to negative cattle herds in the UK can be due to contact with a wildlife reservoir[76]. However, the natural mode of transmission of BVDV to

wild ungulates, and the question of whether wildlife could serve as a reservoir, is not yet clear.

SIGNIFICANCE AND IMPLICATIONS FOR ANIMAL HEALTH

BVDV is a major pathogen of cattle worldwide, with significant economic impact on livestock production. The impact of BVDV-associated diseases on the health of free-ranging wild ruminant populations is currently unknown; there are few confirmed cases of pestivirus-caused disease in these species but no evidence that these viruses have significant population impacts[65].

CLASSICAL SWINE FEVER

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Classical swine fever (CSF) is a highly contagious, febrile, viral disease of pigs. It was first described in the early 19th century in the USA and was termed 'hog cholera'. Later, a condition in Europe named 'swine fever' was recognized to be the same disease. Both names continue to be in use. In general the disease is now called CSF to distinguish it from African swine fever (ASF), which is clinically indistinguishable but caused by an unrelated virus. CSF affects domestic pigs and wild boar (*Sus scrofa*). Owing to their severe economical impact, outbreaks of CSF in domestic and wildlife populations are notifiable to the OIE.

AETIOLOGY

CSF is caused by a small, enveloped RNA virus in the genus *Pestivirus* of the family *Flaviviridae*. *Classical swine fever virus* (CSFV) is antigenically related to the other *Pestiviruses*, namely *Bovine viral diarrhea virus* of cattle and *Border disease virus* of sheep. The latter two viruses are widespread in domestic as well as wild ruminants and can occasionally infect pigs. Usually, ruminant pestiviruses do not cause disease in pigs, and they are eliminated after a few days. However, these infections have diagnostic consequences because serological cross-reactions with CSF virus do occur and may lead to false-positive laboratory results. In these cases additional tests are required to differentiate CSF antibodies from antibodies induced by ruminant pestiviruses.

Under natural conditions CSFV infects only Suidae, i.e. domestic pigs and wild boar, although the virus can be transmitted experimentally to other species. It will grow in porcine cell cultures but does not generally cause a visible cytopathic effect. The virus has only one serotype, and only some limited antigenic variability between strains can be shown. Strain typing for epidemiologic mapping purposes had been done by sequencing of the viral RNA combined with phylogenetic analysis. A large database of CSF viral sequences is available at the Community Reference Laboratory for CSF at the University of Veterinary Medicine in Hannover[77].

The virus is moderately fragile and does not persist in the environment or spread long distances by the airborne route. It can survive for prolonged periods in moist excretions of infected pigs and also in fresh meat products, including ham and salami-type sausages. Low temperatures extend viral survival times, and the virus has been shown to remain viable after several years in frozen pig meat, or months in chilled or cured meat. However, it is readily inactivated by detergents, lipid solvents, proteases and common disinfectants.

EPIDEMIOLOGY

CSF has worldwide distribution. It is endemic in parts of Latin America, some Caribbean islands and pig-producing countries of Asia. In 2005 South Africa reported to the OIE the first occurrence of CSF in mainland Africa since 1918. Australia, New Zealand, Canada and the USA are free of CSF, as is most of Western and Central Europe, although sporadic outbreaks occur in some countries.

Data on the geographic distribution of CSF in Europe is incomplete. The World Animal Health Information Database (WAHID) of the OIE indicates that the countries of the EU are CSF-free and that outbreaks have been reported in the Russian Federation, although some other non-EU countries have not supplied information to OIE. It is known that in some western Balkan countries CSF occurs in domestic pigs. The epidemiological situation in wild boar in these countries is not known.

CSF virus readily infects European wild boar, and direct and indirect transmission of the virus is comparable to that

in domestic pigs. In countries that are free of CSF in domestic pigs, epidemics in wild boar are typically initiated by deliberate or accidental feeding of contaminated pork in garbage at rest sites or landfills. Although the feeding of swill is prohibited in EU countries, knowledge of this and awareness of the risks is often inadequate among farmers, hunters and tourists. In countries with CSF in domestic pigs, wild boar may become infected largely through indirect contact with infected pigs (e.g. manure). Once introduced in this way, the virus can spread within wild boar populations by direct contact and contact with contaminated excretions and carcasses. PI piglets can contribute to virus circulation in wild boar populations. Transmission to domestic pigs may arise as a result of direct or indirect contact with wild boar. Contamination of hunting equipment, livestock feed, swill and shared environments with wild boar excretions or carcasses provide opportunities for the transmission of CSF virus. There are no known animal vectors.

The first indication of an outbreak of CSF in a wild boar population is usually a mortality event. Effective spread of the virus within wild boar populations requires transmission both within and among social groups. Within social groups, the virus is transmitted relatively rapidly by direct and indirect contact, particularly between piglets. By contrast, rates of direct contact among boar from different social groups are lower, and hence transmission occurs mainly by contact with contaminated excretions and carcasses. Direct transmission between groups may be enhanced during the rutting season through dispersing males, and when new social groups are being established. The outcome of CSF infection in individual boar resembles that in domestic pigs, in that a high percentage of young pigs succumb to the infection, but older animals survive. Convalescent pigs mount a sufficiently strong immune response to protect them from future CSF virus infection. There are two possible courses for an epidemic of CSF in wild boar: either the epidemic is self-limiting or it becomes endemic. For some time historical experience of CSF outbreaks suggested that epidemics of CSF in wild boar are always self-limiting. Typically, acquired immunity in a proportion of the population in combination with reduced host density arising from disease-induced mortality, decreased the number of susceptible animals and the infection died out. However, during the last two decades epidemics in some regions have been observed to become endemic with CSF virus persisting in wild boar populations for prolonged periods without signs of self-limitation. Possible reasons for the emergence of endemicity are the increasing density and size of wild boar populations and the involvement of viral strains of low virulence[78]. The principal reason is most likely to be the recent increase in the abundance and distribution of wild boar populations throughout Europe[79]. This is at least in part the result of improvements in nutrition due to intensified agriculture and the effects of climate change, which have led to prolonged breeding seasons. Changes in hunting practices are also likely to have contributed. The increase in wild boar abundance allows the establishment of endemic CSF, because there are at any time sufficient susceptible animals to sustain the chain of infection, i.e. viraemic animals are always able to transmit the virus to at least one other susceptible wild boar (and so the basic reproductive rate of the pathogen is $R_0 \geq 1$). The more susceptible animals belong to a wild boar population and the higher the animal density in a certain area is, the higher is the probability that $R_0 > 1$ will persist for a prolonged time. During initial outbreaks and epidemics all animals are susceptible, whereas in endemic situations it is mainly young animals that are susceptible. Due to the high reproductive rate of wild boar, there is a constant supply of young animals that become susceptible after the waning of maternal immunity. This young age class constitutes a reservoir for the virus and maintains the infection in the population. By contrast, CSF outbreaks in smaller populations are likely to be self-limiting. For example, in populations of about 1500 to 2000 animals, the virus may disappear within 1 year[80].

Epidemiological links between CSF virus infections in wild boar and domestic pigs have been reported repeatedly. Between 1993 and 1997 80% of the 92 primary outbreaks of CSF in domestic pigs in Germany were located in geographic regions where CSF was endemic in wild boar. In 60% of these cases a direct or indirect contact with infected wild boar or wild boar meat was established[81].

PATHOGENESIS, PATHOLOGY AND IMMUNITY

CSF in wild boar has not been studied as extensively as in domestic pigs. Therefore most of the theoretical and practical considerations regarding the disease in wild boar are mainly based on knowledge of CSF in domestic pigs. However, a few experimental studies conducted on wild boar indicate that the clinical course of CSF is similar to that in domestic pigs[82,83].

Infection with virulent CSFV results in an acute haemorrhagic disease of pigs, characterized by disseminated intravascular coagulation, thrombocytopenia and immunosuppression. CSFV replicates in macrophages and vascular endothelial cells of pigs and it is able to suppress interferon production and apoptosis of infected cells.

The course of CSFV infection may follow two different pathways, depending on the time of infection: the animal either gets infected prenatally as a fetus when the immune system is not fully developed; or the infection takes place postnatally when the immune system is already developed (for review see [84]). The two courses differ in their pathomechanisms and have different consequences for the clinical disease, as well as for the perpetuation of the virus within a population of susceptible hosts.

Prenatal Infection

Like all other pestiviruses, CSFV has the ability to cross the placental barrier and infect the fetus in utero. This happens if a pregnant sow, which is not protected by CSF-specific antibodies, undergoes a transient infection with CSFV (see the section on postnatal infection, below). Early infections of susceptible pregnant sows (within the first 6 weeks of gestation) usually result in abortions and stillbirths, whereas later infections yield persistently viraemic piglets. Stillborn and/or mummified animals may be present along with viraemic and non-infected piglets in one litter. PI piglets shed virus permanently until they die. These PI animals can play an important role in the spread of CSF virus in the domestic pig or wild boar population. Although PI piglets are weaker than non-viraemic ones, some of them may survive for several weeks or even months. In domestic pigs the longest period of persistent viraemia reported so far was 11 months and for wild boar piglets 39 days[83]. PI piglets develop the so-called late-onset form of CSF.

Postnatal Infection

The postnatal infection is the common, or 'classical' form of CSF. This can be acute or chronic. The acute course lasts less than 4 weeks and the infected animals either recover completely (transient infection) or die (lethal infection). The mortality rate may be as high as 90%, depending on a number of factors. The chronic form has been defined as lethal disease with a duration of 30 days or more. Under field conditions different forms of CSF occur simultaneously.

The outcome of postnatal CSF has often been related solely to the virulence of the particular virus causing the infection. However, virulence on its own is a confusing and misleading parameter. The age of the pig at the time of exposure, its immunological status, the dose of the infecting virus and the breed are other factors of importance for the outcome of the infection. In adult animals CSFV often induces only transient infections with few mild clinical signs and lesions and low mortality. In pregnant sows reproductive failure frequently occurs (see also the prenatal infection section, above). By contrast, the disease in young animals is generally more serious, leading to high mortality rates. These observations are also valid for wild boar[85]. In wild boar only the acute course of disease has been described so far, and no experimental or field data are available for the chronic course. It may be assumed that the chronic form occurs in wild boar; however, survival time of chronically sick animals might be shorter than in domestic pigs owing to harsher environmental conditions.

Under natural conditions, the mode of entry of CSFV into the animal is the oronasal route. The tonsils are primary sites of virus replication, and CSFV can be found in the tonsils as early as 7 hours post-exposure. Interestingly, also after intramuscular and subcutaneous inoculation CSFV was found most consistently and in high concentration in the tonsils. From the tonsils, CSFV is transported via the lymphatic circulation to the regional lymph nodes. After replication in regional lymph nodes the virus reaches all other organs of the body via the blood. High titres of virus have been demonstrated in spleen, bone marrow, visceral lymph nodes and lymphoid structures lining the small intestine. The virus probably does not invade parenchymatous organs until late in the viraemic phase. Virus spread is usually completed within 5–6 days.

Post mortem examination of pigs that have succumbed to acute CSF often show pathological changes in lymph nodes, tonsils, spleen and kidneys. Lymph nodes may be swollen, oedematous and haemorrhagic. Haemorrhages of the kidney may vary in size from hardly visible petechiae to ecchymotic haemorrhages. Similar haemorrhages can also be observed in the urinary bladder, larynx, epiglottis and heart and sometimes widespread over the serosae of the abdomen and chest. A non-purulent encephalitis is often present. Lesions due to secondary infections may

also be seen, which may mislead the pathologist. Pathological lesions induced by chronic CSF are less typical, especially concerning the lesser extent of haemorrhages on organs and serosae. In animals showing chronic diarrhoea, necrotic lesions in the ileum, the ileo-caecal valve and the rectum are common. As clinical signs of chronic CSF are rather non-specific, many other diseases must be considered as differential diagnoses.

Prenatally, at the early phases of ontogenesis, the virus affects organ differentiation, which may lead to malformations. However, this may not be detectable under field conditions in wild boar.

In general the acute form of ASF leads to a very similar clinical and pathological picture. When present, haemorrhages on the skin and ears are easy to detect and lead to suspicions of acute ASF or CSF. The predominant *post mortem* findings consist of haemorrhagic diathesis and swollen, haemorrhagic lymph nodes. However, the severity of pathological lesions can vary widely. Both diseases can be clearly differentiated by laboratory diagnosis.

In the field the most visible signs of CSF (and ASF) are the lesions described above. However, it takes experience and the awareness of the persons handling wild boar, e.g. hunters and gamekeepers, to recognize the typical signs and to interpret them properly.

The immune response of wild boar against CSFV is analogous to the immune response of domestic pigs. Like all pestiviruses, CSFV affects the immune system, causing transient immunosuppression during acute infection. The CSFV has a distinct affinity to cells of lympho-reticular organs and causes severe depletion of lymphocytes, affecting both B and T cells. Thymus and bone marrow atrophy, as well as generalized lymphocytopenia have been recorded with a prominent B-lymphocyte deficiency. Generalized leucocytopenia, which is usually seen before the onset of fever, is considered a primary characteristic of CSF. Little is known about cell-mediated immunity against CSF. In transient acute CSF, neutralizing antibodies do not appear in blood until the animal has recovered from the leucocytopenia, 2 weeks post-infection at the earliest. Pigs that have recovered are protected against CSF at least for 6 months or even for their lifetime. Likewise, pigs that have been vaccinated with modified live vaccines develop a solid and long-lasting immunity. In wild boar, oral vaccination using the live C-strain vaccine, has been demonstrated to be fully protective at the individual level[86,87].

Maternal antibodies have a half-life of about 11 days and last until the piglets are 3 to 4 months old. Passive immunity generally protects piglets against mortality during the first weeks of life, but not necessarily against virus replication and shedding[88]. In PI piglets colostrum-derived antibodies can only be detected for a short time after birth (less than 1 week) compared with non-viraemic littermates[83].

CLINICAL SIGNS

Clinical manifestation of CSF may vary greatly depending on the age of the animal and viral virulence. In domestic pigs, after an incubation period of approximately 4–7 days, animals become febrile and may develop symptoms typical for CSF. Three clinical courses can be distinguished, i.e. acute infection, chronic infection and late-onset CSF.

In acute cases of CSF, the first signs, e.g. high body temperature (>41°C), inappetence and apathy can be seen after the incubation period. The progression of the disease is marked by conjunctivitis, nasal discharge, intermittent diarrhoea, swollen lymph nodes and muscle tremor. The terminal stage in domestic pigs is often characterized by skin cyanosis, mainly on ears, nose, tail and abdomen, and skin haemorrhages of different grades, predominantly over bone protuberances. In wild boar skin lesions are less evident and may be masked by skin bristles. Sick animals show hind-limb weakness and a staggering gait which is often followed by a posterior paresis. Leucocytopenia and thrombocytopenia are common findings. In addition, wild boar exhibit behavioural changes, becoming seemingly tame and roaming during daylight hours[89].

Animals that do not die within 4 weeks post-infection either recover, developing high titres of neutralizing antibodies, or become chronically ill and remain virus shedders until death. Wasting and diarrhoea are the most evident signs in chronic CSF in domestic pigs. Such pigs have skin lesions, and frequently stand with arched backs. Pigs with chronic CSF may survive for more than 100 days. Secondary bacterial and parasitic infections are frequently present. Thus the clinical picture may often be atypical, variable and misleading.

The late-onset form of the disease is seen in piglets that have been infected in utero and that have been born as PI piglets. After a period without clinical signs, they may develop mild anorexia and depression, conjunctivitis, dermatitis, diarrhoea and locomotive disturbances leading to posterior paresis. CSF characteristic lesions, particularly petechial haemorrhages, are not present. Retarded growth

is the most common finding. Although the prenatal intrauterine infection leading to PI offspring is a rather rare event, it is regarded as one possible mechanism contributing to the persistence of CSFV within a population. In wild boar the prenatal form of CSF has only been demonstrated experimentally[83]. Field data on the occurrence of PI wild boar manifesting the late-onset form of disease are not available.

In summary it must be emphasized that the clinical signs of CSF in wild boar – as in domestic pigs – can be extremely variable, depending on different factors. This may impede early recognition in the field.

DIAGNOSIS

Hunters, veterinarians and farmers should always be highly aware of the risk of CSFV introduction. Close observation of wild boar populations is important for the early suspicion and detection of outbreaks. Abnormal mortality, particularly in young animals and sometimes obviously sick wild boar, are early signs of a CSFV outbreak in a population. A tentative diagnosis of CSF can also be made based on gross pathological lesions found in shot animals, e.g. petechial bleedings and/or haemorrhages. Any suspicion must be confirmed by laboratory diagnosis. Suitable samples for virological investigation can be retrieved from animals found dead or clinically sick wild boar that have been shot. Random sampling of shot animals is the method of choice for epidemiological investigations, such as monitoring and serological prevalence studies.

LABORATORY DIAGNOSIS

All laboratory techniques for diagnosing CSF have been compiled in the Classical Swine Fever Diagnostic Manual of the European Commission[90] and in the OIE Manual of Diagnostic Test and Vaccines for Terrestrial Animals[91]. However, unlike laboratory diagnosis in domestic pigs, the diagnosis in wild boar is often hampered by the poor quality of samples. This makes inter-laboratory comparison tests difficult, if not impossible, and error rates might be higher compared with routine diagnosis of samples from domestic pigs.

The laboratory diagnosis of CSF is based on detection of viral antigen, isolation of virus, detection of viral RNA and demonstration of virus-specific antibodies. Tonsils, mandibular lymph nodes and spleen are the most appropriate tissues for virus detection in wild boar. Blood (if available) and homogenized lymphatic tissues are the materials of choice for virus isolation. For antigen detection the direct immunofluorescence test (FAT) is widely used. The test is both rapid and reliable and is applied to detect CSFV in cryostat sections.

Isolation of CSFV in cell cultures is regarded as a very sensitive method; however, it takes a minimum of 3–5 days. Virus isolation can provide confirmation in cases in which the FAT is inconclusive.

For large-scale screening, antigen-capture ELISA have been developed. These tests require less specialized facilities and can be performed much more rapidly than virus isolation. However, their sensitivity and specificity is inferior to FAT or virus isolation on cell culture. Reliable results are obtained only with samples from animals already displaying clinical signs. Therefore the use of antigen ELISA has to be linked with the clinical or pathological examination of the animal. RT-PCR has become a standard method for CSF diagnosis[92,93]. The sensitivity and specificity of RT-PCR is comparable to that of virus isolation, or is even superior to it. The molecular epidemiology based on the above technique has become a useful tool and may support epidemiological investigations. Typing of CSFV isolates of at least each primary outbreak in wild boar could provide useful information on the origin of the virus.

Serological diagnosis of CSF is important for the detection of the infection in wild boar populations, where acute clinical signs are absent. In addition, it is a tool for the monitoring of epidemic and endemic disease situations. Several tests are available for the detection of CSF antibodies. The fluorescent antibody virus neutralization test and the neutralizing peroxidase-linked assay are commonly used techniques. Although the neutralization tests are considered to be the most sensitive and specific tests, they are time-consuming and not suitable for automated systems and large-scale screening. To achieve these purposes, several ELISA techniques using specific monoclonal antibodies have been developed and are commercially available. However, the sensitivity of the ELISA is regarded to be inferior to the sensitivity of the neutralization tests. The ELISA technique requires less specialized facilities and can be performed much more rapidly than the neutralization test due to automated systems. Large numbers of sera can be examined within a short time. As ELISA systems are subject to occasional difficulties in interpretation, it is

essential to have access to a neutralization test for investigation of sera that give inconclusive ELISA results.

In areas where oral vaccination of free-ranging wild boar is carried out, it is not possible to distinguish between vaccinated or naturally immunized individuals.

MANAGEMENT, CONTROL AND REGULATIONS

Awareness and vigilance are essential so that outbreaks in domestic pigs and wild boar are detected early and control measures are instituted rapidly to prevent further spread.

CSF-eradication programmes in recent decades have been successful in North America, Australia and in most European countries. CSF is notifiable to the OIE and to the competent veterinary authorities in member states of the EU. Prevention of CSF in wild boar is regarded as a crucial element for the protection of domestic pigs from infection.

Control and eradication of CSF in wild boar cannot be managed as in domestic pigs (i.e. by systematic culling and movement restrictions). Once CSFV is established in a wild boar population the primary goal of all control measures must be to reduce the number of susceptible animals in the affected area. The threshold level of susceptible hosts must drop to a level where the basic rate of infection R_0 is reduced to below 1 ($R_0 < 1$). The number of susceptible animals in an area will be influenced by many factors including the size of the infected population, disease-induced mortality rates, acquired immunity, hunting pressure, feeding (movement of animals) and other management interventions (e.g. oral vaccination). Furthermore, the response of the population to management interventions is likely to be influenced by population density and structure. Strategy and methods applied for the control of CSF in wild boar populations must take such factors into account[94].

Depending on the size and structure of the affected wild boar population, this goal can be achieved either by 'doing nothing', i.e. banning hunting and limiting all disturbances of the affected population in the case of small and isolated populations. Hunting, oral vaccination or a combination of both are possible strategies in large populations. Feeding should be avoided, as artificial feeding is a potential source of infection, if meat products or kitchen waste are (illegally) included. As feeding encourages boar to aggregate, it may contribute to further spread and perpetuation of the virus in the population. Feeding sites may be visited by animals whose normal home range is far away, thus intensifying direct and indirect contact between wild boar from different groups[95]. The consequence is to enlarge the population at risk. An additional effect of artificial feeding might be increased fertility arising from improved nutritional condition of the females[96].

For several reasons indiscriminate hunting is not an effective control option. It fails to reduce the wild boar population to the threshold level required for transmission to cease because populations that are substantially reduced by hunting exhibit compensatory reproduction. Consequently, wild boar density tends to return quickly to the carrying capacity of the environment. One consequence of this process is that a large number of young susceptible animals are recruited into the population. Furthermore, immune adult animals in endemic areas will be among those shot, and their removal therefore reduces the proportion of immunes in the population[97]. The removal of older animals by hunting also leads to a dispersion of social groups and increased movement of individuals, thus enhancing opportunities for disease transmission.

Theoretically hunting could have a positive effect if only, or predominantly, young wild boar were removed, as this would reduce the number of susceptible animals in the population without disrupting social groups and increasing dispersal. In practice, however, hunting is often neither efficient nor sustainable, mainly due to the inherent difficulties in mounting sufficiently high hunting pressure, particularly in the face of compensatory reproduction in reduced populations. Hunting also requires the compliance of hunters, whose ethics may not necessarily be compatible with the objectives of CSF control. Financial incentives have been used to overcome the reluctance of hunters to shoot young animals. However, despite these limitations, hunting is useful and necessary for collecting samples for laboratory diagnosis[94]. Trapping of young wild boar using cage traps is an efficient method, which could be applied as an alternative or in addition to hunting.

Oral immunization of wild boar is an effective control option and a key tool for the eradication of CSF in dense wild boar populations. However, in member states of the EU, where prophylactic vaccination against CSF is prohibited, emergency vaccination measures in wild boar must be notified to the Commission[98]. After an outbreak of CSF and during the progression of the epidemic, an increasing number of adult animals survive the infection and become immune, thereby reducing the number of susceptible animals. In this situation oral vaccination using

baits containing live modified CSFV can be used to further reduce the number of susceptible animals below a critical threshold, where R_0 drops below 1 ($R_0 < 1$).

Areas to be vaccinated should be designed according to the spatial distribution of wild boar populations, and the ecological characteristics of the landscape (i.e. natural and artificial borders such as motorways, rivers, lakes, mountains, etc.)[98]. Vaccination strategies have also to strictly define the epidemiological and sampling units. The success of oral vaccination campaigns largely depends on the strategy of bait delivery. CSF vaccine baits are distributed by hand at pre-defined feeding areas. The number of baits depends on the population density and typically ranges between 30 to 40 per km^2. Vaccination is repeated 2 weeks later and thereafter this 'double' vaccination is repeated every 4–5 months[99]. This vaccination procedure increases population immunity progressively, and the maximum population immunity is usually reached after three double campaigns per year. The high reproductive rate of wild boar requires that vaccination continues for at least 2 years after the last virus-positive boar had been detected in order to maintain a sufficiently high level of immunity in the population and so to eliminate the CSFV.

In animals older than 1 year immunity reaches 75–90% after 1 year of vaccination (i.e. three campaigns). In contrast often less than 30–50% of young wild boar are immune even after several years of vaccination. One explanation for the significantly lower seroconversion in young wild boar is insufficient bait consumption. Piglets younger than 6 months do not consume the vaccine baits, because: i) animals higher up in the hierarchy eat most of the baits; ii) the baits in current use are too large; and iii) piglets of that age prefer food of a different texture. Despite these limitations, in combination with natural immunity induced by circulating field virus, vaccination was shown to repeatedly reduce R_0 ($R_0 < 1$) sufficiently to successfully clear CSFV from wild boar populations[85,87,99,100].

CSF does not persist in smaller wild boar populations, as subsequent to virus introduction most of the surviving animals become immune, leaving too few susceptible pigs to maintain the chain of infection. CSFV can only persist in a wild boar population in the presence of viraemic animals, which transmit the virus to enough susceptible wild boar to ensure $R_0 > 1$. However, monitoring and understanding demographic processes and the epidemiology of disease in wildlife populations is challenging, and several parameters of interest (e.g. population structure and dynamics, population size or herd immunity) remain unknown or can only be roughly estimated.

In addition to control measures aimed at the elimination of CSF from the wild boar population, strict measures must also be taken to avoid the transmission of virus to domestic pigs. Therefore wild boar and domestic pigs must be separated effectively, by fencing of holdings, strict hygiene measures, movement restrictions for wild boar (no trade of live wild boar) and trade restrictions for wild boar meat (i.e. wild boar carcasses must remain in the affected zone). For domestic pigs, movement restrictions should also be in place and animals should undergo clinical investigation before they leave a zone where wild boar are affected by CSF. In cases of clinical suspicion a virological investigation should be performed immediately. Pigs in outdoor holdings are at greatest risk, and therefore existing outdoor holdings in the affected zone should be reported to the veterinary authorities. These establishments should be organized and managed in such a manner as to reduce the likelihood of direct contact between wild boar and domestic pigs. In particular, outdoor holdings should be enclosed within a double fence with a gap of at least 1 metre. In addition, these farmers should provide sheds in order to be able to move domestic pigs into closed facilities when there is a high risk of CSFV infection (e.g. when virologically positive wild boar are detected in the vicinity of the holding). In times of CSF epidemics in wild boar the establishment of new outdoor holdings for domestic pigs should be forbidden.

Recent CSF epidemics/endemics in wild boar have made it clear that effective control requires a holistic approach that takes into account the characteristics of the disease, the biology of wild boar populations and human activities. Measures to increase public awareness of the disease are also essential for the success of control.

PUBLIC HEALTH CONCERN

CSF, like all other known pestiviruses, is not transmissible to humans or commonly kept pets such as dogs, cats and avian species. Therefore there is no public health concern in the strict sense.

SIGNIFICANCE AND IMPLICATIONS IN ANIMAL HEALTH

CSF can cause devastating epidemics in domestic pigs, particularly in countries that are free of the disease but have a fully susceptible pig population. In these countries,

vaccination is generally only permitted in emergencies. In the event of an outbreak, strict measures (e.g. culling of infected and suspect pigs and movement restrictions) are enforced in order to control the spread of the disease. This can have severe consequences for the pig industry, especially in densely populated livestock areas. Several outbreaks in member states of the EU in recent years have caused very high economic losses. For example, a CSF epidemic in the Netherlands in 1997–1998 cost about 2 billion euros and more than 11 million pigs had to be destroyed[101]. The mass culling of pigs creates an ethical problem, and the economic losses mean that CSF is one of the most important diseases of domestic animals in Europe.

As CSF may become endemic in wild boar populations, they threaten domestic pig holdings as a possible source of infection, with considerable economic consequences. Therefore the main aims of controlling CSF in wild boar are to reduce the risk of transmission of the disease to domestic pigs, to prevent it becoming endemic, or to reduce the duration of the endemic phase, and finally to eradicate the disease in wild boar.

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CHAPTER

11

PICORNAVIRUS INFECTIONS

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INTRODUCTION

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The *Picornaviridae* family is one of five families in the order *Picornavirales*. There are currently 12 genera assigned within the *Picornaviridae*. Other picornaviruses for which genome sequence data have recently become available, but which have not yet been assigned, include two new groups containing picornaviruses of bats, wild birds, reptiles, seals and fish. Picornaviruses are small, roughly spherical, viruses with a capsid of approximately 30 nm in diameter. Within this capsid is a single copy of positive-sense RNA approximately 7–9 kb in length, including a small, virus-encoded, polypeptide (VPg) covalently linked to the 5′ terminus. This genome includes a single long open reading frame (ORF) preceded and followed by an untranslated region (UTR). The 5′ UTR includes various RNA elements involved in virus replication and the initiation of protein synthesis. The 3′ UTR contains elements that also are involved in virus replication and is followed by a poly(A) tail. The ORF is translated into a polyprotein, which is processed by one or two virus-encoded proteinases. Picornaviruses produce only 11 to 14 multifunctional proteins. In addition, some of the processing intermediates also have distinct functional roles.

In wild animals in particular, aphthoviruses (*Foot-and-mouth disease virus*) and cardioviruses (*Encephalomyocarditis virus*) are recognized as pathogens. They can cause a large range of diseases, ranging from acute, sometimes fatal, to mild and even inapparent infections in many hosts. Others have been associated with syndromes for which their involvement in disease causation is unclear.

FOOT-AND-MOUTH DISEASE

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Foot-and-mouth disease (FMD) is caused by a picornavirus and is predominantly a disease of domestic ruminants (cattle, sheep and goats) and pigs. Now that rinderpest has been globally eradicated, FMD is considered the most important viral pathogen affecting production animals. Although not a cause of significant mortality within herds, FMD is debilitating, and affected animals rapidly lose production. Most importantly, FMD spreads rapidly

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within susceptible animal species. The Food and Agricultural Organization of the United Nations considers FMD to be a transboundary disease, namely:

> *a disease that is of significant economic, trade and/or food security importance for a considerable number of countries; which can easily spread to other countries and reach epidemic proportions; and where control/management, including exclusion, requires cooperation between several countries.*

Because of the global concern over FMD and the success of many countries in eradicating the disease, many species of wildlife have been recorded to be susceptible to FMD virus either through natural or experimental infection. When FMD is confirmed in domestic animals, questions inevitably arise as to the role of wildlife in possibly introducing and maintaining the disease. However, with one important exception (the African Cape buffalo (*Syncerus caffer*)), wildlife species are only occasionally infected with FMD, even when there is an extensive epidemic in domesticated livestock.

There are several reports of FMD in wildlife in Europe[1], but most date to the early part of the 20th century, when the disease was endemic in Europe and diagnosis was usually based only on clinical signs. It is known that European species of deer are susceptible to experimental infection with FMD virus[2]. During the extensive epidemic of FMD in 2001 in the UK, the Republic of Ireland, France and the Netherlands, there were several reports of small numbers of deer with clinical disease highly suggestive of FMD, but none could be confirmed by laboratory tests. In late December 2010, a single wild boar (*Sus scrofa*) was identified as being affected with FMD in Bulgaria, near to the Turkish border.

In summary, although FMD has the potential to cause disease in European wildlife, history indicates that it has never been an important pathogen of wildlife in Europe. Apart from the recent report of the infected wild boar in Bulgaria, there are no recent reports in the English literature of naturally occurring cases of clinical FMD in European wildlife that have been confirmed by virus isolation or other techniques, and – in contrast with the situation in Africa – there is no known reservoir of the virus in any wildlife species.

AETIOLOGY

FMD virus is classified within the order *Picornavirales*, the family *Picornaviridae* and the genus *Aphthovirus*, with FMD virus being the type species in the genus and the Greek descriptor 'Aphtho' referring to vesication, which is the characteristic clinical sign of FMD.

The viral aetiology of FMD (i.e. distinct from bacterial) was established by filtration in 1898 by F.A.J. Loeffler and P. Frosch working with Robert Koch. Foot-and-mouth disease virus was the first virus discovered that affected vertebrates. Since that epochal discovery, FMD virus has been intensively studied. The virus particle is non-enveloped, very small (27 nm in diameter), has an icosahedral symmetry and a single-stranded, positive-sense, RNA genome. FMD viruses are relatively resistant and, dependent upon environmental conditions, can survive several days or more on fomites and even longer in animal products; however, FMD viruses are labile at pH values below 6 and above 9, which simplifies disinfection.

X-ray crystallographic analysis of purified virus has shown that the virions are constructed from 60 copies each of four capsid proteins: VP1, VP2, VP3 and VP4. Amino acid substitutions of VP1, 2, and 3 correlate with antigenic variation.

There are seven serotypes of FMD virus: O, A, C, Asia 1 and SAT 1, SAT 2 and SAT 3. The first FMD serotypes were designated O (Oise) and A (Allemagne) and serotype C was named in the anticipation that a logical alphabetical nomenclature would be established. That was not achieved, although the three serotypes recognized in the South African territories were systematically named.

The serotypes form two groups, as assessed by RNA sequencing with serotypes O, A, C and Asia 1 in one group and the three SAT serotypes in the other. Applying the criteria for recognition of a serotype, infection or vaccination with one virus serotype does not confer protection against another serotype. There is considerable antigenic variation within serotypes, and at one time subtypes were recognized. However, this practice has been replaced by molecular and antigenic comparison of a new isolate to the availability of vaccines within the serotype that will provide clinical protection against infection with the new isolate.

EPIDEMIOLOGY

GEOGRAPHICAL DISTRIBUTION AND HOSTS

The control and eradication of FMD has improved markedly in recent years, particularly in Europe and in some countries in Southeast Asia and South America. However,

the disease remains endemic and at a high prevalence in many countries in Africa, the Middle East, Asia and South America. The countries of Europe, North and Central America, the Pacific nations and the Caribbean are free of the disease. Serotypes O and A are the most prevalent and occur in parts of South America, North and West Africa, and the Middle East. In recent years, serotype O viruses have spread widely in Asia, infecting countries previously free of disease. These serotype O viruses present a threat to other regions, as exemplified by the pan-Asian serotype O virus that spread first to South Africa and then to Europe in 2000 and 2001, respectively.

Serotypes SAT 1 and 2 are present in several countries in sub-Saharan Africa, with SAT 3 currently restricted to southern Africa. Asia 1 is currently restricted to eastern parts of Asia. Type C is possibly extinct in the wild, the last case being in the rainforest of Brazil. Information on the current geographical distribution of FMD virus serotypes may be found at the World Reference Laboratory for FMD[3], the World Organisation for Animal Health (OIE) website (WAHID)[4] and the Food and Agriculture Department of the United Nations (FAO) website[5].

FMD affects a wide variety of cloven-hoofed domestic species. Although the horse is refractory to infection, cattle, water buffalo, sheep goats, llamas, camels, and swine are susceptible to infection and develop clinical signs of varying severity.

More than 70 species of wildlife in 20 different families have been reported to be susceptible to infection with FMD virus[1,6,7]. Table 11.1 gives further details of the historical record of FMD in wildlife species[7]. Many are historical reports and based only on clinical signs with no confirmatory diagnosis, and several relate to experimental infections (both by injection and direct contact with animals with FMD) even though FMD has never been recorded in that particular wildlife species under natural conditions. Some relate to animals in zoological collections or captive situations where there was close contact with domestic animals. Accordingly, the following descriptions will focus on recent reports where there is proven infection with FMD virus in free-living wildlife species.

Antelope are classified within the family Bovidae, so it is not surprising that the most recent and significant reports of clinical FMD infections in free-living wildlife (i.e. as determined by frequency and confirmation of the viral aetiology) are in their loosely defined subfamily.

In terms of geography, most reports of confirmed cases of FMD in free-living species are reported from South Africa – more specifically the Kruger Park, where FMD in wildlife has been studied for many years. Herds of African Cape buffalo in southern and East Africa are commonly infected with FMD virus, but clinical disease is rarely reported. The buffalo are, however, the source of infection for antelope, particularly impala (*Aepyceros melampus*)[8]. Foot-and-mouth disease has also been reported, but less frequently, in other species in South Africa: free-living kudu (*Tragelaphus strepsiceros*), bushbuck (*Tragelaphus scriptus*), warthogs (*Phacochoerus africanus*) nyala (*Tragelaphus angasi*) and giraffe (*Giraffa camelopardalis*).

FMD is endemic in domestic livestock in most areas of Africa. The discovery of the persistence of FMD virus in African Cape buffalo in the 1970s has led to many excellent epidemiological studies demonstrating that wildlife are an occasional source of infection for domestic cattle. Transmission of FMD virus from Cape buffalo to other species is probably a rare event, as they have been found to be infected in areas where FMD does not occur in cattle. It seems likely that impala, which share watering holes in the dry season with Cape buffalo, become infected and transmit the disease to domestic cattle. Readers wishing to learn more of the ecology and control of FMD in wildlife in Africa should consult Vosloo et al. (2004 and 2009)[6,8].

In an outbreak of FMD in mountain gazelle (*Gazella gazelle*) in Israel[9], where cattle were believed to be the source of the infection, the case fatality rate among the gazelle was over 50%, with at least 1,500 animals dying. Death was considered to be due to myocarditis. Fortunately such events are highly unusual, and there have been no further reports of the disease in free-living populations of mountain gazelle.

Historical reports indicate that several species of deer worldwide may be susceptible to FMD, including most of the deer currently found free-living in Europe, but there are no descriptions in the recent literature of the clinical presentation of confirmed FMD in naturally infected deer of any species. However, because of the concern that free-living and farmed deer could play an important role in the epidemiology of FMD in Europe and North America, red deer (*Cervus elaphus*) fallow deer (*Dama dama*), roe deer (*Capreolus capreolus*), muntjac (*Muntiacus muntjak*), sika deer (*Cervus nippon*)[2], white-tailed deer (*Odocoileus virgianus*)[10] and American elk (*Cervus elaphus nelsoni*)[11] have been experimentally infected.

FMD in white-tailed deer in the USA is reported to be similar to that seen in cattle. Although natural cases of

TABLE 11.1 Wild animal species naturally or experimentally susceptible to FMD virus.

Common name	Scientific name	Type of infection	Common name	Scientific name	Type of infection
Agouti-(Brazilian agouti)	*Dasyprocta leporina* *Dasyprocta agouti*	Experimental	Hedgehog (East African)	*Atelerix prurei hindu*	Experimental
			Hippopotamus	*Hippopotamus amphibuis*	Experimental
Armadillo (hairy armadillo)	*Chaetophractus villosus*	Experimental	Ibex	*Capra ibex*	Natural
			Impala	*Aepyceros melampus*	Natural/experimental
			Kudu	*Tragelaphus strepsiceros*	Natural/experimental
Babirusa	*Babyrousa babyrussa*	Natural/experimental	Llama	*Lama glama*	Natural/experimental
Bear	*Ursus horribilis*	Natural	Alpaca	*Lama pacos*	Natural
Bear (Asiatic black bear)	*Ursus thibetanus*	Natural	Marsh deer	*Blastocerus dichotomus*	Experimental
			Moose	*Alces alces*	Natural/experimental
Bear (brown bear)	*Ursus arctos*	Natural	Mountain gazelle	*Gazella gazella*	Natural
American bison	*Bos americanus*	Natural	Mule deer	*Odocoileus hemionus*	Natural
Brown brocket deer	*Mazama gouzoubira*	Natural	Nilgai (antelope)	*Boselaphus tragocamelus*	Natural
Buffalo (African buffalo)	*Syncerus caffer*	Natural/experimental	Nyala	*Tragelaphus angasii*	Natural
			Porcupine	*Hystrix galeata*	Experimental
Buffalo (Indian water buffalo)	*Bubalus bubalis*	Natural/experimental	Red brocket deer	*Mazama americana*	Experimental
			Red deer	*Cervus elaphus*	Natural/experimental
Bushbuck	*Tragelaphus scriptus*	Natural	Reedbuck	*Redunca arundinum*	Natural/experimental
Bushpig	*Potamochoerus porcus*	Natural/experimental	Reindeer	*Rangifer tarandus*	Natural/experimental
Capybara	*Hydrochoerus hydrochaeris*	Experimental	Roe deer (western roe deer)	*Capreolus capreolus*	Natural/experimental
Chamois (Alpine)	*Rupicapra rupicapra*	Natural/experimental			
Collard peccary	*Tayassu tajacu*	Natural	Sable antelope	*Hippotragus niger*	Natural
Columbian deer	*Odocoileus columbicus*	Natural	Sable antelope	*Ozanna grandicornis*	Natural
			Saiga antelope	*Saiga tatarica*	Natural
Dromedary (Arabian camel)	*Camelus dromedarius*	Experimental	Sambar deer	*Cervus unicolor*	Natural
			Sika deer	*Cervus nippon*	Experimental
			Southern pudu	*Pudu pudu*	Natural
Duiker	*Sylvicapra grimmia*	Natural	Spotted deer	*Axis axis*	Natural
Eland	*Taurotragus orys*	Natural/experimental	Tapir (Brazilian tapir)	*Tapirus terrestris*	Natural
Elephant (African elephant)	*Loxodonta africana*	Natural/experimental			
			Tapir (Malayan tapir)	*Tapirus indicus*	Natural
Elephant (Asian elephant)	*Elephas maximus*	Natural	Thamin	*Cervus eldii*	Natural
			Tsessebi	*Damaliscus lunatus*	Natural
Elk	*Alces machlis*	Natural/experimental	Vicuna	*Vicugna vicugna*	Natural/experimental
Fallow deer	*Dama dama*	Natural/experimental	Warthog	*Phacochoerus aethiopicus*	Natural/experimental
Gaur	*Bos frontalis* (*Bos gaurus*)	Natural	White-lipped peccary	*Tayassu pecari*	Natural
Gayal (mithun)	*Bos frontalis domesticus*	Natural			
Gemsbok, oryx	*Oryx oryx gazella*	Natural	White-tailed deer	*Oedocoilleus virginianus*	Natural/experimental
Giraffe	*Giraffa camelopardalis*	Natural			
Gnu (blue wildebeest)	*Connochaetes taurinus*	Natural/experimental	Wild boar (*Sus scrofa*)	*European Sus scrofa*	Natural/experimental
Grant's gazelle	*Gazella granti*	Natural	Waterbuck	*Kobus ellipsiprymnus*	Natural
Guib antelope	*Tragelaphus striptus*	Natural	Yak	*Bos grunniens domesticus*	Natural
Hedgehog (West European)	*Erinaceus europaeus*	Natural/experimental			

FMD have never been recorded in American bison (*Bison bison*), this species has been shown to be highly susceptible to FMD and under experimental conditions can transmit disease through contact with domestic cattle[11].

Although history and experimental studies indicate that many wildlife species should be considered susceptible, until the recent confirmation of FMD in a wild boar in Bulgaria there have been no other reports of FMD in wildlife in Europe since the continental eradication of FMD from domestic livestock in Europe in the 1990s. No confirmed cases of FMD were reported in wildlife species during the extensive FMD epidemic in Northern Europe in 2001. Limited serological surveillance of European wild boar and deer following this epidemic indicated no evidence of infection[12].

The wild boar (*Sus scrofa*) is the ancestor of the domestic pig and is therefore closely related. There are historical reports of FMD in wild boar in Europe. In late December 2010, type O FMD virus was identified in samples of vesicular tissue from foot lesions affecting a wild boar in south east Bulgaria. The boar was one of three shot by hunters approximately 2 km from the border with the European area of Turkey, where FMD in domestic animals has been recently reported. Outbreaks of FMD in cattle, sheep, goats and pigs have since been confirmed in the same area of Bulgaria. A limited survey of wild boar in Turkish Thrace indicated that infection had also occurred in this population. Modelling indicates that FMD virus could remain endemic in the wild boar population in the region for up to 2 years. As of May 2011, epidemiological investigation has failed to identify how the wild boar became infected. Wild boar are omnivorous, and ingestion of infected meat inadvertently carried into a country and discarded could theoretically lead to disease in this species. Various reports on the situation can be accessed from the ProMED website[13].

The same type O FMD virus caused disease in cattle in Northern Israel in spring 2011. There are wild boar populations in Northern Israel and in neighbouring areas of Lebanon and Syria. Although there is no direct evidence that the wild boar were responsible for the introduction of FMD into Israel in 2011, it is reasonable to suggest that they may have been involved. Between 1987 and 1999, according to the data of the Israeli Veterinary Services and the national FMD laboratory, 740 sera were collected from wild boars shot in northern Israel. Of these, 108 (14.6%) were found to have antibody to FMD virus. During the same period, 73 oesophageal tissue samples were collected from hunted boar. Two samples, from boar shot in June 1992, yielded FMD virus serotype O. One of the boar was reported to have had typical FMD lesions on its feet. A later survey of 21 boar hunted in the early months of 2007 revealed that 18 had antibody to the non-structural proteins of the FMD virus. Although no virus could be isolated from these wild boar, the serology clearly indicated that the boar had been infected with FMD virus. The results also indicated that infection in the boar in one location seems to have taken place sometime before the outbreaks were recorded in the domestic livestock[13].

EPIDEMIOLOGICAL ROLE OF WILD ANIMALS

Apart from the persistence of FMD in African Cape buffalo in Africa, the disease is not established in any wildlife population in Europe or any other continent.

TRANSMISSION

FMD virus is transmitted to susceptible animals in several ways; through close contact with infected animals and fomites, by inhalation of aerosolized virus and by ingestion of infected meat products. Infected animals produce large amounts of virus in vesicular fluid and in secretions and excretions, such as in milk and faeces, that can contaminate people's clothing, vehicles and equipment. The virus titre in vesicular fluid may be in excess of 10,000,000 tissue culture infectious doses per 1 ml ($10^{7.0}$ $TCID_{50}$ per ml). The virus is also present in the exhaled breath of infected animals. Infected pigs produce significantly larger quantities of virus as an aerosol (~$10^{6.0}$ $TCID_{50}$ per hour) than infected cattle, sheep, goats and deer (~$10^{4.0}$ $TCID_{50}$ per hour). Cattle may become infected after inhalation by as few as 10 $TCID_{50}$ of FMD virus. The virus concentration needed to infect animals by ingestion is much higher (~$10^{3.0}$ $TCID_{50}$ per ml).

PATHOGENESIS, PATHOLOGY AND IMMUNITY

DOMESTIC ANIMALS

The main route of infection with FMD virus is through inhalation of droplets, but ingestion of infected food,

inoculation with contaminated vaccines, insemination with contaminated semen and contact with contaminated clothing and veterinary instruments can all produce infection. In animals infected via the respiratory tract, initial viral replication occurs in the pharynx and respiratory bronchioles, followed by viraemic spread to other tissues and organs before the onset of clinical disease. Viral excretion commences about 24 hours before the onset of clinical disease and continues for several days. Aerosols produced by infected animals contain large amounts of virus, particularly those produced by swine. Large amounts of virus are also excreted from ruptured vesicles and in milk. FMD virus may persist in the pharynx of some animals for a prolonged period after recovery. In cattle, virus may be detectable for periods up to 2 years after exposure to infection; in sheep for about 6 months. Viral persistence does not occur in swine. The mechanisms by which the virus produces a persistent infection in ruminants are unknown. The virus is present in the pharynx in an infectious form, because if pharyngeal fluids are inoculated into susceptible animals they develop FMD. Attempts to demonstrate that carrier cattle can transmit disease by placing them in contact with susceptible animals have given equivocal results.

WILDLIFE SPECIES

There have been no specific studies of the pathogenesis of FMD in wildlife species. Experimental infections of the larger species of deer found in Europe, mentioned earlier, indicate that the pathogenesis is similar to that of FMD in other ruminants, particularly sheep and goats. Fallow and sika deer become persistently infected with infectious FMD virus, which is recovered from the pharynx of some animals for several months after infection, i.e. they become carriers[2]. Red deer only occasionally became carriers. The smaller species of deer (muntjac and roe deer) do not become carriers. Of the various African antelope susceptible to FMD virus, kudu, wildebeest and sable can become persistently infected, but impala do not[1].

The African Cape buffalo was first discovered to be persistently infected with FMD virus in the 1970s[6]. Since then, persistence of the three SAT serotypes of FMD virus in African buffalo has been extensively studied, and it is recognized that the species is a major reservoir of all three serotypes; however, not all African buffalo populations are infected with FMD virus. The different serotypes are maintained in the infected buffalo herds in the absence of any contact with infected domestic cattle. Individual animals may harbour more than one serotype.

FMD is characterized by vesicles (blisters or aphthae) at multiple sites, generally at points of pressure or mechanical stress, such as the mouth and the feet. The oral lesions in cattle can be extensive, and the entire epithelium of the tongue may be desquamated naturally or be detached as a 'slipper' when the tongue is manually exteriorized for examination. Vesicles in the mouth quickly rupture, exposing an eroded area. However, healing is generally rapid, with the epithelium being completely regenerated within a week. Ruptured vesicles present in the interdigital cleft of the feet commonly become infected with bacteria and healing may be more protracted. Vesiculation of the coronary band can lead to defects in the growth of the horn. At necropsy, ruptured vesicular lesions may be seen affecting the pillars of the rumen. Young animals that die of myocarditis may exhibit 'tiger heart' striping, which is characterized histologically by myocardial degeneration and necrosis. (For images of FMD lesions in domestic animals at various times after onset see[5].)

Histologically, FMD vesicles extend only to the *stratum spinosum* of the epithelium and begin as clusters of hypereosinophilic degenerating keratinocytes, which lead to the accumulation of intercellular oedema. Similar pathology may be seen in wildlife. Pancreatitis was reported in the mountain gazelle that died of FMD in Israel.

Recovery from clinical FMD is correlated with the development of antibody. The early IgM antibodies neutralize the homologous type of virus and may also be effective against heterologous types. By contrast, the IgG produced during convalescence is type-specific. Cattle that have recovered from FMD are usually immune to infection with the same virus type for a year or more, but immunity is not considered life-long. Recovered animals, however, can be immediately infected with one of the other types of FMD virus and develop clinical disease.

CLINICAL SIGNS

DOMESTIC ANIMALS

In general, clinical signs are most severe in cattle and swine, but outbreaks have been reported in swine, whereas cattle in close contact with them did not develop clinical disease. The disease in sheep and goats is often mild and subclinical. In cattle, after an incubation period of 2–8

days, there is fever, loss of appetite, depression and a marked drop in milk production. Within 24 hours, drooling of saliva commences and vesicles develop on the tongue and gums. Cattle often open and close their mouths with a characteristic smacking sound. Vesicles may also be found in the interdigital skin and coronary band of the feet and on the teats. The vesicles soon rupture, producing large denuded erosive lesions. Those on the tongue often heal within a few days, but those on the feet and within the nasal cavities often become secondarily infected with bacteria, resulting in prolonged lameness and a mucopurulent nasal discharge. In calves up to 6 months of age, FMD virus can cause death through myocarditis. The mortality in adult cattle is very low, but although the virus does not cross the placenta, cattle may abort, presumably as a consequence of fever. Also, affected animals become non-productive or poorly productive for long periods. They may eat little for a week after the onset of clinical signs and are often very lame; and mastitis and abortion lower milk production further. In endemic areas, where cattle may have partial immunity, the disease may be mild or subclinical.

In swine, lameness is often the first sign of FMD. Foot lesions can be severe and may be sufficiently painful to prevent the pig from standing. Denuded areas between the claws usually become infected with bacteria; this causes suppuration and in some cases loss of the claw and prolonged lameness. Vesicles within the mouth are usually less prominent than in cattle, although large vesicles, which quickly rupture, often develop on the snout.

The severity of the clinical disease in sheep and goats is dependent upon the strain of the virus, the breed of the animal and environmental conditions. In general, the disease is usually milder than in cattle and is principally characterized by foot lesions accompanied by lameness. The mortality rates in young lambs and kids may be high.

WILDLIFE SPECIES

As there are no recent descriptions of naturally occurring clinical FMD in wildlife species in Europe, the following descriptions are based on the experimental studies mentioned earlier[2]. The clinical appearance of lesions and the severity of the disease were not affected by the route of infection (inoculation or contact), but were species-dependent. FMD in roe and muntjac deer was similar and severe, with elevated temperatures and development of generalized disease; some animals died. There were no subclinical infections. Vesicular lesions developed on the dental pad and tongue and between the claws of the feet. In contrast with the severe disease seen in the roe and muntjac deer, disease in the red, fallow and sika deer was generally mild and similar to that seen in sheep. Small vesicles developed on the tongue and dental pad of some deer and in the interdigital cleft and bulbs of the heel. Colour photographs from the above studies of the disease in roe, muntjac, fallow and sika deer may be found at The Deer Initiative[14].

The lesions of FMD reported in antelope in Africa are similar to domestic livestock. The severity of clinical disease in impala can be highly variable and many are only subclinically affected. Lameness is the most likely clinical indicator of disease, as affected antelope rarely exhibit excessive salivation, even when there are lesions in the mouth. Animals that have recovered from FMD may show faults in the hoof wall as the hoof grows. This may be used in impala to estimate when infection occurred, as it usually takes 5–6 months for the defect to grow out.

DIAGNOSIS

Any vesicular disease in domestic or free-living artiodactyla, or a history of sudden death in a large number of young cloven-hoofed animals, should immediately raise concern that it could be FMD. Failure to act can be disastrous, as an epidemic may quickly generate.

The acronym for foot-and-mouth disease – FMD – also denotes an important characteristic of the epidemiology that directly relates to diagnosis, namely that of a 'fast-moving disease'. The incubation period is frequently no more than 2–3 days, and virus is highly infectious; hence rapid diagnosis of FMD is of paramount importance for early containment of the outbreak. This is particularly important in countries and regions normally free of infection.

While the history of the disease and the involvement of different species may be valuable pointers during the field investigation, there are other diseases that can be clinically confused with FMD, and thus it is not uncommon for specimens to be collected for laboratory diagnosis. Specimens are usually couriered in person to the laboratory to minimize delay. National and international laboratories that are designated as reference centres for the diagnosis of FMD are on constant standby and, when alerted, examine samples immediately upon arrival.

The portfolio of laboratory tests currently in use to diagnose FMD is very sophisticated. The OIE Terrestrial Manual 2009 describes in detail those tests that are internationally recognized for the diagnosis of FMD[4]. Tests based on the reverse transcription polymerase chain reaction (RT-PCR) are used in parallel with enzyme-linked immunosorbent assays (ELISA) to confirm that the disease is caused by FMD virus and to identify which serotype is involved. Assuming the samples were collected from animals in the early stages of disease and were appropriately protected in transit to the laboratory, a diagnosis can be available within a few hours of receipt. Laboratories that are equipped to conduct sequencing of the gene product generated by the RT-PCR tests can usually generate sequence data within 24 hours that are of epidemiological value. The lineage of the virus can often be deduced from the sequence data and thus gives pointers to: i) the most likely source of the virus; and ii) the closest vaccine match. Although the isolation and characterization of the virus in cell culture remains the diagnostic 'gold standard', confirmation of an outbreak of FMD is now based on the results of RT-PCR and ELISA technology.

For serological surveillance, a variety of ELISA are available[4].

MANAGEMENT, CONTROL AND REGULATIONS

FMD is a reportable disease in most countries (i.e. there is a legal responsibility on owners of stock and land to report any suspicion). Upon receiving such reports, the state veterinary authorities of most countries will immediately dispatch a veterinarian trained in the clinical recognition of foreign animal diseases to examine the affected animals. Because FMD virus is so easily spread by contaminated instruments, clothing, etc., care must be taken to avoid inadvertent spread of virus from affected animals while waiting for the diagnostician to arrive. Boots and equipment should be disinfected. Rather than move any dead animals, it is better to protect them from scavengers and leave them at the site where they were found. The movement of apparently normal animals away from the area should be prevented, and visits by people should be controlled until a diagnosis is established.

Notwithstanding the wide range of species that are considered susceptible to FMD virus, viewed globally, the epidemiology of FMD indicates that wildlife species are only rarely affected. With the exception of the African Cape buffalo in sub-Saharan Africa, there is no reservoir of FMD virus in any other wildlife species. Other than in situations that can be linked to African Cape buffalo, FMD in wildlife is an extension of disease in livestock.

The control of FMD both in domestic animals and wildlife depends upon the FMD status of the country. When FMD occurs in countries and regions normally free of FMD, the attention of the veterinary authorities to wildlife is secondary to controlling and eradicating disease in the domestic livestock. Where the disease is endemic in domestic livestock, such as in parts of Asia, South America and Africa, little attention may be given by the veterinary authorities to any possible cases of FMD in wildlife.

There are many routes by which FMD virus can enter a country free of FMD. In contrast to humans, who are generally free to travel between countries without extensive health checks, the international movement of domestic livestock and ungulate wildlife is strictly regulated and often forbidden. Nowadays, most introductions of FMD to non-endemic countries can be traced to the unintentional feeding of infected meat products to domestic pigs. If the disease goes unnoticed and spreads rapidly within the pig population on the affected farm, a large volume of aerosolized virus is generated and is blown as a plume to infect susceptible livestock downwind. This was the series of events that led to the 2001 epidemic of FMD in the UK and other European countries.

As mentioned previously, cattle may become infected after inhalation by as few as 10 $TCID_{50}$ of FMD virus. Although sheep, goats and deer have similar susceptibility to aerosolized FMD virus, they have a smaller respiratory volume than cattle and are less likely to be infected when the concentration of virus in the plume is low. However, once infected, the disease in these smaller ruminants is less likely to be noticed clinically.

While the evidence implicating wild boar in the introduction of FMD into Bulgaria and Israel is circumstantial, these incidents and the increase in boar populations across Europe justify increased surveillance for FMD in wildlife.

In Western Europe, most attention is given to FMD in deer. The larger herding species of free-living deer, particularly red, fallow and sika, often share common pastures with cattle and sheep. The smaller species, such as roe and muntjac, are more secretive and if affected with FMD are more likely to seek cover than graze pastures. During the time when disease is active in the domestic livestock,

observation of wildlife for clinical signs suggestive of FMD is important, but it is generally accepted that more aggressive surveillance in the absence of any concern is unwarranted and more likely to spread disease by dispersing potentially infected deer. When the disease has been controlled in the domestic livestock, selected populations of those wildlife species considered to be susceptible to FMD should be examined for any prior or current evidence of subclinical infection.

PUBLIC HEALTH CONCERN

FMD has been reported to affect humans, but the disease is benign and there have been very few cases. FMD is characterized by fever, anorexia and the development of vesicular lesions at the site of viral exposure (e.g. skin abrasions) or systemically on the hands and feet and in the mouth. No cases of human FMD were reported during the extensive 2001 epidemic in Europe. The disease of hand, foot and mouth disease, as seen predominantly in children, is caused by a different virus from FMD virus.

SIGNIFICANCE AND IMPLICATIONS FOR ANIMAL HEALTH

There is no question that under natural conditions several free-living wildlife species are: i) susceptible to infection with FMD virus; ii) can develop clinical disease; iii) are capable of transmission of FMD virus to domestic species; and iv) can represent a reservoir of FMD virus through persistent infection.

The reservoir of FMD virus in African Cape buffalo is of economic concern to several countries in sub-Saharan Africa, as they develop an export market for beef. There is no known or suspected wildlife reservoir of FMD virus other than the African Cape buffalo. Apart from southern Africa, the disease rarely affects wildlife, and when it does it is an extension of disease in domestic livestock.

SWINE VESICULAR DISEASE VIRUS

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Swine vesicular disease virus (SVDV) belongs to the genus *Enterovirus* of the family *Picornaviridae*. SVDV is a single-stranded RNA virus ranging from 20 to 32 nm in size. Currently four antigenic variants are known, all of them placed in a single serotype. SVD is clinically indistinguishable from other porcine vesicular diseases caused by *Foot-and-mouth disease virus*, *Vesicular stomatitis virus* and *Swine vesicular exanthema virus*.

Swine vesicular disease (SVD) was first diagnosed in Italy in 1966 in domestic pigs and was later reported from several European countries. Currently in Europe SVD is enzootic only in some areas of Italy[15]. European wild boar populations from Lithuania, Slovenia, Sweden, the Czech Republic and the Netherlands have been monitored for anti-SVDV antibodies and only a single reactor was found in Sweden in 2008[16]. Unlike *Foot-and-mouth disease virus*, SVDV does not affect ruminants, although sheep can become seropositive after exposure to the virus. Until now, the only species found to become infected, display clinical signs and excrete SVDV is the domestic pig. The wild boar (*Sus scrofa*), being the same species as the domestic pig, may be susceptible to SVDV infection.

SVDV in the environment is highly resistant and is able to survive up to 2 years in meat products. It resists destruction by common disinfectants. The domestic pig is assumed to be the natural reservoir of SVDV. Wild boar are potential reservoirs; however, no evidence of SVDV infecting wild boar has been found in Europe so far, except for the single reactor found in Sweden.

Transmission takes place by direct contact, through fomites and by ingestion of infected meat products. The gastrointestinal tract is the main site of entry. Infection by the oral route may require up to 300,000 infectious units, whereas infection through skin abrasions or wounds requires a much lower dose, of 100 infectious units[17]. The virus first replicates in the gastrointestinal tract. Viraemia peaks at 2–4 days after infection and the virus persists for up to 7 days in blood and various tissues. Experimentally the first replication site of SVDV in the epithelium is the *stratum spinosum*. SVDV is excreted by oronasal secretions from 48 hours before the onset of clinical signs to 2 weeks after it. Faecal excretion may last for 4 months. Vesicle formation is the only gross pathologic finding. Microscopic lesions in the epithelium consist of hydropic degeneration and intracellular oedema followed by necrosis.

Clinical manifestations of SVD appear 1–5 days after infection, and affected animals usually recover 7 to 14 days after this. Clinical signs may vary, depending on the individual, the viral strain and the infectious dose. Animals develop a high fever, followed by lameness and formation

of vesicles in the mouth, snout, feet and teats. Vesicles may affect the whole coronary band, resulting in loss of the hoof. A subclinical status has occurred frequently in Italian domestic pigs[15].

A presumptive diagnosis is based on clinical signs; however, some cases show no clinical manifestations. The diagnosis of SVDV is confirmed by virus isolation in cell cultures, or virus detection by the antigen-capture ELISA followed by RT-PCR to avoid inhibition of the polymerase activity. ELISA serology is useful as well, but some infected animals may be seronegative. The performance of serological tests has not been evaluated in wild boar.

SVD is a notifiable disease, because it is clinically and pathologically indistinguishable from foot-and-mouth disease in pigs. Where SVDV is diagnosed in animals, eradication by slaughter is performed, with consequent pig-trade restrictions.

The low virulence of SVDV, together with its proven absence in many European wild boar populations, suggests that there is no effect on the population dynamics of wild boar.

ENCEPHALOMYOCARDITIS

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AETIOLOGY

Encephalomyocarditis virus (EMCV) is an RNA virus and belongs to the genus *Cardiovirus* of the family *Picornaviridae*. The encephalomyocarditis (EMC) virions contain a single strand of RNA of 2.6×10^6 daltons, and are enclosed in a protein capsid shell. In contrast to the high antigenic stability, the D region of EMCV (coding for capsid protein VP1) displays considerable genetic variability. A single mutation in a nucleotide sequence can be involved in attenuation or responsible for the diabetogenic nature of a particular EMCV strain[18]. Limited variability between pig and rodent EMCV isolates has been noticed[19].

EPIDEMIOLOGY

Little is known about the distribution of EMCV in European wildlife. A research project funded by the EU detected evidence of exposure in rats (*Rattus rattus*, *Rattus norvegicus)*, voles (*Apodemus sylvaticus*, *Microtus arvalis*), mice (*Mus musculus*) and wild boar (*Sus scrofa*). The seroprevalence in wild boar varied between 0.6 and 10.8%, and a study in Belgium found a prevalence of viral infection of 3.3%[20]. Even though field samples from red foxes (*Vulpes vulpes*) were negative, animal experiments proved foxes to be susceptible to EMCV infection[21].

Rodents are considered to be the natural hosts of EMCV. *Rattus rattus*, *Rattus norvegicus*, *Apodemus sylvaticus*, *Sciurus vulgaris leucorourus*, *Microtus arvalis*, *Myoxus glis* and *Mus musculus* were found to be susceptible to EMCV. In these rodents, the virus usually persists without causing disease. Conversely, African multimammate rats (*Mastomys* spp.) develop clinical disease and die. Infected rodents show high levels of the virus in their tissues, and excrete the virus in faeces and urine. Rodents are thought to play a role as reservoir, introducing and spreading the virus via their faeces or maintaining the infection as infected carcasses. As rodents are the natural hosts, the presence of EMCV or EMC antibodies often indicates virus circulation among a wide range of other species. EMC viruses have been isolated from over 30 host species, including mammals, birds and insects. In mammals, the host range includes monkeys, chimpanzees, elephants, lions, squirrels, mongooses, raccoons and swine (domestic and wild boar). An episode of lion deaths at a zoo was found to be due to feeding carcasses of African elephants that died of EMCV infection[22]. From all outbreaks in wildlife those in elephants are most documented, including an outbreak in Taronga Zoo, Australia[23] and in wild elephants in the Kruger National Park[24].

EMCV has been isolated from a variety of arthropods, including mosquitoes, ticks, houseflies and fleas. Experimental infection of arthropods and arthropod cells with EMCV failed to show any virus replication; however, in some cases virus could be detected for up to 3 months (R.S. O'Hara, L. Bell-Sakyi and N.J. Knowles, unpublished data).

In certain European countries a seasonal pattern of the outbreaks in pigs, with peaks in autumn, was noticed[20], but similar data are not available for wildlife.

Experimentally, EMCV may be transmitted by the oral, intranasal, aerosol, intratracheal and parenteral routes. Infection appears to be influenced by virus strain, viral dose, passage history and susceptibility of the individual animal[19]. In elephants, rodents have been demonstrated to be the source of infection. Feed, including grass, and

water contaminated with EMCV by rodent excreta, or infected rodent carcasses and tissues from infected animals, are considered important sources of natural infection.

PATHOGENESIS, PATHOLOGY AND IMMUNITY

Natural infection is most likely to occur by the oral route. After experimental oral infections in young pigs, virus was isolated at 6 hours post-inoculation (hpi) from the intestinal tract and lymph nodes and was observed in macrophages in the tonsils. The tonsils were considered to be the portal of entry of the virus and migration of infected macrophages the route or viral dissemination to all organs. At 12 hpi EMCV was observed by immunolabelling in scattered degenerated myocardiocytes; myocarditis developed subsequently and progressed in severity. The virus replicated intensively in the cytoplasm of myocardiocytes, and this was followed by viraemia. In this second phase of viral replication, some animals died with typical *post mortem* lesions, including discoloration of the heart. EMCV was observed by immunohistochemistry in the tonsils and in the heart. Three days post-inoculation (dpi), virus was also isolated from liver, kidneys, spleen and lungs. Animals that survived the infection produced EMCV antibodies. After antibody formation, the virus detection decreased[25].

The pathogenesis of transplacental infection with EMCV in pregnant suidae is not well understood. In rats experimentally infected with a myocardial EMCV strain, no clinical or macroscopic lesions were observed in any organs. Virus was isolated most frequently from Peyer's patches and thymus, indicating that these tissues represent a site of persistence after oral infection[26]. In mice, certain strains cause predominantly fatal encephalitis, widespread myocardial damage or even specific destruction of pancreatic beta cells, but there are no studies of pathogenesis in wild rodents.

Not all suidae dying from the acute phase of cardiac failure show gross lesions – the only lesion may be epicardial haemorrhage. Hydropericardium, hydrothorax and pulmonary oedema develop as a result of cardiac dysfunction and are frequently observed at necropsy. The heart is usually enlarged, soft and pale. The most striking lesions are found in the myocardium and consist of multiple greyish-white foci of variable size. Infected fetuses usually appear normal but can be haemorrhagic and oedematous. With some strains, fetuses can become mummified in various states of development, depending on the time of infection. Macroscopic myocardial lesions are exceptionally rare[19]. Some EMCV strains can induce encephalitis, myositis or pancreatitis. However, a species predilection for the development of lesions in particular organs has been reported[27].

Histopathologically, the most significant findings are seen in the heart, the target organ of EMCV. The positive immunohistochemical reaction is mainly localized in the cytoplasm of myocardial cells as a fine and granular signal. Sometimes, the antigen is detected in the Purkinje fibres and in the endothelial cells next to those. Mineralization of myocardium is common but not always present. In the tonsils, the antigen is located in necrotic debris filling the crypts and in the cytoplasm of monocyte-macrophage lineage cells. This last finding also occurs in lymph nodes[25]. In the brain, congestion, meningitis, perivascular infiltration by mononuclear cells and neural degeneration may be observed.

CLINICAL SIGNS AND TREATMENT

Infection with ECMV is generally subclinical in a wide range of species. Sometimes animals are found dead without prior signs of illness. In young pigs, in fast-growing fattener pigs and in elephants the most striking clinical signs are acute congestive heart failure with massive pulmonary oedema. In other age groups dyspnoea can be noticed. In breeding females clinical signs may vary from none to various forms of reproductive failure, including abortion and increased numbers of mummified and stillborn fetuses[19].

There is no treatment but, in the acute phase, mortality may be minimized by avoiding stress or excitement.

DIAGNOSIS

A conclusive diagnosis of EMCV is demonstrated by virus isolation. BHK-21 cell culture is the most sensitive method for virus isolation. Infected cell monolayers show a rapid and complete cythopathic effect. Serial passage of EMCV in cell culture can alter *in vitro* growth characteristics, reduce virulence and affect haemagglutinating activity[28]. The development of nucleic acid probes or RT-PCR for the detection of EMCV has been reported[19]. Of all serological tests virus neutralization (VN) and ELISA are the

most frequently used methods and have been shown to be specific[28]. For VN, antibody titres of >1:16 appear to be significant. VN-antibody reaction starts as soon as 7 dpi and may persist for an extended period of 6 months or one year[19].

MANAGEMENT, CONTROL AND REGULATIONS

An inactivated EMCV vaccine for pigs is commercially available in the USA. The vaccine appears to be effective, as high humoral immunity is detected in vaccinated pigs. Vaccinates were protected from clinical disease when challenged with virulent EMCV that killed 60% of unvaccinated controls, but its efficacy in wildlife has not been documented. A safe and effective aziradine-inactivated vaccine containing high levels of viral antigen and an oil adjuvant was developed following the EMC epidemic in free-ranging elephants in South Africa[24].

PUBLIC HEALTH CONCERN

Currently, the impact of EMCV on public health is believed to be minimal. Despite the frequency of infection in swine, no association between infection and transmission of disease to humans has been recorded[28], even in persons at the greatest risk (veterinarians, animal caretakers, laboratory staff). A serological survey in Austria demonstrated a seroprevalence in hunters of 15% and of 8% in the urban control group. In the light of the ubiquitous presence of EMCV around the world in several animal species, including primates after a rodent plague[29], secondary infections in immunocompromised persons may possibly be anticipated. Recently, EMCV has been isolated from cases of febrile illness in humans in Peru[30]; interestingly, these viruses were most closely related to EMC viruses isolated from European pigs.

SIGNIFICANCE AND IMPLICATIONS FOR ANIMAL HEALTH

Several reports show the role of wild rodents in the transmission of EMCV to pigs, but no data are available to prove the spread from EMCV from wild boar to domestic pigs[21]. Transmission from wild animals to other domesticated species has not been reported.

LJUNGAN VIRUS INFECTION

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Ljungan virus (LV) is a member of the Parechovirus genus in the family *Picornaviridae* that was first isolated in 1998 from bank voles (*Myodes glareolus*) in Sweden. It has now been identified in wild voles, lemmings (*Lemmus lemmus*) and laboratory rats in the USA. A recent study in Northern Italy using RT-PCR confirmed LV in 50% of bank voles and 10% of yellow-necked mice (*Apodemus flavicollis*) sampled. The presence of the virus has been demonstrated in these species together with striped field mouse (*A. agarius*), long tailed field mouse (*A. sylvaticus*), harvest mouse (*Micromys minutus*) and house mouse (*Mus musculus*) in Germany.

Experimentally LV has been shown to induce type-2-like diabetes, uterine reabsorptions, malformations and neonatal death in laboratory house mice (*Mus musculus*). Recent studies have shown that the incidence of intrauterine fetal deaths (IUFD), type-1 diabetes, Guillain-Barré Syndrome and myocarditis in the human population in Sweden varied with the rodent population cycle. This might suggest a possible but unproven association.

The presence of LV has now been confirmed in human fetal brain tissue from elective abortions, due to hydrocephaly and anencephaly, in Sweden. It has been further identified that children with newly diagnosed type-1 diabetes have significantly increased levels of LV antibodies compared to controls. It has also been suspected as the cause of hydrocephalus in red fox (*Vulpes vulpes*) cubs in Great Britain. The presence of LV was indicated by immunohistochemistry (IHC). Red foxes would prey on small rodents and could act as a sentinel species for infection in them.

These preliminary studies indicate that LV could be geographically widespread in wildlife and it may have a role as a zoonotic agent causing reproductive problems and diabetes in man. However only limited studies have so far been carried out and the testing protocols are experimental and have not been fully validated consequently these possible health risks for humans remain speculative.

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CHAPTER

12

PARVOVIRUS INFECTIONS

NICOLA DECARO, CANIO BUONAVOGLIA, MARIE-PIERRE RYSER-DEGIORGIS AND CHRISTIAN GORTÁZAR

INTRODUCTION

NICOLA DECARO AND CANIO BUONAVOGLIA

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Parvoviruses (family *Parvoviridae*) are small (18–26 nm in diameter), non-enveloped viruses, with a linear, single-strand DNA of 5,000–5,200 nucleotides. The icosahedral capsid consists of 60 capsomers that are formed by two major structural proteins, VP1 and VP2, whereas a third capsid protein, VP3, arises from enzymatic cleavage of VP2. Parvoviruses replicate in the cell nuclei and, as a result of the limited genetic information contained in the small DNA molecule, for an optimal viral replication most of them need rapidly dividing cell populations in the synthesis (S) phase of the cell cycle. Consequently, parvoviruses are able to infect a variety of tissues with high replication rates, such as several tissues of fetuses and neonates and haematopoietic (bone marrow, lymphoid organs) and epithelial (intestine) tissues of adults. Their replication *in vivo* is generally associated with the appearance of nuclear inclusion bodies, whereas the cytopathic effect induced in parvovirus-infected cell cultures is not always evident. All parvoviruses are highly stable in the environment, as they are extremely resistant to pH and temperature changes and to treatment with lipid solvents, trypsin and most disinfectants. Virions can be inactivated by formalin, sodium hypochlorite, beta-Propiolactone, hydroxylamine, oxidizing agents and ultraviolet irradiation. Several parvoviruses are able to agglutinate erythrocytes of different mammal and bird species, and some diagnostic tests for parvovirus infections rely on this haemagglutination activity.

The family *Parvoviridae* comprises two subfamilies, *Parvovirinae* and *Densovirinae*, which infect vertebrates and arthropods, respectively. Currently, five genera are included in the subfamily *Parvovirinae*: namely *Parvovirus*, *Erythrovirus*, *Dependovirus*, *Amdovirus* and *Bocavirus*. Unlike other parvovirus genera, dependoviruses, also known as adeno-associated viruses, do not have an autonomous replication, but require co-infection with either an adenovirus or a herpesvirus for productive infection in cell cultures. Parvoviruses causing disease in wildlife are members of the genera *Parvovirus* (*Canine parvovirus 2*, *Feline panleukopenia virus*, *Porcine parvovirus*) and *Amdovirus* (*Aleutian mink disease virus*). Recently, novel parvoviruses closely related to human parvoviruses 4 and 5 have been detected in swine and cattle herds in Hong Kong. These viruses, tentatively assigned to a separate genus, *Hokovirus*, have also been found to circulate in wildlife, and antibodies against *Porcine hokovirus* have been detected in wild boar in Germany.

Infectious Diseases of Wild Mammals and Birds in Europe, First Edition. Edited by Dolores Gavier-Widén, J. Paul Duff, and Anna Meredith.

CANINE PARVOVIRAL ENTERITIS

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AETIOLOGY

Canine parvovirus 2 (CPV2) is an autonomous parvovirus that is genetically and antigenically unrelated to the first described parvovirus of the dog, *Canine parvovirus 1* (CPV1), or minute virus of canines. CPV2 belongs to the feline parvovirus group within the genus *Parvovirus*, together with *Feline panleukopenia virus* (FPLV) and other parvoviruses of carnivores. The original type CPV2, firstly identified in the late 1970s, was completely replaced a few years after its emergence by two antigenic variants, CPV2a and CPV2b, and no longer circulates in the field. CPV2a and CPV2b are variously distributed in the canine population worldwide. More recently, a third antigenic variant, CPV2c, has been reported, firstly in Italy, and has subsequently been shown to be distributed worldwide.

EPIDEMIOLOGY

The three antigenic variants have a variable distribution in domestic dogs in different areas of Europe, with an approximately equal detection of CPV2a and CPV2c in Italy, Germany, Spain and France, and detection of CPV2a in Belgium and eastern Europe and of CPV2b in the UK[1], (Figure 12.1).

CPV2 infects primarily domestic dogs (*Canis familiaris*), and the target canine population is represented mainly by pups aged between 4 and 12 weeks, when the maternally derived antibodies (MDA) wane. However, recently multiple outbreaks of CPV2 disease have been reported in adult dogs, and even in dogs regularly vaccinated. In contrast to the original CPV2, the antigenic variants can infect and cause disease in domestic and wild cats. CPV2 has been reported in European wild species of the *Canidae* family, especially wolves (*Canis lupus*) and red foxes (*Vulpes vulpes*). Clinical outbreaks of CPV2 infections in wolves (*Canis lupus*) have been reported in the USA, and parvovirus infection has been associated with a decline in the wolf population in Minnesota. In European free-ranging wolves, the virus has been detected only sporadically[2] while the same CPV2b strains were found to circulate in both domestic and wild canids[3]. However, the circulation of CPV2 in European wolves has been proven by serological investigations. The CPV2 seroprevalence in these canids was 62.2% in Spain[4] and 32.% in Portugal[5]. Red foxes were found to be infected by viruses intermediate between CPV2 and FPLV, which accounts for their closer phylogenetic relatedness to felids with respect to other *Canidae*. Accordingly, parvoviruses isolated from blue foxes (farmed *Alopex lagopus*) and named *Blue fox parvovirus* (BFPV) were characterized as true FPLV[6]. European red foxes displayed lower CPV2 seroprevalence rates than wolves, with values of 5–12% in Spain[4,7], of 9.1% in Portugal[5], and of 9–13% in Germany[8]. CPV2a sequences were identified in a stone marten (*Martes foina*)[6], whereas an outbreak of CPV2 infection occurred in farmed raccoon dogs (*Nyctereutes procyonides*) in Finland[9]. Exposure to CPV2 or to related viruses has been also demonstrated in free-ranging Marsican brown bears

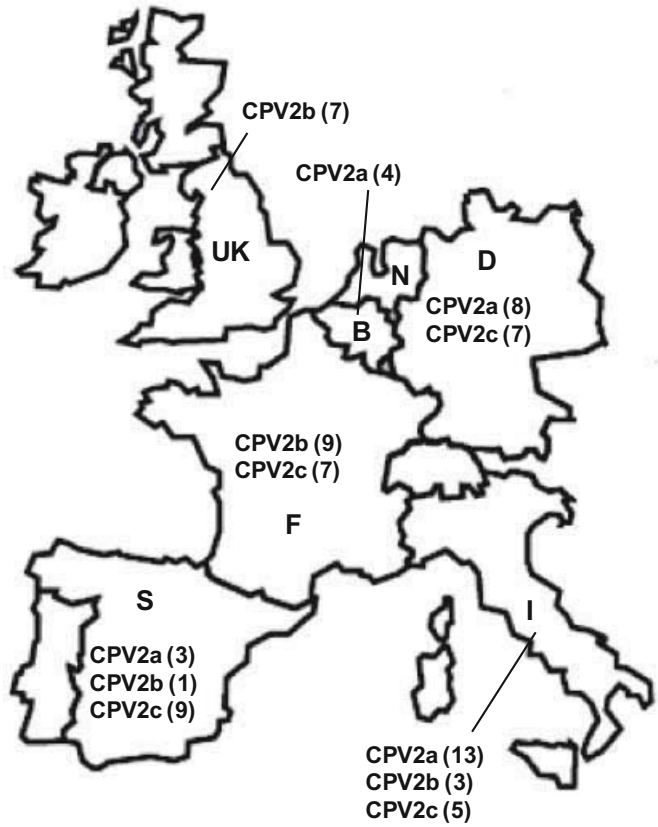

FIGURE 12.1 Geographic distribution of the antigenic variants of *Canine parvovirus 2* (CPV2) in domestic dogs in Europe (adapted from Decaro et al, 2011[1]). The CPV variants are indicated for each country by numbers in parentheses.

(*Ursus arctos marsicanus*) in Italy[10], raccoon dogs and pine martens (*Martes martes*) in Germany[8], Egyptian mongooses (*Herpestes ichneumon*) and common genets (*Genetta genetta*) in Spain[7].

CPV2 is highly contagious and can be transmitted through direct contact with infected animals or – most frequently – by indirect contact, with their faeces. The faeces of the infected animals are highly infectious and may contain billions of CPV2 virions per gram, thus representing the main source of environmental contamination. The faecal shedding may persist for several weeks and, due to its exceptional stability, the virus can remain infective for some months in the environment.

PATHOGENESIS, PATHOLOGY AND IMMUNITY

The pathogenesis of the CPV2 infection in wildlife is poorly known, but it has been extensively investigated in domestic dogs. The target tissues are the intestinal crypts and the lymphoid organs. The virus penetrates through the oronasal route and replicates primarily in the lymphoid tissues of the oropharynx, mesenteric lymph nodes and thymus. After this first-round replication, CPV2 causes leucocyte-associated viraemia and the virus disseminates to the germinal epithelium of the crypts of the small intestine which produces the cells that migrate to the intestinal villi and develop to mature enterocytes. The onset of diarrhoea is due to the massive lysis of these cells and subsequent impairment of the cell turnover at the tips of the intestinal villi. The virus also infects white blood cells, mainly lymphocytes, and lymphoid cells in thymus, lymph nodes and bone marrow. The virus-induced acute lymphopenia (often associated with neutropenia) is responsible for the impairment of the immune response against common pathogens. This impairment may lead to secondary infections, which in turn exacerbate the clinical course of the disease. In 2- to 3-week-old pups, CPV2 is also able to replicate in the cardiac cells, inducing a fatal myocarditis.

Post mortem findings of CPV2 infection in domestic dogs are represented by haemorrhagic enteritis of the small intestine. Mesenteric lymph nodes and Peyer's patches are usually haemorraghic, while the thymus can be atrophic. In the cardiac forms, the heart walls can be flaccid and pale streaks are often evident in the myocardium. Sometimes, areas of patchy consolidation are visible in the lungs as a consequence of pneumonia induced by opportunistic pathogens. Histopathology reveals the presence of multifocal crypt necrosis, intranuclear inclusion bodies in the small intestine and necrosis of lymphoid tissues with depletion of lymphocytes in Peyer's patches, lymph nodes, spleen and thymus. Myocarditis with viral inclusion bodies may be present in young pups. Gross and microscopic lesions in wild canids are similar to those observed in domestic dogs.

Antibodies appear very rapidly after infection, peak 7–10 days post-infection and persist for at least 2 years. Haemagglutination-inhibiting antibody titres ≥1:80 protect dogs against CPV2 infection and disease, whereas titres between 1:20 and 1:40 are able to protect against disease but not against infection. The cell response against CPV2 is poorly studied; however, protection of pups recovered from CPV2 infection is life-long.

CLINICAL SIGNS AND TREATMENT

In domestic dogs, haemorrhagic enteritis is the predominant clinical form observed during CPV2 infection, but the severity of the disease may vary on the basis of titres of MDA still present in the infected pups. After an incubation period of 3–7 days (which can be shorter for the antigenic variants), the infected pups display loss of appetite, depression, vomiting and diarrhoea, which can range from mucoid to bloody. Dehydration and fever can also occur. Currently myocarditis in young pups is only sporadically observed, as most pups have MDA titres that protect them in the early stage of life. Leucopenia is a constant finding of CPV2 infection, with white blood cell (WBC) counts dropping below 2000–3000 cells/μl of blood. Both neutrophil and lymphocyte counts decrease, but lymphopenia is more pronounced. Sometimes the total WBC counts are within normal ranges, as a result of the simultaneous onset of lymphopenia and neutrophilia. The impairment of the immune response can also predispose to bacterial infections of the respiratory tract, with the occurrence of respiratory distress. Subclinical and unapparent infections are frequently detected, mainly in pups with intermediate MDA titres and in adult dogs. The mortality rates can be high (up to 70%) in pups, but are usually less than 1% in adults dogs. Although some viral isolates from European wild carnivores have been characterized at the molecular level, there are no reports on the clinical findings of CPV2 infection in free-ranging animals

and only one report exists on the disease observed in farmed raccoon dogs in Finland. In these outbreaks, 3- to 5-week-old pups were affected, displaying acute gastroenteritis with vomiting and diarrhoea of variable severity[9]. Other wild canids were found to be clinically affected in North America and the clinical signs observed were the same as in domestic dogs. A suspected (but unconfirmed) case of CPV2 acute myocarditis preceding the onset of canine distemper was reported in a 1.5-year-old Eurasian badger (*Meles meles*) in Austria[11].

Treatment of CPV2 infection in infected carnivores is essentially based on the administration of fluids to restore the electrolyte balance and antibiotics when secondary bacterial infections occur. Administration of hyperimmune sera is not recommended to reduce the clinical course of CPV2 infection but only to confer a short-term protection in the case of possible contacts with infected animals.

DIAGNOSIS

Canine parvoviruses are highly stable in the environment and are able to cause haemagglutination *in vitro* only at temperatures below 5°C, and this property is routinely employed for diagnostic purposes. CPV2 can replicate *in vitro* on cell lines of canine and feline origin, such as canine mammary fibroma (A-72), Crandell feline kidney (CrFK) and *Felis catus* whole fetus (fcwf) cells.

Haematology can be useful for detection of the CPV2-induced lymphopenia, which may have prognostic relevance. A definitive diagnosis of CPV2 infection is mainly based on the detection of the virus, viral antigens or nucleic acid in the faeces of the animals with clinical signs. Virus detection in faecal samples is traditionally made by using electron microscopy, virus isolation on susceptible cell lines, haemagglutination in the presence of pig or cat erythrocytes, and in-house tests based on immunochromatography. As these assays have been proven to be poorly sensitive, especially in the late stages of infection, molecular assays have been developed for detection of the CPV2 DNA in clinical samples. Apart from conventional polymerase chain reaction (PCR) protocols, a real-time PCR assay exists for detection and quantification of CPV2 DNA. More recently, type-specific minor groove binder (MGB) probe assays were developed for specific characterization of the various CPV2 variants and for discrimination between vaccine and field strains. Serological methods (haemagglutination inhibition and virus neutralization tests) are not useful for CPV2 diagnosis, but they can be successfully employed for the titration of MDA interfering with active immunization of pups and for seroepidemiological surveys in wildlife.

MANAGEMENT, CONTROL AND REGULATIONS

Prophylaxis of CPV2 infection in domestic dogs is carried out by means of extensive vaccination. Both inactivated and modified live virus (MLV) vaccines are available. Killed formulations have been proven to induce only short-term immunity, so MLV vaccines are more widely used in domestic dogs. CPV2 vaccines are prepared by using either the original type CPV2 or its variant CPV2b. Currently, there are some concerns about the complete efficacy of type-2-based vaccines against the antigenic variants[12]. Vaccines should be administered only after waning of MDA in order to avoid the interference with active immunization of pups. Vaccination of free-ranging carnivores is usually not possible, but it should be performed every time there is an opportunity and is mandatory when a captive-born canid is reintroduced into its natural habitat. Vaccination is also recommended for wild canids living in peri-urban areas that may have multiple contacts with CPV2-infected domestic dogs[6].

SIGNIFICANCE AND IMPLICATIONS FOR ANIMAL HEALTH

There are not enough data to assess whether CPV2 circulates independently in wild carnivores or whether the virus circulation in these animals is limited to sporadic contacts with infected domestic dogs. A role for wildlife reservoirs in the emergence of CPV2 has been proposed, but conclusive evidence is not yet available.

FELINE PANLEUCOPENIA

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Feline panleukopenia virus (FPLV) is a parvovirus closely related to CPV2, exhibiting the same virion structure and

physico-chemical properties. Unlike CPV2, which causes haemagglutination at wide pH values and in erythrocytes of various species, FPLV is able to agglutinate pig red blood cells only at pH values lower than 6.0. At the genetic level, FPLV has a nucleotide identity to CPV2 of more than 98%, differing only in six to seven amino acid residues of the VP2 protein. These mutations may be responsible for the different haemagglutination properties and for the host-receptor specificity. FPLV can replicate *in vitro* on cell lines of feline origin but not in canine cells, inducing the appearance of intranuclear inclusion bodies; however, a cytopathic effect is not always evident. Apart from CPV2, there are wild-carnivore parvoviruses of the feline parvovirus subgroup that are more closely related to FPLV: namely mink enteritis virus (MEV), raccoon parvovirus (RPV) and blue fox parvovirus (BFPV). Currently, these viruses are considered host variants of FPLV rather than separate parvovirus species.

EPIDEMIOLOGY

FPLV has been known since the first decades of the 20th century and is now widespread in all parts of the world, including Europe. The natural host is the domestic cat (*Felis silvestris catus*), and kittens aged between 6 and 14 weeks are highly susceptible to FPLV infection. FPLV is also frequently observed in newborn kittens as a consequence of prenatal infections. FPLV can also infect wild felids (including large felids) and members of the families Mustelidae and Procyonidae. Active virus infection was demonstrated in a free-ranging Iberian lynx (*Lynx pardinus*) in Spain[7] and in a Eurasian lynx (*Lynx lynx*) reintroduced into Switzerland[13]. FPLV antibodies were detected in the Iberian lynx in Spain[7] and in the Eurasian lynx in Sweden[14], as well as in wildcats (*Felis silvestris*) in Portugal[5], Scotland[15], France, Switzerland and Germany[16]. FPLV antigens were also detected by immunohistochemistry in European hedgehogs (*Erinaceus europaeus*) affected by acute gastroenteritis; the source of infection was suspected to be domestic cats[17]. Due to the close relationship between FPLV and CPV2, investigations based on antibody detection are not useful to determine the specificity of the immune response and thus to discriminate between antibodies raised against FPLV and those induced by CPV2. Consequently, parvovirus antibodies detected in some European wild carnivores (see the section on canine parvoviral enteritis) may have been actually induced by FPLV instead of CPV2. The recent detection of the CPV2 variants in domestic and non-domestic felids further complicates the epidemiology of carnivore parvoviruses in wildlife, considering that these viruses are responsible for many feline panleucopenia outbreaks in felids.

FPLV is shed predominantly in the faeces, but urine and saliva can contain a certain amount of infectious particles as a consequence of the high-titre viraemia. Pregnant queens can infect their offspring through vertical transmission. In postnatal infections, susceptible kittens are infected mainly by the oronasal route.

PATHOGENESIS, PATHOLOGY AND IMMUNITY

After a primary replication in the regional lymphoid tissues, the virus spreads through the bloodstream to target tissues, i.e. the epithelium of the intestinal crypts, the stem cells of bone marrow, and the lymphoid cells. FPLV pathogenesis is similar to that of CPV2, but the effects of FPLV replication in the bone marrow are of much greater severity in comparison with CPV2. In prenatal infections, the virus is transmitted vertically by the infected queen to the fetuses and rapidly spread to the Purkinje cells of the cerebellum, causing abortion or, more frequently, birth of kittens with cerebellar hypoplasia, hydrocephalus and retinal degeneration. Like CPV2, FPLV induces an early immune response, with antibody titres peaking shortly after the onset of clinical signs, and recovered cats probably have a life-long protection against reinfection.

Gross lesions observed during FPLV infections are haemorrhagic or fibrinonecrotic enteritis, enlargement of lymph nodes and atrophy of the thymus. The bone marrow can be gelatinous and liquefactive. Microscopic changes include necrosis of the mucosa of small intestine, with presence of intranuclear inclusion bodies in the epithelium, myeloid destruction in the bone marrow and depletion of lymphocytes in the lymphoid tissues.

There are no reports on the clinical signs and pathology of FPLV infections in European free-ranging felids. However, two fatal cases of FPLV infection were described in two captive felines, a 1.5-year-old Eurasian lynx and a 3.5-month-old European wildcat, living in the same wildlife park in Germany[18]. The gross lesions and microscopic changes were undistinguishable from those usually observed in domestic cats, and the source of infection was proven to be the feral cat population living in the park.

By sequence and phylogenetic analysis of the VP2 protein gene, the virus was found to be very closely related to a typical FPLV strain detected in France.

CLINICAL SIGNS AND TREATMENT

Postnatal infections can have a subclinical or an inapparent course, mainly in adult cats, or lead to peracute forms with sudden death, especially in young kittens. The incubation period can vary between 2 and 10 days, and clinical signs are usually characterized by anorexia, depression, abdominal pain, vomiting and fluid – sometimes bloody – diarrhoea. Fever can be succeeded by hypothermia, especially in the terminal stages of the disease. WBC counts may be particularly low, reaching values less than 500 leucocytes/μl of blood. Unlike CPV2, which may cause only acute lymphopenia with normal neutrophil counts, FPLV induces the reduction of all WBC, including neutrophils, as a consequence of the bone marrow involvement. Respiratory distress and nasal discharge may appear as a consequence of secondary bacterial infections. The outcome of prenatal infections depends on the stage of pregnancy when infection is acquired. In the early stages of pregnancy, embryonic death and resorption are frequent, whereas abortion or fetal mummification is more common in the later stages. Prenatal infections occurring in the last 2 weeks of pregnancy are usually associated with the birth of kittens affected by neurological signs consisting of hypermetria, dysmetria and incoordination. Neurological disorders can also occur in infected kittens aged less than 2 weeks.

Treatment of FPLV infection in infected carnivores is supportive only, based on fluid therapy to rehydrate and restore electrolyte balance and antibiotics for secondary bacterial infections. Prognosis is generally poor.

DIAGNOSIS

FPLV infection should be suspected in the presence of a severe leucopenia, especially in young animals. Confirmatory tests are the same as those used for detection of CPV2 infection. Recently, a MGB probe assay for discrimination between CPV2 variants and FPLV was developed, which is potentially useful considering the increasing number of feline infections caused by the canine viruses[19].

MANAGEMENT, CONTROL AND REGULATIONS

FPLV prophylaxis in domestic cats is based on the systematic vaccination of kittens after the wane of MDA immunity using killed or MLV vaccines. According to the relatively high frequency of detection of the CPV2 variants in felids with clinical signs of feline panleucopenia, combined FPLV/CPV2 vaccines should be developed. Although this strategy is hardly applicable to free-ranging wildlife, vaccination of felids to be reintroduced into the wild is recommended. In fact, release into the wild of non-domestic felids bred in captivity would bring about a high risk of introducing of FPLV infection to free-ranging wildlife populations. FPLV can infect a wide range of carnivores but it is not a human pathogen.

ALEUTIAN DISEASE

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Aleutian disease (AD; also known as Aleutian mink disease, mink plasmocytosis or infectious plasmocytosis) was first described in farmed American mink (*Mustela vison*) with Aleutian coat colour. The two main AD forms are: i) a fatal acute pneumonia in neonatal kits born from non-immune females; and ii) a chronic immune complex-mediated disease involving various organ systems and characterized by increased mortality and reduced fertility in adult mink.

Aleutian mink disease virus (AMDV) is a single-stranded DNA virus in the genus *Amdovirus*, subfamily *Parvovirinae*, family *Parvoviridae*. Several strains have been described, ranging from non-pathogenic to highly pathogenic.

The host range of AMDV comprises the mustelids, as well as the raccoon (*Procyon lotor*), common genet (*Genetta genetta*), striped skunk (*Mephitis mephitis*) and red fox (*Vulpes vulpes*)[20,21]. Rare cases of fatal human infections have been reported[22]. All mink are susceptible, but in non-Aleutian mink the disease is generally less severe or inapparent.

AD is globally distributed in mink farms. In Europe, it has been reported as important in the Netherlands and Fennoscandia[23]. The occurrence of antibodies to AMDV and/or viral sequences has been documented in free-ranging native European mustelid species and common genets in Spain and France, and in feral American mink in Spain, France and southern England; clinical disease has only rarely been documented/suspected[20,24–26]. AMDV infection appears to be particularly widespread in American mink, which is thought to be the original host of the virus, supporting the hypothesis that AMDV has been introduced from North America to the rest of the mink-breeding countries.

Transmission occurs horizontally, by direct or indirect contact, and vertically. The virus is shed in urine, faeces and saliva; aerosol spread has also been proposed. AMDV is highly persistent in the environment and resistant to physical and chemical treatments.

Depending on the species, genotype and immune status of the host, and on the virus strain, infected animals may develop: i) a progressive severe disease similar to that in Aleutian mink; ii) a persistent infection with high antibody titres in the absence of lesions; or iii) a non-persistent infection with low antibody titres, no lesions, and eventual clearance of the virus. While antibodies can have a protective role in AMDV-infected mink kits, and the passive transfer of maternal antibody to kits infected after birth prevents the development of pneumonia, antiviral antibody exacerbates disease in AMDV-infected adult mink. Following infection with AMDV, antibody binds to virus and forms immune complexes, which are deposited in tissues and cause inflammation and fatal organ failure.

Kits less than 2 weeks old display respiratory distress and die. Clinical signs in adults include apathy, poor pelt, anorexia and weight loss, diarrhoea and melaena, infertility, polydipsia, neurological disorders, blood-clotting abnormalities and, rarely, ocular lesions. Affected animals display anaemia and uraemia, together with hypergammaglobulinaemia, plasmocytosis, high levels of anti-AMDV antibody, and viraemia. Susceptibility to other diseases increases. In ferrets (*Mustela putorius furo*), which are affected by a distinct virus strain, the disease course is milder, and clinical signs include a wasting syndrome or posterior ataxia and paresis, but immune complex disease is not seen[6].

Acute interstitial pneumonia in kits is characterized by parenchymal haemorrhages, extensive atelectasis and hyaline membrane formation. Adults with classical AD typically present splenomegaly and lymphadenopathy[27]. Histologically, the main lesions include interstitial lymphoplasmacytic infiltration in the renal cortex, systemic perivascular lympho-plasmacytic infiltration often associated with necrosis in kidney, liver and lung, mononuclear hepatitis with bile duct proliferation and liver cell necrosis, and necrotic foci in the spleen. Meningo-encephalitis and chorioiditis are also common.

Diagnosis of AD in mink is based primarily on the clinical signs and detection of AMDV antibodies. In particular, the presence of hypergammaglobulinaemia with the concurrent presence of AMDV antibodies in American mink or ferret is strongly suggestive of AD[28]. For detection of anti-AMDV antibodies in serum, counter-current immune electrophoresis (CIE) is the assay of choice and regarded as the 'gold standard' for diagnosis of AMDV infection. A recently developed enzyme-linked immunosorbent assay (ELISA), with possibly higher sensitivity, was proposed as a suitable alternative[29]. For screening of tissues by PCR, the spleen should be the first choice; lymph nodes are also appropriate[28].

There are no effective vaccines or treatment for AD. The only effective means of control on farms is diligent culling of infected animals. Control of feral American mink populations, prevention of escapes from fur farms and strict protocols for disinfecting equipment during trapping programmes are recommended[20,21].

AD is a major infectious cause of economical losses in mink farming. Its prevalence and significance in free-ranging carnivores is largely unknown. It has been proposed that because of the persistent nature of the disease and the negative effects on reproductive success, AMDV may have negative impacts on wild populations of native susceptible mustelids[6,20].

PORCINE PARVOVIRUS INFECTION IN WILD BOAR

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Porcine parvovirus (PPV) is classified in the genus *Parvovirus*. This ubiquitous and resistant virus causes porcine parvovirus infection, a transmissible infection producing reproductive losses in pigs and Eurasian wild boar (*Sus scrofa*)[30].

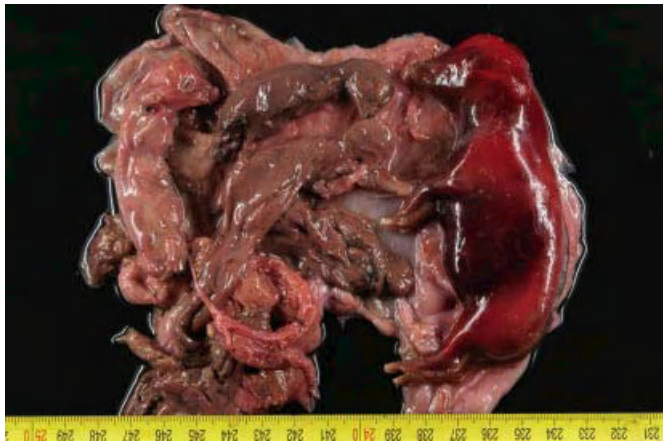

FIGURE 12.2 European wild boar, Spain, abortion and reproductive losses caused by porcine parvovirus infection. The abortion material has several mummified fetuses, which have died at different gestational ages. This type of loss is characteristic in PPV abortions in domesticated pigs.

PPV has a worldwide distribution. In wild boar and feral pig populations, seroprevalence ranges from 14 to 77%. It is highly transmissible and exhibits high seroprevalence at high population densities.

The infection is acquired by the oronasal, transplacental and venereal routes. In immune adult animals, the reproductive losses are generally not seen; however, the tropism of the virus for the reproductive tract in naïve females may lead to mummified fetuses, resorptions and abortion (Figure 12.2), particularly in their first pregnancy and when infection takes place before mid-gestation. Acute infection of postnatal pigs is usually subclinical[30]. Maternal antibodies last for about 3 months[31].

No lesions are detected in PPV-infected adult animals. Affected wild boar fetuses appear oedematous, and may show marked differences in size.

Screening is based on serum antibody detection (e.g. ELISA). PCR or virus isolation are the main antigen detection tools, frequently used on mummified fetal tissues.

Risk factors include fencing and feeding of wild boar populations, increasing density and spatial aggregation, and hence the risk of disease transmission. Natural management is therefore preferred.

Contact with PPV has been linked with lower ovulation rates in wild boar[32]. PPV infection can be associated with post-weaning multisystemic wasting syndrome (PMWS), possibly triggering this disease in some cases. As PPV seroprevalences are usually higher in domestic pig herds than in wild boar populations, it is unlikely that the wild boar will frequently act as a PPV reservoir for domestic pigs. Nevertheless, transmission between domestic and wild populations could take place in both directions.

OTHER PARVOVIRUS INFECTIONS

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Avian parvoviruses belong to the two distinct genera *Dependovirus* (including *Goose parvovirus* (GPV) and *Muscovy duck parvovirus* (MDPV)) and chicken and turkey parvoviruses[33]. Virus transmission is via faeces and infected eggs. GPV causes gosling plague (Derzsy's disease). Clinical signs and lesions vary depending on the age of the geese and can include mild respiratory disease, ataxia, necrotic hepatitis and myocarditis, enteritis with diarrhoea, feather abnormalities and growth retardation. Histologically, intranuclear inclusion bodies are typically found in the liver[34]. MDPV, to which geese are resistant, causes a similar disease in Muscovy ducks. MDPV has also been involved as the cause of short beak and dwarfism syndrome in mule ducks (mallard (*Anas platyrhynchos*) × Muscovy duck hybrids)[35]. The chicken-origin parvovirus (ChPV) and two turkey-origin parvoviruses (TuPV) have been implicated as causes of poultry enteric disease syndromes[33]. Parvovirus-associated disease and mortality has occasionally been reported in wild geese species in zoological parks, suggesting that wildlife may be implicated in the epidemiology of GPV[34], which was further supported by the high prevalence of antibodies to GPV documented in free-ranging geese in Germany[36]. Parvovirus-like infections have been also reported in captive or exotic wild bird species[34]. Parvovirus infection in birds is typically demonstrated by serology or PCR. Loop-mediated isothermal amplification (LAMP) assays are inexpensive and are sensitive alternatives suitable for field conditions[37]. In farmed waterfowl and the poultry industry, parvovirus infections result in high morbidity and mortality associated with great economic losses worldwide. Vaccination is a common preventive measure.

Minute virus of mice (MVM) and *Mouse parvovirus* (MPV) are among the most prevalent infectious agents in

colonies of laboratory mice. Various clinical disease syndromes in mice have been associated with MVM infection, and both MVM and MPV can have immunomodulatory effects[38]. Exposure to these viruses is common also in wild populations, as shown for example in house mice (*Mus domesticus*) in England[39]. Hamster parvovirus (HaPV) is one of the several novel rodent parvovirus strains that have been discovered over the past decades. HaPV was first isolated in a commercial colony of Syrian hamsters (*Mesocricetus auratus*) that experienced high neonatal morbidity and mortality. Hamsters are thought to be aberrant hosts for HaPV, while mice have been proposed as its natural rodent host[38].

Knowledge about parvovirus infections in marine mammals is very limited. Disease has not been reported so far. Two free-ranging Steller sea lions (*Eumetopias jubatus*) from Alaska were seropositive positive at low titres for *Canine parvovirus 2* antibodies by the haemagglutination inhibition test, indicating possible exposure of sea lions to this virus[40].

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CHAPTER

13

POXVIRUS INFECTIONS

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INTRODUCTION

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Poxviruses are a family of large DNA viruses. Within the poxviruses (family *Poxviridae*) there are two subfamilies: *Chordopoxvirinae*, affecting vertebrates, and *Entomopoxvirinae*, affecting insects. There are nine recognized genera of *Chordopoxvirinae*: *Avipoxvirus*, *Orthopoxvirus*, *Parapoxvirus*, *Capripoxvirus*, *Cervidpoxvirus*, *Leporipoxvirus*, *Suipoxvirus*, *Molluscipoxvirus* and *Yatapoxvirus*; some poxviruses are not yet assigned to any of these genera. The viruses affecting birds are all contained within the avipoxviruses, whereas mammalian poxviruses are assigned to the different genera based on cross-protection in hosts, neutralization assays and genomic DNA hybridization studies. Various poxvirus diseases are important in domestic animals, and some are important zoonoses.

Virions contain a dumbbell-shaped core and lateral bodies, enclosed in an outer membrane. Orthopoxviruses and most other poxviruses are 200–400 nm long, brick-shaped and covered with irregularly arranged tubular elements. The genome is linear double-stranded DNA (both a positive and a negative strand) ranging in length from 134 kb in the parapoxviruses to 330 kb in the avipoxviruses. Virus replication takes place in the cytoplasm of the cell. Transmission of poxviruses usually occurs through contact of infective material with abraded skin or via the upper respiratory tract.

Poxviruses are usually named according to the hosts/species affected, e.g. *Fowlpox virus*, *Sealpox virus* (although it is now accepted that the main hosts of cowpox are rodents). Pox diseases generally involve development of relatively mild lesions of the skin (e.g. a rash) and/or mucous membranes, although systemic, often fatal, disease occurs in some host–virus combinations.

AVIAN POX

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AETIOLOGY

The poxviruses affecting birds are all within the genus *Avipoxvirus*. Avipoxviruses include *Canarypox virus*, *Fowlpox virus*, *Pigeonpox virus*, *Quailpox virus*, *Sparrowpox virus* and *Starlingpox virus*; many avipoxviruses have not

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been fully characterized. Some avipoxviruses appear to infect only one host species, whereas others have a broader host range; some of the avipoxviruses show extensive serological cross-reaction and cross-protection.

EPIDEMIOLOGY

Avian pox has been reported practically worldwide, although there are to date no reports from the Arctic or Antarctic. There have been reports of avian pox in wild birds for more than a century, from more than 15 countries across Europe (Table 13.1).

More than 270 bird species from at least 23 orders have been confirmed as hosts of these viruses, and pox has been reported in at least 60 native species in Europe. In general, birds endemic to remote islands (including the Spanish Canary Islands), which have encountered poxviruses only recently, tend to show more severe forms of the disease. Also, young birds generally show more severe disease than do adults. Several recent reports of pox in great tits (*Parus major*) in Austria, Hungary and the UK indicate that this may be an emerging infectious disease in the Paridae[7,5].

Prevalence of avian pox is higher in warmer regions with adequate available water providing suitable conditions for

TABLE 13.1 European bird species in which avipoxvirus infections have been recorded.

Order	Family	Species	Common name	Country reported	References
Podicipediformes	Podicipedidae	*Podiceps cristatus*	Great-crested grebe	Switzerland	1, 2
Procellariformes	Procelariidae	*Puffinus puffinus*	Manx shearwater	UK	1, 2
Ciconiiformes	Ciconiidae	*Ciconia ciconia*	White stork	Switzerland	2
		Ciconia nigra	Black stork	Switzerland	1, 2
Anseriformes	Anatidae	*Anser anser*	Greylag goose	Germany	1, 2
Accipitriformes	Accipitridae	*Accipiter gentilis*	Northern goshawk	France, Germany	1, 2
		Aquila chrysaetos	Golden eagle	Germany	1, 2
		Aquila adalberti	Spanish imperial eagle	Spain	1, 2
		Buteo buteo	Common buzzard	Austria	1, 2
		Circus pygargus	Montagu's harrier	Germany	1, 2
Falconiformes	Falconidae	*Falco peregrinus*	Peregrine falcon	Germany	2
		Falco tinnunculus	Common (Eurasian) kestrel	Germany	2
Galliformes	Phaesinidae	*Tetrastes bonasia*	Hazel grouse (common hazelhen)	France	1, 2
		Lyrurus (Tetrao) tetrix	Black grouse	Denmark	1, 2
		Alectoris rufa	Red-legged partridge	Spain	1, 2
		Perdix perdix	Grey partridge	Austria, Denmark, Germany, Italy, UK	1, 2
Gruiformes	Gruidae	*Grus grus*	Eurasian crane	Germany	1, 2, 3
Otidiformes	Otidae	*Otis tarda*	Great bustard	Romania, Germany	1, 2
Charadriiformes	Haematopodidae	*Haematopus ostralegus*	Eurasian oystercatcher	UK	1, 2
	Charadriidae	*Vanellus vanellus*	Northern lapwing	Denmark	1, 2
		Pluvialis apricaria	European (Eurasian) golden plover	Denmark	1, 2
	Scolopacidae	*Calidris alpina*	Dunlin	UK	1, 2
		Numenius arquata	Eurasian curlew	Germany	1, 2
	Burhinidae	*Burhinus oedicnemus distinctus*	Stone curlew	Canary Islands (Spain)	4
	Lariidae	*Larus canus*	Mew (common) gull	Denmark	1, 2
		Larus argentatus	European herring gull	GB	1, 2
Columbiformes	Columbidae	*Columbia livia*	Common pigeon (rock dove/ feral pigeon)	Austria, The Netherlands	1, 2
		Columbia junoniae	Laurel pigeon	Canary Islands (Spain)	2
		Columba palumbus	Common wood pigeon	UK, Germany, Norway, Sweden	1, 2, 5

TABLE 13.1 *(Continued)*

Order	Family	Species	Common name	Country reported	References
Strigiformes	Strigidae	*Asio otus*	Long-eared owl	Italy	1, 2
		Bubo bubo	Eurasian eagle-owl	Italy	1, 2
Passeriformes	Alaudidae	*Alauda arvensis*	Eurasian skylark	Denmark	1, 2
		Galerida cristata	Crested lark	Spain	1, 2
		Calendrella rufescens	Lesser short-toed lark	Canary Islands (Spain)	2
	Motacillidae	*Anthus berthelotii*	Berthelot's pipit	Canary Islands (Spain)	2
	Troglodytidae	*Troglodytes troglodytes*	Wren	Denmark	1, 2
	Prunellidae	*Prunella collaris*	Alpine accentor	Austria	1, 2
		Prunella modularis	Dunnock	France, UK, Slovakia Poland* (*probable pox)	1, 2, 6
	Turdidae	*Turdus merula*	Common (Eurasian) blackbird	Italy	2
		Turdus philomelos	Song thrush	Denmark	1, 2
		Turdus pilaris	Fieldfare	Italy	1, 2
		Turdus viscivorus	Mistle thrush	Italy	1
	Sylviidae	*Sylvia curruca*	Lesser whitethroat	Denmark, Poland[a]	1, 2, 6
		Sylvia atricapilla	Eurasian blackcap	Austria, Czech Republic, Slovakia	2, 6
	Paridae	*Parus major*	Great tit	Germany, Norway	1, 2
		Poecile (Parus) montanus	Willow tit	Poland[a]	6
	Laniidae	*Lanius collurio*	Red-backed shrike	Poland	6
	Corvidae	*Coloeus (Corvus) monedula*	Jackdaw	The Netherlands	1, 2
		Corvus frugilegus	Rook	Denmark	1, 2
		Corvus corax	Northern (common) raven	Denmark	1, 2
		Corvus corone	Carrion crow	Denmark, UK, Germany	1, 2
		Corvus cornix	Hooded crow	Germany	1
		Pica pica	Eurasian magpie	Denmark, Norway	1
	Sturnidae	*Sturnus vulgaris*	Common (European) starling	Austria, Germany	1, 2
	Passeridae	*Passer domesticus*	House sparrow	Germany, Norway	1, 2, 5
	Fringillidae	*Fringilla coelebs*	Common chaffinch	Germany, UK	1, 2
		Carduelis spinus	Eurasian siskin	Austria, Germany	1, 2
		Carduelis carduelis	European goldfinch	Germany, UK	1, 2
		Carduelis chloris	European greenfinch	Germany, UK	1, 2
		Carduelis (Linaria) cannabia	Common (Eurasian) linnet	Germany	1, 2
		Pyrrhula pyrrhula	Eurasian (Common) bullfinch	Austria, Germany, The Netherlands	1, 2

[a] probable pox

the insects (mainly various species of mosquitoes), which act as mechanical vectors for the virus. Outbreaks occur mainly during periods when these vectors are numerous and active.

Transmission of avipoxviruses occurs by contact of virus to scarified, lacerated or otherwise injured skin. This may occur by mechanical transmission by insect vectors, or by direct or indirect contact between an infected bird and a potential host. The virus cannot penetrate intact skin, but even small breaks in the cutaneous barrier, including those caused by mosquitoes, fleas etc. when feeding, allow infection. When the virus is present in an aerosol, it can reach and penetrate apparently intact mucous membranes; aerosols of feathers and dried scabs containing virus may allow both cutaneous and respiratory infection.

In wild birds, the main mode of infection is mechanical transmission by mosquitoes feeding on an infected bird and then on a susceptible individual. Once a mosquito has bitten an infected bird, infectious virus may remain in the mosquito's salivary glands for 2 to 8 weeks. Territorial interactions leading to skin damage allows direct transmission between birds. Additionally, transmission of infection may be increased where birds are fed by humans, a situation which increases local bird density and the possibility of direct or indirect contact via contaminated bird tables or bird feeders. Transmission is also increased in wild birds maintained in captivity for short periods (e.g. during rehabilitation) or for longer periods such as game-bird rearing or breeding for reintroduction. Because avipoxvirus is relatively resistant it can persist, for example on contaminated bird feeders or perches, for long periods. In situations where there are large numbers of birds housed in close quarters, such as canary aviaries, transmission may occur by inhalation of virus-laden dust.

PATHOGENESIS, PATHOLOGY AND IMMUNITY

Following entry into the host epithelium, the virus penetrates cell membranes within an hour and uncoats. Whereas non-pathogenic or low pathogenic strains remain localized at the site of entry, causing localized lesions, with pathogenic strains, following an initial viraemia, the virus becomes distributed to the liver and bone marrow, with further replication, secondary viraemia and generalized clinical disease.

The initial incubation period varies from as short as 4 days in poultry and pigeons to as long as several months reported in some wild birds, and depends on both the virus strain and the host species. Additionally, the process of development of lesions, regression and healing may take longer in wild birds than in domestic poultry.

In 'dry' pox (cutaneous pox), initial nodules may be white, rapidly growing and turning yellow, then grey or brown, sometimes coalescing with adjacent lesions. The nodule base becomes inflamed and haemorrhagic after about 2 weeks. The scab, covering a haemorrhaging granulating surface with a moist seropurulent exudate layer, may last for 1 or 2 weeks, after which the degenerated epithelial layer desquamates to leave a smooth scar, not always visible. In 'wet' pox, necrotic, moist diphtheritic lesions or membranes are present on the mucous membranes of the mouth, upper respiratory tract (pharynx and larynx, rarely the bronchi) and sometimes the upper gastrointestinal tract (oesophagus and crop). Initially lesions are discrete, whitish and slightly raised; later coalescing lesions may form a yellowish cheesy pseudomembrane.

Histologically, there is localized epithelial hyperplasia and hypertrophy. In the layers above the *stratum germinosum*, maturing cells show hypertrophy and large granular eosinophilic (acidophilic) intracytoplasmic inclusions (Bollinger bodies) are present. Often, there is necrosis in the centre of the lesion and the lesion may be covered by a crust. In the septicaemic form (seen mainly in canaries), the bronchial and parabronchial epithelium show marked proliferation.

Cell-mediated immunity is most important in the host's response, although antibodies can also play a role in the immune response to the virus. Birds that recover from infection are generally immune to reinfection with that strain of avipoxvirus, but they may be susceptible to a different strain.

CLINICAL SIGNS AND TREATMENT

Clinical signs of avipoxvirus infection in birds vary depending particularly on the host susceptibility and virus virulence, but also on distribution of lesions and complicating factors such as traumatic damage to lesions, and secondary bacterial or fungal infection.

In dry pox, nodular, wart-like lesions are found on featherless areas of the body: around the beak and eyelids, on the wattles and/or comb (in gallinaceous birds), the

featherless parts of the legs and feet and sometimes parts of the wings. In perching birds, early lesions are smooth reddish domes, which crack or burst, developing into the typical proliferating lesions. In birds with webbed feet, nodules appear along the blood vessels of the webs, mainly on the plantar surface, with initial focal proliferation and later sloughing. Usually, there are only one or two nodular lesions in wild birds, for example on one or two toes, and they regress spontaneously with no visible scars. However, lesions may be easily damaged with subsequent secondary bacterial or fungal infections. Mortality rates with cutaneous lesions are generally low; however, they may be higher if secondary bacterial infection occurs. Additionally, large lesions, and those around the eyes in particular, may interfere with feeding, leading to debility and predation or death; in some cases lesions around the eyes may blind the bird. Loss of digits may occur as a result of infection.

In wet pox, grey to brown necrotic lesions develop on the mucous membranes of the mouth, pharynx and larynx; if the diphtheritic membrane over the lesions is disturbed, severe bleeding is likely. This form has a higher mortality rate than with dry pox. Combined development of both cutaneous and mucous membrane lesions can occur. The relative paucity of reports of diphtheritic pox in wild birds may indicate lower occurrence or lower detection of this form in free-living birds.

A third, systemic, form of avipox infection is seen mainly in canaries and is rarely reported in wild birds. This presents with acute onset anorexia, somnolence, ruffled feathers and cyanosis; most affected birds die within 3 days of showing signs, and cutaneous lesions are rare.

Treatment of free-living birds is not practical. Where wild birds are in human care, supportive care includes ensuring affected birds have access to food and water; fluid therapy and assisted feeding may be necessary in some individuals. Ophthalmic ointment may be used to protect the bird's eyes and keep them moist, and antibiotic treatment may be used to prevent secondary infection, particularly if lesions become traumatized.

DIAGNOSIS

Diagnosis of avian pox, as for other pox diseases, is usually based on compatible clinical signs in conjunction with the presence of typical poxvirus Bollinger bodies on histopathological examination and detection of typical pox virions using transmission electron microscopy (TEM). There have been recent advances in molecular based detection and identification assays, including polymerase chain reaction (PCR) tests, restriction fragment length polymorphism (RFLP) analysis and use of oligonucleotide probes[8]. With cutaneous avian pox, the skin lesions are characteristic but should be differentiated from other diseases. *Ante mortem* diagnosis of the septicaemic form is difficult. Culture of the virus may be required for confirmation of the septicaemic form. Because the host's immune reactions are mainly cell-mediated, humoral antibodies may not be produced following infection; if present, they may be detected by a virus neutralization test.

MANAGEMENT, CONTROL AND REGULATIONS

Control requires preventing transmission of the virus. This may be possible in a small, confined population but is more difficult in larger areas. Control of vectors, particularly mosquitoes, is important; breeding and resting sites for mosquitoes should be identified and eliminated, and pesticides may be used to control adult mosquitoes. Removal of heavily infected individuals reduces the source of virus for vectors to transmit, as well as reducing direct contact transmission. Where outbreaks occur around wild bird feeders, disinfection of the feeding site and feeders, e.g. using 5% sodium hypochlorite solution (domestic bleach), emptying, drying and refilling of bird baths daily, and temporary discontinuation of feeding (e.g. for a month) before resuming with a smaller quantity of food is recommended. In captive situations such as rehabilitation centres, perches, cages/enclosures, clothing, debris etc. need to be treated with an effective disinfectant such as 5% bleach solution.

PUBLIC HEALTH CONCERN

There is no public health concern associated with avian pox.

SIGNIFICANCE AND IMPLICATIONS FOR ANIMAL HEALTH

Some avipoxviruses of wild birds may also infect domestic poultry, intensively reared game birds, domestic waterfowl and domestic pigeons. Poxvirus infections of wild birds

are generally not significant on a population level. However, they may be more important in populations of wild birds endemic to remote islands; a study of birds captured during 2002–2003 on the Spanish Canary Islands found that 50% of 395 short-toed larks (*Calandrella rufescens polatzeki*) and 28% of 139 Berthelot's pipits (*Anthus berthelotti*) had typical poxvirus lesions, mainly on the legs, sometimes with large lesions and missing nails or digits; there were few lesions on the head. One bird died after being handled and was found to have diphtheritic pox[9]. In free-living red-legged partridges (*Alectoris rufa*) in Spain, 41% of radio-tagged juveniles (but no adults) were found to have pox-type lesions when captured. While only a few deaths directly due to pox were recorded, and there were no significant differences between survival of juveniles with and without lesions, sample sizes were small; it was noted that indirect effects (reduced weight gain, increased susceptibility to predation) might also contribute to mortality[10].

SQUIRRELPOX

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Squirrelpox disease (SQPD) (synonyms squirrel pox viral disease (SQPVD), squirrel distemper, squirrel myxomatosis, squirrel parapox disease and squirrel pox) is confined to the red squirrel (*Sciurus vulgaris*) and the introduced grey squirrel (*S. carolinensis*), where they occur together in the UK. A disease of red squirrels, almost certainly SQPD but not confirmable at that time, was first described in England around 1930.

The disease has conservation importance, as slowly but efficiently it extirpates (destroys) the red squirrel metapopulation (a series of continuous populations linked over a geographically large area) in mainland Britain. Complete metapopulation destruction caused by infectious disease is unusual even in global terms.

AETIOLOGY

Squirrelpox virus (SQPV) causes SQPD. As a typical poxvirus, SQPV is robust, large (300 × 80 nm), spherical or bullet-shaped, with basketweave-like cross striations that cover the surface of virions and are obvious on electron microscopy. The virus is considered to be resistant to chemicals and some disinfectants and, based on the properties of other poxviruses although not yet confirmed in SQPV, the virus is assumed to survive for some time – perhaps days or weeks – in the environment. The genome has been characterized and classifies virulent SQPV on its own in a separate clade of the poxviruses[11] as a previously unrecognized genus of the *Chordopoxvirinae* and not a parapoxvirus as was originally thought. Other pox-like viruses are found in North American squirrel species, but these are not closely related to SQPV.

EPIDEMIOLOGY

The red squirrel has a wide Eurasian distribution, but the disease only occurs in populations of red squirrels in contact with introduced, SQPV-infected grey squirrels. The grey squirrel is the reservoir host or carrier of the virus[12] and is very resistant to disease, with only a single clinical case reported. The combination of both species of squirrel and SQPV together, which leads to the disease, currently occurs only in the UK and Ireland, with the first Irish cases confirmed in 2011 (D. Everest, personal communication). Populations of grey squirrels co-exist with red squirrels in Central Scotland and Italy but are seronegative for SQPV, and consequently neither virus nor disease are present there. Accidental host species have not been identified.

As part of the Veterinary Laboratory Agency Diseases of Wildlife Scheme (VLADoWS) national wildlife disease scanning surveillance, 606 sick or dead red squirrels from northern England were examined between 1998 and 2011, from which 164 cases of SQPD were diagnosed[13] (Duff, personal observations). Findings from these cases are used in this description together with findings published elsewhere. Of the 164 cases, 48 were subadults or juveniles, the remainder being adults. There was a sex bias towards males (107 males, 53 females, 4 not recorded) possibly linked to male behaviour questing for territories and mating opportunities, which render them more likely to encounter infective grey squirrels. A small number of red squirrels survive infection in the wild (2 from the 606; about 1.2% of SQPD cases) but the mechanisms involved are not known.

SQPD may occur in any month of the year, this study showed there were peaks in April (27 cases in total) and

July (20 cases), with an average of 12 cases in each of the remaining months. The April and July maxima may correspond to the two breeding seasons in the UK. There was an increased frequency during the summer as opposed to the winter (103 cases in 6 months from April versus 61 cases in 6 months from October).

EPIDEMIOLOGICAL ROLE OF AFFECTED SPECIES

SQPD is a well-documented example of pathogen-mediated competition between an invasive species acting as a reservoir for a pathogen (the grey squirrel) and a native species that is very susceptible to the pathogen (the red squirrel)[14]. Red squirrels can also be considered as accidental spillover hosts of a virus carried by an introduced species. Over 60% of sampled grey squirrels in England and Wales had antibody to the virus, supporting the reservoir host theory[12]. In the absence of the virus, grey squirrels usually outcompete red squirrels in most habitats; however, the rate of red squirrel replacement by grey squirrels is 17 to 25 times faster in those areas where grey squirrels carry the virus, compared with those areas where they do not[15]. Following introduction from North America, the grey squirrel population, once established, then expanded and has now replaced the red squirrel throughout mainland England south of Liverpool. As the grey squirrel extends its distribution northward into Scotland, an epidemic front of SQPD follows in the wake of the line of population expansion, while behind this epidemic front the red squirrel population has effectively disappeared and has been replaced by the grey squirrel.

SQPD epidemics appear to follow a repeating pattern; grey squirrels first enter an area and after a delay, local sporadic outbreaks of SQPD occur but fade out when the susceptible animals are killed. The local outbreaks become more frequent and eventually the once-continuous red squirrel population is fragmented. In good-quality red squirrel habitat there may be a 'sink effect' where the remaining animals are drawn in from catchment areas and persist for years after the species has disappeared from the rest of the region. Twenty cases of SQPD have now been reported in southern Scotland, and the disease may eventually extend beyond Central Scotland, perhaps coming to a natural barrier in northern Scotland, where only red squirrels survive. SQPV may spare these far-north red squirrel populations if grey squirrels cannot colonize these areas. SQPD exists only in epidemic form and only where red and grey squirrels are in contact, suggesting that red-to-red squirrel transmission does not spatially extend epidemics; that is, the disease has not spread independently of the grey squirrel[16]. However, the increased number of cases during the red squirrel breeding season suggests that red-to-red squirrel transmission may account for some mortality during local outbreaks. Seropositive red squirrels that survive infection are generally in poor condition and appear too scattered temporally and spatially to constitute a viable but possibly immune breeding population.

TRANSMISSION

Direct and indirect transmission of SQPV is thought to occur; however, evidence-based information is lacking. Arthropod parasites may possibly transmit the virus mechanically, but a significant arthropod vector has not been found. Both species of squirrel are solitary and the likelihood of direct contact is relatively low. Indirect transmission may involve virus particles left in shared dreys, on branches or at feeding sites as the possible sources of infection between species. It is unlikely that SQPV is picked up from faeces or urine. The relatively short clinical course and the near 99% case-fatality rate suggest that red squirrels are only infective for a period of days. Supplementary feeders used by squirrels, if virus contaminated, are likely to be a source of infectious virus for other squirrels.

PATHOGENESIS, PATHOLOGY AND IMMUNITY

The means of acquisition of infection are not known but may occur when poxvirus penetrates small breaks in the continuity of the skin. Bilateral lesions on the inner aspects of the front feet seem to be associated with the animals rubbing their facial lesions, and these suggest that the virus may invade intact skin. PCR testing has detected low titres of virus in internal organs. From experimental infection studies of red squirrels, disease was produced by a virus titre of 100,000 virus particles per millilitre given in 0.2 ml doses by skin scarification and as subcutaneous injections. SQPV causes exudative erythematous dermatitis and severe dehydration. The infection typically presents as a necrotizing purulent dermatitis, which often extends into the underlying subcutis, producing a deeper-sited cellulitis. In the VLA study[13], analysis of 157 red squirrels with pox lesions, which were considered well enough preserved

for critical evaluation, the head was invariably affected with pox lesions on the eyelids, lips, ear and face. These had often expanded and coalesced to cover much of the face, which became alopecic. All but five (97%) had unilateral or bilateral conjunctivitis with a yellow ocular discharge and eyelid oedema. Lip lesions were seen in 113/157 (72%), usually extending onto the chin or nares. Lesions on the digits and feet were seen in 59/157 (37%). There was variable but usually lesser involvement of skin on neck, proximal limbs, scrotum, anus, dorsum and flanks. Preputial lesions were seen in 34% of males. Permanent skin scarring was not detected, but this was not unexpected as the survival rate from SQPV is very low.

The early acute uncomplicated primary histopathological lesions consist of multifocal areas of ballooning degeneration and spongiosis concentrated in the mid to upper epidermal layers. In contrast to pox infections in many other animal species, which are classically associated with strongly staining inclusion bodies, only small numbers of poorly defined weakly eosinophilic intracytoplasmic structures are seen with SQPV. As the disease progresses, there is extensive loss of infected epidermis on the skin surface, often extending into the hair follicles and associated adnexal glands. The marked mixed dermal inflammatory cell infiltrate is predominated by neutrophils and histiocytes closely associated with progressive erosion and ulceration. These ulcers quickly became colonized by opportunistic mixed bacterial species – predominantly staphylococci. A thick adherent layer of serocellular debris, bacteria and plant material often covers well-established lesions. The cellular reaction becomes more mononuclear in character in the subacute stages with prominent plasma cell populations. Deeper extension into the underlying subcutis occurs in many cases with resultant purulent or pyogranulomatous cellulitis tracking along fascial planes. Dermal and subcutaneous blood vessels within these areas show marked activation of endothelium, neutrophil diapedesis and adventitial cuffing. Thrombosis and associated haemorrhage are frequent sequelae related to local bacterial toxin production *in situ*. Early activation of angioblasts and fibroblasts may be detected if the animal survives long enough for attempts at healing to occur. A similar pattern of reaction is seen in infected non-hairy locations such as conjunctivae. Pox-associated lesions have never been convincingly described within internal organs.

Secondary staphylococcal infections of pox lesions and septicaemia are important sequelae, and of the study cases cultured, *Staphylococcus aureus* was isolated from 48 animals and *S. scuiri* from 19 cases. In total, 56 animals had staphylococcal infections of internal organs indicative of septicaemia. In a significant number of animals however, mild pox lesions and the absence of cultured bacteria makes it unclear precisely how the disease killed the animal. Affected animals lose weight and are significantly dehydrated. Of the164 study animals, 62 were judged in poor body condition, 86 were in normal condition (condition not recorded in 16).

The humoral antibody response appears protective to a degree, the few surviving animals have had very high enzyme-linked immunosorbent assay (ELISA) titres. How these antibodies neutralize virus is not known. The persistence of protective humoral antibody again is not known, or if it confers immunity to reinfection.

CLINICAL SIGNS AND TREATMENT

In the VLA survey[13], 70/164 red squirrels were found dead, 6 were found in-extremis and quickly died, 3 were reported as 'curled in a ball' lying on the ground for up to 6 hours before dying, 15 were euthanized on capture (or shot to prevent infection of others), and 39 were easily caught and submitted for veterinary treatment, but of these all but 6 eventually died – on average 5 days after submission. The 6 treated animals made a full clinical recovery and were rehabilitated in captivity. Of those listed that were observed alive, the clinical signs most frequently reported were dullness, weakness, lethargy, dehydration, anorexia and inanition. Blindness was reported in 2, however corneal opacity in a further 3 of those found dead, and in addition others in this group, in which the eyelids were matted together by thick dried crusts indicated effective blindness. Rarer signs were gasping and incoordination. Published reports described poor ability to climb, difficulty in moving, dullness and rubbing the facial lesions with their paws. The animals may remain for long periods around feeders and still occasionally eat. Captive animals can be briefly active and aggressive when handled.

Treatment is difficult, as gauntlet handling is essential for medication and therefore euthanasia must be considered at the outset. Rehydration is an immediate priority, and antibiotics effective against staphylococci are required; however, these bacteria can still be readily cultured from lesions well into the course of treatment. Interferon has been used frequently but with equivocal results. The patients do not adjust well to captivity, and animals appar-

ently responding to treatment for several days with resolving lesions may die unexpectedly. It is not known whether recovered animals remain infective; consequently they usually are not released.

DIAGNOSIS

Diagnosis is based on finding the range of typical clinicopathological findings in cases where both squirrel species co-exist. Typical pox lesions in the areas of skin mentioned are clearly visible and have characteristic pox lesion histopathology. Laboratory confirmation by electron microscopy of scabs and removed skin lesions has proven effective even in autolysed animals. Although electron microscopy is sensitive, PCR for viral nucleic acid is probably a more sensitive detection test. The virus has been cultured on cell lines. ELISA serology is used to detect antibody and has been useful in epidemiological studies, for example to assess whether populations of squirrels are naïve for the virus.

MANAGEMENT, CONTROL AND REGULATIONS

The current multi-agency strategy in the UK is to preserve the red squirrel in defined areas of good-quality red squirrel habitat (usually coniferous woodland) and in these to exclude the presence of grey squirrels by trapping. Surveillance for the disease is essential, and the local public in England strongly support surveillance, management and control initiatives. When SQPD occurs in an area, removal of garden feeders is generally advised. Disease modelling has predicted that a grey squirrel population control of >60% effective kill was needed to stop the decline in red squirrel populations[15]. Vaccine has a reasonable chance of conferring immunity. Repeated administration of killed vaccine may be necessary, and it is unlikely that the commercial orf vaccine would cross-protect. Live attenuated vaccine is unlikely to be licensed while live recombinant vaccine incorporating a gene coding for immunogenic portions of the SQPV may have a use, optimally by oral delivery.

There are no EU regulations and no international regulations regarding SQPV.

PUBLIC HEALTH CONCERN

No disease threats to humans or farmed species are apparent in the 80-year history of the disease; however, the precautionary principle should be applied and protective clothing used if animal handling is essential. There is considerable public concern over the disappearance of the once familiar red squirrel from the British countryside. The story of SQPD is a salutary lesson in the dangers of introducing non-native wildlife. Risk assessments on grey squirrels had they been done at introduction were unlikely to have predicted a virus like SQPV in the absence of disease expression.

SIGNIFICANCE AND IMPLICATIONS IN ANIMAL HEALTH

SQPD is a disease of conservation importance related to the threatened destruction of red squirrel populations where they, grey squirrels and SQPV occur together; there are no known threats to other species.

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MYXOMATOSIS AND OTHER LEPORIPOXVIRUS INFECTIONS

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Viruses within the genus *Leporipoxvirus* cause disease in rabbits and hares, including most importantly myxomatosis, caused by *Myxoma virus*, but also hare fibromatosis, caused by *Hare fibroma virus*. *Squirrel fibroma virus* of squirrels in North America is also included in this genus.

MYXOMATOSIS

AETIOLOGY

In native species of rabbit hosts in the New World (*Sylvilagus brasiliensis* and *Sylvilagus bachmani*), myxoma viruses (Brazilian strain in *Sylvilagus brasiliansis*, Californian strain in *Sylvilagus bachmani*) cause only a mild, self-limiting

cutaneous fibroma. However, in *Oryctolagus cuniculus*, the European wild rabbit (including the domestic rabbit), it causes a severe, often-fatal illness characterized by blepharoconjunctivitis and oedema around the head, particularly the eyes, and the perineal area, together with listlessness and fever; later (if the rabbit has not died in the first 48 hours) the classical subcutaneous swellings develop. The severity of disease varies with the strain of the virus and with host resistance. Other leporipoxviruses affecting leporids are *Hare fibroma virus* (see below), *Rabbit fibroma virus* (Shope's fibroma virus) in North America, which is cross-protective with myxomatosis, and *African hare fibroma virus* (not necessarily distinct from *Hare fibroma virus*).

EPIDEMIOLOGY

Originating in the New World, myxoma viruses are now endemic in Europe and Australia as well as North and South America. Both in Australia and Europe, Brazilian strains of the virus were introduced. Within Europe, myxoma virus was first introduced onto a private estate in France in 1951. Spread was rapid, and by the end of the following year it was found across France, Belgium, The Netherlands, Luxembourg, Germany, Spain and England. The main species infected in Europe is the European wild rabbit, although hare species are occasionally affected.

Initially, myxomatosis killed more than 99% of infected *Oryctolagus cuniculus*. However, over time, wild rabbits developed a degree of immunity, and less virulent strains of virus predominated over the most virulent strains, although as rabbits have become more resistant, somewhat more virulent strains of the virus have become more prevalent. Death rates in infected wild rabbits have decreased; nevertheless outbreaks continue to occur and studies on rabbits in Britain and Spain show that myxomatosis continues to regulate rabbit populations[17,18]. Strains of moderate virulence are most likely to be transmitted, as these have the longest periods of time for which infectious titres of virus are present in the skin.

In France, there is a definite seasonal pattern, with epidemics occurring in summer associated with high vector mosquito populations. In the UK, early reports found infected rabbits in all months of the year, but it is generally accepted that there is a major peak in prevalence in autumn, associated with the high rabbit populations following the breeding season, and a minor peak in spring (when fleas become more active). In Spain, myxomatosis is endemic with epizootics associated with recruitment of young, susceptible rabbits to the population; in a study in north-eastern Spain, a delay in breeding one year was followed by a delay in the annual myxomatosis outbreak[18].

The main host and species affected in Europe is the European rabbit. Individual hares (*Lepus europaeus* (European brown hare) and *Lepus timidus* (mountain hare)) can become infected and occasionally develop clinical disease. Reduction in mortality associated with myxomatosis in free-living *Oryctolagus cuniculus* in Europe (and in Australia) appears to be associated with natural selection for innate resistance in the wild rabbits, as well as emergence of reduced-virulence strains of the virus.

Within *Oryctolagus cuniculus*, susceptibility decreases with age; very young rabbit kittens die only 5–6 days after intradermal inoculation, with few external signs of disease (eyelid margins may be thickened), whereas adults survive longer (9–11 days post-inoculation) with development of obvious clinical signs: swelling of the head, closure of the eyes, conjunctival discharge, oculonasal discharge and development of multiple skin nodules. Susceptibility of kittens is reduced if they have passive immunity transmitted by their immune mother, and kittens with passive immunity survive longer if they do become infected[19]. In a study in a wild population in north-eastern Spain, clinical cases were seen only in young rabbits. All older rabbits maintained high antibody levels, while prevalence of antibodies in young rabbits was low initially, gradually increasing during the year[18]. There is no sex difference in susceptibility.

For rabbits infected with moderately virulent strains of myxoma virus, severity of disease is reduced and survival is higher in high environmental temperatures than in low temperatures (experimentally in Australia, survival was highest in rabbits housed at 37–39°C, lowest in those kept at 0–4°C). This appears to be associated with reduced spread of the virus from the primary lesion to the lymph nodes and reduced systemic spread of the virus. With highly virulent strains, mortality is high in all environmental conditions[20].

Transmission of myxoma virus by arthropod vectors is mechanical, occurring when a blood-feeding arthropod feeding on affected skin areas of an infected host (e.g. the eyelids, ear base), where virus is present in high concentrations, then feeds on susceptible individuals. A wide variety of parasitic arthropods – mosquitoes, fleas, simuliidae, biting flies, mites, lice etc. – can act as mechanical vectors. There is no evidence that the virus replicates in mosquitoes

or in any other arthropod. In most of the world, mosquitoes are the most important vectors, for example during summer epidemics in France, as well as in Australia. Within the UK, myxomatosis is transmitted mainly by the rabbit flea *Spylopsyllus cuniculus*, in which the virus can persist for several months. Mosquitoes may play a role in the UK, for example in transmission of the virus between sites, although in the early spread of the disease (1950s–1960s) human translocation of infected rabbits was considered more important. Virus is excreted in ocular discharges and from oozing skin lesions, and transmission by direct contact can occur in close-contact situations, which may occur within rabbit burrows. Transmission of the rare amyxomatous form, characterized by respiratory rather than skin infection, is thought to occur by direct contact[21].

PATHOGENESIS, PATHOLOGY AND IMMUNITY

Following skin inoculation of susceptible rabbits, initially the virus replicates locally in MHC-II (major histocompatibility complex) dendritic-like cells, within which it moves to the local draining lymph node within 24 hours, and replicates there mainly in T lymphocytes, high titres being found by 3 to 4 days post-infection. It then disseminates in leucocytes to the epidermis of the skin, mucocutaneous junctions, and to the lungs, spleen, testes and other internal organs. At this time, clinical signs are restricted to the primary lesion and slight reddening of the eyelids. Replication in the testes and epididymis results in orchitis, epididymitis and reduced fertility of surviving male rabbits. Myxoma virus produces several immunomodulatory proteins, resulting in apoptosis of lymphocytes within lymph nodes, and systemic immunosuppression. In resistant rabbits, although initial replication occurs in the skin at the site of inoculation, titres of virus in the lymph node draining the inoculation site are much lower than in fully susceptible individuals, and virus replication in tissues distal to this lymph node is controlled[22]. Immunosuppression due to myxomatosis may increase the susceptibility of affected rabbits to other diseases such as viral haemorrhagic disease[23].

The primary lesion, forming at the site of infection, is a subcutaneous mass, developing about 3–5 days after virus inoculation. Subcutaneous swellings are mucoid, not fibromatous. With amyxomatous myxomatosis in experimentally inoculated New Zealand white rabbits, external lesions included conjunctivitis, diffuse ear swelling, sometimes flat swellings on the ears or nose, sometimes with crust formation, but there were few gross internal lesions. Gross lung lesions, which were not always present, involved light to dark red consolidated areas of the anteroventral lobes. In some rabbits, splenomegaly was noted[24].

Microscopically, within the subcutaneous masses, large stellate mesenchymal cells proliferate within a seromucinous homogenous matrix; a few inflammatory cells are present. Endothelial cells show hypertrophy and proliferation; in severe disease, this results in narrowing of the lumen of capillaries and necrosis of the myxomatous lesions. In the epidermis overlying the masses, hyperplasia or degeneration may be noted. In the affected epidermis, as well as epidermal cells of the conjunctiva, intracytoplasmic inclusions may be found. Additional findings may include lymphoid depletion of the spleen (common), alveolar epithelial proliferation, hypertrophy and hyperplasia of reticulum cells in the lymph nodes and the spleen, focal necrosis, haemorrhage and proliferative vasculitis.

Antibodies can be detected starting from 8–13 days and remaining (as detected with complement fixation (CF)) for 6–8 months if there is no further contact with the virus.

CLINICAL SIGNS AND TREATMENT

Clinical signs include a primary subcutaneous mass as described above, blepharoconjunctivitis and oedema around the head, particularly the eyes (the swollen eyelids may be completely closed, with an obvious seropurulent discharge), and oedema of the perineal area, together with listlessness and fever. Multiple secondary skin lesions (subcutaneous swellings) develop, particularly on the eyelids, nares, lips and ears, as well as elsewhere on the body. In males, scrotal oedema is a common finding, with enlarged or shrunken testes. Severely affected rabbits appear blind and may show respiratory distress, but may continue to eat and drink. With some strains, skin lesions are flattened. In peracute disease (with very virulent strains and in very young kittens), rabbits may die before the development of external signs other than swelling of the eyelids and/or reddening of the conjunctiva; rabbits may die as soon as 5–6 days post-inoculation with virulent virus. The clinical course is longer when rabbits are infected with more attenuated strains. Exactly what causes death is unknown.

In the amyxomatous form of the disease, which has been described in wild rabbits in France, Spain and Belgium, mainly respiratory signs develop, with few, small skin nodules. In New Zealand white rabbits experimentally

infected with strains isolated from natural amyxomatous infections, blepharoconjunctivitis leading to closure of the eyes was the most prominent clinical sign, sometimes with diffuse swelling of the ears; one strain also produced acute respiratory distress in the rabbits, and abundant seromucous nasal discharge was noted.

Regarding treatment, for individual wild rabbits presented to rehabilitation centres, maintenance at moderately high temperature (28°C) may be beneficial, together with general nursing care, non-steroidal anti-inflammatory drugs, and antibiotics to reduce the risk of secondary bacterial infections. Use of corticosteroids is contraindicated, due to their immunosuppressive effects. Opioid analgesics do not appear to reduce signs of pain in affected rabbits. However, the prognosis is poor and euthanasia is often preferable on welfare grounds.

DIAGNOSIS

Diagnosis of myxomatosis is based on the typical clinical signs of the disease. In atypical cases, virus isolation and further identification are required. Examination of a portion of lesions using negative-staining electron microscopy reveals the typical poxvirus particles (but does not distinguish from Shope's fibroma virus). A portion of lesion (particularly the eyelids) is used for infection of appropriate cell culture such as rabbit kidney cells (primary culture or established cell line), with a typical cytopathic effect generally developing after 24–48 hours of incubation at 37°C (up to 7 days may be required for some strains).

In the agar gel immunodiffusion (AGID) test with standard antiserum, several, usually three or less, lines of precipitation appear with myxoma virus but only one line if Shope's fibroma virus is present (heterologous reaction)[21]. The fluorescent antibody test can also be used on frozen tissue sections. PCR is also useful for rapid diagnosis.

In addition to CF, the indirect fluorescent antibody test, AGID and ELISA may be used for serology; ELISA is the most sensitive[21].

MANAGEMENT, CONTROL AND REGULATIONS

When myxomatosis first occurred in England, control was attempted by local rabbit population elimination, but failed. In much of Europe, in situations in which wild rabbits were considered pests, control measures were not thought necessary. Experimentally in the UK, where the virus is transmitted mainly by rabbit fleas, insecticide treatment to eliminate the fleas also reduced myxomatosis in the treated population. Vaccines developed for use in domestic rabbits are effective in reducing mortality when wild rabbits (particularly juveniles) are vaccinated, but their use has been limited by the need to catch and hand-inject rabbits[25]. It has been suggested that vaccination campaigns have little effect and are not cost-effective[26]. Recombinant vaccines against both myxoma virus and rabbit haemorrhagic disease (RHD) (based on naturally attenuated myxoma virus, also expressing RHD major capsid protein VP60), with limited rabbit-to-rabbit transmissibility, have been developed. Laboratory trials have demonstrated safety and efficacy, with safety and positive serological results in a limited field trial[27]. Insecticide treatment of rabbit warrens may also be useful[23].

Myxomatosis is a very serious disease of domestic rabbits as well as wild rabbits, and it is one of three diseases of leporids notifiable to the World Organisation for Animal Health (OIE).

PUBLIC HEALTH CONCERN

Leporipox viruses do not infect humans.

SIGNIFICANCE AND IMPLICATIONS FOR ANIMAL HEALTH

In many parts of Europe, reductions in wild rabbit populations due to myxomatosis when it was first introduced were considered to be beneficial, reducing crop losses. However, there have been widespread, complex and long-term effects on ecological communities. Of particular importance is the effect on European rabbits in their original habitats in the Iberian Peninsula. Here, rabbits are an important staple prey for a variety of mammalian and avian predators, including endangered species such as Iberian lynx (*Lynx pardinus*) and Spanish imperial eagles (*Aquila adalberti*). Reductions in rabbit populations due to myxomatosis and, more recently, RHD, have had severe negative consequences for several predators in Spain, exacerbated in some cases by increased persecution of predators by rabbit hunting interests[28].

HARE FIBROMA VIRUS INFECTION

This *Leporipoxvirus* infection causes hare fibromatosis, a disease that has been reported uncommonly in European

brown hares in France, Germany, Italy and the UK. This disease was first recognized in the early 1900s, decades before the introduction of myxomatosis into Europe. Unlike myxomatosis in the European rabbit, but similar to that disease in its natural hosts, mortality is low, with most affected individuals showing spontaneous recovery. Morbidity may be high; during an outbreak on a hare farm in Italy in 2001, about 25% of the population was affected[29]. Affected individuals can act as sources of virus for a month or more and transmission is thought to occur mechanically via arthropods but may also occur through direct contact, with skin micro-lesions or trauma predisposing to infection. Outbreaks in wild hares have occurred in late summer and autumn only[29–31].

Lesions are solid skin tumours, 1–3 cm diameter, single or multiple, mainly on the ears, the head (particularly the eyelids) or the legs, but also found on the flank. The tumours spontaneously reduce in size and crust over after 4 to 6 weeks, and may spontaneously detach; at this stage, dry crusts or bleeding scars may be seen. Gross pathology reveals nodular swellings, with the base up to 3 cm in diameter and sometimes forming finger-like projections 4–5 cm long. Histologically, connective tissue proliferation is prominent, containing large spindle-shaped or star-shaped fibroblasts with large nuclei and in the cytoplasm, periodic acid-Schiff (PAS)-positive inclusions. Vascular hyperplasia is present, the new blood vessels having dilated walls and containing large numbers of red blood cells. Electron microscopy reveals typical poxvirus particles with randomly arranged surface tubules, both in crusted material and more abundantly in homogenized material from a nodular tumour. In inoculated embryonated chicken eggs, focal 1–2 mm yellowish-white pocks developed on the chorioallantoic membrane (CAM) after three passages, with poxvirus particles visible in material from the CAM[31][29].

SEALPOX AND CETACEAN POX

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The viruses causing sealpox are parapoxviruses, although they are not yet accepted in virus taxonomy. A recent study has suggested that there are several lineages of cetacean poxviruses and that they are distinct from recognized poxvirus genera[32].

SEALPOX

Sealpox lesions were first reported in captive Californian sea lions (*Zalophus californicus*) in Canada in 1969, and the ultrastructural morphology of the virus was described the following year[33]. Sealpox is generally a self-limiting disease characterized by localized skin lesions, but some severe cases have been seen. Within Europe, pox virus lesions have been described in grey seals (*Halichoerus grypus*) from the Netherlands[34] and the UK[35–38] and harbour seals (*Phoca vitulina*) from the German North Sea[39].

Lesions vary from a few localized lesions on the skin of the head and neck, one or two digits, or the oral cavity, to multiple lesions along the mucocutaneous border or the lips, and multiple lesions, becoming confluent, along much of the ventral neck and body. Small lesions heal spontaneously in about 6 weeks, whereas in extensive cases, skin swellings develop to circumscribed lesions 1.5–2.5 cm diameter and become suppurative. Oral lesions in a severe case started as ulcers, which, as they started healing, were replaced by roundish granulomatous lesions about 1.5 cm diameter[36]. Histologically, the *stratum corneum* shows hyperkeratosis and parakeratosis, and the *stratus spinosum* shows hypertrophy, hyperplasia, cytoplasmic vacuolation and, in degenerating cells, large eosinophilic intracytoplasmic inclusions, mainly at the margins of lesions. The underlying dermis is infiltrated by neutrophils, with fibroblastic hyperplasia also evident.

Diagnosis is initially based on the clinical appearance of the lesions and on histopathological characteristics as described above. In most cases, the virus involved has been tentatively identified as a parapoxvirus, based on appearance from electron microscopy studies. In one case, both parapoxvirus-like and orthopoxvirus-like particles were seen and an orthopoxvirus was isolated[34]; in another case, calicivirus particles were detected alongside typical parapox viruses. Parapoxvirus-specific antibodies were detected in sera of harbour and grey seals from the UK and the Netherlands, ringed seal (*Phoca hispida*) from the Netherlands, Baikal seal (*Phoca siberica*) from Lake Baikal, Russia, and grey and harbour seals from Canada[38]. In recent years, partial genetic characterization has confirmed a parapoxvirus, distinct from parapoxviruses of ruminants, in European seals, Antarctic seals and in Pacific and Atlantic pinnipeds from the coasts of North America[39,40].

Seal parapoxvirus infection is a potential zoonosis. In one outbreak in grey seals in Canada, two handlers

developed lesions on their fingers similar to 'milker's nodules', which can occur with other parapoxvirus infections in humans; the lesions were self-limiting, although recurrent over several months in one individual[41].

CETACEANPOX

Poxvirus infections of a variety of cetaceans result in lesions of the skin, which are usually referred to as 'tattoo lesions'. These have been seen in cetaceans worldwide, generally as an incidental finding, and have occasionally been described in cetaceans in European waters. Tattoo and paisley (print-like) lesions, similar to those confirmed as poxvirus infections in other cetaceans, were described as incidental findings in five of 41 harbour porpoises (*Phocoena phocoena*) found on British coasts. In the tattoo lesions, a dark line about 1 mm wide enclosed an area, slightly depressed and sometimes containing small punctiform or linear black ulcers, while the paisley lesions involved several concentric dark lines with normal-appearing skin between the lines. Lesions were usually 2–5 cm in diameter, and irregular in shape. Histologically, cells showed ballooning and vacuolation, particularly in the *stratum intermedium*, with some of the cells in this layer and many cells just superficial to the germinal layer, containing pale, spherical or irregular intracytoplasmic inclusion bodies[42]. Similar tattoo lesions, 2–5 cm diameter, were described in three of four striped dolphins (*Stenella coeruleoalba*), one white-beaked dolphin (*Lagenorhynchus albirostris*) and one long-finned pilot whale (*Globocephala melaena*)[43] from British waters. In 445 harbour porpoises from the German North Sea and Baltic Sea, no pox lesions were found[44]. In a study of 55 harbour porpoises from the coasts of Belgium and northern France, six animals were noted to have 1–3.5 cm diameter white to grey lesions with a grey to black border, on the head or body[45]. Diagnosis of cetaceanpox is based on the typical histopathological appearance of the lesions (thickened stratum externum, ballooning degeneration of the *stratum intermedius*, with characteristic eosinophilic intracytoplasmic inclusions) and finding of poxviruses on electron microscopy.

COWPOX

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The virus of cowpox is somewhat misnamed. Although it can cause lesions in cattle, the main hosts are thought to be small rodents, and it has been suggested that the true reservoir hosts may be bank voles (*Microtus agrestis*) and field voles (*Clethrionomys glareolus*). The geographical area within which fully characterized cowpox virus has been isolated is 'an area approximately bounded by Norway and Northern Russia, Moscow, Turkmenia, Northern Italy, France and Great Britain'[46]. There are unpublished reports of cowpox in cats in Ireland[47], where voles were historically absent until the recent accidental introduction of bank voles. It has not been reported in the Iberian Peninsula or in southern Italy, also areas where these voles are not found. Further east, cowpox virus has been isolated from wild great gerbils (*Rhombomys opimus*) and yellow susliks (*Citellus fulvus*) in Turkmenia, from the red-tailed gerbil (Libyan jird, *Meriones libycus*) in Georgia and from a root vole (*Microtus oeconomus*) in northern Russia (as well as from laboratory rats in Russia).

In the UK, there is serological evidence of infection in bank voles, field voles, wood mice (*Apodemus sylvaticus*) and house mice (*Mus musculus*), with seroconversion peaking in autumn[48,49]. In Norway, serological evidence suggests that bank voles, wood mice and Norway lemmings (*Lemmus lemmus*) may be reservoir hosts (serological prevalences 17%, 30% and 56%, respectively), with occasional seropositive individuals in field voles, brown rats (*Rattus norvegicus*) and common shrews (*Sorex aranus*)[50]. Additionally, orthopoxvirus DNA was detected in one or more organs, particularly the lungs, of bank voles, grey-sided voles (*Clethrionomys rufocanus*), northern red-backed voles (*Clethrionomys rutilus*), wood mice and common shrews in Norway[51]. A survey in Belgium detected antibodies to orthopoxvirus only in bank voles and wood mice, not in any carnivores or ungulates[52] (although orthopoxvirus antibodies were detected in foxes (*Vulpes vulpes*) in the German state of Brandenberg[53].

Experimental studies support the view that the orthopoxvirus found in voles and other small mammals in Europe is cowpox virus and not ectromelia virus, because bank voles are resistant to ectromelia virus (no clinical signs nor seroconversion following inoculation). Inoculation of cowpox virus into voles produced only localized lesions (footpad swelling, sometimes ulceration) and seroconversion[54].

Although previously seen as a disease of dairy cows, cowpox is now seen as a clinical disease mainly in domestic cats and occurs most commonly in autumn. Typically, a single primary lesion (ulcerated nodule with crust) is seen

on the face or forelimbs of the cat, with secondary lesions appearing 1 to 2 weeks later, as macules or small erythematous nodules, which ulcerate then scab over and heal; scars are left. In about 20% of cases there are concurrent oral lesions. Sometimes there are systemic signs – low-level pyrexia, depression and occasionally diarrhoea, with pneumonia a less common but more serious complication. Atypical presentations have included oral lesions without skin lesions, large areas of skin necrosis, severe oedema and loss of digits[55]. Cowpox has not been reported as a cause of clinical disease in wild European felid species, but it has been seen in various large felids in zoos and should be considered as a possible diagnosis if similar lesions are seen on lynx (*Lynx lynx* or *L. pardinus*) or wildcats (*Felis sylvestris*) in the areas where the ranges of these species coincide with the ranges of the reservoir host rodents. Antibodies to orthopoxviruses (probably cowpox virus) have been detected in lynx (*Lynx lynx*) in Sweden and Finland[56].

Diagnosis of cowpox in cats is based on characteristic histopathological findings with eosinophilic intracytoplasmic inclusion bodies in keratocytes (e.g. on biopsy), with confirmation by immunohistochemistry, electron microscopy, PCR or culture.

Cowpox is a zoonosis, usually acquired from cats or from rodents, with one or two cases per year in humans in the UK, for example. Usually only a single lesion develops, on the hands or face, although in about 25% of cases there are multiple lesions. Occasional severe generalized infections occur and fatalities have been reported.

Natural infection does not cause obvious clinical disease in rodents; however, it is possible that infection may have a complex effect on rodents at the population level, through effects on individual survival and fecundity. Experimentally infected bank voles and wood mice showed a significant delay in the onset of reproduction[57] and reduced survival has been noted in naturally infected field voles[58], although an increased survival was noted in infected bank voles, possibly due to host resources being directed towards immune response and away from reproduction[59]. It has also been suggested that increased infection rates in rodents following population peaks may be associated with both increased contact rates and increased vulnerability to infection associated with higher host abundance[58,60].

CONTAGIOUS ECTHYMA

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Contagious ecthyma (CE) (synonyms: orf, contagious pustular dermatitis) is a viral skin disease caused by the *Orf virus* or other closely related viruses within the genus *Parapoxvirus* (Family *Poxviridae*). The disease is common among domestic sheep and goats worldwide, and has been diagnosed in several wild species and humans (it is a zoonosis).

The distribution and host range among wild artiodactyls have been summarized by Robinson & Kerr[61]. Among free-living wild species in Europe, CE has been reported in ibex (*Capra ibex*), chamois (*Rupicapra rupicapra*)[62] and southern chamois (*Rupicapra pyrenaica*)[63] in the Alps and Pyrenees, and in a small Norwegian population of musk ox (*Ovibos moschatus*)[64]. Disease outbreaks have also been reported in semi-domesticated reindeer (*Rangifer tarandus*) in Fennoscandia[65].

Experimentally, calves of moose (*Alces alces*), wapiti (*Cervus elaphus nelsoni*), white-tailed deer (*Odocoileus virginianus*) and other deer species have been shown to be susceptible to CE infection, but the lesions were mild[61]. Presumed infection with parapoxvirus has been reported in free-ranging red deer (*Cervus elaphus*) calves from Germany with the 'labial form' of CE[66]. A pustular dermatitis caused by a unique parapoxvirus has occurred in farmed red deer in New Zealand, but is not reported from Europe.

The parapoxviruses causing CE in musk ox and reindeer in Norway are genetically similar to *Orf virus* isolates from sheep[64,67], whereas the Finnish parapoxvirus isolates from reindeer have been grouped with either *Orf virus* or bovine *Pseudocowpox virus*[68].

Young individuals seem to be more susceptible to CE than adults, and they develop lesions of greater severity; however, older animals are also affected. Morbidity can approach 100%, but mortality is usually low in uncomplicated cases. Secondary bacterial or fungal infections cause increased mortality[64,69].

Transmission of parapoxvirus is directly by contact with affected animals or indirectly through contact with infectious virus in the environment. Virus is shed in scab material and can survive in dry scabs for months or years in a dry environment sheltered from rainfall[69]. The source of infection in free-ranging wild species is usually difficult to identify, but transmission from domestic sheep and goats that graze on the same pastures as wild species is a possibility. Also, subclinical parapoxvirus infections within the wild populations themselves might cause clinical CE outbreaks if triggered by reduced nutritional status, immunosuppression and/or environmental predisposing factors

such as stress, handling and fencing. A dense population with high stress levels, in combination with many calves immunologically naïve to parapoxvirus, were given as a possible explanation for trigging a severe outbreak of CE among free-ranging musk ox in Norway[64].

Orf virus is epitheliotrophic and infects via damaged skin and replicates in epidermal keratinocytes. The skin infection progresses from erythema to papule, vesicle, pustule and then to scab within a week after infection. Additional pustules and scabs form during the following days. The lesions can be highly proliferative, developing into wart-like papillomas. Primary lesions usually resolve within 4 weeks, usually without scar formation. In uncomplicated cases, CE is febrile and self-limiting[69].

Pustular scabby lesions on the lips and in the skin around the mouth and nostrils are characteristic for CE, but lesions may also develop in the skin or mucosa on other parts of the body such as the buccal cavity (hard palate, tongue, gingiva), limbs (coronet, distal limb), udder, genital skin and oesophagus. Lesions are seldom found in other internal organs[69]. There is variation in the severity of outbreaks and in the anatomical distribution of lesions. In severe cases, large coalescing, papillomatous, cauliflower-like lesions are seen (Figure 13.1). The lesions often ulcerate, and secondary bacterial or fungal infection may occur, often with subsequent lymphadenitis. Infestation with fly larvae (cutaneous myiasis) is also seen. Severe lesions on the mouth/oral mucosa and feet interfere with normal feeding and locomotion, causing lameness, starvation and loss of body condition[64].

FIGURE 13.1 A musk ox calf from a free-ranging population in Norway suffering from contagious ecthyma. Severe, multiple to coalescing, ulcerated, papillomatous lesions are seen on the lips and the skin around the mouth.

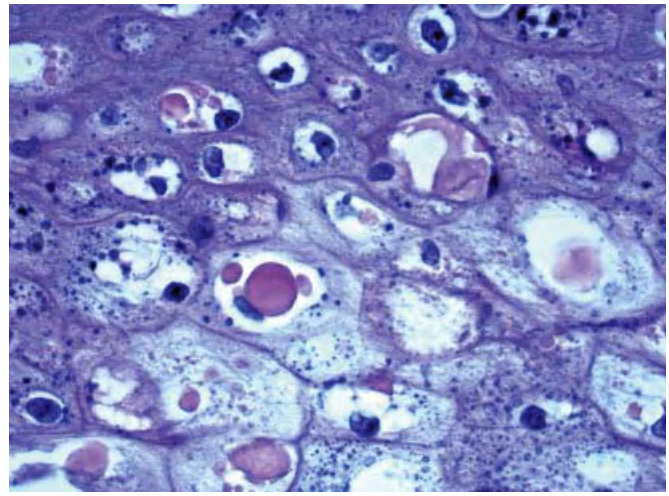

FIGURE 13.2 Microphotograph of a contagious ecthyma lesion in the skin of a musk ox calf showing part of the epidermis. Intracytoplasmic eosinophilic inclusion bodies are seen in degenerating keratinocytes. Haematoxylin and eosin stain. Magnification: 630×.

The histopathological picture seen in CE is a pustular dermatitis characterized by epidermal proliferation, degenerating keratinocytes with intracytoplasmic eosinophilic inclusion bodies (Figure 13.2), vesicopustules, microabscesses and multifocal ulcerations in the epidermis which is covered by a serocellular crust[64].

In sheep, there is an *Orf virus*-specific cellular and humoral immune response after infection, but immunity seems to be short-lived. Thus, the *Orf virus* can repeatedly infect sheep and goats; however reinfection lesions are smaller, less severe and resolve more rapidly than primary lesions[70]. For further reading regarding *Orf virus* infection and host immunity, including viral virulence and immunomodulatory factors the reader may consult Haig (2006)[70].

A diagnosis of CE is made by a combination of clinical signs, pathological findings and detection of the agent. Electron microscopy of tissue samples from CE lesions shows typical parapoxvirus particles and is a reliable method if sufficient intact virus particles are present. PCR methods and sequencing of viral DNA are now becoming the tool of choice for detection of the virus.

A disease outbreak might contribute to a transient population reduction and a temporary change in the age distribution within the population, as seen in a free-ranging musk ox population in Norway. The long-term impact on the population, however, appeared negligible[64]. Severely

affected wild animals should be euthanized for animal welfare reasons.

OTHER POXVIRAL DISEASES

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Sheep pox and goat pox (capripox) are caused by the *Sheeppox virus* and *Goatpox virus* (genus *Capripoxvirus*). These are endemic in Africa north of the equator, the Middle East and parts of Asia and there have recently been several incursions into southern Europe (Bulgaria, Cyprus, Italy, Greece). In native breeds of sheep or goats, these viruses usually cause only a few lesions, but in susceptible breeds there is high mortality. Sheeppox and goatpox are not infectious to humans[71]. Capripox infections have not been reported in wild Caprinae in Europe. Lumpy skin disease of cattle (caused by *Lumpy skin disease virus*) has to date been seen only in Africa.

Swinepox is a worldwide sporadic infection of domestic pigs; it can spread to wild boar (*Sus scrofa*) if they come into close contact with infected pigs. A juvenile male wild boar in Austria showed abnormal behaviour and multiple papules, pustules and scabs up to 'cherry size'. Ballooning degeneration of keratinocytes was visible on histopathology, an orthopoxvirus was visible by negative contrast electron microscopy, and PCR and sequencing confirmed suipoxvirus. It was considered that the numerous lice on the boar may have promoted the infection[72]. Horsepox has not been reported for decades, and there are no reports in wild horses.

Contagious mucocutaneous dermatitis has been described in the mountain hare (*Lepus timidus*). A poxvirus was detected in mountain hares in Finland with acute viral dermatitis. In fully developed acute lesions, although there was only mild epidermal oedema and mild superficial perivascular infiltrate of lymphocytes, the hyperplastic epidermis and follicular infundibulum contained clusters of hydropic, swollen, pale keratinocytes; within these were numerous large intracytoplasmic inclusion bodies. Electron microscopy revealed ovoid virions typical of poxviruses: 300 × 130–150 nm with a biconcave nucleocapsid core, two lateral bodies and an outer lipoprotein capsule. Lesions later became necrotic with variable inflammation, and neutrophils and bacterial cocci in the crusting exudates (secondary pyoderma). Variable dermal fibrosis, epidermal hyperplasia and mild to moderate infiltrate of mainly plasma cells (some lymphocytes and neutrophils) were noted in chronic advanced lesions. Finally, cutaneous scarring developed[73].

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CHAPTER

14

ADENOVIRUS INFECTIONS

NICOLA DECARO, CANIO BUONAVOGLIA, KEVIN EATWELL, KÁROLY ERDÉLYI AND J. PAUL DUFF

INTRODUCTION

NICOLA DECARO AND CANIO BUONAVOGLIA

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Adenoviruses belong to the family *Adenoviridae*, which is divided into four genera, namely *Mastadenovirus*, *Aviadenovirus*, *Atadenovirus* and *Siadenovirus*. These have 19, 6, 4 and 2 serogroups respectively, each organized in several serotypes. The genus-, serogroup- and serotype-specific antigen determinants are localized on the virion surface structures (hexons and fibres). The genus *Mastadenovirus* includes adenoviruses infecting mammals – for example, human adenoviruses, canine adenoviruses, most ruminant adenoviruses and squirrel adenovirus. Most adenoviruses of birds, including fowl adenoviruses and goose adenovirus, belong to the genus *Aviadenovirus*. Viruses of mammalian (some ovine and bovine adenoviruses, possum adenovirus and cervid adenovirus) and avian (*Duck adenovirus 1*, or egg drop syndrome virus) origin with an exceptionally large content of adenine and thymine in their genome constitute the genus *Atadenovirus*, whereas genus *Siadenovirus* comprises adenoviruses of amphibians (*Frog adenovirus 1*) and birds (*Turkey adenovirus 3*, or marble spleen disease virus).

Adenoviruses are medium-sized (80–110 nm in diameter), naked icosahedral particles, with a linear, double-stranded DNA of about 35,800–36,200 nucleotides. The capsid is formed by 252 capsomeres consisting of 240 hexons and 12 pentons at the vertices of the icosahedron. To each penton base capsomere one or two antenna-like fibres are attached, which bind to cell receptors and which, in some adenoviruses, have haemagglutination activity. Five different proteins are associated with the viral DNA, forming a nucleoprotein core. The genome encodes for at least 10 polypeptides, including the structural proteins forming the capsid, fibres and core, and a few non-structural proteins, such as DNA-associated enzymes, the viral replicase and an internal protein. Adenoviruses replicate in the cell nuclei, and their replication is not always associated with a clear cytopathic effect. However, basophilic intranuclear inclusion bodies are observed consistently in the infected cells. Adenoviruses are stable at room temperature and between pH 5–6, and are resistant to lipid solvents. They are usually inactivated at 56°C for 10–15 minutes and by disinfectants containing chlorine and formol.

Adenoviruses can cause various clinical signs involving the respiratory system, gastroenteric and urinary tracts, eyes, central nervous system, spleen and liver. Some avian and bovine adenoviruses are able to induce tumours in

Infectious Diseases of Wild Mammals and Birds in Europe, First Edition. Edited by Dolores Gavier-Widén, J. Paul Duff, and Anna Meredith.

newborn hamsters infected experimentally, but their oncogenicity is usually absent in the natural hosts.

INFECTIOUS CANINE HEPATITIS

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Infectious canine hepatitis (ICH), also known as Rubarth's disease or hepatitis contagiosa canis, is a highly contagious, often fatal, disease of dogs and wild carnivores caused by *Canine adenovirus 1* (CAdV1). Clinical signs were first observed in farmed red foxes (*Vulpes vulpes*), and the disease was named epizootic fox encephalitis because of the neurological disorders observed in the affected animals. ICH was reported in dogs a few years later and, based on the similar clinical course and pathology, Rubarth suggested a common aetiology for the two diseases. The aetiological agent, CAdV1, was isolated a decade later and attenuated through passages on canine and swine cell lines to produce a vaccine. In 1961, another adenovirus of dogs, *Canine adenovirus 2* (CAdV2), was isolated from dogs with laryngotracheitis or tracheobronchitis. The isolate, strain Toronto A26/61, was initially considered to be an attenuated strain of CAdV1, and only subsequently was it proposed as the prototype of a distinct canine adenovirus, designated CAdV2.

AETIOLOGY

CAdV1, a member of the genus *Mastadenovirus* within the family *Adenoviridae*, is highly stable in the environment, surviving several days at room temperature and remaining infectious for months at temperatures below 4°C. The virus is also resistant to various chemicals such as chloroform, ether, acid and formalin. Successful virus inactivation can be obtained with some disinfectants such as iodine, phenol and sodium hydroxide, or with thermal treatment at 60°C for 5 minutes.

CAdV1 is genetically and antigenically related to CAdV2, having a 75% identity at the genetic level and inducing cross-protecting antibodies in the hosts. Although CAdV1 and CAdV2 are strictly related, they have different tissue tropisms and pathogenetic roles. Vascular endothelial cells and hepatic and renal parenchymal cells are the main targets of CAdV1, whereas the respiratory tract epithelium and, to a limited degree, intestinal epithelium, are the targets of CAdV2. CAdV1 is responsible for a severe, often fatal, disease of domestic dogs and wild carnivores. CAdV2 is associated with infectious tracheobronchitis (also known as kennel cough or canine infectious respiratory disease), an acute, highly contagious respiratory disease of dogs affecting the larynx, trachea, bronchi, and occasionally the lower respiratory tract, that is typically a complex of diseases caused by viral pathogens (CAdVs, canine herpesvirus, canine parainfluenza virus, reoviruses) in association with bacteria, mainly *Bordetella bronchiseptica* and *Mycoplasma* spp. The two CAdV types display different haemagglutination patterns: CAdV1 is able to agglutinate erythrocytes of different species, including birds, whereas CAdV2 haemagglutination is restricted to human and rat erythrocytes.

CAdV1 is easily cultivable on a variety of cell lines of different species, including dog, ferret, raccoon and pig. However, the most suitable cell lines used for virus isolation are dog kidney cells, such as Madin–Darby canine kidney (MDCK) cells. Like other adenoviruses, CAdV replicate in the nuclei of the infected cells, inducing intranuclear inclusion bodies.

EPIDEMIOLOGY

CAdV1 has a worldwide distribution in domestic dogs, and several non-domestic carnivore species are susceptible to infection and/or disease. In past decades, epizootic fox encephalitis was very common in European red foxes (*Vulpes vulpes*) housed on northern American farms. In the wild, cases of overt disease have been reported only sporadically in foxes and other wild carnivores belonging to the families Canidae, Ursidae, Procyonidae and Mustelidae. Recently, CAdV1 was isolated in the UK from the internal organs of three free-ranging European red foxes, with gross lesions and histological changes highly suggestive of epizootic fox encephalitis. A serological investigation revealed that CAdV1 infection was probably responsible for a dramatic epizootic in red foxes in England and Scotland[1]. Free-ranging red foxes display the highest seroprevalence rates against CAdV, with values of 35% in Germany[2], less than 20% in Spain[3] and 59.6% in Norway[4]. Arctic foxes (*Vulpes lagopus*) were found to be seropositive to CAdV, with a seroprevalence of 37.8% in the high-arctic islands of Svalbard, and a 67.7% seroprevalence for CAdV was

found in the joint Swedish–Norwegian wolf (*Canis lupus*) population[4]. Antibodies to CAdV were also detected in free-ranging mustelids in south-western France, including European mink (*Mustela lutreola*), polecats (*Mustela putorius*), American mink (*Mustela vison*) and stone martens (*Martes foina*), with seroprevalences ranging from 2 to 10%[5]. A recent serological survey carried out in Spain showed the presence of specific antibodies in the Iberian lynx (*Lynx pardinus*) and Egyptian mongoose (*Herpestes ichneumon*)[3]. ICH was also reported in a captive Eurasian river otter (*Lutra lutra*)[6]. Outbreaks of ICH were also observed in brown bears (*Ursus arctos*) in Hungary[7]. A serological survey carried out on 22 European brown bears from Croatia detected the presence of antibodies against human adenovirus in one animal[8], but a possible cross-reaction with CAdV1 cannot be ruled out definitively. Outside Europe, reports of ICH in non-domestic carnivores are much more numerous.

CAdV1 is shed by animals with active infection through all biological fluids, including saliva, faeces and urine. Recovered animals do not shed CAdV1 in the saliva and faeces, but domestic dogs are recognized to shed the virus in their urine for up to 9 months, so feral dogs may contribute to the spread of the virus to wildlife. Although it is not known how long viruria lasts in non-domestic carnivores, a potential role of these animals as long-term shedders should be considered. The mortality rates are about 10–30% in domestic dogs and farmed foxes, with peaks of 80% mortality in pups. The high seroprevalence rates found in unvaccinated wildlife may account for a lower pathogenicity of CAdV1 in free-ranging non-domestic carnivores. Therefore, these animals may act as reservoirs of the virus in nature, although there are no specific studies assessing the epidemiological role of non-domestic species.

PATHOGENESIS, PATHOLOGY AND IMMUNITY

The main route of infection is by ingestion of virus-contaminated material, although CAdV1 may also be contracted by inhalation of aerosol produced during coughing from animals with CAdV1 respiratory bronchopneumonia. After ingestion or inhalation of infected material, CAdV1 replicates primarily in the tonsils and spreads to the organs and central nervous system via the bloodstream. The main target cells for viral replication seem to be the same regardless of the host species, i.e. the endothelial cells of blood vessels and the hepatocytes. Five days after infection, the virus can be isolated from lungs, liver, spleen, kidney and brain of infected animals. Virus replication in parenchymal cells of liver and lungs and in reticulo-endothelial cells of liver and lymphoid tissues together with infiltration of leucocytes leads to the early appearance of necrotic lesions. CAdV1 infection can cause haemorrhages in many tissues through different pathogenic mechanisms, mainly through extensive vascular damage, impaired hepatic synthesis of clotting factors, thrombocytopenia and disseminated intravascular coagulation. Neurological signs are uncommon in domestic dogs but very frequent in non-domestic carnivores, mainly foxes, as a consequence of cerebral vasculitis. Extensive endothelial damage in the central nervous system may cause hyperacute death without the appearance of observable clinical signs, with the exception of hyperexcitability and convulsions.

An adequate immune response is mounted from 7 days after infection, with production of neutralizing antibodies that are able to clear the virus from the blood and liver, so most animals may recover spontaneously. However, high-titre antibodies can bind to viral antigens, forming immune complexes that precipitate in the renal glomeruli and irido-corneal angle and cause glomerulonephritis, corneal oedema and uveitis, which are common complications. In the late stages of CAdV1 infection, the virus disappears from the glomerular endothelium but can persist in the tubular epithelium for 6–9 months, causing long-term viruria.

Recovery from infection in domestic dogs and foxes is followed by a long-term, probably life-long, protection against CAdV1 disease and infection.

At necropsy, animals that die from hyperacute disease may appear in good nutritional state and with few gross lesions. In acute cases, external examination can reveal ecchymoses and petechial haemorrhages, whereas the abdominal cavity contains abundant clear or serosanguineous fluid. The liver is enlarged, yellowish brown, congested and spotted with small circular areas of necrosis; the gallbladder appears thickened, oedematous and greyish or bluish-white opaque in colour. Oedema of the gallbladder wall is a consistent finding. Congestion and haemorrhagic lesions are observed in the spleen, lymph nodes, thymus, pancreas and kidneys. The lungs show patchy areas of consolidation due to bronchopneumonia. Haemorrhagic enteritis can also be observed.

Histological changes in the liver are characterized by centrolobular necrosis along with neutrophilic and mononuclear cell infiltration and intranuclear inclusions in the Kupffer's cells and hepatocytes. Intranuclear inclusion bodies can also be seen in the vascular endothelium, renal glomeruli and urinary bladder. Multifocal areas of congestion, haemorrhage and leucocyte infiltration can be observed in several organs, mainly in liver and kidney, owing to vascular damage and inflammation. Non-domestic carnivores usually display multifocal haemorrhages in the central nervous system. Interstitial nephritis and iridocyclitis with corneal oedema are present in dogs recovering from ICH, whereas there is little information in wildlife in Europe about the histological changes associated with CAdV1 immune-mediated disease.

CLINICAL SIGNS AND TREATMENT

Clinical signs of ICH are well known in dogs and farmed foxes, whereas there are only sporadic reports in non-domestic carnivores. Virus detection in the faeces of healthy free-ranging wild animals suggests that non-domestic carnivores may become infected without displaying overt disease. Nevertheless, some carnivores (foxes) may be affected by fulminating ICH, especially when a hyperacute neurological form occurs.

The incubation period is usually 4–6 days after ingestion of infectious material. The earliest clinical signs in dogs are represented by a biphasic hyperthermia with temperatures commonly exceeding 40°C. Common clinical signs in all animal species are loss of appetite, depression, vomiting, diarrhoea (often haemorrhagic), abdominal pain, coughing and harsh lower respiratory sounds. Pulse and respiratory rates are accelerated. A serous or purulent ocular and nasal discharge may be present. Haemorrhagic diathesis, as a consequence of the vascular damage, is demonstrated by widespread petechial and ecchymotic haemorrhages and epistaxis. Neurological signs consisting of hyperexcitability, nystagmus and convulsive seizures are rare in dogs but very common in foxes and other non-domestic carnivores. In foxes, the early involvement of the central nervous system may lead to sudden death with few, or no, overt clinical signs. Corneal opacity ('blue eye') and interstitial nephritis may occur 1–3 weeks after recovery as a result of the tissue deposition of immune complexes. However, these immune-mediated clinical signs, commonly occurring in domestic dogs, are infrequently seen in wild carnivores. Haematological changes have been described in detail for dogs, whereas tests in non-domestic carnivores have not been reported. Leucopenia with total white blood cell (WBC) counts <2,000 cells per μl of blood (mainly due to a decrease in neutrophil count) and coagulation disorders associated with disseminated intravascular coagulation (DIC) (thrombocytopenia, altered platelet formation and prolonged prothrombin time) are the most common haematological findings in domestic dogs. Increase in the serum transaminases can be seen only in the severe forms of the disease. Proteinuria (albuminuria) can reach values of >50 mg/dl due to immune-mediated glomerulonephritis.

Treatment of ICH is based primarily on treating the clinical signs together with supportive therapy. Dehydration and DIC require administration of fluids, plasma or whole blood transfusions and anticoagulants. Hyperammonaemia due to hepatic and renal damage can be corrected by oral administration of non-absorbable antibiotics and lactulose, and by oral or parental administration of potassium and urinary acidificants (e.g. ascorbic acid). Supportive therapy may facilitate the clinical recovery of the infected animals, provided that there is sufficient time for hepatocellular regeneration. Broad-spectrum antibiotics can be useful to control bacterial complications.

Passive immunization through administration of hyperimmune sera does not have a therapeutic effect in clinically ill animals, as the onset of clinical signs quickly succeeds to viraemia, and in that stage of infection circulating antibodies are ineffective. By contrast, hyperimmune sera administered to uninfected animals may prevent further spreading of the virus to other animals.

DIAGNOSIS

Clinical laboratory investigations aiming to assess hepatic enzyme activity, WBC counts, haematocrit and proteinuria are of uncertain value in wild species. However, detection of coagulation abnormalities, including thrombocytopenia, altered platelet function and marked prolongation of one-stage prothrombin time, activated partial prothrombin time and thrombin time, may have a certain diagnostic relevance. In dead animals, gross lesions are highly suggestive of CAdV1 infection. Histopathology of liver, spleen, kidney and brain may show hepatic centrilobular necrosis, disseminated vascular damage and intranuclear inclusion bodies. Immunohistochemical techniques

applied to formalin-fixed, paraffin-embedded tissues can detect viral antigens in the liver and other parenchymatous organs. CAdV1 can be readily isolated on cell lines of canine origin, such as Madin Darby Canine Kidney (MDCK) cells. CAdV1-induced cytopathic effects consist of clustering of infected cells, detachment from the monolayer and formation of intranuclear inclusions. However, the cytopathic effect produced by CAdV2 is indistinguishable from that associated with CAdV1 replication, and CAdV2 may be isolated from the same tissues as CAdV1. Immunofluorescence can detect viral antigens in infected cell cultures and in acetone-fixed tissue sections or smears, but cross-reaction with CAdV2 are possible. The adenoviral isolates can be distinguished from their haemagglutination patterns or, more easily, by restriction fragment length polymorphism analysis on viral genomes using the endonucleases *Pst*I and *Hpa*II, which generate differential electrophoretic patterns. Polymerase chain reaction assays have been established that are able to amplify a fragment of the adenoviral genome and to differentiate the two CAdV genotypes.

Serological tests developed for detection of CAdV antibodies are the virus neutralization test, indirect immunofluorescence, haemagglutination inhibition and enzyme-linked immunosorbent assays. These tests are not useful for indirect diagnosis of CAdV1 infection through antibody detection, owing to the cross-reactions with the low pathogenic CAdV2. It is noteworthy that the detection of antibodies in wild animals may reflect the widespread circulation of CAdV1 in wildlife, which is not always associated with clinical disease. In free-ranging wild carnivore populations, serological tests are suitable for epidemiological investigations.

MANAGEMENT, CONTROL AND REGULATIONS

Use of vaccines has greatly reduced the effects of ICH in canine populations. Initial attempts were made with CAdV1-inactivated vaccines, which required repeated inoculations. CAdV1-based modified-live virus (MLV) vaccines proved to be highly effective, but were associated with interstitial nephritis and corneal opacity. Administration of CAdV1 in conjunction with canine distemper virus vaccines was also associated with post-vaccinal encephalitis in domestic dogs. As CAdV1 and CAdV2 are able to induce cross-protection, all current vaccines against ICH are prepared with MLV CAdV2, which does not cause renal or ocular damage.

The CAdV2-attenuated strain Toronto A26/61 is used in most vaccine formulations. In non-domestic carnivores, however, administration of MLV vaccine should be evaluated very carefully, because there have been no extensive vaccination studies to prove the complete safety of MLV formulations. MLV CAdV2-based vaccines have been tested in farmed foxes, proving to be safe and fully protective against CAdV1 infection. A recombinant CAdV2 vaccine expressing glycoprotein G of rabies virus was tested in wildlife species (skunks and raccoons), proving to be completely safe and providing evidence of its promise as a candidate for an oral rabies vaccine[9]. Recently, a DNA vaccine was developed against ICH, but its safety and efficacy were evaluated only in mice[10].

Maternally derived antibody (MDA) is likely to interfere with vaccinal immunity in young animals. Oral administration of CAdV2 to non-domestic carnivores resulted in the development of high virus-neutralizing antibody titres. However, further studies are needed to assess whether oral vaccination of wild animals against ICH is effective and safe. All wild animals that are vaccinated against CAdV infection should, where possible, be tested serologically 15–20 days after vaccine administration to determine whether immunization has been successful.

In domestic dogs, vaccination is usually repeated yearly, although after administration of two doses of CAdV2 vaccine, immunity appears to persist for more than 3 years.

PUBLIC HEALTH CONCERN

Although antibodies against CAdV have been found in humans, this seropositivity could be due to cross-reactions with human adenoviruses rather than infection with CAdV1 or CAdV2. Thus, at the moment, CAdV1 is not considered a zoonotic agent.

SIGNIFICANCE AND IMPLICATIONS FOR ANIMAL HEALTH

A CAdV1 carrier status has been suggested for some wild carnivore species and may play a role in the spread of infection to domestic dogs. Feral dogs may have contact with non-domestic carnivores living in suburban areas, such as European red foxes or grey wolves, causing repeated

virus introduction into wildlife. Epidemiological and pathogenetic studies would help to define the role of wild carnivores as reservoirs for CAdV1 infection, the susceptibility to CAdV1 disease of the different species of European wild carnivores and the impact of CAdV1 infection in wildlife populations.

AVIAN ADENOVIRUS INFECTIONS

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Adenoviruses that infect avian species have been placed in the genus *Aviadenovirus*. These are further subdivided into serotypes based on virus neutralization tests. A variety of serotypes have been identified in chickens, quail, turkeys, ducks, pheasants, pigeons, passerines, psittacines, raptors and waterfowl. These are typically classified into three groups, with a number of isolates remaining unclassified.

Group I causes inclusion body hepatitis and hydropericardium in chickens but also disease in quail, turkeys, pigeons, passerines and raptors. These generally are transmitted both horizontally and vertically and lead to severe diseases in birds under 1 month old.

Group II includes haemorrhagic enteritis virus of turkeys, marble spleen disease of pheasants and splenomegaly of chickens. These generally are spread horizontally and cause diseases in older birds, over 1 month of age.

Group III causes egg drop syndrome of chickens and clinical signs such as tracheitis and bronchitis in goslings.

In many species infection can be subclinical, although it has been suggested that adenoviruses form part of a multiple aetiology, rather than acting as primary agents[11]. In general adenoviruses are more virulent in non-host-adapted species than in their typical host. Some of these viruses cause significant diseases of wild birds in Europe and others could spill into wild populations from captive birds. In many species, such as raptors, the presence and role of adenoviruses in causing disease is largely unknown, although there are a number of case reports outside of Europe. Adenoviral infections are typically acquired via the faecal-oral route.

In captive geese high mortality with no premonitory signs is seen in 4- to 11-day-old goslings. Birds die following a brief period of respiratory distress or diarrhoea. More recently a novel presentation of goose adenovirus has emerged in Hungary[12], where affected birds suffered from hepatitic necrosis, focal myocardial necrosis, haemorrhage and hydropericaridium. Intranuclear inclusion bodies were identified on histopathology in the liver.

Marble spleen disease in pheasants occurs worldwide and was first reported in Italy in 1966. However, this disease has only been reported in captive pheasants. The virus can cause sudden death or lead to a brief clinical history of anorexia, depression, diarrhoea and dyspnoea, most commonly in pheasants between 3 and 8 months of age. *Post mortem* examination reveals splenic enlargement with splenic mottling, and pulmonary oedema. Histopathology reveals reticulum-endothelial cell hyperplasia and lymphoid necrosis. Intranuclear inclusion bodies can be identified in reticulum-endothelial cells.

In Muscovy ducks (*Cairina moschata*) *Duck adenovirus 1* can lead to acute death in captive ducklings up to 35 days of age and is found throughout Europe[13]. It has also been identified in captive animals in Canada[14]. Clinical signs include coughing, dyspnoea and gasping. Exudates and tracheal plugs can be seen on close examination. Tracheal and bronchial epithelium can be hyperplastic and show eosinophilic inclusion bodies[14].

In 1998 an adenovirus was identified in common eider (*Somateria mollissima)* in the Baltic Sea[15]. The only clinical signs noted were of general illness and moving into shallow waters preceding sudden death of primarily male eiders. At the time of the outbreak the males were courting the females. As courting males can lose considerable body weight during this period, this was believed to be a predisposing factor. It was estimated that 1,000 birds, which was 25% of the local population, died over a period of 6 weeks. The females were observed to continue to lay and incubate their eggs and as a result have minimal social interaction, reducing their risk of disease transmission. The signs noted with this outbreak were similar to those reported before in the Baltic Sea. In this outbreak males in poor body condition developed intestinal disease during the breeding season[16]. The birds were in poor body condition with minimal fat reserves. The main lesion, observed in the ten eiders necropsied, was impaction of the proximal small intestine with mucosal necrosis. By histopathology, necrosis of single hepatocytes was observed in two eiders and focal hepatic necrosis in one. No inclusion bodies were seen. There was renal tubular degeneration and congested lungs. Adenovirus was isolated from cloacal swabs from all the dead male birds; it demonstrated a

cytopathic effect in Muscovy duck cells within 6 days. Virus neutralization against reference antisera to adenovirus groups I, II and III led to negative results. Virus was not found in any swabs from live females or healthy eider killed by hunting the previous season. Experimental infection of mallard ducklings with the adenovirus from one of the eiders led to positive antibody titres 14 days after inoculation. These ducklings displayed transient shivering, gasping and watery diarrhoea. They were euthanized, and at necropsy they showed distended small intestinal loops with mild chronic inflammation histologically. It was hypothesized that predisposing environmental stressors required to induce mortality were not present in the experimental set-up.

Confirmation of suspected adenoviral disease in dead birds should initially be based on histopathological changes. Virus isolation and viral neutralization can be used for confirmation in both live and dead birds.

There is currently no specific treatment for adenoviral infections in birds. Supportive care measures, reducing stocking density (in rehabilitation facilities) and ensuring that good basic hygiene standards and biosecurity measures are maintained can together limit the spread of disease. As adenoviruses may not be the primary agents involved with this mortality, appropriate investigation of any deaths should include a thorough evaluation of other pathogenic agents involved.

FIGURE 14.1 Red and grey squirrel adenovirus-positive cases in the UK diagnosed up to October 2009 with scanning electron microscopy and/or polymerase chain reaction (PCR). The one grey squirrel case was subclinical, PCR-positive. Reproduced with kind permission of J. Paul Duff and David Everest of the Veterinary Laboratories Agency and Mark Wilkinson of the Northumberland Wildlife Trust.

ADENOVIRUS INFECTION IN SQUIRRELS

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The *squirrel adenovirus* (SqAdV) is a novel tentative adenovirus species in the *Mastadenovirus* genus of the *Adenoviridae* family, most closely related to guinea pig adenovirus[17]. The virus was first detected by negative contrast scanning electron microscopy (SEM) and characterized phylogenetically based on the hexon gene nucleotide sequence[17]. SqAdV was isolated in mouse L cells, but the maintenance of the isolated strain on subsequent passages was unsuccessful.

The disease caused by SqAdV is characterized by acute enteritis and death in red squirrels (*Sciurus vulgaris*).

Enteric adenovirus infection of wild red squirrels has so far been reported only from various parts of the UK (e.g. Cumbria, Norfolk, the island of Anglesey and Scotland), often associated with translocation/ reintroduction events and in captive animals[18–20] (see Figure 14.1). The fatal disease caused by SqAdV affects red squirrels of all age classes, but the same virus has also been identified in healthy grey squirrels (*Sciurus carolinensis*) on Anglesey. There is no information about the presence of this virus in continental Europe or elsewhere. Reports indicate a tendency towards the clustering of cases, suggesting local outbreaks of disease, and the most likely route of viral

transmission is through direct contact with infected animals. It is unclear yet which species serve as a reservoir of the virus, one possible candidate is the grey squirrel[20] – although other rodents or the red squirrel itself cannot be excluded.

A review of 36 cases of adenovirus enteritis diagnosed from Animal Health Veterinary Laboratory Agency Diseases of Wildlife Scheme data in the UK (Duff, unpublished observations) showed that there was no obvious seasonality in this admittedly limited dataset. From the cases where age was discernable, 21 were adults and 12 were immature, supporting the premise that both young and adult animals are susceptible. The disease appears to be acute in nature – only five animals were found ill, weak and collapsed, and these died within hours of capture; the remainder were found dead, often in nest boxes. An additional indicator of the acute nature of the enteritis was that only 15 of the 36 had diarrhoeic staining of fur around the anus.

The infection appears to affect primarily the mucosae of the small and large intestine, but the presence of SqAdV was also demonstrated by polymerase chain reaction (PCR) in the spleen of a healthy grey squirrel. Gross lesions are few and limited to the gastrointestinal tract. The most consistent findings included mildly congested and distended intestines with profuse yellow coloured watery/mucoid content, often stained with blood. Disc-shaped hyperaemic areas 2–3 mm in diameter were also observed on serosal surfaces of both small and large intestines in a few individuals[21]. In some animals there was splenic congestion, but no other consistent gross visceral lesions were recorded. Histological lesions include acute exfoliating enteropathy associated with villous atrophy, crypt hyperplasia, and the presence of solid amphophylic intranuclear inclusion bodies in the villous enterocytes surrounded with a mild infiltrate of mononuclear inflammatory cells[21]. Acute splenitis and lympholysis can also be observed in some cases.

Laboratory diagnosis is based on the demonstration of SqAdV by SEM, virus isolation and PCR on clinical samples (faeces, faecal swabs). The PCR targets either the hexon or polymerase gene[17,20] and the product is further characterized by sequencing. *Post mortem* examination and histopathology support the diagnosis of SqAdV infection. Serological methods have not yet been developed. Treatment has not been described, and it is not known whether affected animals may respond to supportive treatment.

The risk of spreading SqAdV by translocation and reintroduction of red squirrels should be taken into consideration, along with potential danger to naïve, susceptible animals introduced to an infected area. Adenovirus infection of red squirrels may have significant impact on the survival and conservation of this seriously threatened species in Britain.

ADENOVIRUS HAEMORRHAGIC DISEASE OF DEER

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Infection with a previously unrecognized adenovirus caused a haemorrhagic syndrome that killed thousands of mule deer (*Odocoileus hemionus*) in California in 1993[22]. The virus causes endothelial necrosis in any organ, but vasculitis occurs most frequently in the lungs and intestines, producing pulmonary oedema and haemorrhagic enteropathy, respectively[22]. Adenovirus haemorrhagic disease of deer may be diagnosed on histopathology and with the demonstration of endothelial intranuclear inclusion bodies confirmed by immunohistochemistry. The disease has not been reported in Europe or in European species of deer.

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CHAPTER

15

RETROVIRUS INFECTIONS

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INTRODUCTION

Retroviridae are enveloped, single-stranded non-segmented RNA viruses. Their replication strategy is characterized by reverse transcription of their RNA into double-stranded DNA, which is integrated into the host chromosomal DNA. Retroviruses are classified into seven genera. The alpharetroviruses, betaretroviruses and gammaretroviruses are grouped as simple retroviruses, the genome of which encodes only for the Gag, Pol, Pro and Env proteins. The deltaretroviruses, epsilonretroviruses, lentiviruses and spumaviruses are more complex retroviruses, with a genome encoding an additional array of small regulatory proteins.

The integrated viral DNA or provirus contributes to the ability of the retroviruses to maintain persistent infection, and enter the germ line (cells at the origin of, and including, the gametes), allowing vertical transmission. This integration into the germ line results in endogenous retroviruses. These retrovirus-like DNA sequences, present in most eukaryote cells, are not associated with virus production or disease. Exogenous retroviruses are infectious and commonly associated with disease, including the development of malignant tumours, wasting diseases, neurologic disorders and immunodeficiencies.

MAIN RETROVIRUSES OF WILD MAMMALS

FELINE LEUKAEMIA

Feline leukaemia virus (FeLV) is a gammaretrovirus causing neoplastic and degenerative diseases of the lymphoreticular system, including lymphosarcoma, leukaemia and anaemia syndromes, and feline acquired immunodeficiency syndrome (AIDS).

FeLV is enzootic in domestic and feral cat (*Felis silvestris catus*) populations worldwide, but overall prevalence of FeLV infection is typically low (less than 5%), whereas prevalences of FeLV infection in free-ranging European wildcats (*Felis silvestris silvestris*) and Iberian lynxes (*Lynx pardinus*) reach up to 77% and 18%, respectively. FeLV or FeLV nucleic acids have been identified in both species. Domestic and feral cats are reservoir hosts. To what extent FeLV is transmitted between wild and domestic populations is unclear, and wild felids may also be natural hosts. In domestic and feral cats, FeLV is horizontally transmitted oronasally via secretions and excretions, through bites and intimate contact, and may be vertically transmitted from mother to fetus. Transmission in free-ranging wild felids is probably similar. FeLV is present in high titres in

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the saliva of viraemic cats. It is secreted in semen, vaginal fluids, faeces and urine, but venereal and faecal-oral routes are not important. FeLV is poorly preserved in the environment. Risk factors for free-ranging wild felids are probably similar to those in domestic and feral cats (high density populations, male aggression and free-roaming).

FeLV infection has been studied in naturally and experimentally infected domestic cats. FeLV first replicates in oropharyngeal lymphoid tissues. Cell-associated viraemia spreads the virus to lymphoreticular tissues, bone marrow and intestinal epithelium. Infected cats develop persistent viraemia in 30% of the cases, with infection of mucosal and glandular epithelial cells, causing lifelong viral excretion. In 60% of the cases, a transient latent viraemia develops, followed by a persistent but non-productive infection of the bone marrow. The remaining 10% develop atypical infections, eventually progressing into persistent viraemia or latent infection.

Initially the infection is largely subclinical in most cats, although it may lead to illness or death within 4 to 8 weeks, as a result of leucopenia and acute immunosuppression. Latent infection does not lead to clinical signs or disease, but immunosuppression (e.g. by concurrent *Feline immunodeficiency virus* infection) can cause reactivation of FeLV infection and persistent viraemia. Viraemic cats may develop disease, including progressive immunosuppression, lymphoid and myeloid cell depletion, and finally cell transformation. Eventually these cats develop a plethora of neoplastic and degenerative diseases. Development of persistent viraemia or latent infection of the bone marrow depends on the ability to mount an effective immune response. Persistent viraemia is usually not accompanied by virus neutralizing antibodies, whereas latent infection is, at least in part, kept under control by these antibodies.

Clinical signs in non-domestic felids include lethargy, anorexia, emaciation, dehydration, anaemia and lymphadenopathy. Poor body condition in European wild cats[1] and severe acute anaemia and mortality in Iberian lynxes[2] correlated with FeLV infection.

Gross and microscopic pathology of FeLV-related diseases is described in cats with persistent viraemia. No lesions have been reported in association with FeLV infection in European free-ranging wild felids. Dead Iberian lynxes infected with FeLV had succumbed to bacterial infection in the wild and to a severe acute anaemic disease in captivity[2]. A free-ranging FeLV-infected cougar (*Felis concolor*) presented with generalized benign lymphoproliferative disease and bacterial septicaemia[3].

The infection is traditionally diagnosed by detecting FeLV antigen with immunofluorescent assays (IFA) on blood smears or enzyme-linked immunosorbent assays (ELISA) on plasma or serum. Positive serum antibody tests may indicate latent infection. In addition, polymerase chain reaction (PCR) assays and immunohistochemistry can be performed on a range of organs taken from necropsy, e.g. lymph nodes, spleen, digestive and respiratory tract, kidneys, urinary bladder, salivary glands and pancreas.

There are no regulation, management or control plans for FeLV infection in European free-ranging wild felids. FeLV is not considered zoonotic. The suspected association between severe conditions in European wild cats and Iberian lynxes and FeLV infection indicates that it may pose a threat to these endangered wild felids[4]. Monitoring the prevalence and the impact of FeLV infection in free-ranging wild felids in Europe is indicated.

FELINE IMMUNODEFICIENCY

Feline immunodeficiency virus (FIV) is a lentivirus that replicates in feline T lymphoblastoid cells, inducing immunosuppression[5]. Lentivirus infections are characterized by long incubation periods. Although typically species-specific, lentiviruses may cross species barriers between related species, as has been thought to occur with the primate lentiviruses HIV and SIV.

The prevalence of FIV infection in domestic and feral cats in Europe ranges from less than 1% to 30%. Clinical FIV has not been identified in free-ranging wild felids in Europe and the infection appears rare, although little information is available. Most serological studies did not find evidence of FIV circulation in the European wildcat. In one study in eastern and central France however, 3 out of 38 wildcats had antibodies against FIV[1]. FIV antibodies have not been detected in the European lynx (*Lynx lynx*) or Iberian lynx in Europe, although one study reports Iberian lynxes with cross-reactive antibodies against species-specific strains of FIV[6]. FIV in non-domestic felids worldwide are genetically divergent from domestic feline FIV, and transmission of FIV between felid species is infrequent or non-existent. Yet, opportunities for transmission are likely to exist. In domestic and feral cats, FIV is mainly transmitted through bites. Risk factors for free-ranging wild felids may be similar (older individuals, males, dominant and free-roaming individuals).

The pathogenesis of FIV infection has been studied in naturally and experimentally infected domestic cats.

Infection typically follows a bite via infected saliva. FIV replicates primarily in lymphocytes, macrophages and other non-T cells, including renal cells and cells of the central nervous system. Virtually all lymphoid tissues and the salivary glands are infected within 1 week and by 3 weeks FIV is present in all non-lymphoid tissues, including the brain. It persists for life in the infected host.

The course of the disease in domestic cats is variable and can be divided into five successive stages.

1. The acute stage develops between 4 and 6 weeks, and lasts several weeks with mild fever, neutropenia and generalized lymphadenopathy.
2. The subclinical phase can last several years, during which FIV is present in saliva, peripheral blood mononuclear cells and plasma.
3. Persistent lymphadenopathy follows with progressive depletion of CD4+ T lymphocytes lasting several months with non-specific signs of disease, such as fever, anorexia and weight loss.
4. The AIDS-related complex (ARC) phase may last several months to years and is associated with chronic secondary infections by non-opportunistic pathogens.
5. The terminal feline AIDS phase can last several months and is characterized by severe secondary infections, often with opportunistic pathogens, as well as neoplastic and neurological disorders.

Serum antibodies against FIV appear 2 to 6 weeks post-infection and tend to persist throughout life.

In domestic cats, multiple haematologic abnormalities are observed, including frequent cytopenia (leucopenia, anaemia or both), especially during the later stages of disease. Haematologic landmarks of FIV infection are a depletion of CD4+ T lymphocytes, and an inversion of the CD4+/CD8+ T lymphocyte ratio. No clinical disease associated with FIV infection has been described in free-ranging wild felids.

Histological lesions in the lymph nodes and spleen of domestic cats include follicular hyperplasia and polymorphism during the initial stages of infection. Both follicular hyperplasia and involution are observed during the ARC stage of the disease. Marked follicular involution and depletion characterize the final feline AIDS stage. Histological lesions in non-lymphoid tissues appear later and include lesions of gliosis, perivascular cuffing, white-matter vacuolation, and choroid plexus fibrosis predominantly in the cerebral cortex, glomerulosclerosis and tubular degenerative changes in the kidney and lesions associated with secondary infection.

The presence of FIV-specific antibodies in wild felids, demonstrating ongoing infection, can be detected by serological assays, most commonly ELISA. ELISA results should be confirmed by western immunoblotting. Viral isolation, molecular methods (e.g. PCR), and immunohistochemical tests for FIV antigen can indicate ongoing infection.

No regulations, management or control plans are in place for FIV infection in free-ranging wild mammals in Europe. There are no indications that FIV has zoonotic potential.

FIV may represent a threat to endangered European wildcats and Iberian lynxes, with domestic and feral cats representing a source of FIV infection[4]. Monitoring of European wildcat and Iberian lynx populations for the prevalence and the impact of FIV infection would be indicated.

ALCES LEUCOTROPIC ONCOVIRUS INFECTION

Since the mid-1980s, large numbers of moose (*Alces alces*) have been found in poor condition or dead in south-western regions of Sweden. Among the numerous names used for the disease, moose wasting syndrome (MWS) was probably the most appropriate. Among its possible causes, diet-related factors (mineral deficiencies) and infection with *Alces leucotropic oncovirus* (ALOV) have been considered[7,8]. MWS has only been reported in moose in Sweden since the mid-1980s, notably in Älvsborg county. The moose is the only species reportedly affected, with the highest incidence in females (84%) and older animals. Although transmission of MWS in moose has not been confirmed, the markedly higher prevalence in females suggests venereal transmission. ALOV may be transmitted to calves from mothers with MWS. The pathogenesis of MWS and ALOV infection in moose is unknown. MWS-affected moose present with apathy, anorexia, emaciation, diarrhoea and neurological signs, such as impaired vision, circling and lack of fear of humans. MWS-associated gross and histological lesions include emaciation, erosive, ulcerative, and necrotic lesions in the mucosa of the digestive tract, and atrophy of lymphoid organs. Diagnosis of MWS is based on clinical features and characteristic erosive lesions. There is no regulation, management or control plan for ALOV infection or MWS in moose in Europe. ALOV has not been shown to infect other animal species,

including humans. MWS has had a major impact on the moose population in some areas of Sweden.

AVIAN RETROVIRUS INFECTIONS

Avian retroviruses cause neoplastic diseases, chronic lymphomas, or immunosuppressive disease syndromes in birds. They have a broad host range, and are well documented in commercial poultry. Avian retrovirus disease has rarely been described in birds other than poultry, with most neoplastic lesions in wild birds being of unknown aetiology[9].

LEUCOSIS/SARCOMA GROUP

Leucosis/sarcoma (L/S) retroviruses are alpharetroviruses. Exogenous L/S retroviruses cause a variety of neoplastic diseases of the haematopoietic system, or mesenchymal tumours. Neoplastic conditions associated with L/S viruses in poultry were named after the tissue or cell type transformed. Endogenous L/S retroviruses do not cause disease in their bird hosts, and have been shown to provide protection against exogenous L/S retrovirus infections in chickens.

Lymphoreticular neoplastic conditions have been described in a number of captive and free-ranging wild birds, but a causal relationship between L/S retroviruses and neoplasia has rarely been documented in wild birds[9]. Retrovirus sequences have been amplified in tumour lesions of a European starling (*Sturnus vulgaris*) presenting as multicentric lymphoma with lesions similar to lymphoid leucosis in chickens[10]. *Gag* genes of endogenous and possibly exogenous L/S retroviruses have been demonstrated in 26 species of wild galliform birds[11]. Domestic chickens are reservoirs of L/S retroviruses. The role of wild birds in the epidemiology of species specific L/S retroviruses is unknown. Co-speciation of endogenous L/S retroviruses with wild galliform species, such as partridges (*Perdix* spp.) and ptarmigans (*Lagopus* spp.), indicates an ancient association with vertical transmission[11].

Infection with L/S retroviruses can be acquired congenitally via the albumen, or via faecal-oral transmission[12]. Infected chickens generally develop a transient viraemia and mount an effective antibody response to the virus but may develop a persistent viraemia without antibody production if infected congenitally. Exogenous L/S retroviruses may cause a variety of neoplastic diseases, affecting mainly cells of the haematopoietic system, such as lymphoid, myeloid or erythroid cell lines or tumours of mesenchymal origin such as sarcomas, endotheliomas or fibromas. Endogenous retroviruses typically do not cause disease in their bird hosts.

Chickens with L/S retrovirus infection may be anaemic, or there may be an increase in immature forms of specific white or red blood cells in the peripheral blood. Peripheral leucocyte changes have been observed in captive and free-ranging wild birds with lesions similar to those of lymphoid leucosis in chickens[9].

In wild birds, lymphoid leucosis lesions are the tumours most frequently reported. These are generally described as soft, smooth, white nodular to diffuse tumours affecting a variety of tissues – typically parenchymal organs, notably the liver, the spleen and the kidneys. Microscopically, lymphoblastic lymphomas are most frequently reported.

In wild birds, a tentative diagnosis can be made based on the presence of neoplastic lesions, and the cell types involved in these tumours[9]. A definitive diagnosis of L/S retrovirus infection can only be made by virological investigation. Besides virus isolation or antigen detection by ELISA, detection of retroviral nucleic acids can be achieved by PCR-based methods. Serological methods to detect antibodies are of little practical use for wild bird species.

There are no regulation, management or control plans for L/S retrovirus infections in European free-ranging wild birds. There are no indications that L/S retroviruses are zoonotic.

The impact of L/S retrovirus infection on European free-ranging wild birds is unknown and likely to be minimal[9].

RETICULOENDOTHELIOSIS GROUP

The reticuloendotheliosis (RE) retroviruses are gammaretroviruses causing acute reticular cell neoplasia, chronic lymphomas and a runting immunosuppressive disease syndrome in commercial poultry, notably turkeys.

RE retroviruses are common in commercial poultry worldwide. Prevalence is typically low, and their role as reservoirs of RE retroviruses is debated[9]. Other avian species with RE retrovirus infection or tumours possibly associated with RE retrovirus infection include wild Palearctic species present in Europe, i.e. grey partridge (*Perdix perdix*), ring-necked pheasant (*Phasianus colchicus*) and rock pigeon (*Columba livia*)[9].

RE retroviruses are transmitted both vertically in poultry with persistent viraemia, and horizontally via a direct or indirect route. Direct contact with infectious virus in faeces, nasal and ocular secretions and contaminated litter is believed to be the main route of transmission in poultry. Mechanical transmission by mosquitoes is documented.

Infection is acquired via the albumen in poultry infected congenitally. These develop a tolerant infection with persistent viraemia and no antibody response. In these birds, rates of vertical transmission are high, as are rates of immunosuppression and tumour development. In older poultry, infection is acquired via a faecal-oral or respiratory route. Viraemia is transient, and an antibody response develops. Non-infectious virus can remain detectable for weeks after disappearance of infectious RE retroviruses. These birds generally do not develop clinical disease or tumours.

RE retroviruses infect cells of the lymphoreticular system. RE retroviruses are detected in plasma 3 to 6 weeks post-infection, and can be isolated from internal organs in diseased poultry.

Acute reticular cell neoplasia is observed only in newly hatched poultry, which do not show major clinical signs, although mortality can be high. Older chickens develop runting immunosuppressive disease syndrome with pale extremities and mucous membranes. Some chickens may have abnormal feather development. This runting syndrome has not been observed in wild birds. Birds with chronic lymphoma may be asymptomatic or may present with general malaise, a wasting syndrome and dehydration. Cutaneous lymphomas, generally on the head, have been described in a few species, including the ring-necked pheasant[9].

Only chronic lymphomas and associated lesions have been described in wild birds. Most lymphomas involve parenchymal organs or develop along peripheral nerves. Tumours are typically smooth enlargements of the spleen, liver, heart, thymus or bursa of Fabricius, or nodules in visceral organs. Microscopically, chronic lymphomas associated with RE retrovirus infection involve uniform blastic lymphoreticular cells.

In wild birds, a tentative diagnosis can be made based on the presence of macro- and microscopic lesions. A definitive diagnosis can only be made by demonstrating the presence of virus, viral antigen, viral nucleic acid or virus-specific antibody. Most of the antibody tests are not suitable for wild bird testing. There are no regulation, management or control plans for RE retrovirus infection in European free-ranging wild birds. There are no indications that L/S retroviruses are zoonotic. The impact of RE retrovirus infections on wild bird populations is unknown.

OTHER RETROVIRUS INFECTIONS

Bovine leukaemia virus (BLV) is a deltaretrovirus that causes enzootic bovine leucosis, characterized by chronic leukaemia or lymphosarcoma in cattle. Enzootic bovine leucosis is a notifiable disease in the EU and has been eradicated from cattle herds in most western European countries. In the early 1980s, a free-ranging European bison (*Bison bison*) bull in Poland was found subclinically infected with BLV[13]. Serological studies performed on cervid populations have failed to detect BLV infection in red deer (*Cervus elaphus*), roe deer (*Capreolus capreolus*) and fallow deer (*Dama dama*) populations in Germany[14] and in chamois (*Rupicapra rupicapra*) populations in Italy[15]. However, lymphosarcoma has been described in red deer, roe deer and brown hare (*Lepus europaeus*).

Bovine immunodeficiency virus (BIV) is a lentivirus that is associated with leukocytosis, lymphadenopathy and meningoencephalitis in cattle. Seropositive cattle are present in many European countries. There are no known reports of BIV infection in free-ranging wild ungulates, including bison, in Europe.

Small ruminant lentiviruses (SRLV) include *Visna/maedi virus* and *Caprine arthritis encephalitis virus*. These viruses cause interstitial pneumonia and mastitis in sheep, and arthritis and encephalitis in goats. Hybrids of sheep and mouflon (*Ovis aries musimon*) have been experimentally shown to be susceptible to infection with SRLV[16]. Natural SRLV infection has been reported in ibexes (*Capra ibex*) in the French Alps[17]. Other serological studies have failed to detect SRLV circulation in chamois in Italy[15], in red deer, roe deer and fallow deer in Germany[18] and in mouflons in central Spain[19].

Jaagsiekte sheep retrovirus (JSRV) and *Enzootic nasal tumor virus* (ENTV) are betaretroviruses of small ruminants. JSRV causes pulmonary adenocarcinoma in sheep and ENTV causes nasal adenocarcinoma in sheep and goats. Ethmoturbinate adenocarcinoma potentially caused by a lentivirus has been described in a Persian fallow deer[20] and has occurred for many years in moose and roe deer in Scandinavia[21]. Endogenous retroviruses related to JSRV have been detected in the genome of many species of ungulates, including mouflon, ibex, European bison, moose, reindeer (*Rangifer tarandus*) and Przewalski's horse

(*Equus caballus przewalski*)[22,23]. Their impact on these populations is unknown.

Equine infectious anemia virus (EIAV) is a lentivirus causing severe anaemia in equids. There are no known reports of EIAV infection in wild or semi-wild equids in Europe.

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CHAPTER

16

PAPILLOMAVIRUS AND POLYOMAVIRUS INFECTIONS

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INTRODUCTION

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Both papillomaviruses and polyomaviruses are small, non-enveloped, icosahedral viruses, 50–55 nm and 40–45 nm in diameter, respectively. They contain a circular, double-stranded DNA genome of 7.4–8.6 kbp in papillomaviruses and around 5 kbp in polyomaviruses. The *Papillomaviridae* and *Polyomaviridae* constitute independent families, but they were previously assigned to a common *Papovaviridae* family.

Papillomaviruses are epitheliotropic viruses, causing predominantly benign mucosal or cutaneous tumours. Lesions vary from papillomas consisting almost exclusively of epithelial cells to fibropapillomas and fibromas containing a very pronounced connective tissue component. Papillomaviruses are highly host-specific and infect a wide range of mammals, but only a few avian and reptile species. The *Papillomaviridae* family currently contains 16 genera. The phylogeny of papillomaviruses reflects their host-linked evolutionary history, but it has been also influenced by other evolutionary processes such as adaptive radiation, host-switching events (switching of a pathogen to a novel host) and genetic recombination. As papillomaviruses cannot be isolated in tissue culture, their characterization is based on the analysis of their genomic sequence, their pathogenesis and tissue preference.

Polyomaviruses have been isolated from both mammals and birds; they are less host-species-specific than papillomaviruses and they also infect a wider range of cell types and tissues. A common feature of most polyomaviruses is that they are capable of maintaining latent, chronic infections in some host species while inducing acute disease in others. Polyomaviruses may cause a wide range of lesions and have high affinity for the kidney, which probably plays a significant role in the maintenance of latency. There are currently 12 recognized species belonging to the *Polyomaviridae* family, but similarly to papillomaviruses, there is also an increasing number of novel candidate species. Captive breeding of birds (especially psittacines and passerines) provides an excellent opportunity for the intensive circulation and evolution of polyomaviruses, which pose a potential threat for indigenous wild bird populations and hinder *ex-situ* conservation efforts.

ROE DEER AND OTHER UNGULATE PAPILLOMAVIRUSES

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Deer fibropapillomatosis or deer fibromatosis is a cutaneous benign neoplastic disease occurring in a wide range of cervid species, caused by deltapapillomaviruses of cervids.

Ruminants, including many deer species, are infected with the second most numerous and diverse range of papillomaviruses after humans. Of the three artiodactyl-specific papillomavirus genera, deltapapillomaviruses infect the widest host range, encompassing ovine, bovine and several cervid species.

AETIOLOGY

Papillomaviruses have been identified in skin tumours of European moose (*Alces alces*), reindeer (*Rangifer tarandus*), roe deer (*Capreolus capreolus*), red deer (*Cervus elaphus*) and North American deer species (e.g. white-tailed deer). A common feature of these viruses is that they induce the development of skin tumours with a marked connective tissue component. Cervid papillomaviruses causing cutaneous fibromatosis or fibropapillomatosis share this genus with *Bovine papillomavirus 1* and *2* (BPV1 and BPV2) and *Ovine papillomavirus 1* and *2* (OaPV1 and OaPV2).

The genomes of the following deltapapillomaviruses of cervids have been sequenced, characterized and deposited in GenBank: Deer (White-tailed deer – *Odocoileus virginianus*) papillomavirus (OvPV1), *Reindeer papillomavirus* (RtPV1), *European moose papillomavirus* (AaPV1) and *Western roe deer papillomavirus* (CcaPV1). In addition to the above, characterization of a sequence belonging to the *Red deer papillomavirus* shows that it is also a deltapapillomavirus, very closely related to the *Roe deer virus*[1]. Earlier Southern blot analyses[2] have already indicated this relationship by establishing that *Red deer papillomavirus* genomic DNA cross-hybridizes with BPV1/BPV2 DNA (Figure 16.1).

The size of the non-enveloped, icosahedral deltapapillomavirus capsid is approximately 55 nm in diameter, it is constituted from 72 capsomeres and contains the double-stranded, circular DNA genome of 8–8.4 kbp. All cervid papillomaviruses exhibit homologous genetic structures containing a total of nine open reading frames (ORF) coding early (E) regulatory proteins responsible for replication and transforming properties, the late (L) structural capsid proteins and the ubiquitous, non-coding long regulatory region (LRR). The major capsid protein is the main immunogenic epitope of papillomaviruses. The main oncogene of deltapapillomaviruses is the E5 ORF, but the additional fibroblastic properties typically expressed in deer fibromas may actually be the effect of the E9 ORF identified in all cervid deltapapillomaviruses

The current classification of papillomaviruses is based on the comparison of complete L1 ORF nucleotide sequences. Distinct papillomavirus genera share less than 60% L1 ORF nucleotide sequence identity, whereas papillomavirus 'species' within a genus share approximately 60–70% nucleotide identity.

EPIDEMIOLOGY

Skin lesions caused by papillomaviruses have been described in cervid species from both Europe and North America. In Europe, endemic infections typically occur in roe deer populations of Central-Eastern Europe, i.e. Hungary and neighbouring areas[3] and in European moose throughout Sweden, with high frequency in the middle of the country. Papillomavirus-induced fibropapillomas also occur sporadically in red deer throughout Europe and in reindeer in Sweden (Figure 16.2).

Cutaneous fibromas and fibropapillomas of roe deer were observed in parts of Hungary from the early 1960s, and they received more attention 30 years later because of the allegedly growing incidence of this endemic disease. The prevalence of the infection in endemic areas was found to be between 0.2 and 1.1%, which is similar to the prevalence of OvPV1-induced fibropapillomatosis in North American cervids. A similar 1.1% general prevalence of fibromatosis has been observed in moose in Sweden, but prevalences as high as 50% have been reported from certain areas.

Fibropapillomas are most commonly seen in mature roe deer and European moose – unlike red deer fibropapillomas, which are mostly found in young animals, similar to OvPV1 infection of white tailed deer. Males and females of both roe deer and European moose are equally affected by fibromatosis, but the prevalence is much higher in white-tailed deer bucks in North America.

Papillomaviruses are highly host-specific. The endemic nature of both roe deer and European moose (also named European elk) fibropapillomatosis suggests the possibility that additional, genetically determined susceptibility factors of the hosts may have contributed to the evolution of these diseases within certain subpopulations of each species. In the case of CcaPV1, this possibility is supported

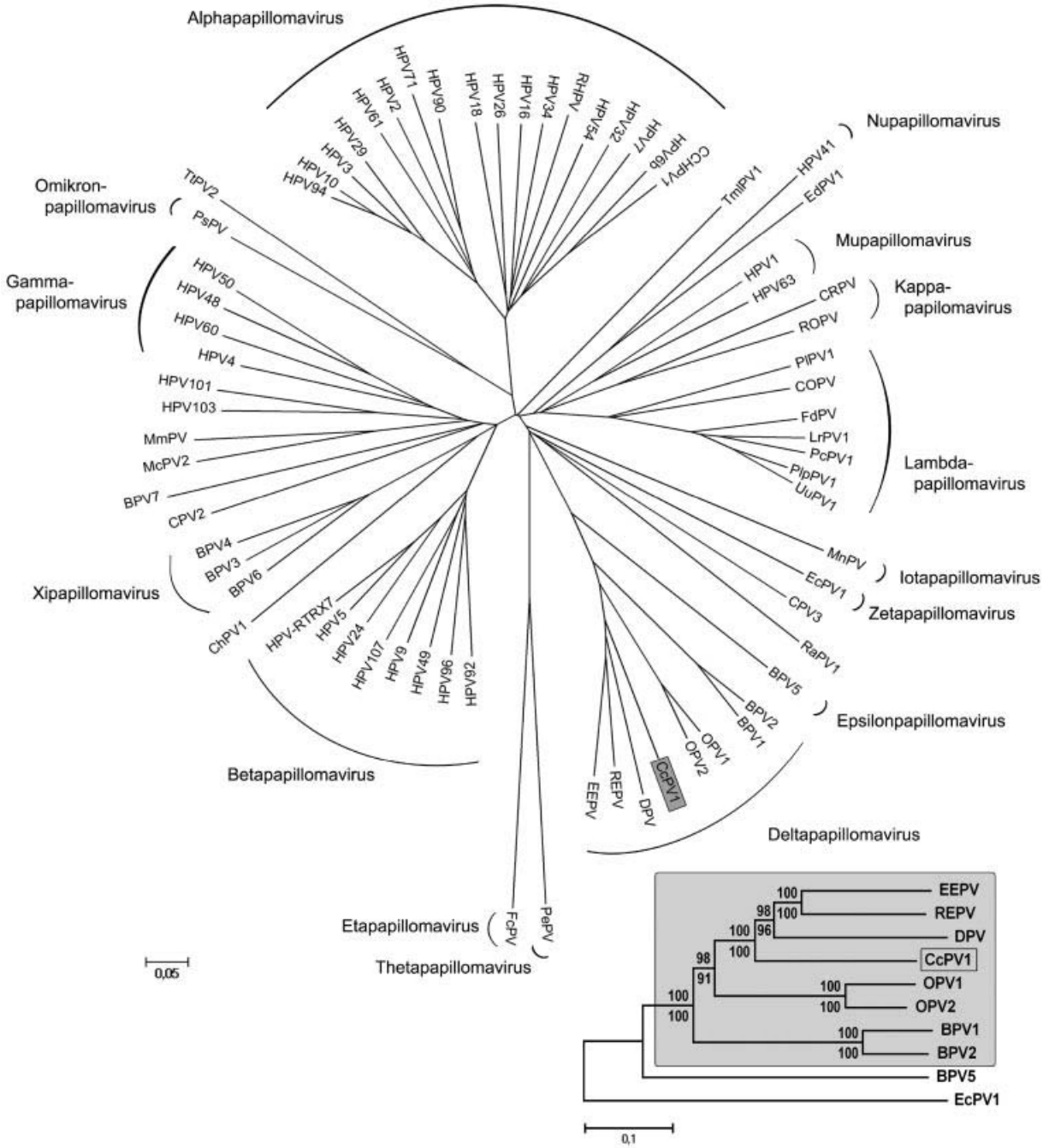

FIGURE 16.1 Molecular phylogeny of the deltapapillomaviruses of cervids.

by the genetic differentiation pattern of European roe deer populations, as the boundary between the two major genetic clusters of European roe deer (southern and eastern European versus western, central and northern European) corresponds to the westernmost distribution limits of roe deer fibropapillomatosis[4].

The exact method of cervid papillomavirus transmission in nature are not known. The primary transmission route of cutaneous papillomaviruses in general is through direct contact with a contaminated environment or infected animals, which shed the virus from the epithelial surface of tumours. As viral particles have to penetrate the

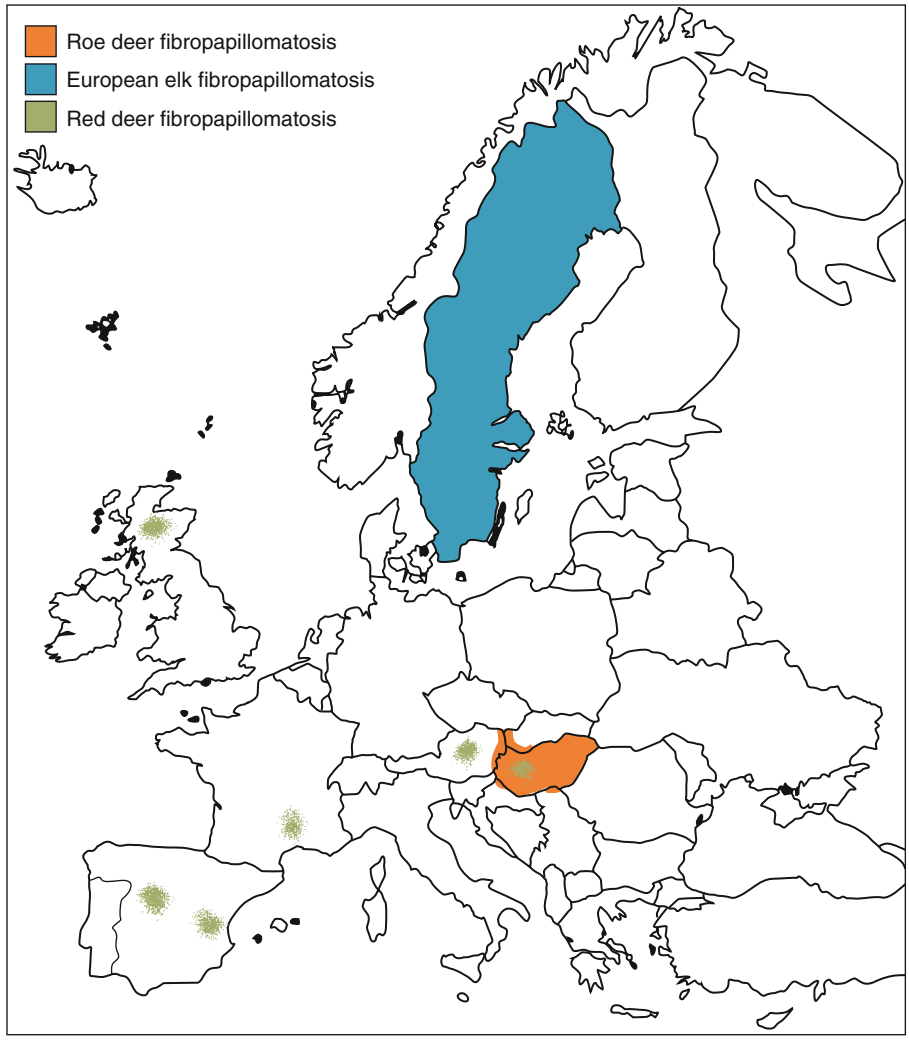

FIGURE 16.2 Distribution map of deltapapillomaviruses of cervids in Europe.

superficial epidermis to cause infection, it is assumed that infection is acquired predominantly through superficial skin lesions. Several aspects of deer behaviour, such as group-formation, scent marking, rubbing of antlers on trees during velvet shedding and fighting, may contribute to transmission. The common localization of lesions on the head, neck, extremities and belly supports these possibilities.

It has been suggested that arthropod vectors may also play a part in the epidemiology of deer fibromatosis. Circumstantial evidence from an epidemiological study of roe deer fibropapillomatosis does not rule out the potential role of arthropods in papillomavirus transmission[3]. The spatial distribution of the disease was correlated with the presence of waterways, habitats especially suitable for arthropod vectors, independently of host density. Alternatively, this correlation could also be the potential effect of seasonal roe deer aggregation in these habitats. The arthropod transmission hypothesis still requires confirmation.

PATHOGENESIS, PATHOLOGY AND IMMUNITY

All deltapapillomaviruses induce the development of cutaneous fibropapillomas and fibromas[5], and OvPV1 and AaPV1 infections are also associated with sporadic cases of nodular pulmonary fibromatosis[6]. An active papillomavi-

rus infection is established when viral particles enter basal epithelial cells. Viral genomes are present in low numbers within the nuclei of basal epithelial cells (keratinocytes) in episomal form and they maintain low-level replication, using the normal cell cycle. After the transcription of early regulatory genes, substantial papillomavirus genome replication and transcription only occurs in an advanced state of epithelial cell differentiation. This process culminates in capsid formation and virus assembly taking part in the stratum granulosum. Simultaneously with the epithelial changes, the proliferation of fibroblasts is induced in the underlying dermis, resulting in the development of the main tumour mass and the clinical manifestation of the disease.

Lesions induced by deltapapillomaviruses are limited almost exclusively to the epidermal squamous epithelium and the connective tissue of the dermis. Apart from the infrequent occurrence of lung fibromatosis, these viruses do not cause lesions in other organs. Deer fibromas and fibropapillomas do not undergo malignant transformation and the nodular lung lesions induced by AaPV1 and OvPV1 are not considered true tumour metastases, as they are probably the consequence of fibroblastic transformation induced by disseminated viral DNA.

Both the progression and regression phase of cutaneous fibropapillomatosis are protracted processes. Experimental infections with OvPV1 in white-tailed deer resulted in the development of fibropapillomas at the inoculation site within 2–3 weeks, and their regression within 7–10 weeks, post-infection. Natural infections with AaPV1 and CcaPV1 are believed to have a course of several months or even years. Although the majority of papillomavirus infections induce certain levels of humoral immune response, cell-mediated immunity plays a major role in clearing the infection. Tumours get progressively infiltrated by lymphocytes and macrophages, and marked hyalinization is also observed during the regression process.

Fibropapillomas of roe deer are conspicuous skin tumours. They are firm, round, raised or pedunculous, most often multiple skin tumours of varying size, typically localized on the head, neck, belly and legs of infected animals (Figure 16.3). In European moose (elk), fibromas are found mainly on the head, neck, shoulders and sides and on the extremities.

Typical fibropapillomas of roe deer are firm, round and hairless, and covered with smooth or verrucous epidermis, but some tumour surfaces may exhibit rough, papillary structure. Tumours are often pigmented and the surface of larger lesions is frequently eroded and ulcerated. The cut surface of the tumour mass exhibits the compact, shiny, white appearance of firm, compact connective tissue. The lesions are on average 3–5 cm in diameter, but they can sometimes reach 11–17 cm. They most commonly appear on the head, neck, belly and legs of the infected animal. Although the number of fibropapillomas on one animal is usually less than 10, individuals carrying more than 150 tumours can often be found.

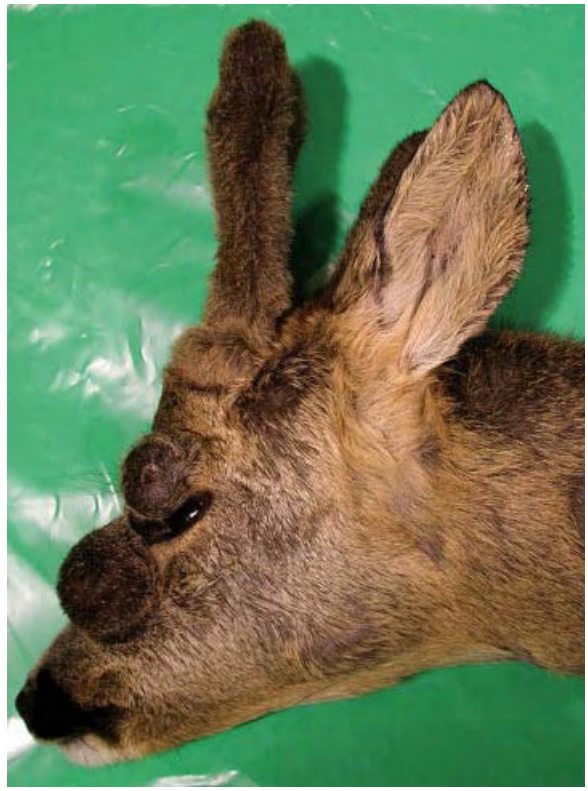

FIGURE 16.3 Macroscopic lesions of roe deer papillomatosis on the face.

Microscopically the main tumour mass consists of groups of proliferating fibroblasts embedded in a mesh of collagen fibres, located in the stratum reticulare of the dermis. Tumours are covered with the looser connective tissue of the upper dermis and the epidermis, showing signs of acanthosis and hyperkeratosis. The cytoplasm of enlarged keratinocytes in the stratum spinosum and stratum granulosum contain aggregations of keratohyaline granules, and their nuclei are vacuolated.

Both papillomavirus L1 antigen and DNA can be visualized in the epidermis covering the tumour stroma.

Papillomavirus DNA homologous to the L1 ORF sequence of CcaPV1 was found to be present in the nuclei of some stratum basale keratinocytes and in large numbers of stratum spinosum and stratum granulosum keratinocytes. Papillomavirus major capsid antigen can be found in both the cytoplasm and nuclei of keratinocytes of the stratum granulosum, but antigen aggregations representing complete papllomavirus particles are often observed between the keratin layers of the stratum corneum.

Nodular lung fibrosis occasionally associated with OvPV1 infection in European moose are multiple, firm, white nodules, 1–10 mm in diameter, formed by bundles of connective tissue located in the alveoli. The presence of AaPV1 DNA was demonstrated in these lesions by *in situ* hybridization, but virus particles were not detected.

CLINICAL SIGNS AND TREATMENT

In the majority of cases the presence of cutaneous fibromas and fibropapillomas does not have additional clinical effects and does not affect the general body condition, particularly in animals with few tumours. However, tumours on the head may obstruct vision or affect the ability of the animal to feed. This may eventually result in the decline of body condition and death of the infected animal due to emaciation. Tumours of extreme size may also hinder the normal movement of animals. It is not uncommon to find an extreme number of tumours on certain animals, coinciding with poor general condition. There is a possibility that these cases occur in immunosuppressed hosts or in animals simultaneously affected by other underlying illnesses.

Similar to the approach used in BPV infections of cattle, the treatment of infected captive deer might be attempted by auto-vaccines prepared from the lesions of the infected animal.

DIAGNOSIS

Papillomaviruses cannot be routinely propagated *in vitro* on tissue cultures. Diagnostic methods are therefore directed towards the demonstration of viral DNA or antigen in clinical or pathological samples. PCR can be used successfully for the demonstration of cervid papillomaviruses[3,4]. The method of rolling circle amplification (RCA) suitable for the detection of circular DNA is widely used for the detection of novel papillomaviruses and the amplification of complete genome sequences. Papillomavirus genomes amplified in RCA reactions can be linearized by digestion with restriction enzymes (e.g. *Eco*RV or *Sac*I in the case of CcaPV1 and red deer PV) and used for subsequent cloning and visualization.

In situ demonstration of papillomavirus DNA or antigen in the epithelial layer of skin tumours by *in situ* DNA hybridization or immunohistochemistry, respectively, can be applied to investigate the association of papillomaviruses with typical lesions (Figure 16.4). The close antigenic relatedness between the large capsid protein (L1) of BPV1 and other deltapapillomaviruses makes it possible to use commercially available anti-BPV1 L1 antibodies for the detection of cervid papillomaviruses by immunohistochemistry[3].

MANAGEMENT, CONTROL AND REGULATIONS

No attempts have been made so far to control fibropapillomatosis in wild cervids. Selective culling of animals with visible skin lesions and reduction of host density may be attempted as a control measure, but the potential efficiency of such intervention is dubious.

PUBLIC HEALTH CONCERN

As papillomaviruses are highly host-specific, deer papillomaviruses pose no risk to human health.

SIGNIFICANCE AND IMPLICATIONS FOR ANIMAL HEALTH

Papillomavirus cross-infections between farmed ruminants, including farmed deer, have not been detected. With the possible exception of OvPV1, all deer deltapapillomaviruses seem to be strictly host-species-specific, circulating within a single cervid species.

CHAFFINCH PAPILLOMA

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Chaffinch (*Fringilla coelebs*) papillomatosis is a disease caused by a papillomavirus, characterized by the develop-

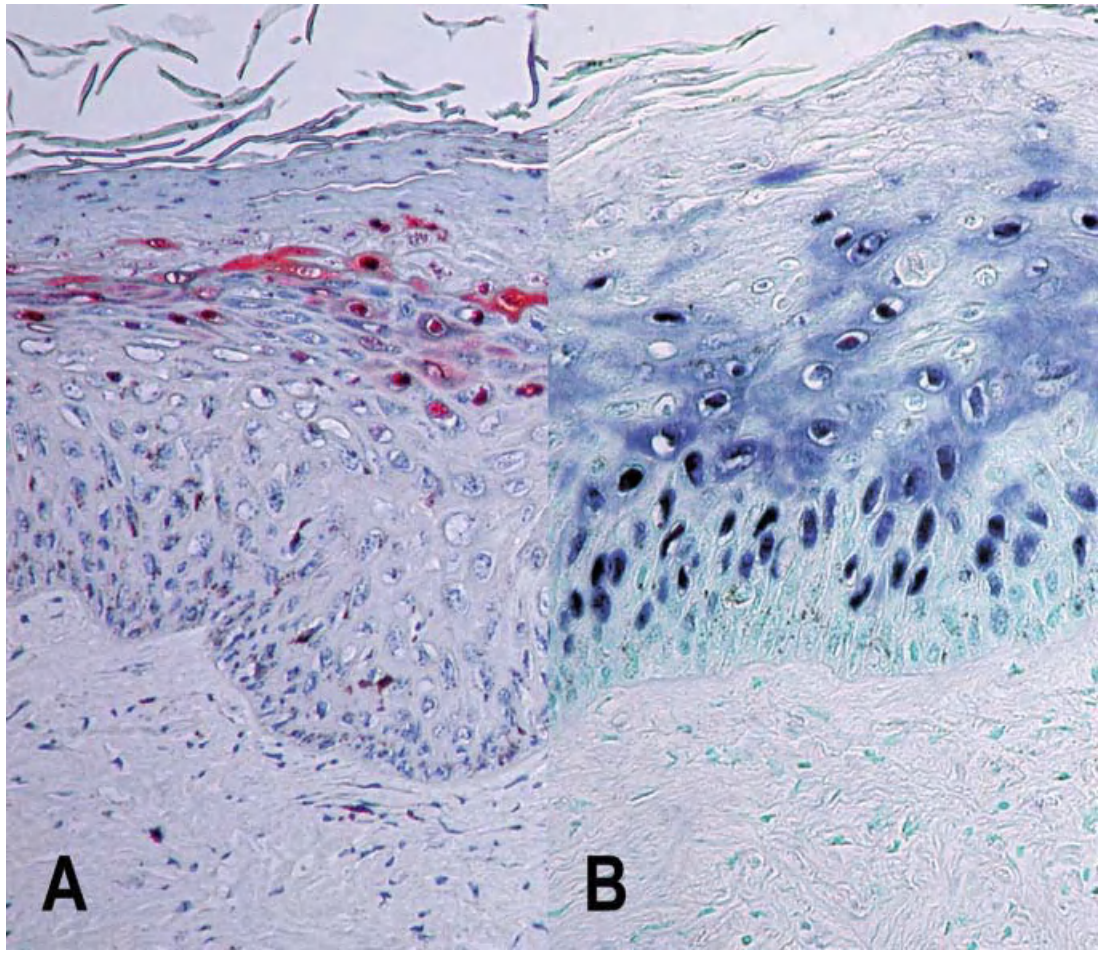

FIGURE 16.4 Roe deer papilloma, *in situ* hybridization (**A**) and histopathology (**B**).

ment of often extensive, benign skin tumours, typically occurring on the feet of infected birds.

The chaffinch papillomavirus, also known as *Etapapillomavirus 1* and the *Fringilla coelebs papillomavirus* (FcPV), is one of the only three avian papillomaviruses identified so far, and the only member of the *Etapapillomavirus* genus within the *Papillomaviridae* family. Its two closest relatives are the *Psittacus erithacus timneh* (African grey parrot) *papillomavirus* (PePV) and the *Francolinus leucoscepus* (yellow-necked francolin) *papillomavirus* (FlPV1). The circular double-stranded DNA genome of the FcPV is 7729 bp long and its genome organization is similar to that of PePV, clearly differing from the genome of mammalian papillomaviruses.

Papilloma-like lesions associated with papillomavirus infection have been observed in chaffinch populations throughout Europe (Czech Republic, Germany, the UK, Sweden, The Netherlands)[7]. Apart from chaffinches, closely related bramblings (*Fringilla montifringilla*) have also been found to be occasionally affected[8]. As papillomaviruses are host-specific, FcPV is assumed to circulate within populations of its host species. The virus is shed with squamous cells, and the route of virus transmission is through direct contact between individuals or with a virus-contaminated environment. Generally the disease occurs at a low prevalence, but the endemic nature of the infection may lead to significant clustering of cases in some areas.

Although first described in 1959, the pathogenesis of chaffinch papillomatosis has not been studied. It is assumed that FcPV induces the proliferation of epithelial cells by mechanisms similar to those used by mammalian papillomaviruses. Viral particles have to penetrate through skin

lesions into the deeper layers of the epidermis, where papillomaviruses replicate in keratinocytes of the basal layer. Papillomavirus virions are released with squamous cells shed from the surface of lesions.

Chaffinch papillomas are typical papillary lesions, characterized by the excessive proliferation of the skin epithelium over a thin core of supporting connective tissue. The epidermis exhibits acanthosis and hyperkeratosis, resulting in multiple layers of keratinocytes containing papillomavirus particles. Papillomas grow slowly, and the disease may progress over several months. Spontaneous regression of lesions probably occurs, as there are reports of ringed birds with lesions that are then not present on subsequent recapture.

The wart-like growths are found on the non-feathered skin areas, usually on the foot or the tarsometatarsus of one or both legs. The size of lesions varies from small nodules to deeply fissured massive papillary growths, which can reach up to 5% of the body mass[8]. The foot, digits and claws may be distorted, eventually resulting in lameness, but the general condition of affected birds is usually good.

Diagnosis may be based on the clinical picture; however, there are several diseases causing similar lesions. Co-infection with both papillomavirus and *Knemidocoptes* mites, which also cause similar leg skin lesions, may also occur. The definitive diagnosis can be established by histopathology, demonstration of papillomavirus virions by electron microscopy or detection of papillomavirus DNA by molecular techniques.

Repeated sightings of individual birds with lesions suggest that the disease does not significantly affect the general condition of infected chaffinches. This is confirmed by reports of good to moderate body condition in birds with lesions and it is often also true for individuals developing more extensive foot deformities. As mortality directly associated with the condition has not been reported, it is likely that spontaneous recovery may occur in a number of cases. There are no known zoonotic risks. As the chaffinch is common in Europe, this disease is not considered to be of conservation importance.

AVIAN POLYOMAVIRUS INFECTION

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Avian polyomaviruses (APV) are small double-stranded DNA viruses and were first described in budgerigars (*Melopsittacus undulatus*) in 1981. Antigenically similar viruses have been since isolated from a variety of passerines and have been associated with high morbidity and mortality worldwide. APV have also been identified in dead wild buzzards (*Buteo buteo*) and a kestrel (*Falco tinnunculus*) in Germany[9].

The acute nature of polyomavirus infection is unusual for papovaviruses. In captive and recently imported fringillid and estrildid finches it has been associated with acute mortality in 2- to 3-day-old chicks, fledglings, juveniles and adult birds. Some birds die peracutely and others exhibit non-specific signs of illness 24 hours prior to death. Clinical signs can include anorexia, weakness, ataxia, diarrhoea and lethargy.

Birds surviving infections can have poor feather development and long, tubular, misshapen beaks. Surviving chicks may have delayed fledging and can also develop persistent infections followed by intermittent shedding of the virus from the cloaca.

Transmission is usually horizontal via faeces, urates, feather dust and respiratory droplets. Vertical transmission may be possible but has only been confirmed in budgerigars[10].

In pied flycatchers (*Ficedula hypoleuca*) in Spain, polyomavirus has been found in larval blowflies in the nesting site, leading to infection in the chicks. Adults are subsequently infected via cloacal shedding from the chicks when nest cleaning[11]. No association was found with other common nest parasites such as *Dermanyssus*. Infected parents did not appear to lead to a higher incidence of APV in the chicks compared with families with non-infected parents, suggesting that routes such as oral transfer or droplet transmission is of less importance in disease transmission[11]. Underlying stress or concurrent diseases may cause subclinically affected birds to shed virus and result in outbreaks of clinical disease.

Affected birds may have no gross or histological lesions, or may show swollen, pale mottled livers, splenic enlargement and internal haemorrhage.

Histological changes can include inflammation and necrosis of the liver, myocarditis, bone marrow necrosis, lymphoid necrosis and plasma cell infiltration of the intestine. Intranuclear inclusion bodies can be demonstrated in the spleen, bone marrow, intestines, kidneys, heart, cloacal bursa and liver. Most isolates are serologically related to the psittacine polyomavirus, but DNA probes used for psittacine virus are unlikely to detect the virus in passerines[12].

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CHAPTER

17

CORONAVIRUS INFECTIONS

DOLORES GAVIER-WIDÉN, MARIE-PIERRE RYSER-DEGIORGIS, NICOLA DECARO AND CANIO BUONAVOGLIA

INTRODUCTION

DOLORES GAVIER-WIDÉN[1] AND MARIE-PIERRE RYSER-DEGIORGIS[2]

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The family *Coronaviridae* belongs to the order *Nidovirales* and includes the subfamily *Coronavirinae*, containing the genera *Alphacoronavirus* (previously known as group 1 coronaviruses), *Betacoronavirus* (group 2) and *Gammacoronavirus* (group 3). *Alpha-, Beta-* and *Gammacoronavirus* are further divided into different viral species[(1)]. A fourth genus, *Deltacoronavirus*, has been proposed to include novel coronaviruses detected in wild birds[(2)]. Among the *Betacoronaviruses*, the species *Severe acute respiratory syndrome-related* (*SARSr*) *coronavirus* includes the virus causing severe acute respiratory syndrome in humans (SARS-CoV) and SARS-CoV-related viruses identified in bats and wild carnivores. Since the severe SARS outbreak in early 2003, many new viruses of the family *Coronaviridae* have been identified in diverse host species, including birds, humans and other mammals.

Coronaviruses are 80–220 nm, spherical, enveloped, and have large (20 nm long) club-shaped spikes, called peplomers, surrounding a core structure with a helical nucleocapsid. These peplomers confer the viruses with a crown-like appearance, and the name 'coronavirus' was derived from *corona* in Latin, meaning 'crown'. The genome consists of a single molecule of linear positive-sense single-stranded RNA, up to 31 kb in size. They contain three to four structural proteins. The virus replicates in the cytoplasm.

The *Coronaviridae* contain numerous pathogens of a wide range of mammals and birds and cause a variety of diseases, although many infections are subclinical. In animals, coronaviruses generally cause respiratory or intestinal infections, but they have also been associated with a wide spectrum of other clinical symptoms, including hepatic, renal, reproductive and neurological dysfunctions. The remarkable diversity of coronaviruses results from their large genome together with their high mutation and recombination abilities. This causes emergence of new virus species and adaptation to new hosts and ecological niches, sometimes causing major zoonotic outbreaks[(3)].

FELINE INFECTIOUS PERITONITIS

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Infectious Diseases of Wild Mammals and Birds in Europe, First Edition. Edited by Dolores Gavier-Widén, J. Paul Duff, and Anna Meredith.

Feline infectious peritonitis (FIP) is a fatal immune-mediated disease of felids, characterized by vasculitis and granulomatous inflammatory reactions[4].

AETIOLOGY

Feline coronavirus (FCoV) is closely related to the transmissible gastroenteritis virus (TGEV) of pigs and *Canine coronavirus* (CCV), and exists in two forms (biotypes): feline enteric coronavirus (FECV), which replicates in enterocytes and is virtually non-pathogenic; and feline infectious peritonitis virus (FIPV), which has a broader cell spectrum, including macrophages, and causes fatal disease. It is widely accepted that FIPV arises by mutation from FECV in the gastrointestinal tract of an infected cat, then spreading systemically and causing FIP; an alternative hypothesis is that two strains, a virulent one and an avirulent one, simultaneously circulate in cat populations[4,5]. Depending on their antigenic relationship to CCV, FCoV strains can be classified into the subtypes serotype I (not neutralized by antibodies against CCV) and serotype II (neutralized by antibodies against CCV), the latter being suspected to have arisen by recombination between FCoV serotype I and CCV. Both serotypes can cause FIP, but most cats with FIP are infected with FCoV serotype I[4,5].

EPIDEMIOLOGY

FCoV is distributed worldwide. Both FIP and exposure to FcoV have been reported in several non-domestic felids, in captivity and in the wild[6,7] (Table 17.1). Cheetah seem to be particularly vulnerable, even more so than domestic cats[15]. In Europe, very low seroprevalences have been reported in free-ranging populations of European wildcat (*Felis silvestris*) and Eurasian lynx (*Lynx lynx*), and infection with FCoV in these species is considered not to be maintained, owing to their solitary social system[10,12]. FCoV is endemic in environments in which many cats are kept together in a small space but is relatively rare in free-roaming cats, because they usually have a predominantly solitary life without close contact with other cats – most importantly, they do not share the same locations (latrines) for their faeces, which is the major route of transmission in multiple-cat households[4]. A recent survey in the Iberian lynx (*Lynx pardinus*), which is also a solitary felid, revealed 16% and 40% seropositive lynx, depending on which population they originated from (Table 17.1). This higher seroprevalence was attributed to higher densities and closer contacts with domestic cats[14]. However, seroprevalence in wildcats is also low, although contacts between wildcats and domestic cats (including hybridization) are relatively frequent. The fact that the Iberian lynx uses latrines may play a major role in the exposure to FCoV in this species[7].

In the domesticated cat the risk of developing FIP is higher for males and for young and immunocompromised cats, because FCoV replication in these animals is less controlled and the critical FECV mutation is more likely to occur. Studies in purebred catteries showed that susceptibility to FIP is heritable, and although not completely understood, genetic factors appear to increase the risk of developing FIP. Environmental factors play an important

TABLE 17.1 Documented investigations of FCoV exposure in free-ranging wild felids in Europe.

Species	Country	Study period	FCoV Ab prevalence, % (N tested)	FCoV viral RNA, % (N tested)	Reference
European wildcat (*Felis silvestris*)	UK (Scotland)	1982–1990	0 (23)		8
	UK (Scotland)	1992–1997	6 (50)		9
	Switzerland	1996–1997	0 (9)	0 (9)	10
	France	1996–1997	6 (34)	1 (22)	10
	Germany	1996–1997	0 (8)	0 (8)	10
	Spain	1991–1993	0 (22)		11
Eurasian lynx (*Lynx lynx*)	Sweden	1993–1999	0 (102)		12
	Switzerland	prior to 2005	4.6 (65)		Ryser-Degiorgis (unpublished data)
Iberian lynx (*Lynx pardinus*)	Spain (Doñana and Sierra Morena)	1989–2000	0% (37)		13
	Spain (Doñana)	2003–2007	16% (44)	0% (45)	14
	Spain (Sierra Morena)	2003–2007	40% (30)	0% (32)	14

role, including re-infection rate in multiple-cat households and stress[4,15]. Concurrent *Feline leukemia virus* (FeLV) infection can greatly increase the clinical incidence of FIP[16]. Although the Iberian lynx has been shown to be vulnerable to feline viral diseases[14] and concern has been raised regarding possible impairment of their immune system, no case of FIP has been documented in this species so far, either in the wild or in captivity, despite relatively high exposure rate to FCoV. By contrast, clinical disease has been reported in captive wildcats[17] and Eurasian lynx[18].

Infection usually takes place oronasally through FCoV-containing faeces, although other routes have also been documented, e.g. through saliva, urine or transplacental infection. FIPV has not been found in secretions or excretions of cats with FIP, and transmission of the mutated FCoV inducing FIP is considered unlikely under natural circumstances. Iatrogenic transmission may occur, however[4,16]. FCoV is a relatively fragile virus, but in dry conditions it has been shown to survive for up to 7 weeks outside the host, and indirect transmission through clothes and other fomites is possible. Many naturally infected healthy carrier cats shed FCoV for at least up to 10 months, sometimes even throughout life[4].

PATHOGENESIS, PATHOLOGY AND IMMUNITY

Approximately 2 weeks after the mutation from FECV to FIPV has occurred, mutated viruses are found in various organs, including spleen, liver and central nervous system. Complement and inflammatory mediators are released from infected macrophages and cause lesions. Two different forms of FIP have been identified. Cats with a poor cell-mediated immune response develop an effusive, exudative wet form, which is an immune-complex vasculitis that causes leakage of protein-rich fluid from the blood vessels into the body cavities. In cats with partial cell-mediated immunity, a non-effusive non-exudative dry form develops, characterized by granulomatous lesions in multiple tissues. Frequently, however, dry FIP is often preceded by a brief episode of wet FIP, and may also become effusive in the terminal stages of the disease, when the immune system response fails[16].

Peritonitis, pleuritis and pericarditis with effusions characterized by very high protein content and granulomatous changes are typical[4–6,17,19]. Involvement of the eyes and/or central nervous system predominates in cats with dry FIP. Generalized synovitis is frequent. Hydrocephalus and orchitis may occur[16]. Histologically, there are mainly perivascular infiltrations of mixed inflammatory cells, granulomas and multifocal necrosis in various organs, and lymphoid depletion. Wet FIP is characterized by perivascular pyogranulomas consisting of aggregates of macrophages filled with virus and surrounded mainly by neutrophils and oedema. The lesions of dry FIP consist of more 'classic' granulomas, mainly located at the surface of organs, with small foci of macrophages in the centre, many of which contain no or small amounts of viral antigen, surrounded by broad bands of lymphocytes and plasma cells[16].

CLINICAL SIGNS AND TREATMENT

Clinical signs appear to be similar in wild felids and domestic cats, although reports are scarce in the former. Most FCoV infections are subclinical. However, after initial FCoV infection, transient mild upper respiratory signs, mild diarrhoea and/or vomiting may be observed in domestic cats. Severe enteric disease with weight loss rarely occurs[4,5].

Clinical signs of FIP are variable and unspecific, because many organs can be involved[4,5,17,19]. In felids with ascites, abdominal swelling is common. Thoracic effusions usually manifest with dyspnoea and tachypnoea, together with muffled heart sounds heard during auscultation. Lethargy, fever, anorexia and weight loss, icterus, diarrhoea, vomiting and obstipation may occur. Abdominal palpation may reveal enlarged mesenteric lymph nodes, thickened loops of intestine, hepatomegaly and irregular shaped or enlarged kidneys. Ocular changes (in retina, uvea and iris) are common. The most frequently observed neurological disorders include ataxia, nystagmus and seizures.

Virtually every cat with confirmed FIP dies[4,17,19]. There is no effective treatment and no effective vaccine.

DIAGNOSIS

Diagnostic options in domestic cats have been reviewed in detail[4,5,16], and information on wild felids is largely in agreement with them. *Ante mortem* diagnosis of FIP is extremely challenging, owing to the lack of specific clinical signs, the lack of pathognomonic haematological and biochemical abnormalities, and low sensitivity and specificity

of routine diagnostic tests. The albumin-globulin ratio, characterized by a decrease of albumin (liver failure, protein loss e.g. due to nephrotic glomerulopathy, or vasculitis) and an increase in globulin, seem to have the best diagnostic value, a ratio lower than 0.6 strongly indicating FIP in domestic cats. For definitive diagnosis, a *post mortem* examination is required, with the demonstration of FIP-positive macrophages by immunostaining in addition to the presence of typical histological changes.

Presence of enteric FCoV can be diagnosed by reverse transcription polymerase chain reaction (RT-PCR) or electron microscopy in faeces, and by immunohistochemical or the immunofluorescent staining of intestinal biopsies. RT-PCR on faeces is useful for documenting viral shedding; the strength of the RT-PCR signal correlates with the amount of the virus present in the intestine. However, as shedding is variable, repeated PCR should be performed daily over 4–5 days for an accurate result. Freezing samples may lead to false-negative results.

Results from serological assays (usually immunofluorescence) must be interpreted with caution. Antibody titres may significantly vary with different methodologies. Also, a large percentage of healthy felids are FCoV antibody-positive with high and rising titres without ever developing FIP, and the absence of antibodies excludes neither infection nor disease. However, a healthy animal without antibodies is considered likely to be free from FCoV. The height of the antibody titre generally correlates well with the amount of virus shed in the faeces. RT-PCR can be performed on blood, but it does not distinguish infection between mutated and non-mutated virus, and false-positive and false-negative reactions may also occur.

MANAGEMENT AND CONTROL

Avoidance of overcrowding and maintenance of a larger proportion of older cats helps to minimize population loads of FCoV. Small groups can spontaneously and naturally become FCoV-free[4].

Antibody testing is recommended to screen felids before their introduction into a FCoV-free environment. Thorough cleaning and sanitizing of potentially contaminated holding facilities is required[4,19,20]. FCoV is destroyed by most household disinfectants and detergents. There should be a 3-month delay before introducing a new, FCoV-free cat into a potentially contaminated environment. Because stress from capture, confinement and transport may compromise disease resistance, translocation procedures should aim to limit the stress associated with human exposure and the holding time of wild felids before release[7,20].

SIGNIFICANCE AND IMPLICATIONS FOR ANIMAL HEALTH

Although FCoV infection has been widely documented in domestic cats and wild felids, FIP appears to be a serious issue only in the captive environment. An impact on free-ranging wild felid populations has not been documented to date[7].

TRANSMISSIBLE GASTROENTERITIS

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Transmissible gastroenteritis (TGE) is a highly contagious, enteric disease of swine caused by a coronavirus (TGEV) closely related to the *Porcine respiratory coronavirus* (PRCV). TGEV is very stable when frozen but thermolabile and photosensitive. It can be spread via faeces on clothing and vehicles, and by subclinically infected pigs, carnivores, birds and insects[21].

In herds of susceptible pigs, morbidity is observed in all age classes, and mortality is high in young piglets (epidemic form), in particular in winter. However, in seropositive herds with frequent farrowing, there are only mild clinical signs and low mortality in piglets (endemic form)[21]. Little is known regarding TGE in European wild boar. Serological investigations have suggested absence or only low prevalence of infection in free-ranging populations[22–24], which are considered unlikely to play a role as a virus reservoir[25].

After being ingested, TGEV infects the mucosa of the small intestine. In domestic pigs, it rapidly causes extensive loss of epithelial cells, resulting in an acute malabsorption syndrome. At necropsy, the small intestine is typically distended by yellow and frequently foamy fluid and the intestinal wall is very thin as a result of villous atrophy.

Piglets display vomiting, profuse watery yellowish diarrhoea containing undigested milk, rapid weight loss and dehydration, and they eventually die, whereas older pigs

usually present only transient inappetence and diarrhoea. Viral detection and/or serology are needed to confirm diagnosis. Cross-reactions with PRCV are common in serologic assays, and results should be interpreted considering clinical signs (enteric versus respiratory signs) and disease history of the herd[21].

TGE is widespread worldwide in domestic pigs and can cause severe economic losses. There is neither specific treatment nor an effective vaccine. As a preventive measure, it is recommended to introduce swine into a herd only if they come from a farm free of TGE, are serologically negative, and/or have been placed in isolation for 2–4 weeks[21].

CANINE RESPIRATORY CORONAVIRUS INFECTION

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Canine respiratory coronavirus (CRCoV), considered a host variant of bovine coronavirus (BCoV), is now included in the unique species *Betacoronavirus 1* within the genus *Betacoronavirus.* CRCoV is unstable in the environment and may be rapidly inactivated by high temperature, ultraviolet radiation and common disinfectants, including solvents of lipids. Like BCoV and its derivatives, CRCoV is able to cause agglutination of mouse and chicken erythrocytes and to replicate *in vitro* on the human rectal tumour cells HRT-18. CRCoV was first isolated in 2003 in the UK from respiratory samples of domestic dogs housed in kennels with a history of respiratory disease. Subsequent virological and serological investigations showed a worldwide distribution of CRCoV in domestic dogs. In Europe, the virus has been proven to circulate in the domestic canine populations of the UK, Ireland, Italy and Greece, but at the moment there are no data about the virus circulation in wildlife.

CRCoV is mainly shed with the respiratory secretions of infected dogs, and the route of infection is typically oronasal. In dogs, CRCoV is responsible for mild respiratory signs and is recognized as aetiological agent of canine infectious respiratory disease, together with mycoplasmas, *Bordetella bronchiseptica*, canine adenovirus type 2, canine parainfluenzavirus, canine herpesvirus and reoviruses[26]. Analogously to these pathogens, single CRCoV infections can have a subclinical or asymptomatic course. However, viral replication in the upper respiratory tract may damage the mucociliar system, leading to concurrent infections by opportunistic pathogens[27]. CRCoV diagnosis is mainly based on virus detection by virus isolation, haemagglutination and PCR-based methods. At the moment, there are no specific therapeutic protocols nor prophylactic strategies against CRCoV infection. CRCoV is a dog pathogen without ascertained epidemiological and pathogenetic roles in wildlife. Although the infection is currently restricted to domestic dogs, a potential transmission to humans cannot be ruled out, considering that other BCoV-like viruses have already passed the species barrier, acquiring the ability to infect human hosts.

CORONAVIRUS INFECTIONS IN BATS

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Bats harbour an enormous diversity of coronaviruses (CoVs) worldwide, including more than 100 viruses belonging either to the *Alphaviruses* or to the *Betacoronaviruses*, and they are suspected to be the reservoir hosts from which all CoV lineages originated. CoVs, including severe acute respiratory syndrome (SARS)-like CoVs, occur in European bat species[28,29] and seem to be predominantly associated with *Microchiroptera*[28].

CoVs of several different genotypes can co-exist in a single bat, and recombination between different bat CoVs could give rise to new CoV genomes, which is believed to have occurred with the SARS CoV. This, together with the great diversity of CoVs, their ability to persist in bat populations and the finding that some CoVs can apparently infect bats of divergent genera, suggests that ongoing evolution of CoVs in bats may pose a continuing threat for emergence of novel CoVs in new hosts[30].

Studies on CoVs in bat populations suggest a peak of infections in spring, prevalence variations from year to year, and higher prevalence in juveniles and lactating mothers. Young bats are possibly more susceptible to infection and may propagate and maintain the viruses within bat colonies[30].

Although CoVs persist within bat populations, individual bats seem to experience only self-limited infections

without apparent illness. Coronaviruses are detected in faecal samples by RT-PCR, followed by sequencing of amplicons and phylogenetic analysis.

CoVs may be particularly significant among potential zoonotic viruses because of their potential to be transmitted via respiratory or faecal-oral routes, resulting in a proven ability to cause major epidemics after host transition.

OTHER CORONAVIRUS INFECTIONS

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Porcine epidemic diarrhea virus (PEDV) belongs to the genus *Alphacoronavirus*, along with FCoV, TGEV and mink and ferret coronaviruses (CoVs). PEDV causes a disease similar to TGE in domestic pigs, characterized by vomiting and acute watery diarrhoea. Outbreaks have been mainly reported from Europe and Asia[31] and may result in serious losses. *Porcine haemagglutinating encephalomyelitis virus* (PHEV) is a member of the genus *Betacoronavirus*, together with *Bovine coronavirus* (BCoV), *Equine coronavirus*, *Mouse hepatitis virus*, *Severe acute respiratory syndrome-related* (SARS) *coronavirus*, and other mammalian CoVs. PHEV causes vomiting and wasting disease or encephalitis in domestic piglets. It is a globally widespread infection, sometimes causing severe economic losses[32]. The European wild boar has not been reported to be susceptible to PEDV or PHEV infection so far.

BCoV and closely related viruses (now included in the unique species *Betacoronavirus 1*) are found worldwide and cause respiratory and enteric infections in cattle – including calf diarrhoea, winter dysentery (WD) and bovine respiratory disease complex – and hemorrhagic diarrhoea in wild or feral ruminants, including water buffalo in Italy[33,34]. Wild ruminants are suspected to play a role as reservoirs for BCoV-like viruses that are able to infect cattle. CoVs similar to BCoVs have also been detected in dogs, birds and humans with clinical disease[33].

The genus *Gammacoronavirus* includes mainly avian viruses (*Infectious bronchitis virus* (IBV), the closely related *Turkey coronavirus* (TCoV), *Pheasant coronavirus* (PhCoV) and IBV-like viruses detected in wild birds), but recently discovered viruses from beluga whale (*Delphinapterus leucas*) and Asian leopard cat (*Prionailurus bengalensis*) also belong to this group[35]. IBV causes an acute respiratory disease in chickens of all ages and diminishes egg production in hens, resulting in severe economic losses in the poultry industry worldwide. A number of strains are nephropathogenic and lead to high mortality. TCoV and PhCoV cause a similar disease in turkeys and pheasants, respectively. A CoV was identified in pigeons exhibiting respiratory symptoms, but its causal role was uncertain[36]. IBV-like viruses and new CoVs have been found in diseased and apparently healthy individuals of several wild bird species in Europe and elsewhere[35,37,38]. A new genus, *Deltacoronavirus*, has been proposed to include three new coronavirus species detected in Passeriformes[2].

CoV infection is most commonly demonstrated by RT-PCR on faecal samples or by serology, but other diagnostic techniques have also been developed. Recent research on coronaviruses in mammals and birds indicates that the geographic distribution, host range and genetic diversity of CoVs are much greater than previously thought, and it is of concern that viruses present in wildlife may have the potential to emerge as human or animal health threats.

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CHAPTER

18

BUNYAVIRUS INFECTIONS

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INTRODUCTION

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The family *Bunyaviridae*, one of the largest viral families with over 300 viruses, contains five genera: *Bunyavirus, Phlebovirus, Nairovirus, Tospovirus* and *Hantavirus.* The viruses have similar morphological features: the viral particles are spherical or pleomorphic, and range from 80 to 120 nm in size. Most of the 300 members of the *Bunyaviridae* family are arthropod-borne, the genus *Hantavirus* being the exception (rodent-borne). Bunyaviruses are single-stranded, enveloped RNA viruses with a genome of negative polarity, divided into three segments. Hantaviruses (genus *Hantavirus,* prototype virus *Hantaan virus* (HTNV), named after the Hantaan River in South Korea) are enzootic viruses carried by wild rodents that cause persistent infections in their natural hosts.

HANTAVIRUSES INFECTIONS

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Haemorrhagic fever with renal syndrome (HFRS) first drew the attention of the medical world in 1951, when more than 3,000 United Nations troops in Korea became ill with a 'new' disease, which had a 10% mortality. However, this 'new' disease, now known to be due to hantavirus infection, had been discovered and rediscovered several times before in many regions.

Earle, and earlier Langdon-Brown, suggested that a condition similar to HFRS was present during the American civil war (1862–1863) called 'trench nephritis'. A similar disease affected German and Allied troops during World War I (1915–1916). A detailed description of what was then called 'war nephritis' was published in 1918. In Korea, Manchuria and the far east of Russia the condition has probably been endemic for centuries. A report of a milder form of the disease in Finland (Puumala fever) was published in 1934. During World War II Japanese troops in Manchuria also fell victim to a similar disease, then called songo fever. In the 1950s, HFRS emerged in former Yugoslavia, Bulgaria, Hungary and Czechoslovakia.

Before 1983 hantavirus disease was referred to with different, usually local, names in Europe and Asia: haemorrhagic nephrosonephritis, epidemic haemorrhagic fever, Korean haemorrhagic fever, nephropathia epidemica, songo fever, Puumala fever, Tula fever, etc. In 1982, the Working Group on Haemorrhagic Fever with Renal Syndrome recommended using the name 'haemorrhagic fever with renal syndrome' (HFRS) to describe the different conditions[1].

Infectious Diseases of Wild Mammals and Birds in Europe, First Edition. Edited by Dolores Gavier-Widén, J. Paul Duff, and Anna Meredith.

AETIOLOGY

Hantaviruses are enveloped viruses with a tri-segmented (S, M and L segment) negative-stranded RNA genome. The small (S) segment encodes the nucleocapsid, the medium (M) segment encodes two envelope glycoproteins (Gn and Gc) and the large (L) segment the RNA-dependent RNA polymerase.

Several authors consider hantaviruses to be one of the best examples of a long-term association between RNA viruses and their hosts. The strong host specificity seems to suggest that hantaviruses co-speciated with the rodents and insectivores they infect, as these mammals last shared a common ancestor approximately 100 million years ago. However, there is scant evidence for a history of co-divergence between hantaviruses and their hosts. Moreover, the divergence times for the hantavirus genus are several orders of magnitude more recent compared with the speciation timescale of their hosts. The phylogenetical similarities between the hantaviruses and their hosts are thus probably the result of a more recent history of adaptation and host switching. The genus *Hantavirus* consists of at least four serogroups or clades, with at least 40 different serotypes. As only about 10% of the more than 2,000 existing rodent species have been examined for their hantavirus carrier status, it is highly probable that the genus *Hantavirus* consists of 300 to 400 serotypes. Of the 40 serotypes known at present, at least 13 viruses can cause one of two major clinical syndromes, sometimes with overlapping features, in humans: HFRS in Europe and Asia, and hantavirus cardiopulmonary syndrome (HCPS) in the Americas. HFRS is caused by *Hantaan*, *Seoul*, *Dobrava*, *Saaremaa* and *Puumala* viruses (HTNV, SEOV, DOBV, SAAV and PUUV, respectively). SAAV and PUUV cause a mild form of HFRS referred to as nephropathia epidemica (NE). HCPS is caused by *Sin Nombre*, *Black Creek Canal*, *Bayou*, *New York 1*, *Juquitiba* and *Andes* viruses (SNV, BCCV, BAYV, NYV, JUQV and ANDV, respectively).

EPIDEMIOLOGY

GEOGRAPHICAL DISTRIBUTION

PUUV, Tula (TULV), DOBV, SAAV and SEOV hantaviruses have been reported as circulating in Europe. NE is typical in PUUV and SAAV infections. HFRS is typical for HTNV, DOBV and SEOV infections. PUUV and DOBV have caused the vast majority of human cases in Europe[2] (Figure 18.1). SAAV has also recently been found to be responsible for human cases in Eastern Europe. TULV has been linked to human infections in the Czech Republic, Switzerland and Germany. No confirmed human cases of SEOV infection have been reported in Europe so far, although an unpublished case, confirmed by focus reduction neutralization tests (FRNT), occurred in France. In Western and Northern Europe only PUUV infections have been reported. From west to east across Europe, the DOBV infection prevalence in humans varies from 3.6% in Southern Germany, to more than 50% in Slovenia and 100% in Greece.

During the past two decades, the number of diagnosed human cases has been increasing constantly in almost all European countries. Since 2002, record case counts have been noted in Finland (2,603 cases in 2002, 2,526 cases in 2005), Sweden (459 cases in 2004), Germany (more than 1,400 cases reported in September 2007) and Belgium (372 cases in 2005). Asymptomatic or mild infections that go unnoticed probably result in an underestimation of the number of hantavirus infections. The ratio ranges of subclinical to clinical infections for PUUV infection is from 5:1 to 10:1 in Europe; it is thus reasonable to assume that the actual number of infected individuals is 5 to 10 times higher than the reported cases. An 8-year study in Finland demonstrated that the estimated ratio of diagnosed HFRS cases was only 13% (4 to 30% for different areas), leaving at least 70% of the PUUV infections undiagnosed because they were subclinical or showed only minimal or atypical symptoms. PUUV, DOBV and HTNV infections are predominant in rural areas, whereas SEOV infections occur in urban settings. Investigations have linked handling wood, farm work, sleeping on the ground, military exercises and lower socio-economic status to increased risk for acquiring hantavirus infection. HFRS is more common in males than in females (the male : female ratio can reach 2:1 to 3:1), and most of the cases occur in the 20–50 age group. In most European countries the seroprevalence rate in the general population ranges from 1 to 5%, with peaks in certain regions (Finland, Bosnia Herzegovina) up to 20% (Figure 18.1).

ENVIRONMENTAL FACTORS

The distribution of hantavirus infections in time and space reflects the distribution range and population densities of

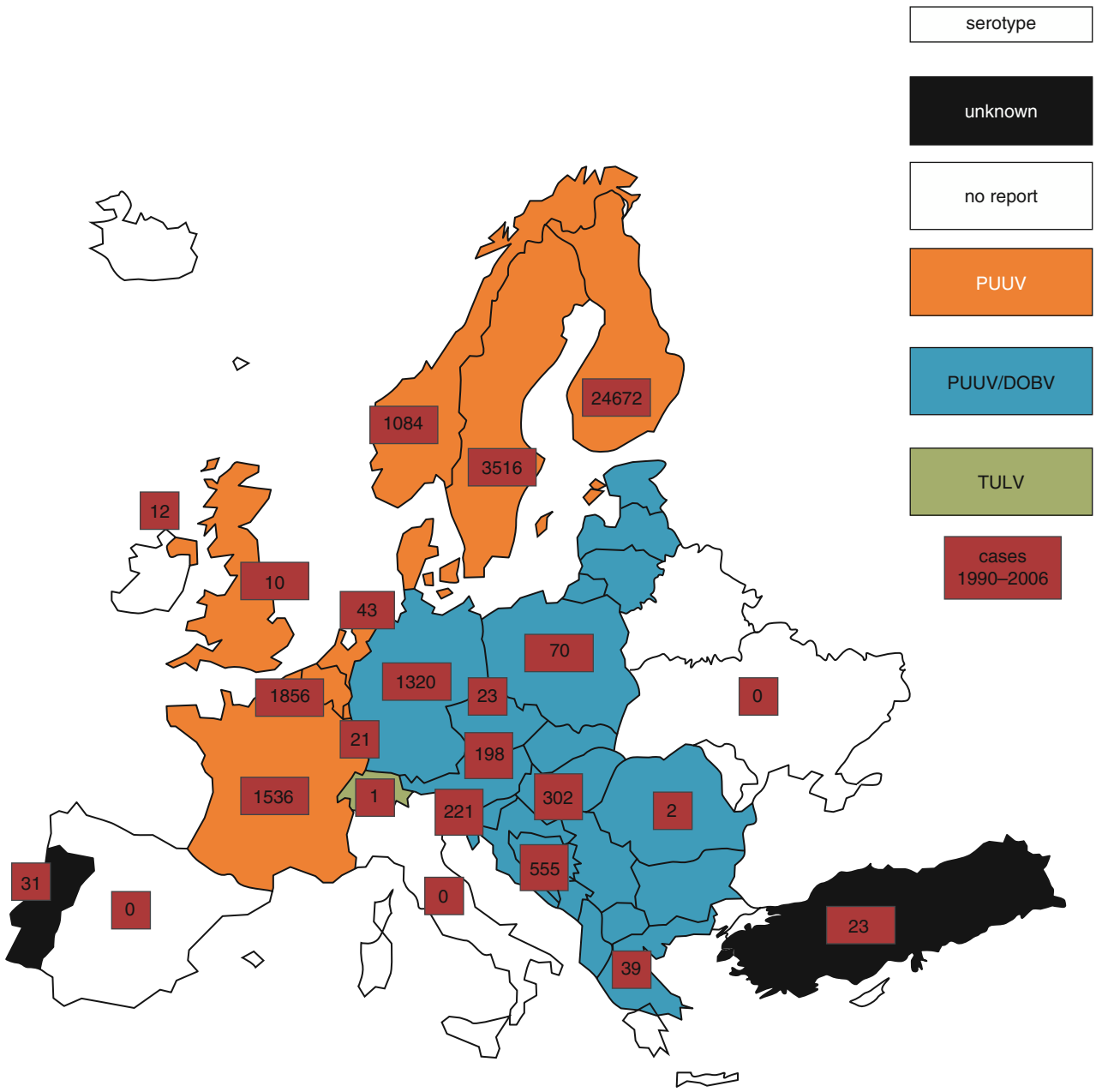

FIGURE 18.1 Human hantavirus cases in Europe (1990–2006).

their rodent reservoir host vector. High reservoir host abundance induces higher transmission rates to humans. In Northern Europe the bank vole (*Myodes glareolus*) population frequently has a 3–4 year cycle of abundance, which coincides with the PUUV infection patterns in the respective regions. These cycles are based on a predator–prey mechanism. In Western Europe, high bank vole population density is related to high tree seed production (mast years), which is triggered by particular climatic conditions. In Belgium, it was demonstrated that NE epidemics were preceded by abundant tree seed production, which in turn was directly linked to climate[3]. The increased hantavirus incidence in humans was preceded by high summer temperatures 2 years earlier and high autumn temperatures 1 year earlier[4].

EPIDEMIOLOGICAL ROLE OF THE AFFECTED SPECIES

Rodents and insectivores act as hantavirus vectors. The viruses are found in the Cricetidae family (subfamilies Arvicolinae, Neotominae and Sigmodontinae) and in the Muridae family (subfamily Murinae). Each hantavirus type is carried by a specific rodent host species, and phylogenetic analysis has revealed that the relationships between hantaviruses parallel the phylogeny of their rodent hosts, although host switches have occurred. Recently, new hantaviruses have been identified in insectivores. It is noteworthy that *Thottapalayam virus* (TPMV), which is carried by an insectivore, *Suncus murinus* (the Asian house shrew, or brown musk shrew), first described in 1971[5] precedes HTNV, which is carried by *Apodemus agrarius* (the striped field mouse) and was first described in 1976[6], although the latter was designated as the prototype virus. In nature, hantaviruses are maintained by horizontal transmission (rodent to rodent, via fighting, biting or grooming) and via aerosolized contaminated excreta.

In Europe, HFRS is caused by four hantaviruses: PUUV, carried by *Myodes glareolus* (bank vole); DOBV, carried by *Apodemus flavicollis* (yellow-necked mouse); TULV, carried by *Microtus arvalis* (common vole); and SAAV, carried by *A. agrarius* (the striped field mouse). In Asia, HFRS is caused by HNTV and the *Amur virus* (AMRV), which are transmitted by *A. agrarius* (striped field mouse) and *A. peninsulae* (Korean field mouse), respectively, and by SEOV, which is transmitted by *Rattus rattus* and *R. norvegicus* (the black rat and the brown rat). Although *R. norvegicus* in Europe was found to carry SEOV, the virus does not seem to be a public health problem at present.

A clear negative effect of high species diversity on both abundance of reservoir hosts and their infection rate with hantaviruses in Panama, South America, was demonstrated[7]. However, a different pattern seems to exist for *Sin Nombre hantavirus* (SNV) in deer mice (*Peromyscus maniculatus)* in North America, where a negative correlation between habitat disturbance and abundance and seroprevalence was noted. In Belgium, a correlation between high host diversity and low rates of pathogen transmission, or disease risk, for PUUV was noted[3]. These findings strongly suggest that high biodiversity contributes to the protection of human health.

All non-rodent mammals, with seropositive populations, are considered dead-end hosts, i.e. not capable of transmitting the infection to conspecifics. Little is known, however, about the presence of hantaviruses in non-rodent mammals. Experimental infection in various non-human primates has led to an antibody response[8]. Antibodies to hantaviruses were detected in domestic cats (*Felis catus)*[9] and dogs (*Canis familiaris*)[10], and in red foxes (*Vulpes vulpes*)[11]. In Belgian dogs and cats, a seroprevalence of 4.9% and 16.9%, respectively, was found. In Sweden, antibodies to PUUV were detected in moose (*Alces alces*) from endemic areas[12].

Coyotes (*Canis latrans*), island foxes (*Urocyon littoralis*) and 25 individuals from seven different mammalian species were all found negative for hantaviruses in California[13]. HTNV was detected in a bird, *Emberiza elegans*, in Russia[14]. Antibodies to HTNV were also detected in bats (Jung, 1995)[15] in South Korea.

The viruses are transmitted to humans by aerosol of infected urine, saliva and faecal material from rodents. In the reservoir species, horizontal transmission occurs, whereas humans and other mammals are dead-end hosts.

PATHOGENESIS, PATHOLOGY AND IMMUNITY

The infectious dose for all hantavirus serotypes is unknown, but it is considered to be low. Presumably, high viral loads cause a more severe infection. Humans become infected when they come into contact with rodents or inhale aerosolized particles. For all but one serotype (the South American ANDV) human-to-human transmission has not been described. Rodent bites may also be a route of infection, but this is rare compared with the inhalation route. Infection via the digestive tract is highly unlikely and has not been reported.

Infected rodents have life-long shedding of virus in saliva, urine and faeces, but the virus shedding is greatest approximately 3 to 8 weeks after initial infection[16]. The virus is also readily detectable in the lungs and the kidneys, but no pathophysiological signs have been described.

In HFRS damage occurs to capillaries and small vessel walls, inducing vasodilatation and haemorrhages. The damage is most severe during the hypotensive and oliguric phases (see below), when widespread haemorrhages can occur. The kidney is usually involved, but pulmonary symptoms are also possible. Although limited amounts of immune complex can be found in the kidney, it is thought that viral destruction of cells plays a more important role than immuno-pathological mechanisms in inducing nephritis.

All hantaviruses induce neutralizing antibody production in the host, which provides life-long protection against reinfection with the same serotype. Cross-protection against infection with other hantaviruses has not been described.

CLINICAL SIGNS AND TREATMENT

Hantavirus infections in rodents and insectivores are persistent and apparently subclinical. However, it was reported that SEOV infection increases aggressive behaviour in male rats (*R. norvegicus*). PUUV infection was recently linked to impaired breeding success and winter survival in bank voles. In mammalian hosts – apart from humans – there are no reports of clinical illness, but the available information is scarce.

HTNV and DOBV cause the most severe forms of HFRS; PUUV, SAAV (nephropathia epidemica) and SEOV cause less severe illness.

HCPS in humans is characterized by fever, myalgia, gastrointestinal symptoms and abrupt onset of respiratory distress and hypotension. The case fatality rate (CFR) can be as high as 40%. Convalescence in all forms of HFRS and HCPS may take weeks to months.

Specific treatment for humans, apart from general supportive measures, is not available for hantavirus infection.

DIAGNOSIS

The diagnosis of HFRS based on clinical examination only is difficult, as hantavirus disease invariably starts with non-specific flu-like symptoms. Diagnostic tests are required for confirmation.

Hantavirus-specific antibodies and antigen can be detected in rodent serum or tissue samples by serological and molecular biology tests, respectively. It is not possible to define the infecting serotype with standard serology tests, such as indirect fluorescent antibody (IFA) test, enzyme-linked immunosorbent assay (ELISA) or western blot, because of the cross-reactions between serotypes. For serological determination of the causal serotype, neutralization tests are required. Neutralization tests, however, are difficult and require at least a biosafety level (BSL) 3 environment.

In humans, hantavirus infection can also be diagnosed by reverse transcription polymerase chain reaction (RT-PCR) applied to blood samples. As the duration of the viraemia depends largely on the hantavirus serotype, RT-PCR must be applied as soon as possible after onset of symptoms. As a rule, higher viral loads are found in more severe hantavirus infections (HTNV, DOBV, SNV and ANDV infections), whereas the level and duration of the viraemia is considerably lower and shorter in PUUV infections. As a consequence, RT-PCR can only detect viral RNA in PUUV infections provided that sampling is done early enough (within the first 7 days after onset of symptoms). The detection of PUUV-RNA in the saliva of NE patients could open possibilities in diagnostic procedures. However, it must be noted that, contrary to what this finding suggests (namely the possibility of person-to-person transmission by close physical contact), PUUV is not known to be transmitted this way.

MANAGEMENT, CONTROL AND REGULATIONS

As hantaviruses are carried by sylvatic rodent species, management and control of populations is hardly feasible. Currently, no nationwide rodent control programmes are in effect. Pest control in agriculture and improved habitat conditions for the natural predators of rodents (mustelids, birds of prey, foxes, feral cats, etc.) may have beneficial effects.

Safe and effective vaccines are needed urgently in order to reduce the incidence of human illness, but to date these are not yet available in Europe or the USA, although M-segment DNA vaccines to HTNV and PUUV are in preparation. A vaccine against HTNV grown in suckling mice brain has been shown to induce protein immunity in mice and humans. It is currently available under the name Hantavax® and is licensed for use in the Republic of Korea, but neither EU nor FDA approval was granted.

Hantaviruses, like most enveloped RNA viruses, are susceptible to 1% sodium hypochlorite (10% sodium hypochlorite for heavily soiled material), 70% ethanol or 2% glutaraldehyde. Infected Vero E6 cells are completely inactivated after heating for 1 hour at 60°C, while about 90% of infectious particles are lost by heating at 56°C for 30 minutes; HTNV has shown sensitivity to a pH of 5. Infectivity of hantaviruses has been reported to persist in neutral solutions for several hours at 37°C and for up to 10 days at lower temperatures. Dried cell cultures remain infectious for up to 2 days. Virus suspensions stored at

–60°C in 1% bovine albumin salt solution remain infectious for over 5 years. Recently, it was shown that PUUV can survive outside the host for at least 10 days at room temperature and probably much longer (weeks or months) at lower temperatures – and, for instance, under snow cover.

Hantavirus disease is a notifiable disease in the majority of European countries; it is not notifiable in Austria, Cyprus, France, Portugal, Romania and Spain. In some countries, mainly in Eastern Europe, the legally required reporting status is not yet determined[2].

PUBLIC HEALTH CONCERN

Hantaviruses are globally important pathogens and cause thousands of human cases each year in Europe, thus having a significant public health impact. Depending on the serotype, the CFR may be high. In Europe the CFR for PUUV is <1% but for DOBV is around 10%. Although in many countries (Europe, Africa and South America) a seroprevalence exists in the general population, hantavirus disease is not always recognized by the medical community. The growing availability of low-cost and user-friendly diagnostic tests will hopefully change this, and there is likely to be a growing demand for diagnostics, as hantavirus infections are emerging infections. Hantaviruses have been reported in new areas of Europe, and their incidence has increased in several endemic regions. The annual number of HFRS cases in Europe has increased during recent years, including in those countries where the disease is known and virus-specific tests have been widely used[2].

SIGNIFICANCE AND IMPLICATIONS FOR ANIMAL HEALTH

Hantavirus disease has not been reported in domestic animals. Nevertheless, wild and domestic animals have been found to be seropositive. All rodent predators and animal species that may come in close contact with rodents, their nests or excreta are in fact candidates for infection through aerosol.

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RIFT VALLEY FEVER

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Rift Valley fever (RVF) is a mosquito-borne viral disease that was first recognized in 1931, affecting sheep in the Rift Valley in Kenya.

RVF, an RNA virus, is classified in the family *Bunyaviridae*. Although predominantly a pathogen of domestic ruminants, the virus is now recognized to affect many mammalian species, including humans, and to be endemic in many countries of sub-Saharan Africa. Since 1931, RVF has been responsible for extensive and devastating epidemics in East Africa and southern Africa, often involving several countries at a time. In 1977, the disease was recognized in Egypt – the first time that it had been reported beyond sub-Saharan Africa. The Egyptian epidemic was extensive in both the domestic animal and human populations. It was estimated that there were 200,000 human cases, with over 500 deaths. In 2000, RVF spread beyond Africa when it was detected in the western provinces of Saudi Arabia and Yemen. Since 2000, extensive outbreaks of RVF have continued to occur in several countries in East Africa and southern Africa, but also in countries in West Africa that border the Atlantic, such as Mauritania and Senegal. It is uncertain whether the virus persists in areas beyond sub-Saharan Africa.

The confirmation of RVF in the Arabian Peninsula, and the 'unexpected' epidemics of several other arbovirus diseases in humans and livestock, such as West Nile fever in the USA and bluetongue in Europe, have led to speculation that RVF could cause similar extensive epidemics in the Americas and Europe. Experimental studies of the susceptibility of different species of North American mosquitoes have established that they are capable of efficient transmission of RVF virus. In the USA, the Department of Homeland Security rates RVF as the third most important foreign animal disease that could be introduced after foot-and-mouth disease and highly pathogenic avian influenza. Countries in Europe are similarly concerned that, were RVF virus to be introduced, an extensive outbreak could occur in domestic livestock and spill over into the human population. There are concerns that RVF could cause disease, possibly dramatic disease, in wildlife populations, but little is known of the susceptibility of North American and European wildlife to RVF.

The ecology of RVF virus is complex and not fully understood. The local climate may influence the life cycle of the virus and endemicity. In eastern and southern Africa, where the virus is recognized to be endemic, it is believed that there are two overlapping cycles, one of low level endemic activity and one of epidemic spread. The climate of this area is characterized by long periods of low rainfall for several years followed by heavy rainfall and floods induced by an El Niño event. The survival of RVF virus during the periods of low rainfall is facilitated by transovarial transmission of the virus by certain 'floodwater' mosquito species. The virus-infected eggs remain viable for long periods (probably several years) following the drying of the sites in which the female mosquito lays her eggs. The eggs hatch when the area floods and the adult mosquitoes are primed to transmit the virus. Local rain events may allow a low level of undetected transmission to occur (endemic activity), but widespread rains, allowing large populations of mosquitoes to develop, may be necessary for widespread epidemics. In areas such as the Nile Delta and the Arabian Peninsula, the absence of mosquitoes species in which transovarial transmission of the virus can occur may explain why the virus has not apparently become endemic.

There is no compelling evidence identifying any one African species of mammal as the natural reservoir of RVF virus. (Indeed, there is arguably no need for a mammalian reservoir host in areas where the virus is vertically transmitted within floodwater mosquitoes and can lie dormant in egg rafts for many years.) Serological surveys of wildlife indicate that several species of ruminants, carnivores and bats can become infected with RVF. Once climatic conditions are favourable for the hatching of the RVF-infected eggs of floodwater species, the African Cape Buffalo (*Syncerus caffer*) may act as an amplifying host, in a similar way to domestic ruminants.

Adult ruminants develop extremely high levels of viraemia, which allow many species of mosquito to be biological vectors. When climatic conditions are favourable for large mosquito populations to build, epidemics can occur over an extensive area, sometimes involving several neighbouring countries.

In domestic ruminants, RVF is predominantly a disease of sheep and goats, characterized by abortions and neonatal death. It is not unusual to see in excess of 90% of newborn lambs dying of RVF during an epidemic. The disease is characterized in domestic livestock by the sudden onset of lethargy, inappetence, high fever, nasal discharge, and diarrhoea. In the acute stages of the disease, viraemia may exceed 10^6 plaque-forming units (PFU) per ml. Necropsy reveals a diffuse hepatic necrosis, splenomegaly and gastrointestinal haemorrhage. Viral antigen is readily detectable in the reticulo-endothelial system and in multiple organs, including the liver, kidneys, adrenal glands, gastrointestinal tract, brain, ovaries and endometrium.

The disease in humans is usually characterized only by fever, but on occasion, by encephalitis, retinitis and/or generalized haemorrhage. Although some human cases almost certainly arise from mosquito bites, RVF is seen most frequently in individuals who are exposed to viraemic blood during the slaughter of animals and the butchering of meat. Veterinarians are at greater risk of acquiring RVF than the normal population, from drawing blood and conducting necropsies.

A comprehensive bibliography on Rift Valley fever may be found in Bird et al. (2009)[17], and specifically in relation to wildlife in Evans et al. (2008)[18].

CRIMEAN-CONGO HAEMORRHAGIC FEVER

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Crimean-Congo hemorrhagic fever (CCHF) *virus* is a member of the genus *Nairovirus* in the family *Bunyaviridae*. CCHF is one of the severe forms of human haemorrhagic fever that is endemic in Africa, Asia, Eastern Europe and the Middle East and is invariably fatal. CCHF virus is an enveloped, spherical, single-stranded RNA virus consisting of three RNA segments, small (S), medium (M) and large (L), which encode the nucleocapsid protein, glycoproteins and viral polymerase, respectively. The virus is tick-borne, and its distribution correlates with that of the *Hyalomma* tick, which is the main vector, although other ixodid species may also be infected. The virus circulates in an enzootic tick–vertebrate–tick cycle, and a wide range of domestic and wild species may act as a reservoir. Antibodies to CCHF virus have been detected in numerous small wild mammals, including European brown hare (*Lepus europaeus*), hedgehog (*Erinaceus europaeus*) and house mouse (*Mus musculus*). These smaller species that harbour immature ticks are believed to act as amplifying hosts and maintain the virus, and mature ticks can then

transmit the infection to larger species, including domestic and wild ruminants, horses and pigs. Ticks have both trans-stadial and transovarial transmission of virus, and humans are generally infected by tick bites, although infection by percutaneous or permucosal routes by contact with animal blood or tissue or drinking unpasteurized milk is also possible.

Clinical disease has only been described in humans, and the recent emergence or re-emergence of CCHF in several Balkan countries, Turkey, southwestern Russia, the Ukraine, northern Greece and Spain has highlighted CCHF disease as a significant zoonotic threat in Europe. Reasons for re-emergence may include climate change and anthropogenic factors such as changes in land use, agricultural practices or hunting activities, and movement of livestock that may influence vector competence and host–tick–virus dynamics. The role of migrating wild birds, particularly ground-dwelling species that are more likely to harbour infected ticks, is unclear but is also considered as a potential route of incursion into new regions in Europe.

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CHAPTER

19

OTHER VIRUS INFECTIONS

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HEPATITIS E

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Hepatitis E virus belongs to the family *Hepeviridae*, which contains two genera: one named *Hepevirus* and one unnamed. The genus *Hepevirus* contains one species consisting of four genotypes (gt1, gt2, gt3 and gt4). A fifth species, *Avian hepatitis E virus* (avian HEV), has not yet been assigned to a genus. The genotypes 1, 2, 3 and 4 exhibit a sequence similarity of up to 75%. The different genotypes can each be further divided into several subgroups. There are indications that genotype 3 and 4 are less virulent than genotype 1 and 2[1]. Hepatitis E virus is small (28–34 nm), rounded and without an envelope. It is resistant to environmental influences and can stay infective for extended periods outside the animal host. The genome consists of single-stranded positive-sense RNA of approximately 7.2 kb (6.6 for avian HEV) that serve as a messenger RNA in the infected cell. The genome consists of three partially overlapping open reading frames.

In the genus *Hepevirus* the gt 1and 2 infect only humans and are known to cause epidemic outbreaks under poor hygienic conditions in developing countries. The gt 3 and 4 are frequently present in pigs and wild boar and cause sporadic infections in humans. The detection of HEV in rabbits has led to the proposal of a fifth genotype of this genus, as it is only approximately 75% similar to the other genotypes[2].

The unnamed genus contains avian HEV, which has been found in poultry and is only 50% similar to the viruses in the *Hepevirus* genus. It can be further divided into three genotypes. There are no indications that these genotypes could infect mammals, including humans.

Furthermore, HEV detected in brown rats (*Rattus norvegicus*) appears to differ substantially from the genus *Hepevirus*, with which it only is 60% similar, and from Avian HEV, with which it shares 50% sequence similarity. Wild boar (*Sus scrofa scrofa*) in Europe have been found to be infected with HEV hepevirus gt 3 in several European countries, including Spain[3], Italy[4], France[5], Germany[6], Hungary[7], the Netherlands[8] and Sweden[9]. Genotype 4 is present in South and Southeast Asia and has not been detected in Europe.

Hepevirus has also been detected in other wild animals in Europe, including brown rats in Germany[10] and roe deer (*Capreolus capreolus*) and red deer (*Cervus elaphus*) in Hungary[7]. Elsewhere, detection of hepevirus has been reported in mongooses (*Herpestes javanicus*) and Sika deer (*Cervus nippon*) in Japan, and in oysters and farmed rex

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rabbits in China. There are also reports on the detection of hepevirus in Asian black bears (*Selenarctos thibetanus*), clouded leopards (*Neofelis nebulosa*), cattle, sheep and horses in China, but confirmation by further investigations are warranted.

This virus is not known to cause overt disease in wild animals, and little is known about the epidemiology. In domestic pigs the virus is most frequently detected in pigs of 3–4 months of age when maternal antibodies are waning and piglets from different sows are mixed. The fact that wild boar tend to live in family groups may have an influence on how HEV is spread in this species, resulting in a different epidemiological situation to that of domestic pigs. Infection with HEV is self-limiting in mammals, including humans and pigs. Excretion in pig faeces lasts for 3–7 weeks[11] and viraemia for 1–2 weeks.

Infection with HEV occurs by the oral route. In poultry, Avian HEV causes disease characterized by enlarged liver and spleen ('big liver and spleen disease'). However, it is not known whether wild birds also can become infected. In pigs HEV infection causes mild to moderate hepatitis.

Antibodies to HEV have been detected in several animal species such as dogs, cats, sheep, cattle and goats, as well as in non-human primates, but confirmation by direct virus detection methods is lacking and the presence of HEV in these animals has therefore not been confirmed. Another reason for caution is the difficulty of achieving consistent results for HEV antibody detection by enzyme-linked immunosorbent assays (ELISA) in different animal species.

The clinical signs in infected pigs are either inapparent or mild, and although this may also be true for wild boars it has not been studied. In humans, the clinical outcome of the infection is apparently dose-dependent[1]; it may be sub-clinical or may cause mild to grave clinical symptoms and lead to death in approximately 0.5–4% of infected individuals due to hepatic failure. The mortality in pregnant women is significantly higher and may reach 25%.

As wild animals infected with HEV probably do not display signs of disease, similar to domestic pigs, the diagnosis is based on laboratory findings. Direct diagnostic methods include reverse transcription polymerase chain reaction (RT-PCR) or real-time RT-PCR for detection of HEV RNA in samples of faeces, liver or bile, whereas indirect methods such as ELISA can be used for detection of antibodies to HEV. Isolation of virus in cell culture is currently not possible. Although one research team[12] report successful replication *in vitro*, others have not been able to reproduce the results.

At present, detection of HEV infection in animals is not notifiable, whereas infection in humans is notifiable in many countries. Infection with HEV is not possible to treat but it is self-limiting in animals and humans, although a few reports suggest the possibility of persistent infection in immunosuppressed humans. Vaccines for animals are not available. Vaccines have been developed for humans but are not yet commercially available.

Several studies have demonstrated that HEV from infected humans can be highly similar to HEV detected in pigs and wild boars, indicating that the infection is zoonotic and can be transmitted to humans by a food-borne route from these animals. The strongest evidence of such a route of infection comes from Japan, where human infection has been linked to the consumption of under-cooked pig and wild boar liver, as well as venison, and from Corsica, France, where human infection has been linked to the consumption of lightly smoked sausage containing pig liver and pig meat[13]. However, so far the routes of infection from animals to humans have not been sufficiently clarified.

ROTAVIRUS INFECTIONS

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Rotaviruses are classified into a single genus within the family *Reoviridae*. They are 70–75 nm in diameter, icosahedral, triple-layered and non-enveloped, and structurally resemble a wheel, hence their name. The rotavirus genome contains 11 segments of double-stranded RNA, which encodes six structural and six non-structural viral proteins. It is characterized by genetic variability, including point mutations, genomic reassortment and genome rearrangements, leading to considerable diversity within the genus and the continuous emergence of new strains. Seven serological groups (A-G) are recognized, and it is the group A rotaviruses (GARV) that are the most clinically significant as pathogens.

Rotaviruses are one of the most important aetiological agents of severe diarrhoeal disease in animals and humans worldwide. Rotavirus-associated enteritis is a major problem in domestic livestock, particularly in young

calves, piglets and foals, but sheep and goats can also be affected. Calves are generally affected within the first 4 weeks of life, and disease involves other diverse infectious agents (bacteria, viruses and protozoa) in addition to rotavirus. Other factors such as herd management, environmental conditions, host nutrition and immune status are also important in determining the severity of disease. Rotavirus has been associated with disease in farmed deer and bison, and gastroenteritis associated with rotavirus and *Helicobacter*-like organisms has been reported in a 5-month-old farmed reindeer in Japan[14]. *Porcine GARV* are associated with weaning and post-weaning enteritis in piglets, which can occur in both enzootic and epizootic forms in commercial piggeries. Again, co-infections with other enteric pathogens increase disease severity. A serosurvey of wild European hogs (*Sus scrofa*) in the USA failed to find evidence of rotavirus infection. Equine GARV are the major cause of diarrhoea in foals up to 3 months of age. Rotaviruses are not recognized as major pathogens of domestic carnivores, but have been reported in clinically healthy Galápagos sea lions (*Zalophus californianus*) and Galápagos fur seals (*Arctocephalus galapagoensis*) pups[15]. A GARV is the cause of epizootic diarrhoea of infant mice (EDIM), an important disease of laboratory mice, and a high seroprevalence to rotavirus has been found in wild house mice (*Mus domesticus*) in Australia[16], in wild mice and rats on pig farms in France[17] and in healthy wild grey squirrels (*Sciurus carolinensis*) in Wales, UK[18]. Recently, rotavirus has been reported in a red squirrel (*Sciurus vulgaris*) in Scotland, UK, in association with diarrhoea and a colorectal intussusception[19]. Rotavirus causes enteritis in weanling domestic rabbits and antibodies to rotavirus have been demonstrated in wild cottontail rabbits (*Sylvilagus* spp.) and snowshoe hares (*Lepus americanus*) in Canada[20].

Rotaviruses have been reported in many avian species, including domestic poultry, guinea fowl, partridges, pheasants and pigeons[21,22], but no reports from free-living wild bird species can be found.

Transmission is via the faeco-oral route, and rotaviruses replicate in mature villous enterocytes. The incubation period is short (usually 12–24 hours), and the epithelial cells of the duodenal villi are first to be infected. This is where replication in the cytoplasm occurs, with release of significant numbers of virions that attack the mid and distal small intestine. Rotaviruses are generally believed to be unable to multiply outside the intestinal tract or to invade deeper tissues and cause systemic disease, although this may not always be true in neonates[23]. As viral multiplication progresses, mature enterocytes are sloughed off and immature squamous or cuboidal cells from the crypts take over to cover the villous surface, leading to a sudden change in the ratio of absorption and secretion and thus accumulation of fluid in the intestinal lumen. Concurrent inflammatory changes in the intestinal epithelium and hypermotility lead to severe diarrhoea. Enterocytes are then regenerated and the villi recover; thus rotavirus infection is considered self-limiting, provided that the resultant dehydration and acid–base disturbance is not so significant as to cause death of very young animals. Concurrent infections may also increase disease severity.

Diagnosis is based on clinical signs and demonstration of the virus using techniques such as isolation in MA 104 cell lines, electron microscopy, electropherotyping and various serological tests.

Rotaviruses are extremely resistant in the environment and to many disinfectants.

In domestic animals, good management and hygiene, vaccination of the dam, ensuring adequate colostrum intake in neonates and use of probiotics are all employed to control disease. Control in wild species has not been described.

Although most rotaviruses are host-restricted, interspecies transmission has been documented, and animal rotaviruses are regarded as a potential reservoir for genetic exchange with human rotaviruses and a zoonotic threat.

BORNA DISEASE

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There is only one virus species, *Borna disease virus* (BDV), within the genus *Bornavirus* of the family *Bornaviridae*. Recently, several novel viral genotypes were recovered from birds, which have been provisionally assigned to a new *Bornavirus* species, *Avian bornavirus* (ABV).

Infection of susceptible hosts with BDV can lead to a severe neurological condition called Borna disease (BD). ABVs are the aetiological agent of a common psittacine disease, proventricular dilatation disease (PDD) and of clinically and morphologically similar diseases in the canary (*Serinus canaria*), Canada geese (*Branta canadensis*)

and trumpeter swans (*Cygnus buccinator*)[24]. Confirmed cases of BD have been found only in endemic areas of Germany, Switzerland and Austria. The disease mainly affects horses (*Equus caballus*) and sheep (*Ovis aries aries*), but sporadic cases have been found in cattle (*Bos primigenus*), goats (*Capra hircus*), rabbits (*Oryctolagus cuniculus*), dogs (*Canis familiaris*) and certain zoo animals, including alpacas (*Lama pacos*), southern two-toed sloths (*Choloepus didactylus*) and pygmy hippopotamus (*Choeropsis liberiensis*)[25]. Reports of cases of classical BD or detection of bornaviral genome sequences from other parts of the world are a matter of scientific controversy. Also, the association of BDV with disease in other animal species, such as cats (*Felis catus*), lynx (*Lynx lynx*), fox (*Vulpes vulpes*) and ostrich (*Struthio camelus*) is not unequivocally proven. The mentioned lynx was shot in Sweden in 1999 because of highly apathetic behaviour with a staring, expressionless gaze. At pathological examination, the emaciated animal displayed a moderate to severe non-suppurative encephalitis and showed positive results in several diagnostic assays for BD[26]. In endemic areas, the virus seems to be maintained by persistently infected reservoir hosts, such as the bicoloured white-toothed shrew (*Crocidura leucodon*). In Sweden, detection of BDV (not ABV) nucleic acid was reported from faecal samples of mallards (*Anas platyrhynchos*) and jackdaws (*Corvus monedula*), and it was postulated that wild birds could act as reservoirs of the virus[27].

ABV seems to be distributed worldwide, and cases of PDD infected with different ABV genotypes have been reported widely, including from Europe. Psittacine birds seem to be the natural hosts and, as they shed virus in the faeces, transmission between birds is very likely. A condition similar to PDD that occurs sporadically in canaries (*Serinus canaria*) has been attributed to a divergent genotype of ABV, provisionally termed *canary bornavirus*.

In natural hosts, BDV infection most likely occurs via the olfactory tract with subsequent spread of the virus to the brain. Non-suppurative encephalitis in many cases with characteristic intranuclear inclusion bodies, and viral antigen are most prevalent in the rostral brain regions, such as the olfactory cortex, the limbic system and the brainstem. In immunocompetent hosts the virus remains restricted to the central nervous system (CNS) and shedding of virus does not occur. BDV itself is not cytolytic and clinical disease and pathological changes are due to the effect of the immune response. In horses, BDV induces a severe neurological disease, with depression, repetitive behaviour and finally paresis, with a mortality of at least 80%.

ABV have a different distribution pattern, as they are present in the CNS and also in the peripheral and autonomic nerve system as well as in many non-neural cell populations. The major lesions of PDD are non-suppurative encephalitis, and ganglioneuritis of the ganglia and nerves of the upper digestive tract, resulting in proventricular dilatation and upper digestive tract impaction in birds. Psittacine birds with PDD show regurgitation, passage of undigested seeds in the faeces and weight loss. Although specific treatment is not possible, clinical signs can be alleviated in some cases by suppressing inflammation with the use of COX-2 inhibitors such as celecoxib.

Abundant viral antigen or viral RNA are easily demonstrated in the nervous system, in both BDV and ABV infection. In live animals the diagnosis of BDV is difficult, as presence of viral signals in extraneural locations, including blood, has never been convincingly proven and the widely used serological assays are not entirely reliable. In cases of ABV infection, PCR assays for detection of ABV nucleic acid fragments in faeces are a promising approach.

BDV in horses and sheep has become a rare, sporadic disease in recent decades and requires no specific control measures. For PDD, the recognition of the aetiology paves the way towards efficient control measures, such as eliminating the virus from breeding facilities by PCR-based screening programmes.

A potential association of BDV with human psychiatric diseases has been discussed, but convincing proof is missing.

AFRICAN SWINE FEVER

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African swine fever is a haemorrhagic disease of pigs caused by the *African swine fever virus* (ASFV). The disease causes devastating losses in the pig industry.

AETIOLOGY

ASFV is the only member of the genus *Asfivirus* in the family *Asfarviridae*. Some 54 different viral structural

proteins have been identified in ASFV. Twenty-two different genotypes have been identified based on the sequence of the C-terminal end of the *p72* gene (reviewed in[28]). ASFV is highly resistant at low temperatures in an organic medium but is inactivated in 30 minutes at 60°C. The virus can be isolated from serum or blood kept at room temperature for 18 months and may persist and remain infective in pork-meat products for more than 100 days.

EPIDEMIOLOGY

ASFV is currently endemic in many sub-Saharan countries. The virus was first described outside Africa in 1957 when it reached Portugal and, after a second epizootic in Portugal, it was also identified in Andorra, Belgium, France, Italy, Malta, the Netherlands and Spain. Thereafter, ASFV was eradicated from these countries, except from parts of Italy. Nowadays, Sardinia is the only European territory where ASFV is still reported. In 2007, ASFV appeared in the Caucasus via a port in Georgia, and since then it has spread in Georgia, and to Armenia, Azerbaijan and Russia. A recent report from the European Food Safety Authority (EFSA) established a moderate risk of ASFV reaching countries in the EU and becoming endemic in European wild boar populations[28].

ASFV is maintained in Africa by wild suids, domestic swine and soft ticks of the genus *Ornithodoros*[29]. Three African wild suids are infected by ASFV with no clinical manifestations: the warthog (*Phacochoerus africanus*), the bush pig (*Potamochoerus larvatus*) and the red river hog (*Potamochoerus porcus*). The role of another wild suid, the giant forest hog (*Hylochoerus meinertzhageni*) remains unclear. Eurasian wild boar and feral pigs (*Sus scrofa*) are also susceptible hosts for ASFV, although their role as an ASFV reservoir remains unresolved.

Following the introduction of highly virulent ASFV strains to wild boar populations, massive mortality is observed in all age and sex classes.

Several *Ornithodoros* spp. of tick are recognized as reservoirs of ASFV in Europe, Africa and America. The only proven vector of the genus in Europe is the *O. erraticus* group, which is distributed in Italy, Portugal, Spain and Turkey[28]. A wild cycle involving *O. moubata/porcinus* and the warthog is of significant importance for the maintenance of ASFV in Africa. Nonetheless, stable populations of *O. erraticus* have only been found in European domestic pig premises, especially in older, more traditional pig farms in which environmental tick maintenance sites such as crevices and cracks were present. *Ornithodoros erraticus* has never been described in Eurasian wild boar and, in contrast to the warthog, the Eurasian wild boar does not habitually reside in burrows where ticks may be present. Thus, it seems unlikely that a wild cycle involving Eurasian wild boar and *Ornithodoros* spp. ticks would occur in Europe.

The warthog acts as a true ASFV reservoir in that the virus infects the animal without inducing any lesions and *O. moubata/porcinus* are essential for its maintenance[29]. The bush pig is able to maintain the virus, although at low circulation rates, and the significance of this species as a wild reservoir of ASFV is lower[29]. Other wild African suids are considered to be of less significance as ASFV reservoirs in addition to their more restricted habitats, behaviour, abundance and distribution.

Clinical signs occur in both domestic and wild *Sus scrofa* following infection by ASFV, and infection with a highly virulent virus strain is frequently fatal. This occurred following the introduction of ASFV to the Iberian Peninsula, Sardinia and more recently the Russian Federation, where mortality of wild boar was reported. In domestic pigs, infection by low virulence isolates had a subclinical course. Wild boar infected by ASFV in Spain were found to be either subclinically infected, to have recovered from infection or to have been infected by a low viral dose[30]. This together with the reported decrease in virulence of the ASFV isolates circulating in domestic pig herds in the same region (reviewed in[30]) suggests a possible circulation of low-virulence ASFV isolates in wild boar populations in the area at that time. According to reported observations, two different epidemiologic scenarios may occur in Eurasian wild boar populations: i) a scenario in which circulating ASFV maintains high virulence; and ii) a scenario in which circulating ASFV reduces its virulence. In the first scenario, it would be expected that the mortality of wild boar would cause a significant impact in viral transmission, leading to the natural eradication of the virus. However, if large and interconnected wild boar populations are present in a region, the virus may spread rapidly. This seems to have occurred in Russia, where dead wild boar with ASF have been found in areas where the disease has not been reported in domestic pigs[28]. In the second epidemiologic scenario, the Eurasian wild boar would eventually become a reservoir for ASFV, dependent to some extent on the pathogenicity of the ASFV isolate

and on the wild boar population characteristics. This epidemiological scenario seems to contradict the experience in Sardinia, where it has been suggested that in the absence of the virus in domestic pigs, the wild boar is unable to maintain the virus[31]. But the Sardinian situation may be similar to the first epidemiological scenario in which ASFV isolates did not develop reduced virulence.

Most transmission of ASFV is direct between individual animals. Owing to the long persistence of ASFV in organic material, indirect transmission via cannibalism or consumption of infected pork or meat products may occur. Indirect transmission through *Ornithodoros* spp. ticks is also important. Transmission over long distances occurs by movement of infectious meat and meat waste products that are fed to pigs[28]. Wild boar may acquire ASFV infection by: i) direct contact with infected individuals or fomites contaminated by faeces, urine or saliva containing virus; ii) wounds caused during fighting with infected individuals; iii) consumption of infected products (waste meat products, carcasses); iv) rooting in pastures to which manure from infected pig herds has been applied; and v) being bitten by infected *Ornithodoros* spp. ticks. Transmission may also occur between wild boar and domestic pigs in areas where the virus is endemic in the domestic pig and where extensive farming systems with free-roaming pig production is traditional (e.g. areas in Italy, Portugal, Romania and Spain). Wild boar are suspected to have been the source of ASFV in Armenia, Azerbaijan and Chechnya[28,32] as well as the source for a small number of ASFV outbreaks in Spanish pig herds[30].

PATHOGENESIS, PATHOLOGY AND IMMUNITY

ASFV is present in blood, tissues, secretions and excretions. The establishment of infections may depend on the virulence of the viral strain involved, which may also determine the level of excretion, persistence or viral replication.

The first sites of replication of ASFV vary depending on the site of initial infection. In domestic pigs, ASFV is commonly observed to replicate first in mononuclear phagocytic cells of tonsils and mandibular, or retropharyngeal, lymph nodes. Other primary replication sites following infection via aerosols are bronchial, gastrohepatic and mesenteric lymph nodes. From these initial replication sites the virus spreads to the target organs through the lymphatic drainage system and blood. ASFV has also been found infecting megakaryocytes, endothelial cells, glomerulus mesangial cells, hepatocytes, renal tubular epithelial cells, thymus reticulum-epithelial cells, fibroblasts, muscle cells of venules and arterioles and neutrophils[33].

Peracute, acute, subacute, chronic and subclinical manifestations of ASFV infection may take place in both domestic swine and wild boar, for which disease development is highly dependent on the strain of the virus. The clinical manifestation of ASFV infection shows marked variation between individual animals.

Persistent infection has been described in domestic swine and has relevance for control and eradication of the disease, but it is not known if it occurs in wild boar.

Naturally infected Spanish wild boar showed lesions consistent with acute ASF[30]. Gross lesions consisted of severe, diffuse haemorrhages in lymph nodes (mesenteric, gastrohepatic and mediastinal lymph nodes) and the spleen. Similar gross lesions were observed in experimentally infected feral pigs[34]. Microscopic findings consisted of severe necrosis and depletion of lymphocytes in paracortical areas of the lymph nodes and similar but more moderate changes in lymphoid follicles of the lymph nodes and spleen. Macrophages and monocytes appeared with a cytopathic effect.

ASFV produces a strong humoral immune response and high levels of specific antibodies are produced, lasting for a long time after initial infection[33]. However, there is currently little available information on the humoral and cellular immune responses to ASFV in Eurasian wild boar.

CLINICAL SIGNS

Wild boar develop similar clinical signs to those of domestic swine. Experimental infection of feral pigs with high and moderate virulent ASFV isolates resulted in fatal disease[34], in which pyrexia (temperatures >41°C) was the first clinical sign reported, accompanied by lethargy and inappetance. Animals died between 2 and 13 days post-infection depending on the virulence of the ASFV isolate. Clinical signs of ASF in wild boar, which die quickly when infected with moderate and high virulence strains, have not been reported. Chronic manifestations of ASF in wild boar also have not been reported, probably because in

these cases, assumed to be caused by low virulence isolates, the clinical signs are mild.

DIAGNOSIS

Clinical diagnosis of ASF is difficult because of low specificity of the clinical signs. Laboratory diagnostic tests employed to test domestic swine are also recommended to test wild boar[29,35]. Direct isolation/detection of ASFV or indirect detection of specific anti-ASFV antibodies are the two recommended approaches. Virus detection is usually carried out by the haemadsorption test. Some isolates are not haemadsorbent, and in these cases, the immunofluorescence test can be used to detect viral particles in tissues. PCR targeted against highly conserved genetic sequences in the different ASFV isolates offers a quick, sensitive and specific detection method in animal tissue samples. Serological tests for the presence of specific anti-ASFV antibodies can be performed by ELISA, indirect immunofluorescence test, immunoblotting and counter immunoelectrophoresis[35].

MANAGEMENT, CONTROL AND REGULATIONS

No effective vaccine is currently available for the prevention and control of ASF. Control and eradication in domestic swine has been performed by strict biosecurity measures, early detection and removal of positive swine[28]. Control of the wild cycle involving the warthog and soft ticks in Africa is considered impractical at present[29]. Only biosecurity measures preventing the contact of domestic swine with warthogs and ticks have been proposed to avoid transmission from the wild reservoir.

In the case of the Eurasian wild boar there is concern that ASFV could become endemic in large interconnected wild boar populations. Control of ASFV spread in these instances may be difficult, but reducing population densities could reduce viral transmission.

REGULATIONS AND REPORTING

ASF is a notifiable disease to the World Organisation for Animal Health (OIE). Regulation and reporting protocols for ASF in the EU are detailed in the Council Directive 2002/60/EC[36].The suspicion of ASF in pigs or wild boar must be immediately notified to the regional and national animal health authorities and confirmed by regional and national reference laboratories. If ASF is confirmed, the outbreak must be notified to the OIE and to every EU member state.

PUBLIC HEALTH CONCERN

ASF is a disease strictly affecting suids, and as a consequence there are no current known public health concerns.

SIGNIFICANCE AND IMPLICATIONS FOR ANIMAL HEALTH

Highly virulent ASF isolates cause severe losses in domestic swine populations. The effect on wild boar population dynamics seems also to be potentially significant. Recent information from Russia suggests that the virus could have a significant impact on the population dynamics of wild boar. The introduction of ASFV in the dense, continuous wild boar populations of Central and Eastern Europe may be devastating if high virulence is maintained.

PORCINE REPRODUCTIVE AND RESPIRATORY SYNDROME

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Porcine reproductive and respiratory syndrome virus (PRRSV) of swine causes respiratory disease in young animals and reproductive disorders in adults. PRRSV belongs to the genus *Arterivirus* in the family *Arteriviridae*, together with *Equine arteritis virus*, *Lactate dehydrogenase-elevating virus* of mice and *Simian hemorrhagic fever* virus.

PRRSV is an enveloped, single-stranded positive-sense RNA virus. Viruses are 50–65 nm in diameter and replicate in macrophages producing long-lasting viraemia and persistent infections. Two different PRRSV prototypes are recognized, one from North America and one from

Europe, and they show approximately 60% nucleotide homology.

A reproductive and respiratory syndrome first observed in pigs in the USA in 1987 was later recognized in central Europe. More recently, PRRSV has spread over Europe. PRRSV was detected in free-roaming European wild boar from Germany, Italy and France and in farmed wild boar from France. Studies carried out in Croatia, Germany, Russia, Slovenia, Sweden and other eastern European countries did not detect PRRSV infection. Recent investigations in wild boar populations in Spain have shown a low seroprevalence to the virus.

PRRSV is considered as a swine pathogen and has not been detected in other species, including rodent, carnivores and birds. In domestic pigs, spread of PRRSV within a farm may occur in 2 to 3 months; when 85 to 90% of the animals become seropositive. The density of pigs and pig farms influences the rate of spread of PRRSV within a farm or geographic region. In a study in European wild boar the seroprevalence was slightly higher in young (6.4%) than in adult (4%) animals[37], whereas in another study the age and population density did not affect the prevalence[38]. Significant differences in PRRSV prevalence were found between study sites in the German wild boar metapopulation[38], suggesting that transmission patterns depend on environmental and local differences in the wild boar populations.

PRRSV survival in aerosols is higher at lower temperatures and at lower relative humidity[39], which suggests that aerosol transmission in nature is influenced by climate. Arthropod vectors can maintain PRRSV for short periods, but they are not considered to be important reservoirs.

The current panorama of PRRSV status in European wild boar and domestic pig populations suggests that wild boar are spillover hosts of a virus maintained by domestic pigs. However, where wild boar population densities and infection rates are high PRRSV may be maintained in these populations. There is little information on the transmission pathways of PRRSV between wild boar individuals, groups or populations.

In domestic pigs PRRSV is excreted in blood, semen, saliva, aerosols, milk, colostrum and, depending on the study, faeces. Vertical transmission from females to their offspring has also been demonstrated. The routes for PRRSV excretion in wild boar and the infective viral titre are unknown, although the high prevalence found in wild boar lung[38] suggests that respiratory transmission is important.

The PRRSV targets cells within the monocyte/macrophage lineage. After replication of PRRSV in macrophages at the primary site of infection, the virus is distributed by these cells to the rest of body tissues. There is significant variation in the development of infection in individual animals. A humoral immune response appears in infected domestic pigs after a week post-infection (pi), with maximum antibody titres 30 days pi.

PRRSV infection may persist in the host at low titres for a variable period. Vertically infected pig fetuses had PPRSV infection 210 days after birth and sentinel animals developed PRRSV antibodies 14 days later. To date, persistent infection in wild boar has not been described.

Macroscopic lesions associated with PRRSV infection may vary with the age of the host and concurrent infections, and in general consist of moderate to severe lymphadenopathy. Histologically, mild to severe interstitial pneumonia and hyperplasia of lymphatic follicles with cellular necrosis and lymphoid depletion is observed. Other lesions may be observed in piglets, including rhinitis, encephalitis and myocarditis.

No lesions associated with PRRSV infection have been described in European wild boar.

PRRSV causes a marked increase in return to oestrus, late-term abortions, stillborn and weak piglets. In many cases, severe respiratory disease in suckling and weaned pigs also occurs. No clinical cases of PRRS have been described in wild boar.

Diagnosis of PRRSV infection in wild boar may be performed by using common techniques of virus isolation or by RT-PCR. Antibody detection in wild boar has been carried out mainly with ELISA tests designed for the domestic pig, but the tests have not been validated in wild boar.

Prevention of PRRSV introduction to domestic pig premises requires implementation of strict hygiene protocols in order to avoid accidental introduction by fomites or aerosols from nearby infected farms. Once the disease has entered a farm, control can be achieved by testing and slaughter, herd slaughter or vaccination. Currently, control and management measures are not applied to wild boar populations even though there is evidence of low circulation of PRRSV in many populations. Nonetheless, evaluating available commercial vaccines and other control measures in prevention of future outbreaks of PRRSV in wild boar populations should be considered.

There are no known concerns for public health associated with PRRSV.

No link between domestic pig and wild boar with regard to PRRSV prevalence has been demonstrated.

ALPHAVIRUS INFECTIONS

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Historically, the arthropod-transmitted alphaviruses were classified on epidemiological and antigenic criteria as Group A arboviruses, the 'A' of which subsequently inspired the creation of the genus *Alphavirus*. The word 'arbovirus' is an acronym (**AR**thropod-**BO**rne viruses) and for practical reasons is still used as a lumping term for viruses from different families using arthropod vectors. The alphaviruses are positive-stranded RNA viruses and form a genus of approximately 30 virus species within the family *Togaviridae*. There is only one other genus, *Rubivirus*, with its single member, *Rubella virus*, within this family. Alphaviruses cycle between vertebrate reservoir hosts and insect vectors, the majority of which are mosquitoes. Most alphaviruses are geographically restricted in their distribution, and only very few of them have been found in Europe. A number of them are human pathogens, causing rashes, arthritis or encephalitis. Some are also significant veterinary pathogens with implications for wildlife, but none of these viruses is currently recognized in Europe. However, alphaviruses, like other arboviruses may be able to quickly establish efficient transmission cycles in new habitats, sometimes thousands of kilometres from their original ones (e.g. CHIKV, SINV).

SINDBIS VIRUS

Sindbis virus (SINV) has a wide geographic distribution, ranging from Europe, through large parts of Africa and Asia to Australia. SINV regularly causes human disease in northern Europe. Disease is characterized by fever and arthralgia and is known under the names of Ockelbo disease, Pogosta disease, or Karelian fever when found in Sweden, Finland and Russia, respectively[40]. Curiously, larger outbreaks tend to occur at 7-year intervals. In other regions of the world the infection of humans is generally subclinical. The maintenance cycle of the virus is primarily between *Culex* and *Culiseta* mosquitoes and wild birds, mainly of the order Passeriformes[41]. The virus seems to have established a permanent bird–mosquito transmission cycle in Northern Europe, but continuous additional introductions by migratory birds from Africa are most likely. SINV-associated diseases or mortalities in birds or other animal species have never been reported.

EASTERN EQUINE ENCEPHALITIS VIRUS

Eastern equine encephalitis virus (EEEV) is present in North and South America. In North America, its natural transmission cycle involves mainly ornithophilic mosquitoes of the species *Culiseta melanura* and passerine and wading birds of different species. Localized epidemics of equine and human infections occur after heavy rains, when mosquito species with other feeding preferences transfer the virus to these species. EEEV is the most virulent of the encephalitic alphaviruses, with a case-fatality rate of 50–70% in humans and near 100% in horses. The disease is characterized by a diffuse meningoencephalitis with widespread neuronal damage. EEEV has been implicated in neurological disease of several birds, mostly introduced species, such as emus (*Dromaius novaehollandiae*), pheasants (*Phasianus colchicus*), rock partridges (*Alectoris graeca*), house sparrows (*Passer domesticus*), domestic pigeons (*Columba livia*) and whooping cranes (*Grus americana*)[41]. A single fatal case of EEE has been described in a free-ranging white-tailed deer (*Odocoileus virginianus*).

WESTERN EQUINE ENCEPHALITIS VIRUS

Western equine encephalitis virus (WEEV) occurs through most of the Americas. In western North America, it is transmitted enzootically among passerine birds by mosquito vectors, primarily *Culex tarsalis*[42]. Epidemics have become comparatively rare, with no major outbreak recorded in the last 20 years[41]. The virus is able to cause encephalitis in horses and humans with much lower fatality rates than EEEV. A fatal WEEV-associated neurologic disease has been described in emus and turkeys (*Meleagris gallopavo*)[41,43].

A virus closely related to WEEV is *Highlands J virus* (HJV) which is maintained on the east coast of the USA in a transmission cycle involving *Culiseta melanura* as primary vector and birds as reservoir hosts. It is not a

significant pathogen of horses and humans but has been proven to cause encephalitis in turkeys, pheasants, emus, whooping cranes and partridges[41,43].

VENEZUELAN EQUINE ENCEPHALITIS VIRUS

Venezuelan equine encephalitis virus circulates in the tropical and subtropical regions of the Americas. So-called enzootic subtypes are involved in transmission cycles between swamp-living mosquitoes and different rodent species. These cycles go typically undetected and only occasionally direct spillover to humans has been recorded[44]. Major epidemics are associated with epidemic strains that use peridomestic mosquito species, such as *Aedes* spp. and *Psorophora* spp. as vectors and equines (horses, donkeys (*Equus asinus*) and mules (*Equus mulus*)) as amplification hosts. Human fatality rates are at 1–3%[41]. Epidemic strains are also highly pathogenic for equines. Birds do not play a relevant role in the transmission cycles of this virus, and there are no records of natural diseases in other than the already mentioned species.

CHIKUNGUNYA VIRUS

Chikungunya virus (CHIKV) is circulating in Africa, Southeast Asia and the islands of the Indian Ocean. Primates are considered the natural reservoir hosts, and the virus remains largely unrecorded when being maintained in sylvatic cycles. However, peridomestic mosquito species such as *Aedes aegypti* and *Aedes albopictus* represent excellent vectors, and urban cycles involving humans as the sole amplifying host are easily established. This has led to dramatic epidemics causing hundreds of thousands of human infections in the islands of the Indian Ocean and the Indian subcontinent in recent years. The disease is characterized by fever, severe arthralgia and maculopapular rash. CHIKV was introduced to Italy by a viraemic traveller from India in 2007, where it established a short-lived autochthonous transmission cycle using local *Aedes albopictus* mosquitoes as vectors and resulted in several hundred human infections. No cases of animal disease have been reported.

MAYARO VIRUS

Mayaro virus is distributed in northern South America and the Amazon River Basin. It is principally maintained in a sylvatic cycle with forest-dwelling mosquitoes of the genus *Haemagogus* as vectors and most likely non-human primates as amplification hosts. The virus causes sporadic outbreaks of a dengue-like human illness characterized by fever, headache and arthralgia. So far, human infections have always been associated with exposure to forest environments and have to be considered as spillover infections. However, as this virus shares many features with the closely related CHIKV, the possibility of future urbanization and efficient amplification in humans and peridomestic mosquitoes cannot be excluded[41,44].

ROSS RIVER VIRUS

Ross River virus is present in large parts of Australia and is responsible for the most common arboviral disease in this country. It is also distributed in Papua New Guinea and the Solomon Islands. Several mosquito species have been identified as competent vectors, and numerous mammalian species, primarily macropods (e.g. kangaroos and wallabies) have been implicated as hosts. Outbreaks are seasonal and associated with prolonged inundation of salt marshes or localized floodings of arid regions. Human disease is characterized by fever, arthralgia and rash. There is no convincing evidence for disease in animals, although the virus has been isolated from horses with musculoskeletal disease[41].

TORQUE TENO VIRUS

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Torque teno virus (TTV), also named transfusion transmitted virus, or TT virus, is an unenveloped, spherical virus with a single-stranded 2.8–3.9 kb circular DNA genome belonging to the floating genus (genus classification not ascribed) *Anellovirus*. Torque teno viruses have been classified into five different genogroups with at least 39 genotypes identified. TTV-infecting swine are distinct from human TTV, and currently TTV1 and TTV2 genogroups have been described in domestic pigs and wild boar[45].

TTV has a ubiquitous distribution and has been detected in humans, several nonhuman primates, cattle, sheep, camels, water buffalo, swine, dogs, cats, a tree shrew (*Tupaia belangeri chinensis*) and chickens. Swine TTV has

been reported in domestic pigs and also in wild boar (*Sus scrofa*) from Spain[45].

Epidemiological studies of TTV in animals are scarce. Nonetheless, it seems that age is a risk factor for increasing prevalence in humans, domestic pigs and wild boar[45]. TTV has been detected in excretions from humans and animals, and vertical transmission is also possible.

Human TTV seems to replicate in bone marrow, liver and respiratory tract, but shortly after infection nearly all organs harbour TTV. No information on the sites of swine TTV replication is available.

Attempts to link TTV infection with clinical disease in humans have been unsuccessful. Experimentally infected gnotobiotic piglets developed post-weaning multisystemic wasting syndrome when infected by porcine circovirus type 2 (PCV2) after TTV1 infection and TTV1 has been also found associated with porcine respiratory and reproductive syndrome virus in triggering porcine dermatitis and nephropathy syndrome in the absence of PCV2. No clinical manifestations have been found to date in wild boar.

PCR is the most useful diagnostic test for TTV in humans and animals, whereas serological methods are only used in humans.

Information on TTV in wildlife is scarce, and the potential role of the virus in possibly triggering clinical disease is unknown. The role of wildlife in the epidemiology of TTV cannot be ruled out with the available information.

INFECTIOUS BURSAL DISEASE (GUMBORO DISEASE)

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Gumboro disease is a disease of 1- to 6-week-old chickens caused by *Infectious bursal disease virus* (IBDV), an RNA birnavirus that destroys B lymphocytes in the bursa of Fabricius. IBDV infections cause necrotic destruction of the lymphoid follicles of the bursa, and in birds that survive disease this results in a secondary immunodeficiency. Death may either be acute with haemorrhagic lesions in muscles and haemorrhage in the bursa, or subacute and often associated with septicaemia. Although turkeys, ducks, guinea fowl and ostriches may be infected, clinical disease occurs only in (young) chickens. Evidence of IBDV antibody in wild bird seroprevalence studies has been found in the absence of clinical disease, and it is possible that wild birds may be a source of virus to chickens.

LYMPHOCYTIC CHORIOMENINGITIS VIRUS INFECTION

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Lymphocytic choriomeningitis virus (LCMV) is a member of the *Arenavirus* genus in the family *Arenaviridae*. LCMV was the first identified arenavirus, discovered in 1933 as a cause of aseptic meningitis in humans, and now widely recognized as an important zoonosis. Arenaviruses are enveloped single-stranded RNA viruses with a genome consisting of two RNA segments: large (L) and small (S). Virions are spherical to pleomorphic, 50–300 nm in diameter and may have ribosomes present, which gives them their characteristic sandy appearance (*arena* means 'sand' in Latin) when visualized by electron microscopy. LCMV is associated with wild rodents, principally the house mouse (*Mus musculus*), and is the only arenavirus to have a worldwide distribution. Mice infected *in utero* fail to mount an immune response and develop a chronic subclinical infection with high levels of virus that are shed throughout life in urine, faeces, nasal secretions, saliva, milk and semen. Prevalence of infection may reach 100%. LCMV has also been found in domestic mice, hamsters, rats and guinea pigs. In wild rodents, LCMV has also been found in the Algerian mouse (*Mus spretus*), long-tailed field mouse (*Apodemus sylvaticus*), yellow-necked mouse (*Apodemus flavicolus*), striped field mouse (*Apodemus agrarius*), harvest mouse (*Micromys minutus*), bank vole (*Clethrionomys glareoulus*), common vole (*Microtus arvalis*) and grey squirrel (*Sciurus carolinensis*). Serological surveys in wild rodents in Europe include a prevalence of 9% in Spain, 31% in the UK and 5.6% in Northern Italy. In the UK 4/19 (21%) grey squirrels in North Wales were antibody-positive. However, many rodents can be persistently infected in the absence of circulating antibodies, so serology correlates poorly with the presence or absence of live virus. Molecular techniques such as RT-PCR may be used on animal tissue and are more reliable.

In laboratory rodents, LCMV infection can occasionally cause disease, depending on immune status and age at

infection. Clinical signs include piloerection, hunched posture, blepharitis, weakness, tremors and convulsions. At *post mortem* examination all visceral organs are infiltrated with lymphocytes and an immune complex glomerulonephritis is a characteristic feature. However, clinical disease has not been described in wild species.

Humans can be infected by direct or indirect contact with infected rodents. Infection in humans can cause acute CNS disease and congenital malformations, and has also been reported recently as a fatal disease acquired by organ transplantation. Non-human primates, in particular callitrichids (South American marmosets and tamarins) are also at risk from contact with, or ingestion of, infected wild or captive-bred rodents, and develop a fatal callitrichid hepatitis (CH), recognized as an emerging disease in captive populations within zoos.

ORBIVIRUSES, BUNYAVIRUSES AND TOGAVIRUSES IN SEABIRDS

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At least 20 viruses, from the bunyavirus, orbivirus, togavirus and possibly coronavirus groups, have been isolated from the *Ixodes uriae* tick, a widely distributed parasite of seabirds, associated with auks in the northern hemisphere, penguins in the southern hemisphere and other species of seabirds; this tick also occasionally bites humans. Orbiviruses of the Kemerovo serogroup, and a bunyavirus of the Uukuniemi serogroup, have been isolated from *I. uriae* tick pools and from a kittiwake (*Rissa tridactyla*) taken from a seabird colony in Scotland[46]. Genetic reassortment occurs in these viruses, giving rise to new virus 'types'. New viruses may also be introduced by birds visiting from other seabird colonies.

PUFFINOSIS

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In 1946 and 1947 epizootics were observed among manx shearwaters (*Puffinus p. puffinus*) on the island of Skomer off the Welsh coast; further disease outbreaks have been seen in more recent years. The disease was seen in juveniles and was characterised by vesicles on the plantar and dorsal foot web surfaces[47]. Conjunctivitis was also seen in 1946. It was estimated that 90% of the birds with clinical signs died of the disease. The cause is thought to be an as yet unidentified virus. Pox virus infection produces a mild self-limiting disease in shearwaters and is not the cause of puffinosis.

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SECTION

2

Bacterial Infections

CHAPTER

20

MYCOBACTERIA INFECTIONS

DOLORES GAVIER-WIDÉN, MARK CHAMBERS, CHRISTIAN GORTÁZAR, RICHARD DELAHAY, RUTH CROMIE AND ANNICK LINDÉN

INTRODUCTION

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The *Mycobacterium* genus belongs to the family Mycobacteriaceae, which has more than 100 members and comprises saprophytes, opportunists and obligate pathogens. The genus includes the slow-growing *M. tuberculosis* and *M. avium* complexes. The members of the *Mycobacterium tuberculosis* complex (MTBC) are the causative agents of human and animal tuberculosis. The MTBC includes *Mycobacterium bovis,* the aetiologic agent of bovine tuberculosis, *M. caprae,* a species closely related but distinct from *M. bovis*, also causing tuberculosis in animals, *M. tuberculosis,* the most common cause of human tuberculosis, and *M. africanum*, *M. canetti*, *M. microti* and *M. pinnipedii*. The *M. avium* complex (MAC) includes two main species: *M. avium* and *M. intracellulare*. *Mycobacterium avium* comprises the following subspecies of veterinary importance: *M. avium* subsp. *avium*, *M. avium* subsp. *hominissuis*, *M. avium* subsp. *paratuberculosis* and *M. avium* subsp. *silvaticum.*

All mycobacteria are rod-shaped, about 0.5 µm wide, variable in length, aerobic, non-spore forming and characteristically acid-alcohol fast, staining bright red using the Ziehl–Neelsen (ZN) stain. Although not strictly Gram-positive, they are classified as acid-fast Gram-positive bacteria because of their lack of an outer cell membrane. All *Mycobacteria* have a characteristic cell wall, thicker than in many other bacteria, which is hydrophobic, waxy, and rich in mycolic acids and mycolates. This lipid-rich, complex cell wall protects the mycobacteria from being killed in the phagolysosome and plays a role in the survival of the bacteria in macrophages. Parts of the cell wall are immunostimulatory and form the basis for vaccine adjuvants. Biochemical tests and culture characteristics help to differentiate species of mycobacteria but cannot identify them all; therefore molecular methods are used for species classification and phylogeny.

Tuberculosis refers to diseases caused by members of the MTBC, mainly *M. tuberculosis* and *M. bovis*. Mycobacteriosis is the term used to refer to infection by other mycobacterial species: atypical mycobacteria or non-tuberculous mycobacteria.

Infectious Diseases of Wild Mammals and Birds in Europe, First Edition. Edited by Dolores Gavier-Widén, J. Paul Duff, and Anna Meredith.

TUBERCULOSIS, *MYCOBACTERIUM BOVIS* AND *MYCOBACTERIUM CAPRAE* INFECTIONS

In most cases, tuberculosis is a chronic progressive infectious disease with zoonotic potential and a wide host range, including many species of wild animals in Europe.

AETIOLOGY

Tuberculosis in terrestrial wildlife species within Europe is most frequently caused by *Mycobacterium bovis*. Tuberculosis caused by *M. caprae* is emerging in cattle, affects humans and has been reported in wild boar (*Sus scrofa*) and red deer (*Cervus elaphus*) in Spain, and in red deer in the Alps[1]. *Mycobacterium tuberculosis* the cause of human tuberculosis; it is adapted primarily to humans and is able to infect wildlife species, but is thought to only occur as a consequence of close contact with infected humans.

Strain Diversity and Molecular Epidemiology

Members of the *M. tuberculosis* complex can be identified at the species level by growth characteristics and deletion or SNP typing. Further characterization of *M. bovis* (and *M. caprae*) strains is possible by direct variable repeat spacer oligonucleotide typing (DVR-spoligotyping) and can be refined by variable-number tandem-repeat typing (VNTR). Over 1,400 spoligotyping patterns are recorded in the *Mycobacterium bovis* Spoligotype Database website[2]. Typing allows the identification of differences between host species and coincidences in strain occurrence, spatial and temporal changes in the *M. bovis* isolate community, and co-infections of a single host by different strains. Hence, molecular typing can be a valuable tool for epidemiological surveys at different spatial and temporal scales[3]. Some types are very frequent and widespread, while others are rare. However, biological differences between strains remain largely unexplored. Up to 252 patterns have been recorded in a single country (Spain), and 12 out of the 15 most frequent types were present both in cattle and in wildlife[4]. Combining spoligotyping and VNTR, up to eight different *M. bovis* strains have been recorded in cattle and wildlife in one single locality (Doñana National Park). In general in the UK, the genotypes found in wildlife are similar in both kind and frequency to the genotypes found in cattle in the immediate area.

EPIDEMIOLOGY

GEOGRAPHICAL DISTRIBUTION IN EUROPE

Mycobacterium bovis infection of livestock has been eradicated or controlled in large parts of Europe, but remains a serious problem in areas where the presence of wildlife reservoirs of infection hampers eradication. The best-known wildlife reservoirs occur in the Eurasian badger (*Meles meles*), mainly in the British Isles, and in the Eurasian wild boar mainly in the Iberian Peninsula. Locally in southern England and in Iberia deer may be maintenance hosts in areas of high population density. Cases of tuberculosis in wildlife have also been recorded in other European countries, including areas considered free of bovine tuberculosis. In recent years, tuberculosis has been reported in red deer in Austria, France and Portugal. No cases occur presently in Iceland and Scandinavia, where bovine tuberculosis has been eradicated.

HOST FACTORS

Host Species

Infection has been recorded in many free-ranging mammals in Europe, including not only ungulates and carnivores, but also insectivores, rodents and lagomorphs (Table 20.1).

Most wildlife species exhibit low prevalence of infection and act as dead-end hosts. Some cases of spillover to endangered species, such as the Iberian lynx (*Lynx pardinus*), are of concern for wildlife conservation. In domestic animals, cattle are the main host, but goats, pigs and occasionally sheep and cats are also affected. *Mycobacterium bovis* infection of domestic cats was common in Europe in the past, before control measures of tuberculosis in cattle and pasteurization of milk were implemented. Tuberculosis constitutes a serious health and economic problem in animals in captivity, in farmed game species – particularly red deer, fallow deer (*Dama dama*) and wild boar – and in zoos and animal parks, where it affects ruminants and often south American camelids, which are susceptible.

Badgers

Badgers are mostly affected in regions of Ireland and the UK where bovine tuberculosis is endemic. The prevalence of tuberculosis in badgers in South West England can

TABLE 20.1 Some free-living wild mammals in which *M. bovis* infection has been confirmed in Europe since 1990.

Common name	Species
European badger	*Meles meles*
Red fox	*Vulpes vulpes*
Feral cat	*Felis catus*
Iberian lynx	*Lynx pardinus*
Stoat	*Mustela erminea*
Western polecat	*Mustela putorius*
Feral ferret	*Mustela furo*
American mink	*Mustela vison*
Otter	*Lutra lutra*
Red deer	*Cervus elaphus*
Roe deer	*Capreolus capreolous*
Fallow deer	*Dama dama*
Reeve's muntjac	*Muntiacus reevesi*
Sika deer	*Cervus nippon*
European bison	*Bison bonasus*
Eurasian wild boar	*Sus scrofa*
Common shrew	*Sorex araneus*
Wood mouse	*Apodemus sylvaticus*
Yellow-necked mouse	*Apodemus flavicollis*
Field vole	*Microtus agrestis*
Bank vole	*Clethrinomys glareolus*
Grey squirrel	*Sciurus carolinensis*

range from 2% to 37%. Badgers may excrete mycobacteria in sputum, urine and faeces or through draining abscesses and infected bite wounds. Badgers may remain infectious for many years, during which time they may contaminate pastures, or enter farm buildings and contaminate cattle feed. Tuberculosis is sporadically reported in badgers in Spain and France.

Wild Boar

Tuberculosis in wild boar is widespread in Europe[5], with a prevalence of 1 to 52%. Prevalence is high in the southern Iberian Peninsula, where artificially high densities of wild boar (up to 90 per square km) and their aggregation at feeders or waterholes create ideal conditions for maintenance of the infection. In these circumstances, the Eurasian wild boar acts as a tuberculosis reservoir. Because of their high susceptibility and the ease with which they can be sampled, wild boar are also good sentinels of infection. Wild boar are able to breach fences and can act as links between farms and wildlife habitats.

Deer

Infection in red deer can last for years, making this host particularly relevant for maintenance and onward transmission of disease to other susceptible species. Local prevalence of up to 30% has been recorded. Tuberculosis in roe deer (*Capreolus capreolus*) is reported at low prevalence, of approximately 1%, particularly in areas where tuberculosis is endemic in cattle. Wild fallow deer can be important hosts where they are sufficiently abundant. Prevalence varies locally, and in the UK was estimated to be up to 5%, but with local foci of up to 20%[5]. From 1997 to 2004 in a reserve in the Bieszczady Mountains in Poland tuberculosis was widespread in a herd of wild European bison (*Bison bonasus*)[6], which subsequently had to be destroyed.

Carnivores in general (with the aforementioned exception of badgers) show a low susceptibility to develop severe disease and often develop mild forms of tuberculosis, with minimal or no grossly detectable lesions. *Mycobacterium bovis* infection in red foxes (*Vulpes vulpes*) with prevalence of up to 4% is reported in several areas of Europe where there is a high level of infection in wildlife. Rodents are considered to be relatively resistant to *M. bovis* infection. Nonetheless, low prevalence of tuberculosis has been detected in some rodent species in endemic areas in the UK[7].

Age and Sex

The prevalence of tuberculosis increases with age. Being male is a significant risk factor for infection in red deer. Among badgers, higher prevalence of infection has been recorded in adults than in cubs and in males than females. In addition, infected male badgers exhibit higher rates of mortality than females. Among wild boar, lung lesions are more frequent in juveniles, the dispersing age group. This could contribute to disease spread. There is also evidence that younger badgers may develop more severe forms of disease.

Social Group

Population structure can be an important determinant of disease distribution. In high-density badger populations, where individuals are clustered in territorial social groups, *M. bovis* infection has been observed to be aggregated in space and time[8]. Infection risks in badgers may vary with

social group structure and size, with inter-group movements being identified as a risk factor at both individual and group levels[9]. Belonging to an *M. bovis*-infected social group is a significant risk factor for infection in red deer and wild boar, but not for fallow deer[3].

Host Genetics

Variation in inherent resistance of the host to infection or to progression of tuberculous disease has been observed among individuals of the same species. This is better understood in humans and in mice models but has been little investigated in wild animals. Inbreeding and reduced genetic variability are risk factors for tuberculosis among wild boar and red deer. This may be due to the effect of specific immune-related genes rather than just individual heterozygosity.

Environmental Factors

Host aggregation at feeding or watering sites increases the risk of transmission. In the Iberian Peninsula, the north-to-south gradient in wildlife tuberculosis prevalence probably reflects a climate-mediated gradient of host aggregation, particularly during summer. Temperature and humidity also affect the survival of mycobacteria in the environment. In high-density badger populations the concentration of badger excretory products at communal latrines may constitute potential sources of environmental contamination. The relatively constant temperature, high humidity and dark conditions in badger burrow systems (setts) may provide an environment in which *M. bovis* can persist.

Transmission

Mycobacterium bovis is efficiently transmitted by aerogenous dissemination, so high population density and aggregation of hosts enhances opportunities for transmission. In badgers this is further facilitated by their occupation of setts, where they share confined air spaces. Oral infection from ingestion of contaminated feed or water is frequent under certain conditions, such as during artificial feeding of deer. Predators and scavengers may become infected by eating infected prey or carcasses. Another potential route of transmission may be via bite wounding, and it is suspected that this may lead to rapid haematogenous spread of infection in badgers. Male badgers tend to have a higher incidence of bite wounds than females. Not all infected host individuals have the same significance in transmission. Animals in a more advanced stage of disease progression may excrete greater numbers of mycobacteria and potentially act as 'super-spreaders', contributing disproportionately to disease transmission. However, any infected animal has the potential to transmit. The probability of transmission will depend on the number of viable bacilli present, the degree of containment within the lesion, and its anatomical location. The potential for infection of conspecifics or other species is related to host density, distribution and behaviour.

PATHOGENESIS, PATHOLOGY AND IMMUNITY

Infection by the respiratory route is common in most hosts. However, infection by the orophayryngeal route occurs frequently in wild boar and red deer. Inhalation of *M. bovis* is the most frequent source of infection. Low doses of mycobacteria can establish infection by the aerogenous route. Experimental studies in many species, including cattle, badgers, deer, mice and guinea pigs, show a dose of *M. bovis* of less than 10 colony-forming units (CFU), which is sufficient to cause infection by the respiratory route. Establishment of infection via the oral route requires a much higher dose of mycobacteria. Infection can also be acquired percutaneously, particularly through biting. Rarely, transplacental infection results from tuberculous endometritis of the dam. The genital route of infection is rare.

The inhaled mycobacteria that escape the mucociliary clearance of the upper respiratory passages gain access to the pulmonary alveoli, where they are phagocytosed by macrophages. These phagocytes attempt to kill the mycobacteria, but the virulent bacilli often persist. The bacilli multiply and destroy phagocytes, and new macrophages accumulate. A cell-mediated immune response is elicited in 10 to 14 days post-infection, which leads to the development of a granuloma. Macrophages carrying *M. bovis* enter lymphatic vessels and migrate to the lymph nodes, draining the primary lesion. This early focus may evolve into a progressive lesion or may undergo fibrosis and calcification, eliminating or restricting the infection. Foci may maintain a latent infection, which can become reactivated later in life, particularly if the immune defence is weakened. Reactivation causes secondary tuberculosis sometimes several years later or during old age. The lymph

nodes draining primary foci usually also develop tuberculous lesions. From these lesions the mycobacteria spread by lympho-haematogenous dissemination, resulting in new foci in other organs. These are most often located in preferential sites, such as lymph nodes and lungs, but may occur in any organ – for example, mammary glands, uterus, liver, kidneys, joints and others. The tonsils are frequent locations of *M. bovis* infection, but often without gross lesions. Granulomas may erode through mucosae or walls of ducts, resulting in spread and implantation of mycobacteria on the linings, such as the nasobronchial or intestinal mucosae, or of ducts of salivary or mammary glands. Spread along cavities, such as pleural or meningeal may also occur. The miliary form of tuberculosis is characterized clinically by its rapid course and pathologically by numerous solid, white foci, 1 mm to several mm in diameter. It is produced by the haematogenous dissemination of numerous bacilli simultaneously. Depending on the pattern of spread, it can be limited to the lungs or it may affect organs such as the liver, kidneys, spleen and bone marrow.

Infection via the oral route results in uptake of the mycobacteria at the mucosal-associated lymphoid tissues, such as Peyer's patches and tonsils. This results in development of lesions in the draining lymph nodes.

The propensity to develop severe disease varies among species. This is illustrated by the high frequency of severe tuberculous lesions observed in deer, whereas at the other end of the spectrum mild forms of tuberculosis, often with no gross lesions, occur in red foxes. The progress of the infection and the development of lesions are highly dependent on the host mounting a successful immunological response, which controls the multiplication rate of mycobacteria. The outcome of the tuberculous infection depends on a combination of host factors (i.e. the state of acquired immunity, immune competence and natural resistance, age, species, general condition, stress and concomitant infections) and mycobacterial factors (i.e. infection dose, route and virulence). In this way, individuals of any species, including those with lower propensity to develop severe disease, have the capacity to develop generalized or advanced forms of tuberculosis. In most cases the course of infection is dynamic and may result in intermittent shedding of mycobacteria. The tuberculous lesions may grow through the walls of ducts and cavities, eroding mucosae and structures and opening into the lumen of respiratory, digestive or urinary tracts, often excreting mycobacteria. These lesions may later become healed and circumscribed by a fibrotic capsule, thereby interrupting the shedding of mycobacteria to the environment. This may be followed by new cycles of growth and erosion, alternating with healing and containment, resulting in intermittent excretion of *M. bovis*, in a process which may last for years.

In most cases, tuberculosis progresses slowly. Over the course of months or years, the granulomas develop, grow in size and coalesce to form larger lesions. Simultaneously, there may be reseeding of mycobacteria, causing new lesions. Large areas of tuberculous bronchopneumonic consolidation may replace increasingly larger parts of the pulmonary parenchyma. Similarly, the tuberculosis lesions in other organs may also expand, leaving little healthy tissue and ultimately causing organ failure and death.

Tuberculosis has a remarkable spectrum of lesion sizes, from a barely detectable white focus (1 mm) to very large tubercles or completely consolidated lungs. Often, no gross lesions are observed in infected animals, but microscopic lesions may be found. This is termed 'no visible lesion tuberculosis' (NVL). A single gross lesion in a lymph node, particularly of the head or respiratory system, is often the only pathological finding in infected animals (reviewed in[10]). The lesions may be solid (with no necrosis) or may have a centre of coagulative necrosis or caseation, with or without mineralization, or liquefaction. The gross appearance of tuberculosis lesions varies among taxonomical groups (Table 20.2). The classical tuberculous granuloma is described in cattle as formed by macrophages (or epithelioid cells, which are elongated macrophages) surrounded by a zone of lymphocytes and Langhans giant cells, with a fibrotic capsule, central necrosis and mineralization. However, the morphology of granulomas shows considerable variation among species, with some exhibiting very different forms to cattle (Table 20.2). For example, in badgers the granulomas are formed essentially by whorls of epithelioid cells, with lymphocytes and no giant cells (Figure 20.1). Acid-fast bacilli (AFB) in ZN-stained histological sections are easily demonstrated when they are abundant, during active phases of the infection. Granulomas with few AFB still indicate potential infectivity, as a single AFB in a 1 cm^2 area of granuloma indicates that a minimum of 2,000 AFB may be present in that particular granuloma. In many cases of confirmed *M. bovis* infection, AFB are difficult to find or not observed at all in the granulomas.

The immune response to infection is characterized by activation of T cells that produce a range of

TABLE 20.2 Summary of gross and microscopic tuberculous lesions in selected wild mammals.

Species	Predominant location of tuberculous lesions	Gross appearance of tuberculous lesions	Histological features of tuberculous lesions	Comments
Badger (*Meles meles*)	Lungs, tracheobronchial and mediastinal LN. Skin, (tuberculous bite wounds) and regional LN. Retropharyngeal, parotid, mandibular, hepatic, renal and mesenteric LN. Generalized disease in up to 5% of the cases. Kidneys. Liver.	White nodules, often small (1 mm to a few cm), with necrosis and calcification in large tubercles. Enlarged LN with necrotic or solid lesions. Pulmonary consolidation. Miliary tuberculosis.	Rounded granulomas formed by clusters of epithelioid macrophages and peripheral lymphocytes. Discrete circumscribing fibrosis. Central necrosis and sometimes mineralization in larger granulomas. Often difficult to find AFB. No giant cells.	Pulmonary tuberculosis is most frequent (60% of cases). Often develop slowly progressive tuberculosis, resulting in mild forms with small lesions. The NVL presentation is frequent. Severe generalized disease is more frequent in young badgers.
Eurasian wild boar (*Sus scrofa*)	Characteristically in mandibular LN (92–100% of cases). Medial retropharyngeal LN, parotid and tonsils. Lung and thoracic LN (19–83%). Mesenteric LN. Spleen, liver and joints. Generalized lesions.	Tubercles with thick fibrotic capsule and necro-calcified center (Figure 20.2). Variable size, from 1 mm to 15 cm. Small tubercles (<1 cm) are frequent. Several tubercles can be present in a same lymph node.	Epithelioid cells granulomas. Necrocalcific centre, surrounded by cellular layers of macrophages with multinucleated giant cells. Peripheral lymphocytes. Older granulomas are larger, with more extensive necrosis and calcification and thicker capsule. AFB may be difficult to find.	Juvenile wild boar (between 12 and 24 months) usually develop the most severe lesions.
Red deer (*Cervus elaphus*) and fallow deer (*Dama dama*)	Medial retropharyngeal LN. Lungs, tracheobronchial and mediastinal LN. Mesenteric LN and ileo-caecal valve. Tonsils. Generalized lesions in head, abdomen and thorax.	Variable size: 1 mm white foci, round tubercles 1–10 cm diameter with caseous necrosis and sometimes calcification. Large abscesses with liquefied necrosis (Figure 20.3). Pulmonary tuberculous consolidation.	Encapsulated granulomas, with central necrosis sometimes with calcification, peripheral zone of epithelioid macrophages, Langhans or irregularly shaped giant cells, lymphocytes, plasma cells and neutrophils. Variable numbers of AFB, not always observed.	High propensity to develop severe lesions. Intestinal tuberculosis is frequent in wild red deer. Thoracic lesions are more frequent in fallow deer.
Red fox (*Vulpes vulpes*)	Mesenteric LN. Lungs.	No gross lesions in most cases. A few foxes show gross lesions, small tubercles, often calcified.		NVL tuberculosis in the majority of cases. A few foxes develop severe disease.

AFB – acid-fast bacilli; LN – lymph node(s); NVL – no visible lesion

pro-inflammatory cytokines and other mediators, typified by interferon gamma (IFN-γ) in particular. Antibodies are produced during infection with *M. bovis* but are generally easier to detect as the disease becomes more advanced.

CLINICAL SIGNS

Most infected animals do not exhibit clinical disease. Respiratory signs of disease such as coughing or nasal exudates may be observed in severe pulmonary tuberculosis. Tuberculous lesions in any organ or tissue can cause signs of organ dysfunction. Enlargement of superficial lymph nodes containing lesions may be evident, and in some cases form fistulas opening through the skin to drain purulent debris. This is frequently observed in red deer and wild boar. Tuberculous arthritis is observed mostly in wild boar. Slowly progressive tuberculosis in the terminal stages is manifested by progressive emaciation and lethargy. Miliary tuberculosis has a rapid and most often lethal clinical course.

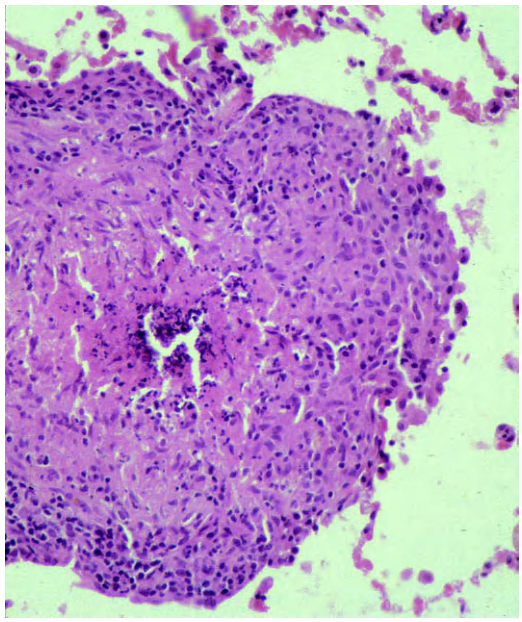

FIGURE 20.1 Badger lung with a small granuloma caused by *Mycobacterium bovis* infection. The granuloma is formed by macrophages/epithelioid cells, with smaller numbers of lymphocytes in the periphery. There is necrosis and mineralization in the centre of the lesion. No giant cells are observed in tuberculous lesions in badgers. Haematoxylin and eosin. Enlargement 400×. Photo: D. Gavier-Widén, SVA.

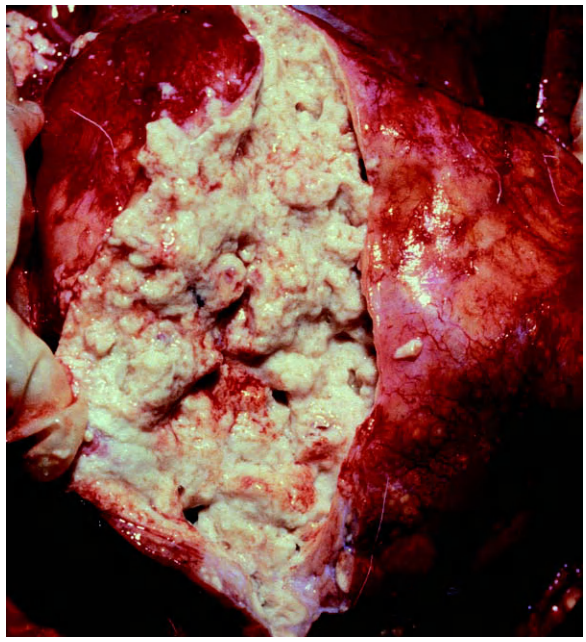

FIGURE 20.3 Bovine tuberculosis in a fallow deer. A large tuberculous abscess with caseo-purulent necrosis replaces lung structure. Photo: Bengt Ekberg, SVA.

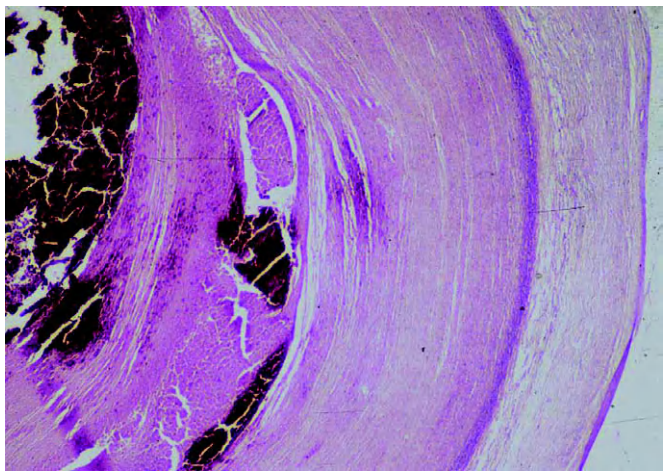

FIGURE 20.2 Histological section of an old tuberculous granuloma of a European wild boar, showing a thick fibrotic capsule and a necrocalcified centre. Photo: D. Gavier-Widén, SVA.

DIAGNOSIS

Post mortem examination: a presumptive diagnosis may be based on the presence of gross lesions consistent with tuberculosis, but the definitive diagnosis requires the demonstration of *M. bovis*. Many cases present with only single or few lesions, which may be discrete. If these are not detected at necropsy and sampled for the demonstration of *M. bovis*, the diagnosis of tuberculosis most likely will remain unconfirmed. For this reason, to increase the chances of detecting lesions, a thin slicing of target lymph nodes and of lungs at necropsy is recommended.

Histopathology can improve detection of small tuberculous lesions and can contribute to the differential diagnosis. Identifying histological lesions typical of tuberculosis for the species and with the presence of AFB in ZN-stained sections support a presumptive diagnosis of tuberculosis but cannot differentiate between infections caused by *M. bovis* and those caused by other mycobacteria – for example, *M. avium*. The main differential diagnosis, grossly and histologically, is the parasitic granuloma, frequently found in mesenteric and respiratory lymph nodes and lungs of cervids and other wild hosts.

The isolation and culture of *M. bovis* from tissues obtained *post mortem* remains the 'gold standard' for the confirmation of tuberculosis. Detection rates are highest where macroscopic lesions are present, but often *M. bovis* may be isolated from tissues with NVL[10]. For greatest

sensitivity, samples for culture from NVL animals should include the most frequently affected lymph nodes in the species. In badgers these are the lymph nodes of the head, respiratory tract and digestive tract; in wild boar, they are the tonsils and mandibular lymph nodes; and in deer, they are the tonsils and medial retropharyngeal lymph nodes, ideally supplemented with the left bronchial and mediastinal lymph nodes and the mesenteric and ileo-caecal lymph nodes. Isolation of mycobacteria from samples taken from live animals, such as urine, faeces, exudates or lavage samples of upper respiratory tract frequently has low sensitivity as a diagnostic procedure, largely due to the intermittent nature of mycobacterial excretion[11].

As well as its low sensitivity, mycobacterial culture is an expensive and labour-intensive process, and it can take up to 12 weeks to confirm a positive result. Bacterial culture has the advantage that *M. bovis* isolates are available for molecular typing using methods such as spoligotyping or mycobacterial interspersed repetitive unit-VNTR (MIRU-VNTR) typing, which may allow greater understanding of the epidemiology of the infection. For many wildlife species there are no proven diagnostic alternatives to bacterial culture.

The confirmation rate of tuberculosis in badgers was increased when more tissues were examined grossly and histologically, when a greater quantity and variety of tissues were cultured and when the culture period was extended[12]. This is likely to be true for other species.

Genetic probes can be used to reduce the time for culture confirmation, but most are only MTBC-group-specific. Polymerase chain reaction (PCR) offers the promise of faster and more specific detection of *M. bovis* from tissues, clinical and environmental samples. However, despite widespread use, a standardized, validated procedure for PCR detection of *M. bovis* does not yet exist, and culture has proven to be more sensitive than PCR for the detection of *M. bovis* from *post mortem* samples.

Diagnosis of tuberculosis is increasingly based on the detection of an immune response to *M. bovis* infection, either in association with, or as a replacement to, culture. The principal immunological response of the host to infection with *M. bovis* is the acquired cellular immune response, exemplified by the proliferation of lymphocytes and the production of cytokines such as IFN-γ. The mainstay of diagnosis of tuberculosis in cattle, the tuberculin intradermal skin test, is a method of detecting the cellular response in *M. bovis*-infected animals, but is impractical for free-ranging wildlife because of the need to examine animals for any cutaneous reaction 24–72 hours after the injection of tuberculin.

An increasing number of alternative *ex vivo* immunological tests are becoming available for the diagnosis of tuberculosis in wildlife. The optimum sensitivity of detection is generally seen through the measurement of IFN-γ by enzyme immunoassay following stimulation of whole blood or isolated peripheral blood mononuclear cells with tuberculin or specific antigens. Such a test has been reported for badgers and red deer[13], but in most cases the reagents involved are species-specific and would need to be generated for each species. Furthermore, for greatest success the test requires blood samples to be processed within 24 hours or less by a specialist laboratory, so in some settings the logistics or costs involved may make it unfeasible to operate the test.

Where low-cost, simple and rapid testing is required, immunological tests based on an antibody (serological) response may be more suitable. A wide variety of such tests have been published in the research literature for their application in numerous wildlife species (reviewed in [14]). Some enzyme-linked immunosorbent assay (ELISA) tests are available commercially for badgers, deer and wild boar. The principal limitation associated with serological tests in general is their relatively low sensitivity. There are exceptions to this, such as those developed for wild boar. Serological tests appear particularly suitable for detecting animals with advanced disease. For the monitoring of tuberculosis prevalence in wildlife populations, a combination of diagnostic tests may prove the most sensitive and cost-effective approach. For example, serology or lesion recording *post mortem*, combined with targeted application of more expensive but sensitive tools such as culture, PCR confirmation, or an IFN-γ test.

Although the presence of gross lesions consistent with tuberculosis is not an ideal tool for estimating prevalence of disease, such information is still valuable for exploring the magnitude and general distribution of infection in wildlife, provided a sufficient sample size is obtained from a large enough area.

MANAGEMENT, CONTROL AND REGULATIONS

Bovine tuberculosis is a reportable disease for the World Organisation for Animal Health (OIE); it is notifiable in

wildlife species in some European countries and is included in the list of diseases in the eradication and monitoring programme of the EU.

However, tuberculosis in free-living wildlife has proven very difficult to control, and is probably impossible to eradicate. There are three main reasons to control tuberculosis infection in wild animals in Europe. Firstly, to prevent wildlife populations acting as a reservoir of infection for domestic animals; secondly, to reduce disease in wildlife hosts (particularly in protected and endangered species); and thirdly, to reduce the potential risks to public health. The principal approaches are reducing host abundance, which is usually achieved by culling, vaccinating host populations, or some form of management intervention to reduce opportunities for transmission either within wildlife populations or onwards to domestic species.

The approaches described here should not be considered in isolation, as the greatest positive impact may result from their use in combination. For example, in some species the effectiveness of a vaccination programme could potentially be increased by the addition of effective fertility control to curtail the recruitment of susceptible young animals. Alternatively, targeted culling may have a role in certain circumstances if employed with other measures such as vaccination and improved biosecurity of domestic animals.

CULLING

A large-scale UK field experiment to test the efficacy of badger culling in reducing transmission risks to cattle identified both negative and positive consequences[15]. The outcome of culling operations may vary geographically, as a similar field trial in the Republic of Ireland identified only a net positive effect of badger culling on cattle herd breakdown rates[16]. Such variation may potentially arise as a result of differences in culling techniques and strategies, and in the density and social behaviour of host populations. Ongoing developments in methods to diagnose tuberculosis and an improved understanding of tuberculosis dynamics in wildlife may increase the potential for targeted culling to be used. A potential alternative to culling wildlife populations is fertility control by immunocontraceptive vaccination, although this approach requires more research on immunocontraceptive agents, the demographic consequences of fertility control, and methods of delivery before its potential can be realized.

VACCINATION

Of the vaccines available, *Mycobacterium bovis* strain bacille Calmette-Guérin (BCG) is the tuberculosis vaccine of choice for use in wildlife in the near future and is currently being developed for use in a variety of domestic and wild animal species. BCG vaccination via subcutaneous, intramuscular and mucosal routes has been shown to confer significant protection to a number of wildlife species, including badgers, farmed red deer and wild boar. An obstacle to effective BCG vaccination of some wildlife populations is a practical means of delivering a stable vaccine preparation to target species in the field. A lipid matrix has been developed in New Zealand, which allows BCG bacilli to be maintained in a viable state suitable for oral delivery. Recent studies in badgers, brushtail possums and white-tailed deer (*Odocoileus virginianus*) have shown that oral vaccination with lipid-formulated BCG can induce protection against *M. bovis* that is comparable to that induced by injected vaccine. Specific baits have also been developed recently for the selective vaccination of wild boar piglets. To license BCG for use in wildlife it is necessary, among other things, to demonstrate efficacy against *M. bovis,* and the safety of a vaccine overdose to wild animals of the target species. These studies have been completed in the UK for BCG in badgers. In March 2010, a licence was granted for the use of BCG in badgers in the UK as an injectable (intramuscular) vaccine – 'BadgerBCG'. This is the first licensed vaccine against tuberculosis in animals to be granted. It will take several years to generate data required to license oral BCG for wildlife in Europe, although work is in progress.

BIOSECURITY – REDUCING CONTACT BETWEEN LIVESTOCK AND WILDLIFE

Tuberculosis transmission between wildlife and domestic animals could be reduced if the two populations could be physically separated. Fences can be used successfully to control movements of larger mammals such as deer. Where transmission risks are concentrated in a particular location (e.g. badgers entering farm buildings), exclusion measures may be targeted accordingly. Exclusion of small mammals or those that burrow is more difficult. In game species, such as deer and wild boar, the management of spatial aggregations at supplementary feeding sites or waterholes, and the safe disposal of viscera by hunters could contribute to reducing tuberculosis transmission risks.

PUBLIC HEALTH CONCERN

The manipulation of live or dead wild animals may present a risk of infection for humans. In Spain, molecular typing of *M. bovis* showed that 6 out of 11 spoligotypes identified in wildlife were similar to those described in human cases. This suggests that hunters, wildlife managers or game-meat consumers may be at risk of infection. *Post mortem* inspection of deer and condemnation of affected organs or whole carcasses markedly reduces the danger of infection by consumption of venison. Infection of semi-domesticated cats and domesticated cats and dogs may represent direct zoonotic potential[5].The demonstration of human-to-human transmission of *M. bovis* indicates the potential public health risks of humans acquiring *M. bovis* infection[17].

SIGNIFICANCE AND IMPLICATIONS FOR ANIMAL HEALTH

The consequences of tuberculosis in wild animals include adverse effects on disease eradication in livestock, and adverse effects on wildlife management and conservation, as the disease can affect the productivity and density of wildlife populations with an economic or recreational value. The potential role of wild animals in the maintenance and spread of *M. bovis* infection in livestock represents the greatest economic impact of the disease in certain European regions. The disease is of particular importance in countries where eradication programmes have substantially reduced the incidence of tuberculosis in cattle but where disease persists and new outbreaks occur. Tuberculosis has an impact on conservation of endangered species. The critically endangered Iberian lynx, which may become infected through consumption of tuberculous carcasses, may be at particular risk.

ACKNOWLEDGEMENT

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AVIAN TUBERCULOSIS

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Avian tuberculosis (synonyms: avian mycobacteriosis, *M. avium intracellulare* disease, *M. avium intracellulare* complex disease or *M. avium* complex disease) is an insidious, chronic, bacterial disease of birds, most commonly reported in waterbirds, gregarious birds, raptors or those associated with agricultural premises. *Mycobacterium avium* is the principle cause of the disease in birds.

AETIOLOGY

As both saprophyte and opportunist facultative pathogen, *M. avium* is a ubiquitous, slow-growing, environmental bacterium favouring acidic conditions rich in organic matter. Much of the organism's ecological success, both in host and environment, is due to the lipid-rich, thick, waxy cell wall, which helps to protect it from desiccation, freezing and ultraviolet radiation, and accounts for its resistance to both antibiotics and disinfectants. In the environment, the hydrophobic cell wall is responsible for the organisms being found at the air–water interface, leading to the reference to the mycobacteria as 'the ducks of the microbial world'[18]. *Mycobacterium avium*'s ability to grow at temperatures between 20 and 45°C allows it to exploit birds as a host.

The classification of *M. avium* and the means by which this can be done are as complex as the organism itself. It has a large number of biochemically identical stable serotypes distinguished by the numbers of polar glycopeptidolipids present in the cell wall. Using classical agglutination serotyping, *M. avium* consists of serotypes 1–3 (the most common avian pathogens), with *M. intracellulare* including serotypes 4–24. To this end *M. avium* is sometimes known as the *M. avium* complex (MAC), *M. avium intracellulare* (MAI) or the *M. avium intracellulare* complex (MAIC). Within this complex lies the unusual mycobactin-dependent strain responsible for the disease in wood pigeons (*Columba palumbus*), often referred to as the wood pigeon bacillus[19]. This mycobactin dependence is shared by the closely related *M. avium* subsp. *paratuberculosis* responsible for Johne's disease. Avian disease caused by these different serotypes within the MAC are indistinguishable. Molecular techniques have been highly valuable for diagnosis and epidemiological investigations and have overturned many epidemiological assumptions. They have also revealed insertion sequences linked to avian virulence: IS1245 and the closely related IS901[20].

EPIDEMIOLOGY

GEOGRAPHICAL DISTRIBUTION AND HOSTS

The disease has a global distribution, with the majority of wild cases being reported in the northern temperate zone. Cases have been reported across Europe and the absence of disease in a region probably reflects lack of reporting. Europe sits within a number of major migratory bird flyways, and for such a chronic slowly progressing disease, cases in Africa and Asia and, to a lesser extent north America, may still have some importance in the epidemiology of the disease in Europe and vice versa.

Mycobacterium avium is the principle cause of avian tuberculosis in wild, domestic and captive birds and also causes infections in an extremely wide vertebrate host range, including free-living European mammals such as: mustelids, e.g. Eurasian otters (*Lutra lutra*)[21]; rodents, e.g. European beavers (*Castor fiber*)[22] and yellow-necked mice (*Apodemus flavicollis*)[23]; cervids, e.g. red deer (C*ervus elaphus*)[24]; suids, e.g. wild boar (*Sus scrofa*)[25]; and members of the Insectivora, e.g. European hedgehogs (*Erinaceus europaeus*)[26] and common shrews (*Sorex araneus*)[23]. In captive situations the host range is even broader, including marsupials, mustelids, primates, a wide range of ungulates and even fish and reptiles.

The disease has been described in a wide range of bird species. This is probably in part because of differences in genetic/inherent susceptibility. This has been suggested from captive studies in which, even when exposed to infection, the majority of birds did not succumb to disease. The genetic basis of susceptibility to mycobacterial infections has been largely determined for laboratory mice and is likely to be similar for birds. This is important for some highly susceptible threatened species in captivity that suffer from founder effects and inbreeding. A high prevalence of avian tuberculosis in free-living threatened whooping cranes (*Grus americana*) in the USA (39% for one population) has negatively affected this conservation programme[27], and genetic factors may be at play.

Of equally great importance in susceptibility and risk of infection are life history, ecology and behaviour. In the wild, the disease is most commonly reported in:

- waterbirds, with a 'usual' prevalence of 1–6% of mortalities
- birds associating with agricultural premises, where prevalence in e.g. starlings (*Sturnus vulgaris*) and sparrows (*Passer domesticus*) has been reported between 5 and 40%[28]; for this group there are two main risk factors, namely contact with potentially infected poultry and high population densities due to abundance of food supply and nest sites; however, it should be recognized that they are also the subject of greater investigation and thus reporting than other bird groups
- gregarious birds: this group incorporates many of the first two categories, but additionally a gregarious lifestyle puts birds at risk due to the density dependence of the disease; as an example, the disease has been diagnosed in domestic and free-living feral pigeons (*Columba livia domestica*) with a prevalence of 1%[29], and in 3% of crows[30]
- raptors and scavengers: for this taxon, population density is less of an issue but cases reflect consumed infected prey or secondary infections of wounds; reports in raptors may reflect the higher rate of discovery of carcasses or sick/injured birds and greater likelihood of investigation given the greater interest frequently shown for this family of birds; European reports estimate between 1 and 4% prevalence of avian tuberculosis[31,32].

In captivity, avian tuberculosis is typically a disease associated with older birds, and given the slow progress of the disease this is likely to be the case for wild birds. The relatively low age structure of many wild populations may assist in keeping prevalence low.

There is no apparent sex predilection for disease susceptibility. The role of stress should not be underestimated, and periods of nutritional or other stress are certain to have an adverse impact on latent infection, leading to conversion to clinical infection or reducing the host's protective responses during the prolonged immunological battle with the host.

ENVIRONMENTAL FACTORS

Appreciating the ecology of *M. avium* allows an understanding of why birds in damp or wet environments are at particular risk. High densities of hosts have the impact of both increasing possible stresses but also increasing the rate of encountering infectious individuals. Contact with potentially infected domestic or captive birds is an obvious and important risk factor. Access to infected prey or food materials is a clear risk factor that is reflected in the numerous reports in predatory and scavenging birds.

The role of exposure to environmental mycobacteria in sensitization of the immune system and subsequent disease

outcome is well recognized in humans[18] and thought to occur in captive birds. It is probably of critical importance to the disease outcome in wild birds but too complex to evaluate or test.

EPIDEMIOLOGICAL ROLE OF WILD ANIMALS

Outcomes of infection are difficult to determine in wild birds. It is assumed that birds either: overcome initial infection (the degree to which this protects from further infection is not known); develop latent infection, which may reactivate e.g. during a later period of stress; or develop progressive disease, during which time they may be infectious and shed bacilli.

Infected and infectious birds play two roles in the epidemiology of the disease, firstly as direct shedders into the environment, and secondly through being eaten and infecting predators and scavengers directly. The debilitating nature of the disease would probably allow disproportionate numbers of tuberculous prey species to be caught by predatory species.

Migratory birds, particularly those with periods of their life cycle in which population densities are high, e.g. wintering or staging waterbirds, or communally winter-roosting species, have the greatest potential for becoming infected and spreading infection across great distances. Raptors, even if migratory, are less likely to be involved in significant onward spread of infection.

TRANSMISSION

Mycobacteria are shed in faeces from lesions in the intestinal tract, and thus the principle source of pathogenic avian tubercle bacilli is a faecal-contaminated environment. Oral ingestion accounts for the majority of cases, and how a bird interacts with its environment during feeding may be key in determining ingestion rates – for example, dabbling waterfowl sifting food from muddy areas at air–water interfaces are likely to ingest more bacilli than selectively grazing geese. Inhalation of aerosols, either from a contaminated environment or directly from infected birds with lesions in the respiratory tract have been suggested as the cause of primary pulmonary infections in domestic or captive birds at least[33].

The role of the egg in transmission is intriguing. It has long been assumed that vertical transmission does not occur, as infected poultry give rise to apparently uninfected young. However, there are a small number of reports of *M. avium*-infected eggs[34]. It is unknown whether or not these would have gone on to develop into healthy embryos, and it is speculated that they may have accounted ultimately for embryo death, but the role of the egg can be viewed as relatively unimportant in the epidemiology of this disease.

Contamination of wounds has been recorded in raptors, and other possible, if less likely, sources or modes of transmission have been suggested, including ticks, rodents, beetle and fly larvae, and coitus.

PATHOGENESIS, PATHOLOGY AND IMMUNITY

Most often *M. avium* enters the body through ingestion of bacteria while feeding. What constitutes an infectious dose has not been defined for wild birds, but it is speculated that infection is probably acquired over time following repeated ingestion of pathogenic strains of *M. avium* from a contaminated environment.

Following either ingestion or inhalation, the lipid-rich cell walls help protect against initial physical and chemical innate defence processes within the host. It is then assumed that bacteria bind to enterocytes in the intestinal mucosa, with eventual haematogenous spread to the most frequently involved organs: the liver and spleen.

Although the intestinal tract, liver and spleen are commonly affected organs, the disease can be generalized, with involvement of practically any organ. Infections in bones are not uncommon. Disease spread within the body is likely to take place over several months.

Host responses ensure that during late stages of disease coalescing granulomatous masses may replace extensive amounts of functional tissue, and death may be as a result of this loss of organ function or due to rupture of an infected organ.

Alternatively this chronic debilitating disease may render the bird more likely to predation or other infectious processes, so avian tuberculosis may not be the proximate cause of death. As an example, the mass mortality of lesser flamingos (*Phoenicopterus minor*) in Kenya reported in 1999[35] was due to other factors, but high levels of background avian tuberculosis were felt to have weakened the birds.

A tuberculous bird is usually in poor condition, with muscle wasting and loss of body fat. Disease may be disseminated or restricted to one tissue. Lesions range in

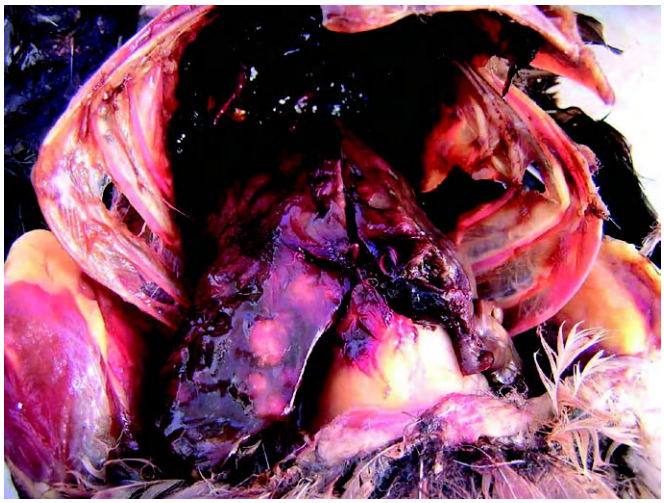

FIGURE 20.4 Multiple tuberculous lesions in the liver of a pink-footed goose (*Anser brachyrhynchus*) shot and opened by hunters in Iceland. Photo: Ruth Cromie.

colour from white through pale grey, cream or yellow to tan and in size from diffuse military foci to coalescing masses, several centimetres in diameter[36]. Although lesions are most typically found in the intestines, liver and spleen, either on serosal surfaces or within parenchyma, any tissue can be affected. Lesions may be discrete and encapsulated or diffuse into the surrounding tissue. Granulomas on the intestinal wall and mucosa may be ulcerated, allowing shedding of bacilli into the lumen. Lung and bone involvement are not uncommon. Infected joints are usually swollen and frequently contain caseous material. Granulomas may contain caseous material or be tumour-like in cross-section. Figure 20.4 shows extensive lesions in the liver of a pink-footed goose (*Anser brachyrhynchus*) shot by hunters in Iceland.

Affected organs may be enlarged and/or uniformly pale in colour. Such findings may also be found in the non-tuberculoid form of the disease, which is typified by histiocytic inflammation, or histiocytosis with diffuse, concurrent amyloidosis[37]. This histiocytic form may be characteristic of early stages of the disease[38].

Microscopically, in early stages of infection there may be scattered foci of epithelioid macrophages and lymphocytes. Macrophages may be seen containing large numbers of bacilli with associated vacuolated/foamy cytoplasm. As the disease progresses, better-defined caseous granulomas may be seen. The most typical form of granuloma involves a central area of necrosis with surrounding epithelioid macrophages and lymphoctyes. Multinucleated giant cells may or may not be present and a capsule of connective tissue may or may not surround the granuloma. Alternatively, lesions may be made up of aggregations of histiocytes. Acid-fast bacteria can usually be demonstrated, either within macrophages or within the central necrotic debris.

In the non-tuberculoid form of the disease the organ enlargement is caused by diffuse infiltration of large foamy histiocytes[37].

Avian tuberculosis is typified by a protracted immunological battle with the mycobacterium's waxy cell wall, responsible for much of the pathogenesis. Mycobacterial immunity is classically dependent on cell-mediated responses with humoral immunity playing a less important role.

Phagocytosis and killing by macrophages is key to host immunity to *M. avium*. Although the precise mechanisms at play within the avian macrophage are not fully understood it is assumed that the mycobacteria can survive and multiply within these cells by inhibiting phagosome–lysosome fusion, thus avoiding proteolytic enzymes and also disrupting cytokine production[37] and inactivating superoxide radicals produced by the host cell[39]. Glycosides from the bacterial cell wall promote the host's granulomatous response and enhance intracellular survival. Appropriate T-cell responses can induce killing of contained mycobacteria or the host response attempts to ensure bacteria can be 'held at bay' within activated macrophages within these granulomas. Host cells expressing mycobacterial antigens may be killed, resulting in tissue necrosis. Loss of protective cell-mediated responses (owing to nutritional stress, for example) can lead to escape of multiplying bacteria from granulomas and spread to other parts of the body.

CLINICAL SIGNS AND TREATMENT

In general there are few specific signs for avian tuberculosis in the field. Clinical presentation is most typically chronic wasting, with birds becoming emaciated and often exhibiting a prominent keel, or breast bone. Birds are usually weak and lethargic, often with poor or ruffled plumage. In late stages of the disease, abdominal distension as a result of liver enlargement and a build-up of ascitic fluid can give an emaciated bird an unusual 'bottom-heavy' appearance. Lameness is relatively common if there is bone

TABLE 20.3 Illustrative levels of leucocytosis for various avian species with avian tuberculosis.

Species	Situation	Normal values	Tuberculous values		Reference
		Range × 10^9/l	Range × 10^9/l	Mean ± sd (if available) × 10^9/l	
Quail	Experimentally infected	4.75	8.6–26.25 n = 32 (mean range)	21.75	42
Domestic fowl	Naturally infected	3.7–11.9 n = 30	10.3–230.0 n = 26	52.5 ± 48.6	43
Raptors	Free-living	11–20 n = 6	5–850 n = 6	170 ± 136	45
Various zoo birds	Captive zoo	Not given	Not given n = 17	62.475	42
Cranes	Captive zoo	2.1–20.1 n = 57	6.2–214.4 n = 17	47.9 ± 45.3	43
Various species of duck	Captive zoo	3.5–13.4 n = 40	18.8–61 n = 6	32.7 ± 18.4	Cromie (unpublised observations)

involvement. Chronic or intermittent diarrhoea is common.

Ceres and other areas of exposed skin may become progressively paler as the disease progresses. There may be cutaneous or subcutaneous lesions, particularly in raptors[32], and even ocular lesions[40]. Respiratory involvement is relatively unusual but this may result in dyspnoea. Involvement of the vertebral or central nervous system may account for neurological signs. Alternatively birds may just be found dead or succumb to another cause of death before these clinical signs are apparent.

Treatment of captive birds with anti-tuberculous drugs is complex and rarely recommended for large-scale use. Immunotherapy has been tried on a small scale but requires further research for treatment of disease in threatened species[41].

DIAGNOSIS

Diagnosis in wild birds, particularly *ante mortem*, is both complex and far from satisfactory and relies on a combination of tests before a positive diagnosis can be made.

HAEMATOLOGY

Studies with both captive and feral birds have found haematological screening to be relatively useful for *ante mortem* diagnosis[42–44]. Avian tuberculosis often results in marked leucocytosis due to monocytosis, and/or heterophilia. There may also be a lymphocytosis, and mild to moderate anaemia. Table 20.3 provides some values of leucocytosis that have been reported as being indicative of avian tuberculosis in various avian taxa.

Haematological changes may reflect the stage of infection, with clinical cases generally exhibiting the greatest haematological abnormalities. However, during late stages of the disease birds can decline into a state of anergy, which may confound this situation.

Although haematological abnormalities such as leucocytosis are not specific for avian tuberculosis, in combination with other data on body condition or other epidemiological factors, they can be useful for suggestive, or preliminary, diagnosis.

Serum Biochemistry

There are no reports of reliable and consistent diagnostic serum biochemical changes during avian tuberculosis. Some authors report elevations in serum aspartate aminotransferase or globulin. Serum biochemistry values are often unremarkable during this disease, at least for pet birds[46].

Haemagglutination

The haemagglutination test is a simple, rapid test that relies on agglutinating antibodies in a drop of serum or

whole blood when mixed with a drop of prepared antigen on a white tile. Results are highly variable, with false positives a particular problem, making it of limited practical use.

Complement Fixation Tests

Poor correlation has been found between clinical status and presence of complement-fixing antibodies – hence this is of limited value for diagnosis.

ELISA

Measurement of humoral responses for diagnosis of active avian tuberculosis is fraught with interpretation problems, mainly because of cross-reactivity of antibodies to environmental mycobacteria. However, some ELISA have achieved both high levels of sensitivity and specificity for some avian taxa[44]. One of these assays had some capability of predicting tuberculous death within a year of testing[41].

Ultrasonography

This can be used to identify changes in organ size and possibly extremely large tuberculous masses but is of limited value as a stand-alone diagnostic test.

Laparoscopy

For lesions on serosal surfaces of organs such as the liver, spleen, intestines, lungs or air sacs, laparoscopy offers a means of both visualization and potential biopsy for confirmative diagnosis, but lesions within the parenchyma or less accessible organs or body parts will go undisclosed. Additionally the need for anaesthesia makes this of limited value for working with wild birds.

Tuberculin Test

In poultry this intradermal test involved injection of avian tuberculin into a wattle or comb of a chicken, with resultant thickening at the site of injection within 48–72 hours. It was a powerful tool in managing the disease in these domestic birds for decades.

Areas of accessible suitable skin, let alone a handy dangling wattle, are absent from most wild birds. The necessity to hold a bird for 48–72 hours coupled with the highly variable results from exotic birds make this test of negligible value for the wildlife diagnostician.

Microscopy

The ZN stain has long been used to identify mycobacteria within lesions, faeces or aspirates. Other staining techniques involve use of fluorescent dyes (such as auramine or acridine orange). Microscopy alone cannot diagnose *M. avium,* merely the presence of acid-fast organisms. Further tests (e.g. molecular techniques or culture) are required for positive identification.

Cytological examination for *M. avium*-like bacilli is a relatively low technology test, but numbers of bacteria within lesions (from which smears may be made) can sometimes be low and therefore easily missed on examination. Faecal samples can be tested, but negative results may indicate a bird not currently shedding. Alternatively 'positive' samples may indicate the presence of other non-pathogenic environmental mycobacteria merely passing through the intestinal tract.

Culture

Culture of the aetiologic agent remains the 'gold standard' for diagnosis. However, in general, culture of mycobacteria requires specialist laboratories, as the bacteria have very specific growth requirements e.g. Lowenstein–Jensen or Middlebrook 7H10 culture medium with particular temperature and oxygenation settings. The BACTEC liquid medium system with radio-labelled reagents has reduced a usual 6-week culture time to approximately 2 weeks. Once cultured, identification can be made on colony characteristics and biochemical testing. Alternatively DNA probes can be used or techniques such as high-performance liquid chromatography[47].

Molecular Probes

Molecular techniques for detection of *M. avium* antigens or gene sequences have greatly improved diagnosis and aided epidemiological investigations. Most involve liberating mycobacterial DNA, DNA amplification by PCR and use of DNA probes to identify sequences. These techniques can be used on tissues (even formalin fixed), biopsies and faeces although the amount of organic material present in the latter can be a problem due to inhibition of PCR.

MANAGEMENT, CONTROL AND REGULATIONS

Avian tuberculosis is difficult to diagnose and treat; it has an insidious nature, with extended shedding periods, a broad host susceptibility and long environmental persistence. Therefore it is important to prevent its establishment in the wild by reducing risk factors, such as contact with infected domestic or captive birds.

The most effective disease control options for domestic and captive animal situations include routine surveillance with resultant culling of infected flocks, cleansing and disinfection (C&D) as happens in poultry farms. Zoo responses often involve euthanasia or quarantine of birds and complete exhibit renovation with C&D. Host immunity can be boosted by reducing stress, better nutrition and even immunoprophylaxis, although the latter is not known to have been tested in wild settings. Risk of infection can be reduced by ensuring good standards of hygiene, aided by use of certain disinfectants[37]. Although *M. avium* is more resistant in the environment than many other bacteria, ultraviolet radiation is a useful sterilizing agent, and exposing substrate (with turning as appropriate) to bright sunlight reduces mycobacterial load (during this time vertebrates should be excluded). Raising pH by the addition of lime is also valuable. Reducing population density (which can also serve to reduce stress) is also recommended. Reedbed treatment systems have been found to remove pathogenic *M. avium* from watercourses and should be considered where appropriate[48].

In wildlife situations the following management options are available:

1. continuous monitoring of wild bird populations, and *post mortem* examination of birds (and other species) found dead or viscera from hunted birds provide useful diagnostic samples
2. poultry, pigs or captive collections of birds should be spatially or physically separated from areas where wild birds congregate
3. free-ranging poultry in e.g. wetland areas should be avoided/discouraged
4. untreated poultry litter poses a risk and should not be used as fertilizer
5. wastewater from poultry holdings or slaughterhouses and sewage effluent should be excluded from wild areas
6. stresses to wild birds such as anthropogenic disturbances should be minimized, particularly during unavoidable stressful times, e.g. cold weather
7. healthy habitat management should aid reduction of nutritional stresses; paradoxically, provision of supplemental food may reduce nutritional stress and reduce feeding from a potentially contaminated environment yet can increase disease risk through increased population density and subsequent environmental contamination; where supplemental food is provided to raptors, this should be checked to ensure it is free of infection (avoiding avian meat is strongly advised)
8. within wetland systems, constructed treatment wetlands can be created to reduce contamination by *M. avium* and other pathogens
9. if there is a serious outbreak at a site, extreme measures to decontaminate and exclude wild birds can be taken, e.g. draining, turning soil/vegetation and substrate to expose to ultraviolet radiation while scaring birds away from the area[49]
10. reared game birds must be subjected to rigorous health screening before release
11. for conservation reintroduction programmes, raising birds in biosecure environments from eggs with thorough health monitoring is advised.

Although not a notifiable disease, avian tuberculosis in wildlife is classified as an OIE reportable disease. Among other reasons, difficulties in diagnosis prevent rigorous specific regulations with respect to trade or movements of domestic or captive birds; however, health certification measures should ensure that those birds showing signs of disease are excluded.

PUBLIC HEALTH CONCERN

The uncommon occurrence of avian tubercle bacilli infection in people even where the disease is common in poultry indicates that, in general, humans are extremely resistant to the pathogen. Given the relatively low prevalence of infection in wild populations of birds and this resistance, the disease in wild birds poses relatively little risk to public health.

People most at risk are those with pre-existing underlying chronic pulmonary problems, neoplastic conditions or immunodeficiencies. The advent of HIV/AIDS has

increased the incidence of human cases of MAC infections significantly. Although the developing world is burdened with co-infections of *M. tuberculosis* and HIV, in the developed world MAC is a relatively common co-infection for persons with AIDS. However, serotyping and molecular epidemiological investigations indicate that these MAC infections are generally associated with other environmental MAC strains rather than those of an animal origin, e.g. MAC from domestic supplies of water and other sources within human habitation. It has been stated that 'for practical purposes all MAI infections in humans are of environmental origin'[19].

Evidence of direct transmission from wild birds to humans is lacking, but high-risk persons, or those handling infected carcasses or working in avian tuberculosis control situations, should take appropriate and prudent health and safety precautions.

SIGNIFICANCE AND IMPLICATIONS FOR ANIMAL HEALTH

Poultry are very susceptible to avian tuberculosis, which can cause both reduced egg production and high mortality with the additional economic impacts of control programmes. Changes in intensive poultry production (such as improved hygiene and, importantly, culling at an early age) have reduced significantly the disease from most European domestic stock. The disease still occurs sporadically in smaller poultry flocks. Free-ranging birds, including farmed ratites, are at greatest risk of infection from wild birds.

In general, disseminated MAC infection in domestic mammals is rare and less progressive than the more frequently seen mammalian mycobacterial infections. The infection is more typically diagnosed at slaughter as localized lymph node lesions in e.g. cattle and pigs. Pigs are readily infected with *M. avium*, with numerous reports of lymphadenitis. It has long been assumed that wild birds have provided the source of infection for pigs; however, like immunocompromised humans, they may just be susceptible to environmental strains[20]. Cattle are relatively resistant to MAC and generalized infections are extremely rare. The greatest significance of MAC to cattle is in its non-specific sensitization to bovine mycobacterial antigens, hence reducing the diagnostic value of the intradermal bovine tuberculin test. This antigenic cross-reactivity has resulted in the widespread use of avian and bovine tuberculins being used as comparative tests in many countries. There have been numerous reports of *M. avium* infection in farmed deer, and these animals are considered reasonably susceptible. Cats and dogs are considered to be highly resistant, and reports of MAC infections in horses, sheep and goats are rare.

Cases of avian tuberculosis in wild birds have been recorded throughout the last century. Whether prevalence in wildlife has changed over this time cannot be determined, owing to paucity of standardized long-term surveillance data across broad geographical scales. The disease rarely causes significant outbreaks, and as such is likely to remain, largely, a persistent but relatively unseen cause of morbidity and mortality in wildlife. The reduction of avian tuberculosis in poultry farms is likely to have reduced point sources of infection for wild birds, although the disease within captive collections of birds remains a problem. Rearing, release and intensive management of game birds poses a risk, although close disease surveillance within the rearing flock should help to minimize risks, as does the young age of the birds at time of release.

Recovery programmes for threatened bird species frequently use captive environments for the birds, which is associated with risk of infection. Translocations themselves have potential risks for introduction of disease that need to be carefully managed. Threatened and insular species in the wild may be particularly vulnerable to the disease owing to loss of heterozygosity, and additionally the death or morbidity of a few key individuals can have a profound effect on a population.

PARATUBERCULOSIS OR JOHNE'S DISEASE

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Paratuberculosis, or Johne's disease, is a chronic granulomatous enteritis of ruminants found worldwide and caused by *Mycobacterium avium* subsp. *paratuberculosis* (Map). The disease remains a subject of concern in many countries because diagnosis and control in livestock are difficult and expensive and as a result of possible zoonotic links with Crohn's disease.

AETIOLOGY

Mycobacterium avium subsp. *paratuberculosis* belongs to the *Mycobacterium avium* complex, (MAC) which includes several species of slow-growing mycobacteria that are isolated from environmental, animal or human samples. The two main species of MAC are *M. intracellulare* and *M. avium*. This latter can be subdivided into four subspecies: *M. avium* subsp. *avium*, *M. avium* subsp. *silvaticum*, *M. avium* subsp. *paratuberculosis* and *M. avium* subsp. *hominissuis*. Map strains are classified into three types (types I, II and III) on the basis of growth characteristics and different molecular typing. These types present host preferences but not host specificity. Type I and III strains (grouped in S 'sheep' type) share similar phenotypic traits and have been isolated mainly from sheep and goats and rarely from wildlife. The type II strains (or C 'cattle' type) are the most common in Europe. They were primarily isolated from cattle but have a very broad host range and have been isolated from small domestic ruminants, different wildlife species and humans. Type II is predominant in wild cervids. A 'bison' type has been described, but this type is currently included in type II. The complete genome sequence of Map K10 strain is published (GenBank accession number: AE016958).

Map is an acid-fast, aerobic, non-spore-forming, non-motile bacillus (1 to 2 μm by 0.5 μm). In ZN-stained smears, the bacilli appear as short, acid-fast rods in clumps, indicative of intracellular growth. Like other mycobacteria, Map possesses a lipid-rich cell wall, thus resulting in acid fastness, hydrophobicity and increased resistance to chemicals such as chlorine. On the commonly used media, colonies are initially round, smooth and white. Map is a very slow-growing, fastidious organism, and many strains require the presence of mycobactin. Map is the slowest growing of the cultivable mycobacteria, with a generation time of over 20 hours, and visible colony formation on solid media may require up to 6 months.

EPIDEMIOLOGY

GEOGRAPHICAL DISTRIBUTION AND HOSTS

Presence of Map in wildlife is documented in ruminant and non-ruminant species in several European countries. In Spain, a recent serologic survey described a widespread distribution of exposure to Map in red deer (*Cervus elaphus*)[50]. A 2-year study (2002–2004) has shown increased cases of paratuberculosis in wild cervids in Austria[51]. Map (or specific antibodies) has also been recorded in free-ranging populations in the Czech Republic, Slovakia, Italy, Greece, France, Belgium, Netherlands, Germany, Norway, the UK and Poland.

Reports of occurrence in European ruminants show different results, depending on the country and the wild species studied. Comparison between countries is difficult, because some studies are large-scale serologic surveys and others are based on isolation and molecular characterization of *Map* from a few targeted wild animals. One study performed in free-ranging red deer in Spain reported a seroprevalence of 30%[50]. Presence of Map or antibodies was also recorded in Italy and Spain in free-ranging red deer[52], fallow deer (*Dama dama*)[53], Cantabrian chamois (*Rupicapra pyrenaica parva*)[54], mouflon (*Ovis aries musimon*)[55] and ibex (*Capra ibex*)[56]. In Norway, antibodies were detected in red deer (3.8%), roe deer (*Capreolus capreolus*) (12.2%), moose (*Alces alces*) (1.9%) and semi-domesticated reindeer (*Rangifer tarandus tarandus*) (3.4%), whereas wild reindeer were seronegative[57]. Red deer with antibodies to Map (2%) were also detected in the Czech Republic[58]. In roe deer, a prevalence (by culture and PCR) of 22% was reported in Italy[52]. In Europe, reports related to the presence of Map in roe deer are less frequent than in red deer.

In North America, many studies demonstrate the presence of *Map* in a large range of free-ranging ruminants, including Rocky Mountain bighorn sheep (*Ovis canadensis*), Rocky Mountain goats (*Oreamnos americanus*), white-tailed deer (*Odocoileus virginianus*), Key deer (*Odocoileus virginianus clavium*), tule elk (*Cervus elaphus nannodes*) and bison (*Bison bison*)[59].

Paratuberculosis is primarily a disease of ruminants, but the presence of Map in several non-ruminant wildlife species (Table 20.4) underlines its multi-host infectivity and suggests that the epidemiology of the disease is complex. In lagomorphs, the presence of Map was suspected for the first time in a hare (*Lepus europaeus*) in the UK. Thereafter, several studies reported the presence of mycobacteria in wild rabbits (*Oryctolagus cuniculus*), first in Scotland, and then in other countries. The most relevant studies were undertaken in Scotland (in areas where paratuberculosis was endemic in domestic ruminants), showing a prevalence of 20 to 60% in subclinically infected rabbits[60]. The most probable way of inter-species transmission is through ingestion of grass with contaminated

TABLE 20.4 Wildlife host range in which Map has been detected in Europe.

Mammals		
Cervidae	Red deer	*Cervus elaphus*
	Roe deer	*Capreolus capreolus*
	Fallow deer	*Dama dama*
	Reindeer	*Rangifer tarandus*
	Moose	*Alces alces*
Bovidae	Mouflon	*Ovis aries musimon*
	Ibex	*Capra ibex*
	Chamois	*Rupicapra rupicapra*
Suidae	Wild boar	*Sus scrofa*
Canidae	Red fox	*Vulpes vulpes*
Mustelidae	Badger	*Meles meles*
	Stoat	*Mustela erminea*
	Weasel	*Mustela nivalis*
Ursidae	Brown bear	*Ursus arctos*
Muridae	Brown rat	*Rattus norvegicus*
	Black rat	*Rattus rattus*
	Wood mouse	*Apodemus sylvaticus*
	Common vole	*Microtus arvalis*
	House mouse	*Mus musculus*
Leporidae	European rabbit	*Oryctolagus cuniculus*
	Brown hare	*Lepus europaeus*
Soricidae	Lesser white-toothed shrew	*Crocidura suaveolens*
Birds	Carrion crow	*Corvus corone*
	Rook	*Corvus frugilegus*
	Jackdaw	*Corvus monedula*
	Black-headed gull	*Larus ridibundus*
	European curlew	*Numenius arquata*
	Ruff	*Philomachus pugnax*
	Common cuckoo	*Cuculus canorus*
	Savi's warbler	*Locustella luscinioides*
	Starling	*Sturnus vulgaris*

faeces from shedding cattle. Subsequently, several methods of transmission have been demonstrated between lagomorphs. The horizontal route (faecal-oral route) is the most frequent; however, isolation of Map in the placenta, fetus and milk suggest that vertical and pseudovertical (through suckling) transmission routes are possible in rabbits [61]. These results demonstrate that Map may persist within populations of wild rabbits even in the absence of infected domestic ruminants. Among other wild species, Map has been detected in predators and scavengers. In the UK, a high prevalence (80%) has been detected in foxes (*Vulpes vulpes*)[60]. The bacteria has also been isolated from the lymph nodes of stoats (*Mustela erminea*) and weasels (*Mustela nivalis*), from faeces of other mammals including rodents and wild boar (*Sus scrofa*), and from birds. Birds and rodents could cause Map contamination of food stored for cattle.

A characteristic of paratuberculosis is the higher susceptibility of young ruminants. This is well known in cattle and farmed deer. Oral experimental infection with a high dose of Map of weaned calves, yearlings and adult red deer showed a strong age-related resistance of adults, which became infected but did not develop clinical or subclinical disease[62]. Genetic susceptibility to Map infection and/or disease is demonstrated in some breeds of cattle and sheep. Similar genetic studies have not been undertaken in wildlife.

ENVIRONMENTAL FACTORS

Map is an obligate intracellular pathogen of animals, but the organism can survive for long periods outside the host (in soil and water). Survival for more than 1 year was observed in a dry, fully shaded environment, and the survival decreased in locations exposed to direct sunlight. In favourable environments (high humidity, low pH and shaded areas), Map may survive for several months in pasture contaminated by shedding animals. Results of these environmental studies suggest that the major threat to Map survival is large temperature variations. The presence of dormancy-related genes have been demonstrated in the Map genome, and other potential survival mechanisms of the bacteria include biofilm formation and aerosolization. Moreover, some studies suggest that nematodes, insects or protozoa could play a role as vehicle for this mycobacterium[63].

EPIDEMIOLOGICAL ROLE OF THE AFFECTED SPECIES

Although Map has been isolated from many wild species, these may be classified into two categories according to their epidemiological significance for livestock. The first group includes wild ruminants and lagomorphs (especially rabbits),which may be considered as maintenance reservoirs. These wild species can maintain infection independently of the presence of infected domestic ruminants, and ecologically they share the same pastures. The second group are dead-end hosts; these are carnivores that may contract the infection but do not maintain it if the primary reservoir is absent. This group also includes some rodents and birds, which may disseminate Map passively in the

environment. In this context, the transmission of Map from wildlife to livestock is assumed to be by faecal contamination of feed and forage, on pastures or in the barns. The interspecies transmission has been demonstrated experimentally; cattle may be infected with a rabbit strain of Map, and conversely.

TRANSMISSION

Map is predominantly transmitted through the faeco-oral route, via mycobacteria-faecal-contaminated colostrum, milk, water or feed. Moreover, contaminated colostrum and milk produced by the infected dam is the major route of transmission for neonates. The bacterium has also been isolated from semen from domestic cattle. Fetal infection is also possible in utero, and the rate of intrauterine transmission has been demonstrated to be higher in farmed red deer than in cattle or sheep. Carnivores may also be infected by eating an infected prey species.

PATHOGENESIS, PATHOLOGY AND IMMUNITY

After ingestion, Map enters the intestinal tissue through the M cells in the Peyer's patches of the small intestine. Regional enterocytes may also be involved in the uptake of the bacterium. The bacterial cells are then phagocytosed by resident subepithelial macrophages or dendritic cells, in which they multiply[64]. Map is able to survive within the macrophages by inhibiting phagosome acidification and phagolysosomal fusion. This is a crucial process in establishment and evolution of the disease. With disease progression, infected macrophages migrate into local lymphatics, resulting in bacterial spread to mesenteric lymph nodes. The infection may be disseminated, with mycobacteria detectable in extra-intestinal lymph nodes (mammary, pulmonary, hepatic and head) and associated organs.

Subsequent to infection, Map is initially controlled by a T helper (Th1) response with IFN-γ production. The progression of the disease from a subclinical to a clinical state is associated with a switch from a Th1 to a Th2 immune response, leading to antibody production that is less effective in controlling infection. The loss of control in some hosts may be due to genetic factors or may be induced by external factors, including parturition, malnutrition or secondary viral or bacterial infections. The interplay between Th1 and Th2 cytokines has been reviewed[64].

The host response to Map infection results in granulomatous lesions in the small intestine in which thickened mucosa and associated lymphadenopathy are characteristic. The proliferation of reactive lymphocytes, epithelioid macrophages and giant cells results in infused, blunted villi with decreased absorptive capacity. Malabsorption in the ileum, exudation from the inflamed intestinal mucosa, and lymphatic stasis all act to overload the absorptive capacity of the bowel, resulting in weight loss associated with a protein-losing enteropathy.

Pathology in Wild Ruminants

Cervids that die with terminal disease present with severe emaciation and faecal staining around the perineum and hind legs (Figure 20.5). Gross lesions and histopathology may be suggestive but may not distinguish between lesions induced by Map, *M. bovis* and *M. avium avium*. The gross lesions are not always present and early cases may be missed at *post mortem* examination. The lesions are mainly observed in the lower part of the jejunum, ileum, ileocaecal junction and associated mesenteric lymph nodes (Figure 20.6). The classical picture is a diffuse thickening of the intestinal mucosa, where transverse folds may be observed. Frequently in wild cervids, the only gross lesion

FIGURE 20.5 Adult female red deer with paratuberculosis, killed because of cachaexia and signs of diarrhoea.

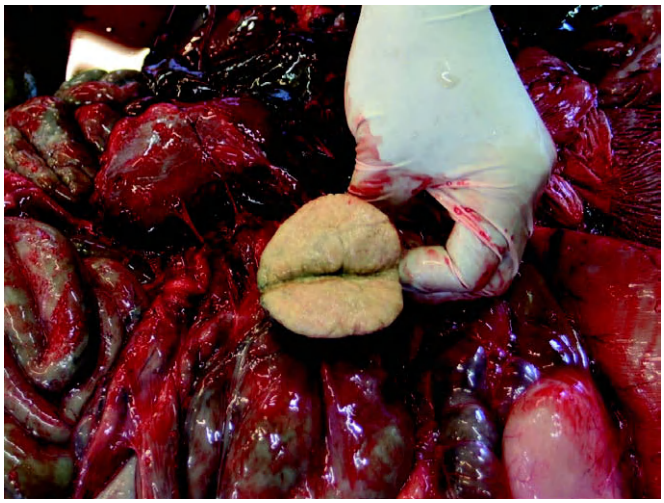

FIGURE 20.6 Cross-section of mesenteric lymph node from an adult male red deer found dead (severe emaciation and signs of diarrhoea), revealing extensive fibrosis and multifocal necrosis. Both mesenteric and mediastinal lymph nodes were enlarged and ZN-positive, PCR IS 900-positive and culture-positive for *M. paratuberculosis*.

present is enlargement of mesenteric lymph nodes, with none or only mild intestinal-wall thickening. Lymph nodes are pale and oedematous with indistinct corticomedullary junctions. Occasionally, they contain cream-coloured caseous exudate. Prominent lymphatic vessels may be observed as thickened cords in the mesentery leading to the mesenteric lymph nodes. Disseminated infection with lesions in the lung and liver has been reported in severe cases in farmed and free-ranging cervids.

Microscopic changes are characterized by granulomatous inflammation in the mucosa of the small intestine and associated lymphatics and lymph nodes. Occasionally, focal granulomas may be observed in extra-intestinal tissues, especially liver, lung and associated lymph nodes. In affected tissues, focal or multifocal granulomas consist of macrophages, variable numbers of Langhans giant cells and mild infiltrations of lymphocytes. In severe cases, epithelioid macrophages may accumulate in lymph nodes and foci of necrosis may occur, but mineralization is rarely observed in wild cervids. As in domestic ruminants, acid-fast organisms may be observed in variable numbers (paucibacillary and multibacillary forms) in macrophages and Langhans giant cells on ZN-stained sections. These two distinct forms are associated with two different immune responses. On the one hand, the multibacillary form (also named lepromatous form) is characterized by numerous intracellular bacteria and epithelioid macrophages. In this form, animals mount a weak cell-mediated immune response, but they develop serum antibodies. On the other hand, the paucibacillary form is associated with increased lymphocytes, a strong cell-mediated immune response and a poor antibody response. In animals with paucibacillary lesions, intracellular bacteria are present in lymph nodes or Peyer's patches but not always in the intestinal mucosa. A histopathological grading system to differentiate these two forms of paratuberculosis in red deer has been developed[65].

Pathology in Non-Ruminant Wild Animals

Typical lesions have only been observed in wild rabbits and in some carnivores. In rabbits, some studies reported thickening of the intestinal wall and enlarged lymph nodes[66] whereas others did not report these gross lesions[60]. In severely affected wild rabbits, gross lesions may be observed in intestines, gut-associated lymphoid tissue, mesenteric lymph nodes and liver. They consist of chronic granulomatous inflammation with numerous macrophages and giant cells and many intracellular acid-fast organisms[60]. In fox, stoat and weasel, positive lymph node cultures associated with mild histopathological lesions suggest that these carnivores may be infected by Map. In comparison with ruminants and rabbits, the lesions in these carnivores are subtle, suggesting that these animals may be 'dead-end' hosts for Map. Similar mild histopathological lesions have also been observed in a crow (*Corvus corone*) and a wood mouse (*Apodemus sylvaticus*)[60]. In other species (Table 20.1), the isolation of mycobacteria was not associated with lesions, suggesting a simple passage of ingested Map through the intestinal tract[60].

CLINICAL SIGNS AND TREATMENT

Clinical paratuberculosis is infrequently diagnosed in free-ranging cervids, but it is more frequently observed in farmed red deer. In advanced clinical disease, animals are weak, emaciated and usually have chronic diarrhoea. This clinical sign is frequent in cattle but not always present in wild ruminants. Most studies report subclinical cases in

which Map is isolated from apparently healthy cervids, or results of serological studies for which there is no information about the health status of the sampled wild animals. In Europe, some clinical cases have been observed in fallow deer in Spain[53], in red deer in Austria, Italy and Belgium[24,67,68], in roe deer in the Czech Republic[69] and in Alpine ibex in Italy[56]. In the majority of cases, these animals were found dead, emaciated and with rough coat hair. Emaciation is progressive and fatal, but the appetite is conserved, and the wild cervids remain alert until the terminal stages. It appears that the progression of paratuberculosis is much more rapid in wild cervids than in cattle and it is not uncommon to isolate Map from young red deer under 2 years old and even in fawns, which may be found dead and emaciated. In farmed deer, two clinical syndromes, both fatal, are described: sporadic cases in adults with progressive weight loss over a few months, and acute cases in young deer (8–15 months old) with profuse diarrhoea and rapid weight loss within a few weeks[70]. Growth of antlers may be adversely affected. In non-ruminant wildlife species, no clinical cases have been associated with Map isolation.

Long-term treatment of paratuberculosis is not considered in individual animals, because antibiotic therapy is cost-prohibitive, and efficacious drugs are prohibited in food-producing animals. *In vitro*, Map strains are susceptible to drugs such as clarithromycin, azithromycin, amikacin and rifampicin.

DIAGNOSIS

Diagnosis of paratuberculosis, especially subclinical cases, continues to be a challenge in domestic ruminants. Poor performances of current diagnostic tools are a major problem for the control of paratuberculosis in livestock. In wildlife, the diagnosis is also difficult because routine tests, especially serologic tests, used in domestic livestock are generally not validated for wildlife.

Thickened mucosa of the distal small intestine and enlarged associated mesenteric lymph nodes are the most frequent lesions observed in infected wild cervids. However, gross and microscopic lesions may be suggestive of paratuberculosis but are not diagnostic. The confirmation is based on culture and/or PCR. For histopathology and isolation of Map, targeted samples are the lower part of the jejunum, ileum, ileo-caecal junction, and associated draining lymph nodes, including other intestinal segments, is advised.

Direct microscopy of ZN-stained smears (faeces, intestinal mucosa or lymph nodes) is a rapid method but is not specific and has low sensitivity. Short, red-staining rods, characteristically in clumps, indicating intracellular growth, is characteristic of Map. The detection of single acid-fast rod bacilli is inconclusive, because they may be environmental mycobacteria.

The isolation of Map by culture (from faeces or gut tissue) is considered as the *post mortem* diagnostic 'gold standard', and this technique is essential if further molecular typing is required. Cultures are time-consuming; however, although slow- and fast-growing Map strains are described and decontamination protocols may lead to false-negative results, the specificity is 100%. The recommended procedures for decontamination and culture are reviewed[71]. Several solid media of choice may be used, all containing the iron-chelating growth factor mycobactin J: i) egg-based media such as Herrold's egg-yolk medium and Lowenstein–Jensen medium; ii) serum-based medium such as Dubos medium; and iii) synthetic medium such as Middlebrook medium. On solid media, primary colonies of Map may appear from 5 weeks to 6 months after inoculation. The environmental mycobacteria appear more rapidly in the same media. For identification, a small inoculum of suspect colonies is subcultured on the same medium with and without mycobactin (dependence test). This criterion must be used with caution because the mycobactin requirement is not confined to Map. Compared with the culture on solid media, the liquid-culture-based BACTEC system reduces the time and is considered more sensitive[71].

Molecular tools give higher sensitivity and faster results than culture. PCR tests may be used to identify specific mycobacteria in faeces or tissues and also to confirm identification of colonies. Several Map-specific genomic sequences such as insertion sequence IS900, f57, ISMav2, ISMap02, ISMap04 have been identified. Of these, IS900 is by far the most widely used target for PCR detection of *Map*. However, the presence of IS900-like sequences in non-Map mycobacteria has lead to caution in the use of IS900 and indicates the need to verify the result with DNA sequencing. In order to improve the specificity and to make differentiation between single and mixed mycobacterial infections, multiplex PCR have been developed with success[72,73].

Among serological tests, ELISA are the most widely used in wildlife. Several ELISA kits are available and, for the majority, sera are pre-absorbed with *M. phlei* to increase specificity. However, these ELISA are not validated for wild species. For serology of clinically affected farmed deer, an IgG1 antibody ELISA using two antigens (Paralisa®, with sensitivity >80% and specificity >98%) has been developed in Australia[74]. Sensitivity of serological tests is lower during the subclinical phase. For free-ranging cervids, an ELISA has been developed in Spain[50].

Tests of cell-mediated immunity such as the intradermal skin test and the lymphocyte transformation test provide poor specificity because of the widespread exposure of deer to environmental mycobacteria. The IFN-γ test is successfully used as an indirect diagnostic tool in the early stage of the disease in cattle. No commercial IFN-γ test for use in deer or other wildlife is currently available.

MANAGEMENT, CONTROL AND REGULATIONS

Control of paratuberculosis is difficult in wildlife. Several management measures related to the wild host and their habitats may be taken in combination to reduce the prevalence of the infection. However, if Map is established in a wild cervid or rabbit population, eradication of the disease is unrealistic.

Management Practices Related To Host and Habitat

If a point source for a paratuberculosis outbreak is detected in wild cervids, selective culling of clinically diseased animals (severe emaciation with/without diarrhoea) may be carried out to eliminate potential excretors and in this way prevent Map from spreading. In some countries, this procedure is legal and is carried out by hunters and forest rangers throughout the year, particularly in 'hot spot areas'. Necropsy of the animals culled is mandatory to confirm paratuberculosis. Selective culling should be limited to focal outbreaks and caution must be taken to avoid the disruption of the social structure of the group, for example by causing dispersal of infected animals. Measures relating to control of host species density are also important. When wild cervids are artificially gathered under higher than natural densities, the risk of transmission of many pathogens, including mycobacteria, is increased. Campaigns may be carried out to inform stakeholders about the infectious risks inherently associated with disease and the overabundance of cervids[75], including the recommendation to reduce artificial feeding in the habitat. During hunting activities in hot spot areas, hunting pressure may be increased and hunters should be encouraged to arrange laboratory analysis of cervids that are found dead. Moreover, in all hunting areas, correct disposal of viscera must be organized to limit the sylvatic cycling of mycobacterial infections. The success of such measures is largely dependent on the participation of field sports authorities, who have an important role in the control of stock densities and also in the early detection of disease cases. Despite these measures, a proportion of undetected shedders will remain as a continuing source of infection. Paratuberculosis has persisted for over 20 years in bighorn sheep in Colorado, despite selective culling and increased hunting pressure in the infected area. As eradication is not possible, minimizing contact between wild and domestic herds, by managing separate areas of grazing, may be considered in some regions. Besides wild ruminants, Map is also extremely persistent in rabbit populations. In the UK, a simulation model of Map dynamics in rabbit populations demonstrated that repeated annual rabbit culls (>40%) over many years are more effective at reducing infection than single (not repeated) and massive (>95%) population reduction[76].

There are several live attenuated and killed Map vaccines for use in farmed deer. Some provide significant protection against clinical disease and reduce faecal shedding, but they do not prevent infection and they also interfere with several bovine tuberculosis diagnostic tests. Vaccination studies are not reported in wildlife. Paratuberculosis is included in the OIE listed diseases affecting wild animals. Annual notification is mandatory.

PUBLIC HEALTH CONCERN

Although not currently classed as zoonotic agent, *Map* is suspected to be involved in the pathogenesis of Crohn's disease (CD) in humans. It is recognized that CD involves the interaction of at least three components: a genetic predisposition, an environmental trigger and an unregulated immune response. The involvement of Map in CD is supported by several factors, including: i) CD and

paratuberculosis share clinical and histopathological similarities; ii) viable Map has been cultured from intestinal tissues, blood and milk of patients with CD; and iii) antibodies to Map antigen have been identified in the blood of Crohn's patients. Moreover, recent meta-analysis studies indicate that there is an association between the presence of Map and CD, but whether this association is causal or coincidental is not known. Arguments against a role of Map in CD are that TNF-γ inhibitors, which would not be effective if Map were the cause of CD, are effective in treatment of CD, whereas anti-mycobacterial treatment has limited efficacy[77].

In conclusion, the infection of humans with Map and possible association with CD remains a controversial issue. In some countries, precautionary control measures are taken in food sectors, as there is a probability that dairy and meat products are contaminated by this mycobacteria. Of major concern are the studies demonstrating that Map has been cultured from pasteurized retail milk and that Map may be present in drinking water[78]. For wildlife concern, the decision to class Map as zoonotic agent or not will have an impact on the level of control in wildlife.

SIGNIFICANCE AND IMPLICATION FOR ANIMAL HEALTH

In the past 10 years, the prevalence of paratuberculosis in livestock appears to be gradually increasing worldwide, despite efforts in expensive control programmes in farm animals. In this context, the existence of wildlife reservoirs with the potential to transmit the infection to livestock may affect the success of these programmes. Several molecular epidemiological studies have demonstrated sharing of Map strains between wild and domestic animal species[79]. However, the risk of transmission from wildlife to livestock has to be put into perspective. In an infected herd, the major problem is young calves infected by their mothers or congeners. Wildlife reservoirs may have epidemiological importance on farms that have eliminated all infected livestock from the premises or on farms free of paratuberculosis but located in the same geographic areas as infected farms. In Europe, some studies suggest that the presence of paratuberculosis in wild rabbits and cervids may represent a risk for livestock. However, dead-end hosts, such as predators and scavengers, are probably not high-risk factors for interspecies transmission.

The implication of paratuberculosis for wild animal health is mainly for captive and endangered wildlife species. Since 1991, paratuberculosis has been responsible for deaths of numerous hoofed mammals housed at the Zoological Society of San Diego[80]. For free-ranging ruminants, it is difficult to determine the effect of paratuberculosis on wild populations. In some populations, there is a high rate of subclinical infection and the effect of chronic infection is difficult to quantify. However, it is unlikely that wild populations are severely affected, because the mortality is generally low. The risks from paratuberculosis should be considered in reintroduction or translocation programmes.

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INFECTIONS BY OTHER MYCOBACTERIA OF THE M. TUBERCULOSIS COMPLEX

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MYCOBACTERIUM TUBERCULOSIS (HUMAN TUBERCULOSIS)

Mycobacterium tuberculosis is the principal cause of human tuberculosis worldwide. Infection by *M. tuberculosis* has occurred throughout the world in a broad range of species in zoos, mostly in primates, but also frequently in Asian elephants (*Elephas maximus*) and additionally in African elephants (*Loxodonta africanum*), black rhinoceroses (*Diceros bicornis*) and other ungulates. Infection of pet psittacine birds also occurs, presumably acquired from their owners, and infection from humans with *M. tuberculosis is* generally considered to be the source of most infections in animals and birds. New drug-resistant strains are emerging. Infection by *M. tuberculosis* in free-ranging wildlife in Europe has not been reported.

MYCOBACTERIUM MICROTI

The main host of *M. microti* is the field vole (*Microtus agrestis*), and the disease is called vole tuberculosis. The infection has been reported to be endemic with high prevalence in field voles in the UK[81]. *Mycobacterium microti* infection also occurs in wood mice (*Apodemus sylvaticus),* bank voles (*Clethrionomys glareolus*), shrews (*Sorex araneus*), domestic cats (mostly of extra urban locations) and occasionally in badgers (*Meles meles*), domestic pigs, cattle, llamas, alpacas and zoo animals in Europe.

In a study in the UK the prevalence of vole tuberculosis, based on detection of gross skin lesions, was estimated to vary from zero to up to 50% of voles infected per site studied. The prevalence increased with the age of individuals and showed apparent seasonality, with higher numbers of cases in the late spring and early summer, which was attributed to a higher proportion of older voles in the spring[81].

The molecular typing of *M. microti* isolates from cats and other animals in the UK has shown a diversity of genotypes, geographically localized and different from isolates obtained from *M. microti* infection clusters in the Netherlands and Belgium[82,83], and did not overlap with the distribution of *M. bovis*[82]. No host specificity for the different spoligotypes of *M. microti* were shown[82].

It has been suggested that field voles act as maintenance host of *M. microti*[81] and cats are spillover hosts that contract the infection from hunting rodents[82].

Mycobacterium microti causes lesions in a wide range of mammalian species, in some of which infection is probably acquired via the skin through bite wounds. Voles show characteristic cutaneous lesions, which consist of subcutaneous granulomas with skin ulcers in the scapular region; these represent late stage of infection and are therefore found in adult voles (mass >17 g)[81]. Lesions may be disseminated and occur also in the lungs, lymph nodes and other organs. In particular, superficial lymph nodes draining skin wounds show granulomas. Lesions in cats are mostly located in the skin and superficial lymph nodes of the head, suggesting infection via bite injuries contracted during hunting of rodent prey[82]. In other species, including alpacas, a llama, a horse and badgers, a range of pathology has been described, from no visible lesions to generalized disease[82]. The frequency of generalized disease in any species is unknown. Histologically, in voles accumulation of macrophages, with necrosis and calcification, and sometimes abundant AFB are observed in the skin and granulomatous lesions occur also in lungs, lymph nodes and other organs.

In a study in voles in the UK, the recognition of clinical signs of vole tuberculosis (characteristic skin lesions and superficial lymphadenitis) detected a prevalence of 7%, whereas *post mortem* examination (with demonstration of caseous abscesses in subcutaneous or internal abdominal organs) detected more cases, a prevalence of 21%[83]. The definitive diagnosis is established by culture and identification of *M. microti*, with molecular methods used for identification and typing of strains.

Mycobacterium microti is a zoonotic agent, and infection has been reported in immunosuppressed and also in immunocompetent humans in several European countries.

MYCOBACTERIUM PINNIPEDII

Mycobacterium pinnipedii infection has been reported in various captive and free-ranging pinnipeds in the southern hemisphere. Terrestrial mammals, including humans, are also susceptible to *M. pinnipedii* infection. An outbreak of tuberculosis due to *M. pinnipedii* occurred in southern sea lions (*Otaria flavescens*) in a zoo in the Netherlands, with granulomatous lesions in bronchial, mediastinal and mesenteric lymph nodes, lungs and other organs. Many zookeepers in close contact with the sea lions contracted the infection[84]. Infection by *M. pinnipedii* has also been reported in other zoos in Europe, causing mostly pulmonary and lymph node lesions in a South American sea lion (*Otaria byronia*), Bactrian camels (*Camelus bactrianus bactrianus*) and Malayan tapirs (*Tapirus indicus*) in Germany[85]. No cases of *M. pinnipedii* infection have been reported in free-ranging wild animals in Europe.

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CHAPTER

21

YERSINIA INFECTIONS

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YERSINIA PSEUDOTUBERCULOSIS AND *YERSINIA ENTEROCOLITICA*

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The genus *Yersinia,* family *Enterobacteriacea,* comprises the three pathogenic species *Y. pestis, Y. pseudotuberculosis* and *Y. enterocolitica.* The causative agents of yersiniosis are *Yersinia pseudotuberculosis* and *Yersinia enterocolitica,* comprising various serotypes and biotypes. The predominant *Yersinia* spp. clinically relevant in Europe are *Y. pseudotuberculosis* serotypes O:1, O:2 and O:3, and *Y. enterocolitica* serotypes O:3, O:8, O:9, and O:5,27[1]. These cause enterocolitis, mesenteric lymphadenitis and, less frequently, septicaemia.

AETIOLOGY

Both *Y. enterocolitica* and *Y. pseudotuberculosis* are Gram-negative coccobacilli, motile at 20°C but not at 35°C, and grow well on MacConkey agar. Biochemically they produce catalase and urease, reduce nitrates and produce acid from cellobiose, melibiose, rhamnose and sucrose under aerobic and anaerobic conditions. They are oxidase-negative.

The pathogenic potential of *Yersinia* is a complex interaction between the plasmid of virulence (pYV) and specific chromosomal virulence determinants encoded by several genes. The virulence of pathogenic *Yersinia* is closely associated with a 64–70 kb plasmid encoding a number of *Yersinia* outer proteins (Yop) and proteins of the *Yersinia* type III secretion apparatus whose expression is regulated by the transcriptional regulator VirF. As Yop are immunogenic in humans and animals, *Yersinia* infections can be detected by anti-Yop antibodies independent of serovars or biovars[2]. Both pathogens are psychrophilic, being able to multiply at low temperatures (2–5°C) and in microaerophilic conditions. *Yersinia* are susceptible to many disinfectants as well as moist and dry heat. Outside the host, *Yersinia* can survive for up to 20 days in water and 540 days in soil.

EPIDEMIOLOGY

GEOGRAPHICAL DISTRIBUTION AND HOSTS

Some aspects of the epidemiology of yersiniosis still remain obscure. *Yersinia pseudotuberculosis* and *Y. enterocolitica* are ubiquitous and carried subclinically by a range of animal species, including wild mammals, birds and rodents. Outbreaks of disease are usually sporadic, but epizootics

Infectious Diseases of Wild Mammals and Birds in Europe, First Edition. Edited by Dolores Gavier-Widén, J. Paul Duff, and Anna Meredith.

causing high mortality have been documented in a wide variety of wild and exotic animals[3]. Yersiniosis has been recorded in farm and domestic animals (cattle, horses, sheep, goats, pigs, rabbits, cats, dogs, guinea pigs, hamsters, etc.), in commercially reared fur-bearers (chinchilla, mink and coypu), in wild mammals (European brown and mountain hares, rats, mice, foxes, voles, deer, marmot, beaver, hedgehogs, etc.), and in mammals in zoological gardens (e.g. monkeys, pumas, kangaroos, agoutis). Epizootics have been reported in sheep, farmed wild fur-bearers and farmed deer[4].

Disease is relatively frequent in domestic and wild birds. Epizootics have been sporadically reported in turkeys, ducks, stock doves, in aviaries of canaries and finches, and wood pigeons. Sporadic infection has been recorded in more than 50 species of bird.

Infection with *Y. enterocolitica* has been observed mainly in mammals. Outbreaks have been reported in hares, chinchillas and captive monkeys, and sporadic infection in dogs, pigs, sheep, guinea pigs, rabbits and cattle[3].

Human and animal infection with *Y. pseudotuberculosis* is essentially of European origin. It is assumed that the appearance of *Y. pseudotuberculosis* in other parts of the world is attributed to the importation of infected animals from Europe. Moreover, based on the knowledge that the acquired resistance to infection with *Y. pseudotuberculosis* protects against plague, it is suggested that the emergence of the enzootics of *Y. pseudotuberculosis* may have contributed to the disappearance of pandemic plague, and may have curtailed plague in Europe during the third and last pandemic[5]. The disease is rarely reported in the Mediterranean region. No human or animal case has been reported from Spain, and in Portugal the infection has been found only in imported guinea pigs and monkeys. In Italy and Greece, only one human case in each country has been recorded. The disease is unknown in the Near and Middle East.

Yersinia enterocolitica has a wider geographical distribution and is found not only in Europe, but also on other continents. The highest concentration of human and animal cases has been found in France, Belgium, the UK, Sweden and Finland. In France, 300 isolates of *Y. enterocolitica* and related *Yersinia* species were isolated by testing 1307 specimens from a terrestrial ecosystem[6]. In another study in France on regional prevalence and intestinal distribution, *Y. enterocolitica* was isolated from 5.7% of 3533 small wild mammals[7]. In Norway, Sweden and Finland 24 isolates of *Y. enterocolitica* were recovered from 551 small wild rodents[8]. In Sweden, *Y. enterocolitica* was isolated in 5.6% and *Y. pseudotuberculosis* in 0.6% of 468 faecal samples from 57 different species of migratory bird[9]. In this study *Y. pseudotuberculosis* was detected in song thrush (*Turdus philomelos*) and redwing (*Turdus iliacus*), and *Y. enterocolitica* in barnacle goose (*Branta leucopsis*) (in highest frequency), redshank (*Tringa totanus*), dunlin (*Calidris alpina*), redstart (*Phoenicurus phoenicurus*), blackcap (*Sylvia atricapilla*) and rough-legged buzzard (*Buteo lagopus*). Of the 29 *Y. enterocolitica* isolates, 10 belonged to bioserotype 3/O:3 (more often associated with human disease), three belonged to 1A/O:5, and one to 1A/O:3. In Finland, a large population of deer and hares on Ahvenanmaa Island were presumable subclinical carriers and reservoirs of *Y. pseudotuberculosis*[9,10]. Twenty-six *Y. enterocolitica* strains were isolated from 178 brown rats (*Rattus norvegicus*) and black rats (*Rattus rattus*) captured in pig houses in the former Czechoslovakia[11]. *Y. enterocolitica* and *Y. pseudotuberculosis* were isolated from Siberian ruddy voles (*Clethrionomys rutilus*) in arctic regions of Russia[12]. Forty-six *Yersinia* strains (21 *Y. enterocolitica* and 25 *Y. pseudotuberculosis*) were isolated from 37 wild animals originating from the mountain areas of Bulgaria, during winter hunting[13].

In the UK, 321 *Y. pseudotuberculosis* strains have been isolated from 72 different animal species, of which 101 were from wild animals living in captivity, and 68 from free-living wild animals[3]. Of the latter, 28 strains were isolated from six species of mammal: coypu (*Myocastor coypus*), brown hare (*Lepus europeus*), mouse (*Mus musculus*), rabbit (*Oryctolagus cuniculus*), red fox (*Vulpes vulpes*) and field vole (*Microtus agrestis*). Sporadic infection has been found in the Scottish blue or mountain hare (*Lepus timidus scoticus*)[14].

Forty *Y. pseudotuberculosis* strains have been isolated from 21 species of wild bird: blackbird (*Turdus merula*), eider (*Somateria mollisima*), fieldfare (*Turdus pilaris*), great tit (*Parus major*), greenfinch (*Chloris chloris*), green woodpecker (*Picus viridis*), hedge sparrow (*Prunella modularis*), hoopoe (*Upupa epops*), house martin (*Delichon urbica*), magpie (*Pica pica*), oystercatcher (*Haematopus ostralegus*), partridge (*Perdix perdix*), pheasant (*Phasianus colchicus*), pied wagtail (*Motacilla alba*), redwing (*Turdus musicus*), swallow (*Hirundo rustica*), swift (*Apus apus*), stock dove (*Columba oenas*), tree sparrow (*Passer montanus*), wood pigeon (*Columba palumbus*) and wren (*Troglodytes troglodytes*)[3]. Other British wild birds in which the disease has been reported include the coot (*Fulica atra*), chough (*Pyr-*

rhocorax pyrrhocorax), goldfinch (*Carduelis carduelis*), jackdaw (*Corvus monedula*), pied flycatcher (*Ficedula hypoleuca*), puffin (*Fratercula arctica*), rook (*Corvus frugilegus*), starling (*Sturnus vulgaris*), skylark (*Alauda arvensis*), sparrowhawk (*Accipiter nisus*), song thrush (*Turdus ericetorum*), swallow (*Hirundo rustica*) and willow warbler (*Phylloscopus trochilus*)[15]. In Continental Europe the disease has also been reported in the tawny owl (*Strix aluco*) and mute swan (*Cygnus olor*).

Of wild animals, hares (*Lepus* spp.) are probably most susceptible to yersiniosis. They appear to be equally susceptible to *Y. enterocolitica* infection as to *Y. pseudotuberculosis* infection. In France and Germany, *Y. pseudotuberculosis* accounts for more leporine deaths than any other bacterial cause, having been isolated from up to 60% of hares in some areas. Similarly high percentages of affected hares have also been found in the UK[3]. Anti-*Yersinia* antibodies were found in 55% of hare sera from Northern Germany[16] and *Y. pseudotuberculosis* was isolated from 13% of shot or found dead hares and *Y. enterocolitica* in 4%[17]. In a Belgian survey, 14% of hares were found to be carriers[18].

In north-eastern Germany, 62.6 % of the wild boars were found positive for anti-Yop antibodies[19]. In the UK, 1% of 3,000 coypus (*Myocastor coypus*) live trapped or otherwise caught and killed in Norfolk and Suffolk over a 6-year period were recorded as having yersiniosis[20]. In wild foxes only one case, from which *Y. pseudotuberculosis* was isolated from many organs, has been recorded in the UK[21]. There are few reports of infection in wild rabbits (*Oryctolagus cuniculus*). The first epizootic among farmed chinchilla (*Chinchillidae* family) imported from California and caused by *Y. enterocolitica* was reported in Germany[22]. In Continental Europe the disease has also been observed in roe deer (*Capreolus capreolus*), wild fallow deer (*Dama dama*), mink (*Mustela lutreola*), martens (*Martes martes*), moles (*Talpa europaea*), hedgehogs (*Erinaceus europaeus*) and marmots (*Marmota marmota*).

ENVIRONMENTAL FACTORS AND EPIDEMIOLOGICAL ROLE OF WILD ANIMALS

The principal reservoirs of *Y. pseudotuberculosis* are rodents and birds. Rats and mice are resistant to natural disease and for this reason play an important epidemiological role in the spread of infection. Experimental *per os* administration of a culture of *Y. pseudotuberculosis* to the rat or mouse does not produce any visible lesions when the LD_{50} is less than 1.10^8 CFU for mice (the rat is totally resistant), but results in excretion of the organism in the faeces over a period of 1–2 weeks[23]. Hares, common voles (*Microtus arvalis*) and water voles (*Arvicola terrestris*) are also known to serve as reservoirs. It is considered that most mammalian species that are susceptible to infection may also become subclinical carriers of *Y. pseudotuberculosis.* Moreover, these animals often have less severe disease compared with domestic species. Latent infection may manifest as clinical disease under certain conditions, such as in the winter months, when animals, particularly free-living species, are exposed to cold and starvation. Although worldwide in distribution, the disease has highest prevalence in temperate climates and is more frequent in winter (December–March) than summer. Yersiniosis is a common disease in Northern Europe, Scandinavia and the mountain areas of the Balkan region.

Human infection with *Y. pseudotuberculosis* and *Y. enterocolitica* is acquired by direct or indirect contact with domestic animals, wild animals, birds or consumption of food and water contaminated with the bacteria.

TRANSMISSION

Yersiniosis is principally transmitted by the faeco-oral route by ingestion of contaminated food and water, at pasture or watering places. Transmission of *Y. enterocolitica* appears to be similar to that of *Y. pseudotuberculosis.* The creation of new interfaces between livestock and wildlife is the most important factor in disease transmission[24]. It is accepted that contaminated soil, green crops, feeds and water sources are important sources of infection for farm and wild animals, rodents and birds. The role of insect vectors remains unclear. Although the rat flea (*Xenopsylla cheopis*) has been found to be a carrier of *Y. pseudotuberculosis,* its capability of transmitting the infection to a susceptible guinea pig was not proved[20]. Milk-borne spread is a possibility from yersinial mastitis. Venereal transmission is possible through semen derived from an infected reproductive tract. Transplacental spread to the fetus has been recorded in several species. Vertical transmission is a possibility in the turkey at least, as seen by the detection of infected eggs[25]. Experimental intranasal infection has been reproduced in guinea pigs and mice[26,27]. It has been suggested that insectivorous birds and mammals may acquire the disease from insects that have fed on the droppings of infected rodents and birds[28].

Although faecal shedding occurs as long as clinical signs persist (usually 2–3 weeks), prolonged sub-clinical

carriage has also been reported. The faeces of wild animals should therefore be considered a potentially important source of *Yersinia* spp.

PATHOGENESIS, PATHOLOGY AND IMMUNITY

Following ingestion, *Y. enterocolitica* and *Y. pseudotuberculosis* attach to the intestinal mucosa, and *Y. enterocolitica* produces an enterotoxin that induces diarrhoea. Both pathogens are able to cross the gut epithelium and proliferate locally in the underlying tissue. The bacteria selectively enter via the M (microfold) cells, and reach the intestinal lymphoid aggregates, the Peyer's patches[29]. The chromosomally encoded Inv protein enables the organism to invade Peyer's patch M cells. Entry into the Peyer's patches leads to an enormous recruitment of phagocytic polymorphonuclear leucocytes, with formation of microabcesses comprising extracellular *Yersinia*, appearance of dead apoptotic cells and, eventually, complete destruction of the cytoarchitecture of the Peyer's patches[30]. Monocytes infiltrate the Peyer's patches and mature into inflammatory macrophages to produce cytokines such as interleukin-12, gamma interferon (IFN-γ) and tumour necrosis factor alpha (TNF-α), which aid in development of the immune response[31]. Professional phagocytes significantly restrict the rate at which *Yersinia* multiplies in the host tissues, thereby allowing the host to develop a specific protective immunity. However, *Yersinia* can respond by impairing phagocytosis, inhibiting killing by phagocytes, triggering apoptosis and suppressing the normal release of TNF-α and other cytokines. Once established in the Peyer's patches, the bacteria can disseminate to the mesenteric lymph nodes and eventually to the liver, spleen and lung[32].

In the host, cytosol effector Yop inhibit several innate immune mechanisms, like phagocytosis, synthesis of proinflammatory signalling molecules and activation of the adaptive immune system[33]. Yop also play a role in inducing apoptosis[34]. CD4 and CD8 T cells play an essential role in the adaptive immune response directed at *Yersinia* infection[35].

There is an increasing amount of evidence suggesting that the O antigen of lipopolysaccharide (LPS) is required for virulence in pathogenic *Yersinia*, including colonization of host tissues, resistance to complement-mediated killing and resistance to innate immunity[36]. A case of coinfection was found in a dead mouflon (*Ovis musimon*) in which *Y. pseudotuberculosis* O:3 was isolated from the heart and *Y. enterocolitica* O:5 from the faeces[13]. Mixed infection is also reported in brown hares from Northern Germany[17]. *Yersinia enterocolitica* was isolated mainly from tonsils and tongue, but *Y. pseudotuberculosis* from lung, spleen, heart and kidney of rabbit, wild boar (*Sus scrofa scrofa*), Asiatic jackal (*Canis aureus*), red fox (*Vulpes vulpes*), European river otter (*Lutra lutra*), beech marten (*Martes foina*), polecat (*Mustela putorius*) and wild cat (*Felis silvestris*).

Gross pathologic changes vary among different host species. Infection by *Y. pseudotuberculosis* and *Y. enterocolitica* causes lesions primarily in the gastrointestinal tract and associated lymphoid tissues mostly in liver, spleen, mesenterial lymph nodes and peritoneum, but may occur in the lungs, kidneys and other organs. Intestinal congestion, acute fibrino-necrotizing (fulminant yersiniosis) and granulomatous enteritis and mesenterial lymphadenitis with enlargement and congestion of mesenteric lymph nodes, ulcers or nodular lesions of the duodenum, splenomegaly, are common findings. Following dissemination numerous granulomatous nodules, small necrotic foci and focal caseous abscesses are found in the liver, spleen, lung, mammary glands and other organs.

Histopathologically there are a large number of intralesional Gram-negative coccobacilli, micro-abscessation or diffuse suppurative or pyogranulomatous inflammation of the intestinal mucosa. Multifocal suppurative mesenterial lymphadenitis and hepatitis are detected too. As an incidental finding or in animals with *Yersinia* infection in other organs, caseous mesenteric lymphadenitis with pyogranulomas containing microcolonies of *Yersinia* surrounded by neutrophils and giant cells may be detected. Lesions may also be observed in other organs. Yersinia infection may cause sporadic pneumonia and septicaemia with lesions in various organs.

Experimental infection of ground squirrels (*Citellus cytellus*) was followed by hyperplasia of the peribronchial lymph tissue and defined catarrhal pneumonia. Small nonreactive necrotic foci were found in the spleen[37]. Splenitis, lymphadenitis and necrotic foci were seen in the spleen and liver. In experimentally orally infected pigs, purulent meningoencephalitis with leucocytic infiltration of the white brain substance and accumulation of lymphoid cells around the blood vessels were established[38]. Miliary

necrotic foci around the lympho-reticular tissue of tonsils, catarrhal pneumonia and lymphoid cell proliferation were well demonstrated in the Peyer's patches.

CLINICAL SIGNS AND TREATMENT

Both *Y. pseudotuberculosis* and *Y. enterocolitica* infections may provoke the same clinical picture[16,39]. The most common clinical manifestations of yersiniosis are enterocolitis, fever, diarrhoea, abdominal pain, nausea, vomiting (in humans), mesenteric adenitis and/or terminal ileitis, and leucocytosis. Acute yersiniosis takes the form of a fulminating septicaemia involving visceral organs (e.g. liver and spleen), enteritis, and death within 1 to 3 days. In the subacute and chronic forms, the disease is characterized by loss of weight over a period of days or weeks due to necrotizing or ulcerative enteritis, increasing listlessness, anorexia, severe diarrhoea, respiratory distress, muscular weakness and incoordination[40].

In wild birds the disease is usually fatal and often takes a peracute course with catarrhal or nodular lesions of the duodenum. The acute and subacute forms of the disease are characterized by diarrhoea and lameness or stiffness of gait.

Interstitial pneumonia and myocarditis in farmed deer infected with *Y. pseudotuberculosis*, as well as abortions and epididymo-orchitis in sheep and cows have been observed too[4,41]. Latent infections of wild animals are also possible[13].

Yersiniosis is often self-limiting. Antibiotics may shorten the duration and severity of clinical signs when administered orally or parenterally – for example, aminoglicosides, doxycycline, potentiated sulphonamides or seftriaxone.

DIAGNOSIS

Laboratory diagnosis of infection, with or without clinical signs, includes isolation of *Y. enterocolitica* or *Y. pseudotuberculosis* from faeces, throat swabs, mesenterial lymph nodes, peritoneal fluid or blood. Faecal cultures are generally positive during the first two weeks of illness. Positive *Yersinia* serology by agglutination test or enzyme-linked immunosorbent assay (ELISA) are of diagnostic support, as there can be difficulties in bacteriological isolation of *Yersinia*. Polymerase chain reaction (PCR)-positive identification of several key genes (*ail*, *inv*, *virF*, *yopB* and *yopH*) indicate *Yersinia* infection. A western blot kit is commercially available – *recom*Blot *Yersinia* IgG/IgA (Microgen, Martinsried, Germany). The kit is based on five recombinant *Yersinia* outer proteins, which are specific for the serodiagnosis of yersiniosis. By using the more specific protein-based western blot strips, cross-reactivity with tularaemia and brucellosis can be excluded. The kit has been successfully applied for detection of anti-*Yersinia* antibodies in a study of the prevalence of yersiniosis in wild boars from north-eastern Germany[19].

MANAGEMENT, CONTROL AND REGULATIONS

Surveillance for yersiniosis is strictly regulated for domestic animals in EU countries, where National Food Agencies monitor this food-borne disease. In addition, investigations of case reports and outbreaks of yersiniosis are conducted to control them and to learn more about how to prevent this infection. Council Directive 92/117/EC, also referred to as the Zoonoses Directive, introduces and outlines the requirements for the surveillance, monitoring and reporting of certain zoonoses on a national and EU level, e.g. yersiniosis.

No specific and effective preventive measures are known. Normal hygienic measures, regular disinfection and effective rodent control programmes in animal farms, slaughterhouses, zoological collections, etc. help to prevent transmission and diminish the risk of incidence of yersiniosis. Additional protection may be provided by prevention of access to food supplies for humans and animals by rodents, other small wild mammals and birds.

Infection may be introduced into hitherto yersiniosis-free areas and countries by the importation of animals – for example, for re-stocking hunting reserves or for breeding purposes[3].

PUBLIC HEALTH CONCERN

As a zoonosis, yersiniosis is of concern to the economy and public health. The highest rates are reported in cooler climates – Northern Europe and Scandinavia, where *Y. enterocolitica* is the third most important food-borne pathogen. The infection is more frequent and severe in children. Approximately 65% of *Y. enterocolitica* infections

occur in infants and young children, and the 5–20 age group accounts for 75% of *Y. pseudotuberculosis* infections. In a small proportion of cases, complications such as skin rash, joint pains or septicaemia can occur. Risk factors for human infections are related to local disease ecology, including animal reservoirs. Wildlife can be the source of human disease transmitted directly or indirectly via domestic animals. Ingestion of contaminated food and water, contact with wild and domestic animals, including household pets, are potential routes of exposure and infection. Pigs are the main sub-clinical reservoir for pathogenic *Yersinia* strains, being isolated predominantly from the tonsils[42]. Consumption of raw pork and pork products including cold cuts presents an increased risk of infection by *Y. enterocolitica* because of its ability to multiply under refrigeration and vacuum packaging. The health risks associated with the consumption of game meat are unknown.

SIGNIFICANCE AND IMPLICATIONS FOR ANIMAL HEALTH

Yersiniosis is a relatively uncommon disease that generally affects the intestinal tract, where the inflammation may be self-limiting. In some animals there is a more protracted course with extra-intestinal infection, which may spread to other tissues. This dissemination of infection is usually fatal and may develop in elderly, stressed and immunocompromised animals. The significance and implications of yersiniosis for animal health are likely to grow with increased densities and range expansion of susceptible wild species. The disease prevalence in wildlife is also likely to increase where there is an increased risk of contact with livestock. The impact of *yersiniosis* on wildlife is not clearly known; however, this disease is a matter of concern in domestic livestock, semi-domestic herds, farmed cervids and as a food-borne infection in humans.

BUBONIC PLAGUE

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Bubonic plague, or sylvatic plague, is an acute, highly virulent zoonotic disease. Three classic manifestations have been described – bubonic, pneumonic and septicaemia plague, which are primarily seen in susceptible non-rodent species and humans[43].

AETIOLOGY

Yersinia pestis, the causative agent of plague, is a small (0.5–0.8 × 1–3 μm), Gram-negative, non-spore-forming, facultative anaerobic, facultative intracellular, pleomorphic rod. Like other *Enterobacteriaceae*, *Y. pestis* is oxidase-negative, capable of fermenting glucose, and usually nitrate reductase-positive. Multilocus sequence typing of housekeeping genes has revealed that *Y. pestis* resembles a clone derived from the enteric pathogen *Y. pseudotuberculosis*[44]. Among 11 species of *Yersinia*, four are considered primary pathogens, but *Y. pestis* is the most important agent in wild mammals[43].

EPIDEMIOLOGY

In Europe, *Y. pestis* occurred for a long period in the past, but is now notably absent from Western and Central Europe (as well as Canada and parts of North and South America). Natural foci of plague exist in the southern and south-eastern regions of the former Soviet Union, i.e. Armenia, Azerbaijan, Georgia, Kazakhstan, Kirghizia, Russia, Tadjikistan, Turkmenistan and Uzbekistan[45]. In Mongolia, highly active plague foci have been described from the western parts of the country[46]. For more recent information about the occurrence of plague in animals worldwide, see the World Organisation for Animal Health (OIE) World Animal Health Information Database (WAHID) Interface[47].

Plague is fundamentally a disease of rats and other wild rodents. Humans, domestic animals and other wildlife are considered accidental hosts. Sylvatic plague occurs in wild rodents other than rats. At least 38 species of wild rodents, including marmots and squirrels, have been found to be susceptible; however, the organism has been found in association with more than 200 species of wild rodents throughout the world, except Australia[3]. Of these rodent species, only 30–40 serve as permanent reservoirs of plague, with the remainder being temporary or epizootic hosts. Mammalian hosts can be divided into different epidemiologically important categories based on susceptibility to *Y. pestis*:

1. enzootic or maintenance, reservoir rodent hosts (e.g. *Microtus* spp., *Spermophilus* spp., *Onychomys* spp.)
2. epizootic or amplification rodent hosts (e.g. *Cynomys* spp.)
3. resistant non-rodent hosts (e.g. ungulates, rodent-consuming carnivores)
4. susceptible non-rodent hosts (e.g. primates including humans, *Felis* spp., *Mustela nigripes*)[48].

Certain features have been identified that are characteristic for the different mammalian host categories: e.g. a high versus a low resistance to plague morbidity and mortality in enzootic versus epizootic hosts, a high reproductive potential and multiple litters in enzootic hosts and, as the death rate is high, a large population density in epizootic hosts. Birds, lagomorphs, carnivores and primates may play an occasional role in disseminating infection[43]. Siberian marmot (*Marmota sibirica*), Pallas's pika (*Ochotona pallasi*), Brandt's vole (*Lasiopodomys brandti*) and souslik (*Spermophilus* spp.) are the main hosts for plague in Mongolia, but *Y. pestis* has also been detected in Daurian pika (*Ochotona daurica*), grey marmot (*Marmota baibacina*), long-tailed marmot (*M. caudata*), vole (*Alticola* spp., *Microtus* spp.), gerbil (*Meriones* spp., *Rhombomys* spp.), Siberian five-toed jerboa (*Allactaga sibirica*), Russian hamster (*Onychomys leucogaster*), Siberian polecat (*Mustela eversmanni*), mountain weasel (*M. altaica*) and the Northern wheateater (*Oenante oenante*)[45,46]. A correlation between the feeding behaviour of carnivores and omnivores (i.e. prey species and their susceptibility to *Y. pestis*) and their exposure to plague has been observed. Predators feeding on enzootic and epizootic hosts probably contract plague.

Yersinia pestis requires an animal host for its long-term survival. The bacterium is transmitted by fleas, which become infected during their blood meal on infected rodents. *Yersinia* ingested by fleas multiplies in the midgut. When the flea bites, aspirated blood containing *Y. pestis* is regurgitated from the flea into the bite wound. More than 1,500 flea species have been described as potential vectors. Host specificity and host range of fleas, geographic distribution, flea activity throughout the year, and the flea life cycle (nesting versus body flea) account for flea vector competence. Seeking for substitute host species, if the primary host is not available ('straggling'), could contribute to amplification of infection during plague epizootics. The vector efficacy is influenced by the degree of bacteraemia and the level of antibodies in the host at the time of the blood meal. In addition, transmission of *Y. pestis* by fleas is affected by host animal behaviour, social structure (group of animals versus the solitary species) and feeding habits. Lagomorphs and insectivores sharing habitats with sylvatic rodents may enhance their exposure to plague-transmitting fleas. Enzootic plague foci are maintained by certain rodent–flea associations[43].

PATHOGENESIS, PATHOLOGY AND IMMUNITY

Temperature and iron acquisition are important for *Y. pestis* virulence. Depending on the host and cell type infected, other stimuli may also induce the expression of virulence factors. Growing at low temperature inside the flea host results in little or no expression of the F_1 capsular antigen, one of several components used by *Y. pestis* for evading phagocytosis by neutrophils and macrophages. Other virulence factors include the plasminogen activator (Pla), which is required for dissemination from the inoculation site to the surrounding lymph node, and a type III secretion apparatus, which injects virulence proteins into the cytosol of target cells.

The principal route of transmission in mammals is via flea bites. Less commonly seen is ingestion of, or exposure to, another mammal infected with *Y. pestis*. Inhalation of aerosolized bacteria has also been described. Close contact to recently deceased infected animals such as during skinning or *post mortem* investigation has been shown to cause infection and death in humans[49]. Transmission of the infection by the latter routes differs significantly from flea-borne infection: the bacterial inoculate is much greater and *Y. pestis* growing in the mammalian host expresses virulence factors that assist in evading the host's immune response. These *Yersinia* are more virulent than flea-borne *Y. pestis*[43].

The lesions seen in *Y. pestis*-infected mammals vary greatly according to the mode of transmission and susceptibility of the host. Highly susceptible rodents often die so quickly that the lesions may be subtle. Most animals develop a bubonic form of the disease with varying degrees of internal organ involvement and bacteraemia. After an infected flea bite, susceptible animals form an almost immediate red areola around the bite wound, followed by a papule within 2 to 3 days. *Yersinia pestis* spreads from the wound to the regional lymph nodes, causing the formation of a bubo (swollen lymph node). Oral inoculation can also lead to buboes in the submandibular, cervical,

retropharyngeal and mesenteric lymph nodes. The infection can be contained at this level (bubonic plague), or the bacteria multiply greatly and spread, via the bloodstream, to the liver, spleen and other organs, including other lymph nodes, where secondary buboes may develop (septicaemic plague). Septicaemia is frequently followed by death approximately 2 weeks after infection, as a result of disseminated intravascular coagulation, endotoxic shock and widespread haemorrhages and thrombi formation. Pneumonic plague is characterized by severe alveolar oedema and vascular haemorrhage, in addition to fulminant necrotizing pneumonia caused by massive bacterial replication and inflammation.

Histologically, necrosuppurative inflammation associated with very large numbers of *Y. pestis* bacilli is seen – a central area of bacteria, haemorrhage and necrotic debris is surrounded by dense neutrophilic infiltration in affected lymph nodes, liver, spleen and pneumonic lesions. It is unclear what determines resistance or susceptibility to *Y. pestis* infection in rodents and other animals, and current understanding of immunity to *Y. pestis* is based largely on studies involving *Y. enterolitica* and *Y. pseudotuberculosis.* Both cellular and humoral responses are evoked, but antibodies are not always protective against infection.

CLINICAL SIGNS AND TREATMENT

Clinical signs vary depending on the susceptibility of the host, and in highly susceptible species such as many rodents, rapid death may be the only sign. Carnivores are generally resistant to plague, and ingestion of infected rodents causes inapparent to mild disease and seroconversion. However, orally infected domestic cats become acutely ill with a high fever, depression and anorexia. They develop buboes, septicaemia and pneumonic plague, and approximately one-third of cats die within 10 days of infection – similar susceptibility is suspected in wild felines but has not been proven. Although plague is relatively rare in wild ungulates, mule deer (*Odocoileus hemionus*) with systemic plague revealed bronchopneumonia and lymphadenitis[50] and ocular plague has been described in a black-tailed deer (*Odocoileus hemionus columbianus*) in the USA[51]. The black-tailed deer was blind and, histologically, had moderate to severe necrotizing and fibrinopurulent endophthalmitis, with varying degrees of keratoconjunctivitis with abundant intralesional coccobacilli[51]. Numerous firm, white foci within the lungs and on pleural surfaces, and necrotic areas within enlarged lymph nodes, were noted in mule deer[50]. Microscopically, the lung lesions constituted of necrotic foci containing large, pale basophilic bacterial colonies surrounded by neutrophils. Extensive necrosis was also found in the medulla of mandibular and pharyngeal lymph nodes. In addition, a severe degenerative myopathy was described[50].

Treatment of wild animals with plague is generally not possible or practical. However, supportive care and antibiotic therapy with doxycycline, tetracycline or potentiated sulphonamides can be effective in uncomplicated cases.

DIAGNOSIS

Diagnosis of plague is restricted to reference laboratories, because *Y. pestis* is a highly virulent and zoonotic agent that must be processed under biosafety level 3 conditions. Attending staff should use standard barrier precautions, including gloves, FFP3 masks and gowns while examining and treating suspect animals. Surgical masks may not provide protection from respiratory droplet exposure via inhalation, and a well-fitted FFP3 mask is recommended. Gross and microscopic lesions may be suggestive of plague but culture of *Y. pestis* is necessary for definitive diagnosis. Fluorescence antibody tests and detection of *Y. pestis* DNA by PCR are also frequently used.

PUBLIC HEALTH CONCERN

Plague is a zoonotic disease. People working with wildlife and in veterinary practice as well as hunters in plague-endemic areas should be aware of the signs and symptoms suggestive of plague and the risks associated with handling possibly plague-infected animals. Appropriate hygiene is recommended in order to minimize the risk of disease transmission to humans. Treating suspect animals and *post mortem* investigations should be carried out using appropriate personal protective equipment (gloves, FFP3 masks, gowns) and in biosecure cabinets.

SIGNIFICANCE AND IMPLICATIONS FOR ANIMAL HEALTH

The effect of plague on wild animal populations depends on the susceptibility of the host species. In reintroduced Canada lynx (*Lynx canadensis*), six of 52 fatal casualties

died from pneumonic plague. The disease appeared as a significant mortality factor that warrants further investigation to determine its potential for impact on the recovery of lynx in their habitat range[52]. Epizootic plague affects prairie dogs (*Cynomys* spp.) and black-footed ferrets (*Mustela nigripes*) and is a major factor limiting recovery of the highly endangered black-footed ferret in the USA. Flea control as well as vaccination studies have been initiated in ferrets[53].

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CHAPTER

22

TULARAEMIA

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Tularaemia is a highly infectious septicaemic disease of rodents, lagomorphs, other wildlife species, farm animals, and sometimes humans. Historically it was known as plague-like disease of rodents, *harpest* (Sweden), *leemands soet* (lemming fever – Norway), *sibiriskaia iazva* (Siberian ulcer – Russia), deerfly fever (USA) and *yato-byo* (hare disease – Japan).

AETIOLOGY

Francisella tularensis, the aetiological agent of tularaemia, was first isolated and characterized in 1912 during an outbreak of a 'plague-like' disease in ground squirrels in Tulare County, California. Tularaemia is a zoonotic disease, and *F. tularensis* is on the list of Class A biothreat agents, as a potential agent of biological warfare.

The *Francisella* genus is the sole member of the *Francisellaceae* family. This genus comprises five species; *F. hispaniensis, F. noatunensis, F. piscicida, F. philomiragia* and *F. tularensis*. *F. tularensis* is small (<1 μm), obligate aerobe, Gram-negative, non-motile, pleomorphic coccobacillus, covered by a carbohydrate-rich capsule. It is oxidase-negative and weakly catalase-positive, and cysteine is required for its growth. Four subspecies of *F. tularensis* are recognized: the highly virulent *F. tularensis* subsp. *tularensis* (Type A), the moderately virulent *F. tularensis* subsp. *holarctica* (Type B) and *F. tularensis* subsp. *mediasiatica* and the low virulent *F. tularensis* subsp. *novicida*. Utilization of glycerol is an effective tool to differentiate *F. tularensis* subsp. *tularensis* (glycerol-positive) and *F. tularensis* subsp. *holarctica* (glycerol-negative).

EPIDEMIOLOGY

GEOGRAPHICAL DISTRIBUTION AND HOSTS

Tularaemia in Europe is caused by *F. tularensis* subsp. *holarctica*. This subspecies is found throughout the northern hemisphere and occurs throughout much of Europe, except the UK, Ireland and Iceland. Endemic Type B tularaemia has occurred in focal areas of Sweden, Finland and the former Soviet Union since early in the 20th century. Today, and in particular during the last decade, its geographical range with reports in animals and/or humans has extended to include Norway, Germany, Turkey, Kosovo, France, Spain, Hungary and Switzerland[1]. Tularaemia is today considered an emerging infection in several areas – for example in Spain, where it was probably introduced by translocation of infected hares. *Francisella tularensis* subsp. *tularensis* has been found almost exclusively in North America. The distribution of *F. tularensis* subsp. *mediasiatica* is restricted to Central Asia. *Francisella tularensis* subsp. *novicida* have been linked to waterborne transmission in Australia and the USA.

Infectious Diseases of Wild Mammals and Birds in Europe, First Edition. Edited by Dolores Gavier-Widén, J. Paul Duff, and Anna Meredith.

Francisella tularensis has a remarkably broad host range – probably the broadest of all zoonotic agents. Natural infections with *F. tularensis* have been reported in a range of vertebrates, including mammals, birds, amphibians and fish, and in certain invertebrates. Despite the broad host range, tularaemia is primarily a disease of the genera *Lagomorpha* and *Rodentia*. The European brown hare (*Lepus europaeus*) is a common host of tularaemia in Central Europe, where it is also an important game species, so causing a public health problem. Tularaemia occurs frequently in mountain hares (*Lepus timidus*) in Scandinavia and Russia; in some of these regions, both species of hare are affected. The European wild rabbit (*Oryctolagus cuniculus*) and the domestic rabbit are both relatively resistant to tularaemia. Rodents are of great importance for maintaining enzootic foci for tularaemia in Eurasia. Voles (*Microtus* spp., *Arvicola amphibius, Myodes glareolus*) are most frequently involved in tularaemia epizootics, but infection in other rodent species (*Ondatra zibethicus*, *Cricetus cricetus*, *Castor fiber*, *Lemmus* spp., *Rattus rattus, Mus musculus, Apodemus flavicollis, Tamias sibiricus, Sciurus vulgaris,* etc.) has been detected[2].

ENVIRONMENTAL FACTORS

The ecology and epidemiology of tularaemia is complex. *Francisella tularensis* subsp. *holartica* is often associated with aquatic ecosystems (rivers, lakes, ponds, etc.) and their hosts, such as water voles. It may persist silently in the environment, including in water and sediment[3] and cause cyclic outbreaks of mortality when conditions are favourable. Outbreaks of tularaemia in humans often parallel outbreaks in wild animals. One of the factors related to the triggering of outbreaks is the relative abundance of wildlife hosts.

The environmental conditions that favour outbreaks in mammals and humans are poorly understood. One study performed in the USA suggested that there is a subtle shift in geographical distribution of tularaemia with climate change. A warmer climate in the period 1965–2003 was associated with a northward movement of tularaemia[4]. Illustrating the complexity of tularaemia ecology, the geographical trend of tularaemia reports in Europe are less consistent with a northward movement.

Haematophagous arthropods have substantial roles in both maintenance of *F. tularensis* in nature and disease transmission. Ticks are believed to be the most important arthropods in the ecology of tularaemia. They are both mechanical and biological vectors, the latter by amplifying the number of bacteria contributing to re-transmission and by maintaining the bacterium throughout its multiple life stages. Ticks are true reservoirs that may perpetuate specific enzootic foci during inter-epizootic periods. The tick *Dermacentor reticulatus* plays an important role in maintenance and transmission of *F. tularensis* among small and medium-sized mammals in Central Europe. Other ticks – *Ixodes ricinus*, *I. persulcatus*, *D. marginatus*, *Rhipicephalus rossicus* and *Haemaphysalis concinna* – have also been found to be naturally infected with *F. tularensis*[5]. Other blood-sucking arthropods transport tularaemia mechanically. Mosquitoes belonging to the genera *Aedes*, *Culex* and *Anopheles* are historic vectors in the northern boreal forest of Scandinavia and Russia. Horse flies (Tabanidae) have also been observed to serve as a route of infection in the former Soviet Union. *Francisella tularensis* has been frequently isolated from haemotophagic gamasid mites (Gamasidae) collected from rodents. Fleas (*Siphonaptera*) are considered of minor importance for transmission and maintenance of *F. tularensis*[5].

EPIDEMIOLOGICAL ROLE OF THE AFFECTED SPECIES

The European brown hare is moderately sensitive to *F. tularensis* infection and can possibly maintain tularaemia for a longer time than the mountain hare, serving as a reservoir[6]. Infection in mountain hares is often fatal. The water vole (*Arvicola amphibius*) and the common vole (*Microtus arvalis*) in addition to being highly susceptible to tularaemia, may also become chronically infected, thereby serving as disease reservoirs during periods between epizootics. Voles are hosts for immature stages of several important tick species as well. Mouse species (*Mus musculus*, *Apodemus* spp.), because of their high susceptibility and sensitivity to tularaemia, are probably not important reservoir hosts[7], but their role in the epidemiology of tularaemia in Europe has not been studied. Carnivores and scavengers are not considered to have a major role in the maintenance of *F. tularensis* in nature; their high seroprevalence indicates former exposure and a probable ability to survive infection[7]. Birds are not considered to be important components in the ecology of tularaemia[7]. Potentially, their most significant roles are in the transport of infected arthropod vectors to new areas, as indicators of

tularaemia activity in their prey species, and by contamination of surface waters through body discharges. It was found that a species of protozoan (*Acanthamoeba castellanii*) might be an important aquatic-environment reservoir of *F. tularensis*. This organism can persist for a long time in watercourses, and at low temperatures in the terrestrial environment[5].

TRANSMISSION

There are two known cycles of tularaemia: the terrestrial cycle and the aquatic cycle. In the terrestrial cycle, hares and rodents are the most important mammalian hosts, whereas haematophagous arthropods play a role as vectors. Hares and rodents can contaminate the environment through their body discharges. Different routes of transmission have been described in hares in Europe. Aerogenous infections may be frequent in European brown hares[6], whereas the alimentary route in mountain hares in Scandinavia is important in winter[8]. Stress-related aggression could promote transmission, and cannibalism could be a route of transmission among voles, especially in populations at high density[7]. In the aquatic cycle, voles and possibly muskrats and beavers serve as the main host species, and could shed live bacteria into the environment. Carcasses of infected animals can further contaminate the water.

PATHOGENESIS, PATHOLOGY AND IMMUNITY

Francisella tularensis is a highly infectious pathogen. It can enter the body in several ways: via inoculation by haematophagous arthropods, through skin lesions, across ocular-mucous membranes, by inhalation of infected aerosols or by ingestion of contaminated meat (cannibalism) or water. Only a small inoculation dose (LD_{50}: <10 colony forming units (CFU)) of *F. tularensis* subsp. *tularensis* is required in order to cause fatal infection in mice, guinea pigs or rabbits, and a small dose is enough to initiate a severe, sometimes fatal, infection in humans. *Francisella tularensis* subsp. *holarctica* causes lethal infections in mice and guinea pigs, with a similarly small inoculation dose; however, a higher dose is needed to induce the disease in rabbits (LD_{50}: $>10^6$ CFU) or humans (LD_{50}: $>10^3$ CFU)[9].

After entering the body the bacteria multiply locally, causing ulceration and necrosis, and then invade the blood and lymph vessels and spread to the lymph nodes and organs, including liver, spleen, lung, kidney, the serosal membranes and bone marrow, causing multiple foci of coagulative necrosis. *Francisella tularensis* is a typical intracellular pathogen with a high predilection for growth in macrophages but can infect many other cell types, such as epithelial cells, hepatocytes, muscle cells and neutrophils.

Francisella tularensis septicaemia occurs as an end stage of the disease, when the bacteria invade the blood vessels without lesions indicative of a tissue response. This septicaemic form is seen in highly sensitive species and animals, which die within 2–10 days.

The pathology of tularaemia differs considerably between different animal species and gross lesions are not always observed. In Scandinavia, acute forms of tularaemia have been described in mountain hares[8], whereas in Central Europe infection of European brown hares apparently has a more chronic course[6]. In mountain hares, the most characteristic necropsy finding is the enlarged spleen. Multiple white foci of coagulative necrosis can be seen in the spleen, liver and bone marrow in some, but not all, acute cases. Haemorrhagic enteritis and typhlitis can be found as well, particularly during winter. The mucosa in the jejunum and caecum is congested and occasionally necrotic. The crypts and villi of the intestine may show focal necrosis. Histologically, the focal lesions are initially characterized by apoptosis, absence of inflammatory cell infiltrate and thrombosis of small vessels. In subacute cases the lesions are inflammatory and granulomatous, with necrosis and a proliferative reaction of macrophages and giant cells.

In subacute cases in European brown hares, numerous, randomly distributed, well-demarcated, greyish-white or yellowish-white foci, with a diameter of 0.1 to 1 cm, can be observed most frequently in the lungs (Figure 22.1), the pericardia (Figure 22.1) and the kidneys[6]. Foci are frequently observed on the serosal surfaces of the lungs and kidneys. These foci and those in the pericardium are raised, dome shaped or flattened, and their surfaces are dry and granular. Foci can sometimes be seen in the testicles, bone marrow and mammary glands. Gross lesions are usually not found in the spleen and liver. Histologically, the foci are randomly distributed and correspond to focal or coalescing granulomatous inflammation, which replaces the normal tissue structure. Macrophages are the dominant

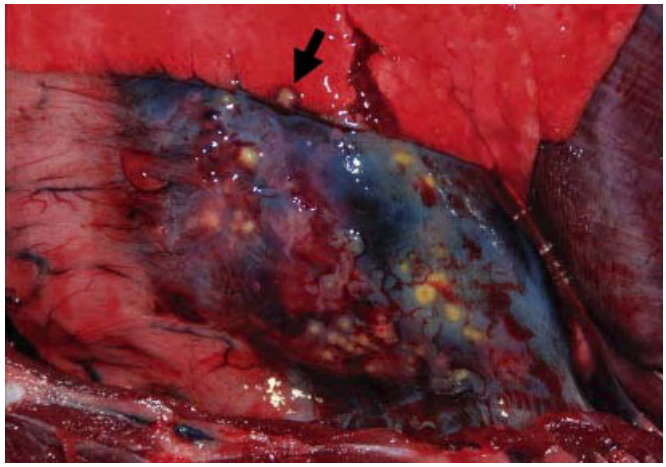

FIGURE 22.1 Pericardium and lung of a European brown hare. Note the numerous yellowish-white, flattened foci of different sizes on the pericardium and one greyish-white nodule surrounded by a dark, hyperaemic area in the lung lobe (arrow).

constituent cell type, but other cells – including lymphocytes, heterophil granulocytes, multinucleated giant cells and fibrocytes – are also found occasionally. Focal or multifocal necrosis is often observed in the centre of these lesions. Granulomatous inflammation can also be detected as microscopic lesions in the mediastinal lymph nodes, liver and spleen.

The pathology of tularaemia in rodents depends on the sensitivity of the species. The usual macroscopic finding is the enlarged spleen and, less frequently, the liver. Pinpoint white foci can be seen on these organs. Microscopically multifocal coagulation necrosis is characteristically found in the spleen, liver, lymph nodes, bone marrow and lungs. Karyolysis, pyknosis and the presence of inflammatory cells such as macrophages and heterophils are observed in less acute cases.

Little is known about the immune response of the host to *F. tularensis*. Cell-mediated immunity has long been believed to be critical for protection. The importance of humoral immunity is also now recognized. Synergy between antibodies, T-cell-derived cytokines and phagocytes appears to be critical to achieving immunity against *F. tularensis* and clearing infection. In humans, an antibody response is measurable by the second week post-infection. Antibody levels are highest during the second month after infection, and decline gradually thereafter.

CLINICAL SIGNS

Clinical cases of tularaemia are infrequently observed in free-ranging wildlife, as infected animals are usually found moribund or dead[7]. Non-specific signs include depression and pyrexia. Local inflammation or ulceration at the portal of entry and enlargement of the regional lymph nodes may be observed. Highly sensitive animals develop fatal septicaemia and may be non-responsive before death. In hares depression, stupor, loss of body weight and lack of fear, facilitating capture, are observed in the late stages of the disease. Relatively resistant wild species survive the infection and develop immunity.

DIAGNOSIS

Detailed description of laboratory diagnostics and protocols are found in the World Organisation for Animal Health (OIE) *Manual of Diagnostic Tests and Vaccines for Terrestrial Animals*[10] and the World Health Organization (WHO) *Guidelines on Tularaemia*[11]. Field examination of carcasses is not recommended when tularaemia is suspected, because of the real potential for human exposure and the risk of contaminating the environment. There is a high risk of infection of humans by direct contact with *F. tularensis*-infected tissues. Special precautions, including the wearing of gloves, masks and eyeshields are recommended when handling infective materials. Procedures should be performed within secure biosafety containment facilities (biosafety level 2 or 3). Diagnosis is based on the combined results of necropsy findings and the demonstration of *F. tularensis* from the samples or tissues.

F. tularensis appear as numerous small, Gram-negative bacteria in impression smears or in histological sections of spleen, liver, lung, kidney, bone marrow and lymph node as well as in blood smears. *Francisella tularensis* can be demonstrated specifically by direct or indirect fluorescent antibody tests. Immunohistochemical assay is a very useful and sensitive method for the detection of *F. tularensis* in domestic and wild animals[6]. Abundant bacterial antigen can be observed, often extracellularly, in foci of necrosis. Intracellular *F. tularensis* is found in macrophages and giant cells and less frequently in other cell types.

Francisella tularensis can be identified by culture. The preferred samples for culture are, in acute cases, heart blood, spleen, liver or bone marrow and in subacute/

chronic cases, the granulomatous lesions. Owing to the highly fastidious culture requirements of *F. tularensis,* isolation can be difficult, as it grows poorly on ordinary culture media. Francis medium, McCoy and Chapin medium or modified Thayer-Martin agar are recommended. The colonies are small, round and do not appear within the first 48 hours of incubation at 37°C. Isolation of *F. tularensis* from carcasses may be difficult because of overgrowth of other bacteria. Penicillin, polymyxin B and cycloheximide can be added to prepare selective culture media. If it is difficult to isolate *F. tularensis* on primary culture, it may be isolated following inoculation of tissue suspension from suspect cases into laboratory animals, such as mice or guinea pigs.

A variety of polymerase chain reaction (PCR) methods have been described for the detection of *F. tularensis* DNA in both clinical and environmental specimens. The gel-based PCR assays target the genes encoding the outer membrane proteins, *fopA* or *tul4*, show good specificity and allow for the rapid detection of *F. tularensis* in specimens. However, *Francisella*-like endosymbionts of ticks (bacteria closely related to *F. tularensis* found in ticks) can produce non-specific positive results in these assays. Real-time PCR methods show no evidence of cross-reactivity with non-*F. tularensis* bacteria (environmental bacteria and vector-borne organisms) and the detection limit of very low numbers of organisms increases the likelihood of detecting *F. tularensis* in environmental samples in which the number of organisms is low[11]. Molecular methods are also applied to cultured *F. tularensis* to provide resolution among the *Francisella* species, subspecies and within-subspecies strains[5].

Serology can be carried out for investigating exposure to *F. tularensis* in species that are relatively resistant to the disease, such as sheep, cattle, pigs, moose (*Alces alces*), dogs, cats or birds[10]. A slide agglutination test, using one drop of stained bacteria and one drop of whole blood, is a widely used field method for screening the European brown hare populations in Central Europe. The standard serological test is the tube agglutination. Commercially available antigen for slide and tube agglutination tests is available (Bioveta Inc., Ivanovice na Hané, Czech Republic). Possible cross-reaction with *Brucella abortus*, *B. melitensis*, *B. suis*, *Legionella* spp. and *Yersinia* spp. may occur. The enzyme-linked immunosorbent assay (ELISA) allows an early diagnosis of tularaemia[10], and a commercial kit is also available (Serion Immundiagnostica GmbH, Würzburg, Germany).

MANAGEMENT, CONTROL AND REGULATIONS

Francisella tularensis has an extremely broad host range and very complex ecological transmission cycles; therefore it is difficult to control. Monitoring and surveillance of wildlife, arthropod vectors and surface water for tularaemia activity in enzootic areas provides useful information for wildlife managers and public health authorities. Monitoring focuses on only a small number of primary species, despite the broad host range. At present (in 2011), there are no EU regulations specifically for reporting tularaemia, although it is a notifiable disease on the World Animal Health Information Database. A country is considered free from tularaemia when the disease has not been present for at least 2 years and when bacteriological or serological surveys (both hosts and vectors) in previously infected zones have given negative results[12]. Translocating hares can introduce tularaemia into new areas. Screening of hares before they are exported is necessary, but the regulations about this vary and are usually based only on bilateral agreements between countries. The quarantine of individual hares for 15 days and their screening with the slide agglutination test at the beginning and at the end of the quarantine period, at least, should detect most infected animals with a view to preventing the introduction of tularaemia into new areas. Treatment of the hares against blood-sucking parasites (ticks) during the quarantine period is a further measure to prevent the transport and introduction of infection. The lack of any licensed vaccine and the very broad host range and complex disease transmission routes of *F. tularensis* renders vaccination in wildlife as a theoretical management method only.

PUBLIC HEALTH CONCERN

Humans are highly susceptible to *F. tularensis*. The virulence of the strain, dose and route of exposure are all important factors influencing the clinical form and severity of the disease in humans. People can be infected via several routes, such as: bites from infected arthropods; handling of infectious animal tissues or fluids; wounds or small cuts; direct contact with, or ingestion of, contaminated water, food or soil; through the conjunctiva; and inhalation of infective aerosols. Laboratory-work poses a significant risk of contracting tularaemia, for example by

aerosol exposure. Human infection often occurs during hunting, trapping or skinning infected wildlife. Hay, grain and water supplies contaminated by rodents have been the source for numerous human cases. Drinking water contaminated with *F. tularensis* is also an important source of human infection. Recent outbreaks of oropharyngeal tularaemia in humans in Norway have been attributed to contamination of water wells after snow thaw with dead rodents or their excreta[13].

Outdoor activities expose people to infected animals, bites by infected arthropods or contact with contaminated surface waters. The most frequent presentation of clinical signs in humans are inflammation and later ulceration at the primary site of infection, with swelling of regional lymph nodes, which may become abscessed. Generally, the course of the clinical disease includes sudden onset of fever, generalized aches, inflammation of the upper respiratory tract with nasal discharge, vomiting, malaise and anorexia. Seven clinicopathological forms of tularaemia have been described in human medicine: ulceroglandular, glandular, oculoglandular, oropharyngeal, pneumonic, typhoidal and septicaemic[14].

Infection of humans by *F. tularensis* is treatable with antibiotics. People can minimize their potential exposure to *F. tularensis*. Publicizing epizootics and providing information on how to protect oneself are important. To prevent contact transmission, rubber gloves should be worn by trappers or hunters when skinning those species commonly associated with tularaemia. As for arthropod transmitted infection, the use of insect repellent, protective clothing and frequent body searches with prompt removal of ticks can greatly reduce the risk of infection. Meat from potentially infected animals should be well cooked. Untreated water from lakes and streams should not be consumed. Vaccination has generally not been widely applied, but vaccines have been used for high-risk situations, typically for laboratory researchers. Development of various vaccine candidates, including acellular subunit, killed whole cell and live attenuated, has taken place in recent years, but a licensed vaccine does not exist yet.

SIGNIFICANCE AND IMPLICATIONS FOR ANIMAL HEALTH

Francisella tularensis occurs naturally in certain ecosystems. It can cause large epidemics among hares and rodents. The role of tularaemia as a population-regulating factor for small rodents is often considered to be beneficial. Tularaemia rarely occurs among domestic animals or in zoological collections. Among domestic animals, sheep and cats are infected most frequently[7]. Outbreaks generally occur among sheep in spring, in the lambing season. The result of tularaemia infection of dogs and cats varies from an absence of clinical disease, to death. These pet animals can be involved in the transmission of tularaemia by bringing infected ticks into the household. Mechanical transfer of *F. tularensis* to people occurs from the contaminated mouths and claws of these pets that have recently fed on diseased rodents or hares. Zoo primates hunt and consume small prey species such as rodents that enter their enclosures and may contract tularaemia from their prey.

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CHAPTER

23

PASTEURELLA INFECTIONS

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INTRODUCTION

The term 'pasteurellosis' refers to infection with bacteria of the genera *Pasteurella* and *Mannheimia,* which occurs in animals and humans. It has been also called 'septicaemic pasteurellosis', 'haemorrhagic septicaemia', or in birds 'avian cholera', 'fowl cholera' or 'pasteurellosis of fowls or ducks'. The classification of these pathogens is under continuous review. Infection with these bacterial species is frequently subclinical, and the bacteria can also act as opportunistic pathogens. Some species and strains of *Pasteurella* and *Mannheimia* can also act as primary pathogens, and can occasionally cause epidemics of pneumonia or septicaemia, with significant associated mortality in domestic and, more occasionally, wild animal species. *Pasteurella* spp. and *Mannheimia* spp. are of worldwide distribution, and most vertebrate species are susceptible to disease from one of these bacteria, which infect most mammalian families and a wide range of non-mammalian species.

Pasteurella and *Mannheimia* are members of the order *Pasteurellales*, family *Pasteurellaceae.* They are small, pleomorphic Gram-negative rods or coccobacilli; they exhibit bipolar staining, are non-motile, facultatively anaerobic or micro-aerophilic, produce oxidase and/or alkaline phosphatase, ferment glucose without gas production and reduce nitrate to nitrite. These organisms are mucosal commensals of the oropharynx and gastrointestinal tract of healthy reptiles, birds and mammals and usually do not survive for a long time outside the host.

Pasteurella multocida is the most ubiquitous of these organisms and has been investigated since 1880 by Pasteur (after whom the name *Pasteurella* derives) as the causative agent of avian cholera or fowl cholera – despite the fact that, as the species name suggests (*multocida* – 'killer of many'), it can infect many species.

Factors that allow commensal strains to become virulent are incompletely understood, and a disease outbreak could be the result of 'activation' of a commensal strain of the organism or the introduction of a new *Pasteurella* sp. strain into a given host population.

PASTEURELLA INFECTIONS IN WILD MAMMALS

AETIOLOGY

Pasteurella multocida has been differentiated into three subspecies: *P. multocida multocida*, *P. multocida septica* and *P. multocida gallicida,* based on their ability to ferment D-sorbitol and trehalose. *Pasteurella multocida* is a normal

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commensal of the oral and pharyngeal bacterial flora and can be easily distinguished from the other *Pasteurellaceae* because it does not cause haemolysis on blood agar medium and does not grow on MacConkey agar. Pathogenic strains of *P. multocida* are encapsulated and the colonies are smooth and iridescent. The classification of *P. multocida* is based on five capsular serogroups (A, B, C, D, E) and 16 somatic serotypes (1–16). Although all capsular and somatic serotypes cause enteritis, pneumonia and/or septicaemia, serotypes B2 and E2 in addition cause haemorrhagic septicaemia.

Mannheimia haemolytica (formerly *Pasteurella haemolytica* biotype A) was established in 1999 and includes all trehalose-negative strains of the *P. haemolytica* complex that ferment mannitol, but not D-mannose. *Mannheimia haemolytica* is the most common and studied of the five species included in the genera. Although *M. haemolytica* ferments arabinose, strains that ferment trehalose are now classified as *Pasteurella trehalosi.* This last species includes isolates previously classified as *P. haemolytica* biotype T and serotypes 3, 4, 10 and 15, whereas isolates previously classified as *P. haemolytica* biotype A 1, 2, 5–9, 12–14, 16 and 17 are now classified as *M. haemolytica*.

EPIDEMIOLOGY

Pasteurella multocida serogroup A is mainly associated with avian cholera, rabbit pasteurellosis and pneumonia in ruminants. It is a common isolate from the respiratory tract of ruminants, where it can be found in the absence of lesions, but could also have the capacity to invade the hosts' mucosae as a secondary infection following pneumonia caused by viruses or other bacterial pathogens. In pneumonia outbreaks in wild bovids, *P. multocida* is frequently found in the lungs of animals with bronchopneumonia, especially in young animals (kids or yearlings); however, it is difficult to understand whether this is the result of secondary invasion or the primary cause of the disease. In rabbits and hares serotype A is known to cause a variety of syndromes, including purulent rhinitis, sinusitis, bronchopneumonia, conjunctivitis, otitis and skin abscesses. It is also the serotype frequently isolated from the oral mucosa of felines. The commensal nature of many strains is confirmed by the fact that in the domestic rabbit it has been shown that neonates become colonized during parturition or in first days of life by contact with infected does. Disease is triggered by environmental factors. Pasteurellosis is not uncommon among hares (*Lepus europaeus*) used for re-stocking hunting areas.

Pasteurella multocida serotype D is a commensal and may cause disease in stressed animals, whereas serogroups B and E cause haemorrhagic septicaemia. Endotoxaemia is probably the cause of haemorrhages and necrotic foci as well as of disseminated intravascular coagulation, all of which are frequently reported. Serogroup E is usually a non-commensal bacterium and is associated with acute and highly fatal clinical forms of haemorrhagic septicaemia that mainly affect species belonging to the *Bovidae* family.

Mannheimia haemolytica is one of the most important pathogens in domestic bovids, where it is the causative agent of pneumonic pasteurellosis – known as 'shipping fever' because of its occurrence in translocated animals. Field evidence suggests that viral and/or mycoplasmal agents predispose the host to pneumonia by impairing host defences, allowing a rapid proliferation of *M. haemolytica* in the nasopharynx. However, experimental infection of cattle with *M. haemolytica* alone has reproduced the disease. Serotype A1 is the predominant strain associated with pneumonia in domestic Bovidae, accounting for approximately 60% of the isolates, followed by serotype 6 found in about 25% of pneumonia cases.

Pasteurella spp. and *Mannheimia* spp. are distributed worldwide. *Pasteurella multocida*, can cause severe disease, such as haemorrhagic septicaemia and pneumonia in ungulates (*Dama dama, Cervus elaphus, Odocoileus virginianus, Odocoileus hemionus, Ovis musimon, Rupicapra rupicapra, Capra ibex*, *Rangifer tarandus*), avian cholera in birds, atrophic rhinitis in pigs and wild boar (*Sus scrofa*), and pneumonia or septicaemia in foxes (*Vulpes vulpes*), mink (*Mustela vison*), coypu (*Myocastor coypus*), rabbits (*Oryctolagus curiculus*), hares (*Lepus europaeus*) and wild brown rats (*Rattus norvegicus*). *Pasteurella multocida* serotype A is frequently isolated from healthy or dead bats belonging to several bat species of the genera *Pipistrellus*, *Plecotus, Vespertilio, Myotis* and *Eptesicus*. It has also been reported to cause severe outbreaks in captive fallow deer (*Dama dama*) herds[1–4]. Haemorrhagic septicaemia, an acute form of *P. multocida* infection of wild and domestic animals, killed 273 fallow deer out of a population of 1,800 during 1992–1996 in a large deer park in Denmark[5].

Mannheimia haemolytica and *P. trehalosi* are commensals of the nasopharynx and are frequently isolated from Bovidae such as Spanish ibex (*Capra pyrenaica*)[6], chamois (*Rupicapra rupicapra*), Alpine ibex (*Capra ibex*) and moufflon (*Ovis musimon*), as well as from European brown

hares – where they cause pneumonia and septicaemia, with both single case reports or, more frequently, disease outbreaks (Ferroglio, unpublished data).

Mannheimia haemolytica is frequently isolated from small domesticated ruminants and also from wild ruminants. It is a cause of septicaemia and pneumonia, usually in young animals rather than in adults.

The commensal nature and the ubiquitous species distribution of *Pasteurella* spp. and *Mannheimia* spp. suggest that the pathogen is acquired during the early stages of life. Stressors, such as weather changes, poor condition and overcrowding, can trigger outbreaks, with high morbidity and mortality in wild animals.

In wild ruminant disease-free herds, the subclinical *P. multocida* carrier rate is usually very low (3–5%), even though after outbreaks up to 50% of animals harbour this bacteria in their nasopharyngeal or tonsillar mucosae. In a survey of healthy Spanish ibex (*Capra pyrenaica hispanica*), *M. haemolytica* was isolated from 11.5% of the ibex tested, while 8.1% and 3.4% of animals were positive for *P. multocida* biotype A and *P. trehalosi*, respectively[6]. So it is plausible that wild species can act as maintenance host for these bacteria.

Pasteurellaceae spread, directly or indirectly, to susceptible animals through saliva or nasal discharges from diseased animals. Contamination of food and water can occur and vectors – ticks and fleas – are probably capable of transmitting infection. Wound infection due to animal bites or scratches has been reported in humans, cats and dogs, as demonstrated by the fact that 54% of birds with bite wounds attributable to cats were infected with *P. multocida*. The presence of numerous *P. multocida* strains in bats suggests that interspecies transmission, following cat bites, is probably the most frequent source of infection in bats in Europe[4].

PATHOGENESIS, PATHOLOGY AND IMMUNITY

It is considered that disease is caused by the invasion by commensal *P. multocida* as a result of the decrease in the host immunity caused by stressor factors; however, aerosol or direct contact may give rise to transmission in addition to vectors, such as ticks and fleas. In *P. multocida* infection septicaemia occurs as a result of the bacterial penetration of the pharyngeal mucous membranes by pathogenic bacteria, and it has been demonstrated that serotype A virulence is correlated with the capacity to avoid phagocytosis.

Fallow deer that die of *P. multocida* infection show oedema in the pharynx, larynx and the tongue, with necrotic mucosae covered by mucoid or fibrinopurulent exudates. There are haemorrhages and oedema in mucosae and serosae, and haemorrhages are also present in lungs, lymph nodes, spleen and other organs. When pneumonia occurs, pulmonary lesions are similar to those observed in many domestic and wild ruminants and are represented by acute fibrinous pneumonia with pleuritis localized in the cranioventral part of the lung.

In European brown hares, *P. multocida* causes purulent or catarrhal conjunctivitis, pneumonia, peritonitis, endocarditis and enlargement of the spleen. In hares pneumonia can also be due to *M. haemolytica*, and usually gross lesions include purulent exudates in the trachea and bronchi, bronchiolitis and purulent pneumonia.

Mannheimia haemolytica inhabits the nasal cavity and the tonsillar crypts. Immunosuppression, caused for example by stress, results in the inability of the host to control the replication of *M. haemolytica.* After invading the lungs, *M. haemolytica* infects the lower respiratory tract and alveolar epithelia, resulting in rapid development of fibrinous pleuropneumonia, with deterioration of lung structure and function, by infiltration of neutrophils, fibrin and blood in bronchi, bronchioles and alveoli.

The virulence factors involved in infection include a leucotoxin that prevents phagocytosis and is lethal for leucocytes and platelets. The enzymes released by these lytic processes recruit further leucocytes by means of chemotaxis. A lipopolysaccharide (LPS) is also produced and stimulates the production of cytokines, oxygen radicals and proteases that damage the lung parenchyma. The LPS also acts to decrease cardiac output, thereby reducing blood circulation to the lungs. Other factors are released that act in concert to impair the innate defences of the lungs. Haemorrhage and thrombosis in the lungs may be due to platelet lysis, which is also caused by leucotoxin[7].

As is also the case for *Pasteurella trehalosi,* the main virulence factors are the leucotoxins, and all strains that exhibit β-haemolysis on sheep blood agar have the structural leucotoxin gene.

Mycoplasma ovipneumoniae, which produces dysfunction in the ciliary activity of the epithelial cells of the upper respiratory tract, may predispose bighorn sheep to *M. haemolytica*[8]. Development of immunity in wildlife is poorly understood, but animals that have recovered from pasteurellosis usually remain healthy during subsequent outbreaks[5].

CLINICAL SIGNS

Fallow deer infected with *P. multocida* show swelling of the head and the neck that can reach the shoulder and the sternum, with subcutaneous gelatinous oedema and scattered petechial haemorrhages. In outbreaks of septicaemic disease, affected animals have fever and depression and may have oedema of the head and neck and bleeding from orifices. Clinical signs of pneumonia are the same for both *M. haemolytica* and *P. trehalosi*, and affected animals show dyspnoea and coughing, and mucous exudate is frequently visible from nostrils. Animals may recover from this infection, but with reduced lung capacity.

Clinical signs of pasteurellosis in hares depend on the route of infection, and include oculonasal discharge with snuffling, conjunctivitis and pneumonia in the case of respiratory route infection, torticollis in the case of otitis, and vaginal discharge due to metritis. When subcutaneous abscessation occurs, often as a result of wound infection usually by bite wounds, it is common to see swelling in several sites.

DIAGNOSIS

Pasteurellosis is suspected from typical clinical signs and gross lesions, and is confirmed by isolation and identification of bacteria from representative lesions on blood agar or glucose agar plates supplemented with serum. Colonies after 24 hours' incubation and growth are 1–2 mm wide, smooth, greyish and variably β-haemolytic. When septicaemia is present, bacteria can also be easily recovered from cerebrospinal fluid. Species differentiation is based on phenotype, but recently molecular methods have allowed a better characterization of isolates, providing greater value in epidemiological studies[4]. Assessing the clinical significance of *Pasteurella* spp. or *Mannheimia* spp. isolates from diseased animals in combination with other pathogens presents difficulties, because it is not possible to trace lesions to a given pathogen. Also assessing the potential clinical significance of *Pasteurellaceae* when isolated from healthy animals is very difficult. Serology has limited value in clinical diagnosis; however, it has an important role in evaluating the immune status of animals and the epidemiology of the infection at the population level.

MANAGEMENT, CONTROL AND REGULATIONS

Only indirect measures can be adopted to control pasteurellosis in wildlife. Action to increase the nutritional status of ungulate herds (i.e. artificial feeding) has been suggested by some authors, but overcrowding at feeding places could be a major risk for transmission of these pathogens. Considering that in some cases, especially in mountain ungulates on extensive natural pasture shared with wild ungulates, domestic animals can also be the source of infection, reducing the contact and thereby mutual transmission between domestic and wild animals is probably the most effective management action.

PUBLIC HEALTH CONCERN

Wild mammal pasteurellae are potentially zoonotic; however, reports of human disease linked with wild mammal infections are rare. A young farmer in England died from *P. multocida* septicaemia thought to have come from a freak skin scratch he suffered from a wounded wild rabbit that he had shot. He had been in good health prior to the incident[9].

SIGNIFICANCE AND IMPLICATIONS FOR ANIMAL HEALTH

Pneumonia is one of the main health concerns in mountain ruminants, where pneumonia outbreaks are relatively frequent, with an important impact on the affected species at population level. *Pasteurellaceae*, mainly *M. haemolytica* and *P. trehalosi* – occasionally *P. multocida* if it is also isolated from diseased animals – are considered to be the main agents. In Alpine chamois (*Rupicapra rupicapra*) and Alpine ibex (*Capra ibex*) – in which pneumonia outbreaks can cause up to 30% mortality in herds, affecting mostly kids and yearlings – 30% of affected animals tested positive for *P. trehalosi,* whereas 11% were positive for *P. multocida A* (Ferroglio, unpublished data).

In bighorn sheep in North America, it seems that *P. trehalosi* is more frequently isolated than *M. haemolytica* from healthy animals[10,11]; however, the role of domestic sheep and cattle as carriers of *M. haemolytica* to bighorn has been proven[11,12].

AVIAN CHOLERA

GENERAL INTRODUCTION

Avian cholera, or fowl cholera, is caused by *Pasteurella multocida* and is a significant cause of mortality, especially

in waterfowl, in many parts of the world, including the Americas, Europe, Asia and Africa.

AETIOLOGY

Pasteurella multocida serotypes 1, 3 and 4 are usually isolated from wildfowl, and type A are the most virulent strains owing to their capacity to resist phagocytosis.

EPIDEMIOLOGY

Outbreaks of fowl cholera in wild birds are reported from many countries, and disease occurs in many major flyways, with reports of outbreaks more frequent in North America's wildfowl populations. Extensive epidemics of avian cholera involving hundreds or thousands of aquatic birds are probably more likely to be recognized and reported than the many smaller disease foci. Some species suffer greater mortality than others, and cases in coots and swans usually precede mortality in mallards (*Anas platyrhynchos*) and teal (*Anas crecca*). There is no consistent evidence for differential susceptibility by sex or host age, even though greater mortality in a particular sex or age has been reported in different publications.

Molecular characterization of *P. multocida* isolated from wild and domestic birds in Denmark indicates that isolates from wild birds have indistinguishable patterns, irrespective of species, origin and year of isolation, whereas several clones exist among domestic poultry[13]. Spillover from wildfowl to poultry, or vice versa, is usually not clear.

Geographical Distribution and Hosts

Pasteurella multocida is highly infectious and produces septicaemic and respiratory diseases in more than 180 species of wild birds, such as pelicans, cormorants, wading birds, herons, flamingo, swans, geese, ducks, eagles, hawks, falcons, gallinaceous birds, cranes, rails, coots, shorebirds, gulls, auks, pigeons, doves, owls, swifts, jays, crows, thrushes, starlings, sparrows and finches (see [14] for further detail on species), but its host range may be still greater because many cases remain unreported or unconfirmed. It has been stated that probably all bird species could be susceptible to avian cholera under particular circumstances. In Europe avian cholera has been reported in doves, crows, partridges, sparrows and pheasants (*Phasianus colchicus*), but epizootics in species other than wildfowl are uncommon. In Europe, cases are reported from Mediterranean countries to Scandinavia and from the British Isles to the far east of Eurasia.

In the UK, a corvid respiratory disease (CRD), which affects primarily rooks (*Corvus frugilegus*) at breeding colonies and roosts and which has been recognized for 20 years, is now thought to be caused by *P. multocida* infection, possibly in association with other, as yet unknown, pathogens. Birds may be seen ill, weak and dyspnoeic, as the condition is chronic in nature. Dead birds have pneumonia and airsacculitis, from which *P. multocida* can be cultured. Little is known of the epidemiology; however, in one incident approximately 30 birds died over a 9-month period from a flock of about 200–300 birds. The *P. multocida* isolates from these birds were all capsular type F, but it is not known if this pathogen poses a risk to domestic poultry[15].

Environmental Factors

In the past avian cholera was considered a disease of winter, but in recent decades outbreaks have been observed in spring and during the nesting season, and cases are recorded now throughout the year. Probably the concentrations of birds that collect in the winter period, for example in wetland habitats or spring staging grounds (migration stop-over localities), makes mortality more noticeable, thus biasing data collection. In colonial breeding birds, such as eider (*Somateria mollissima*) and cormorant (*Phalacrocorax carbo*), the breeding season leads to high bird densities, which facilitate transmission between close-flocking individuals. This could explain why outbreaks of avian cholera in eiders are reported only in breeding colonies[13]. However, it could also be easier to detect outbreaks when birds are concentrated in breeding areas. Poor nutritional state of birds has usually been considered a predisposing factor for avian cholera; however, many birds that die during avian cholera epizootics are in good body condition, suggesting that nutritional status is not the main predisposing factor.

Many authors report that *P. multocida* can persist for a long time in wetlands after avian cholera outbreaks and that bacteria survival in the environment is linked to water quality (calcium, magnesium, chloride, sodium and sulphate concentrations), with survival increased by the presence of salt and organic matter. However, field studies have shown that the bacteria can be isolated from water samples for only a few days, or at the most a few weeks, after an avian cholera outbreak. During an outbreak in Denmark

no *P. multocida* isolate was obtained from water from a small water pond[13].

Probably the environment is not a good reservoir of *P. multocida* in the absence of birds, and only the introduction of infected birds can trigger outbreaks. Even though *P. multocida* can survive in water for more than 1 year under experimental conditions, field conditions in waterfowl habitats are very different, and the recovery of the bacterium is very rare in ponds and wetland water. Considering the wide species distribution of the pathogen, it may be continually introduced to an area as carrier birds arrive. In wetlands the presence of ill birds can initiate an epidemic because infected carcasses may lead to a short-term accumulation of *P. multocida* and thus act as a source of infection for susceptible birds. It is likely that many outbreaks involve small numbers of animals and are undetected. High bird density is probably the most frequent and important factor in initiating outbreaks[14].

Epidemiological Role of Wild Animals

Data collected from 1996 to 2003 in Denmark suggest that a *P. multocida* strain has survived during several years among wild birds in that country[13], and this supports the hypothesis that wild birds can act as a reservoir for *P. multocida* – as does the fact that disease often occurs in areas where no sick animals have been introduced and wild birds are capable of spreading the infection to new areas. There are reports of infection in poultry due to the same strain present in wildlife, but because of the wide diffusion of the pathogen it is difficult to evaluate the concrete role of wild birds in the domestic cycle.

Transmission

Invertebrates have been considered as a possible maintenance source for *P. multocida*; however, even if transmission of infection through ingestion of infected invertebrate or arthropods species, such as maggots or flies, has proven to be possible, it is highly unlikely that this represents an important source of transmission[14]. Ectoparasites have been found to transmit *P. multocida*, and in the soft tick *Argas persicus* the bacteria can survive for at least 1 month and may have a 1,000-fold increase. Among ectoparasites, poultry mites (*Dermanyssus* spp.) and lice have been demonstrated to be able to transmit the bacteria.

Apart from these routes, transmission mainly occurs through inhalation of aerosols. Aerosols are created by waterfowl splashing, and in the aerosol the bacterial concentration could be 10 to 1,000 times higher than the water from which it originates. This occurs because animal movements lead to air bubble rupture, which ejects the bacteria present in the water surface microlayer into the atmosphere. Birds taking flight in large rafts by running across the water has been considered as a predisposing factor, particularly as coots, which run in large rafts, are usually the first to show disease and have a higher morbidity. Considering that bacteria are present in larger amounts at the water surface, species that graze frequently at the surface, such as coots, could also more easily ingest *P. multocida*. Transmission by predators or scavenging of infected birds could also be important in disseminating infection. The risk of *P. multocida* infection by ingestion of contaminated food or soil is mainly due to the shedding of *P. multocida* by diseased birds in faeces, or from the carcasses of dead birds, which increases the environmental contamination, facilitating the transmission of the bacteria.

PATHOGENESIS, PATHOLOGY AND IMMUNITY

Pasteurella multocida enter avian tissues through the mucous membranes of the pharynx, upper respiratory passages and conjunctivae, and in some cases through cutaneous wounds. Septicaemia results from this invasion when *P. multocida* avoids the host phagocytosis, and the predominant lesions, haemorrhages and necrotic foci are caused by endotoxaemia. At necropsy, in acute forms birds are in good body condition. Gross lesions consist of petechial haemorrhages on the heart surface and focal necrosis in the liver and other internal organs. Mucoid enteritis with necrotic-diphtheritic lesions, is also found in chronic forms, as well as mucoid inflammation of the upper respiratory tract with mucopurulent exudate and cytoplasmatic vacuolation, loss of cilia and desquamation of individual cells. In some areas there is accumulation of numerous bacteria, surrounded by neutrophilic granulocytes in the lung parenchyma.

Birds that recover from infection are usually protected from reinfection, even if some authors report a low prevalence of birds with detectable antibodies after an outbreak.

CLINICAL SIGNS AND TREATMENT

Clinical signs are difficult to detect, particularly in acute disease, and the death of the birds maybe the only visible

sign. In peracute or chronic forms, when birds are found alive, infected individuals show fever, depression and anorexia, mucoid oral discharge, ruffled feathers, diarrhoea, conjunctivitis and torticollis due to nervous system manifestations. In many cases respiratory function is compromised and dyspnoea with tachypnoea is frequently seen in chronic infection.

Treatment is based on the use of sulfonamides, and antibiotics are commonly used. Sulfaquinoxaline sodium in feed or water usually controls mortality, as do sulfamethazine and sulfadimethoxine. High levels of tetracycline antibiotics in the feed (0.04%), drinking water, or administered parenterally may be useful. Penicillin is often effective for sulfa-resistant infections.

MANAGEMENT, CONTROL AND REGULATIONS

Monitoring for waterfowl mortality is probably the most important recommendation, because this allows detection of disease foci at their earliest stage and the implementation of control actions in order to reduce transmission and prevent high losses. It has been suggested that one diseased bird may be sufficient to start an outbreak, owing to contamination of water and environment from the fluids of the dead animal, allowing transmission of the bacterium to susceptible birds. Scavenging infected carcasses and invertebrate dissemination can obviously increase the dissemination of the pathogen. Considering that carcasses probably represent the major source of *P. multocida*, carcass removal can be the most successful management strategy to reduce the contamination and persistence of *P. multocida* and, even if no conclusive study has been done, it seems to be effective in controlling avian cholera outbreaks. Habitat manipulation could also be used to control outbreaks by selective drainage or water pumping and diversion in order to modify waterfowl species distribution and densities, and also to dilute the pathogen contamination of the water. Clearly the reduction in wetlands increases the crowding of waterfowl and can favour transmission of the pathogen in crowded populations.

Pasteurella multocida vaccines are available for poultry; however, few cases of wildfowl vaccination have been reported, and this option is likely to be feasible only in small, captive flocks.

PUBLIC HEALTH CONCERN

Disease in humans caused by *P. multocida* is not uncommon, and *P. multocida* may be considered a zoonotic organism. No reports exist of direct transmission from wild birds or poultry to humans or vice versa, but the possibility for such infections cannot be excluded.

SIGNIFICANCE AND IMPLICATION FOR ANIMAL HEALTH

Avian cholera outbreaks can cause severe mortality in some areas, and reports from North America seem to indicate that during such outbreaks mortality can cause a population loss of about 0.2% in ducks and of 3.9% in swans. In common eider avian cholera outbreaks can have an important impact on post-hatching survival, and this affects recruitment into the breeding population and thus population dynamics[16].

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CHAPTER

24

BRUCELLOSIS

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Brucellosis is a major zoonotic bacterial disease, widely distributed in both humans and other mammals. When studying the disease in wildlife, the issues of whether wildlife represent a significant reservoir of infection for livestock or are just spillover hosts, and the zoonotic implications of wildlife infection have to be considered.

AETIOLOGY

Brucella spp. are Gram-negative, facultative intracellular bacteria. They are coccobacilli measuring 0.6 to 1.5 µm long and 0.5 to 0.7 µm wide. They are generally single, and rarely arranged in groups of two or more. *Brucella* spp. can resist weak acidic treatment and will therefore appear red after Stamp staining.

Ten species are recognized within the genus *Brucella*: the six 'classical' *Brucella* species, some of which include different biovars: *Brucella abortus* (biovars 1, 2, 3, 4, 5, 6, 7, 9), *Brucella melitensis* (biovars 1, 2, 3), *Brucella suis* (biovars 1, 2, 3, 4, 5), *Brucella ovis*, *Brucella canis* and *Brucella neotomae*[1] and the recently described *Brucella ceti* and *Brucella pinnipediae*[2], *Brucella microti*[3] and *Brucella inopinata*[4].

The classification of the classical species was mainly based on differences in phenotypic characteristics, host preference(s) and in pathogenicity. Distinction between species and biovars is currently performed by differential laboratory tests.

EPIDEMIOLOGY

GEOGRAPHICAL DISTRIBUTION AND HOSTS, EPIDEMIOLOGICAL ROLE OF WILD ANIMALS

Worldwide, the main pathogenic species for livestock are: *B. abortus* (all biovars), responsible for bovine brucellosis; *B. melitensis* (all biovars), the main aetiologic agent of small ruminant brucellosis; and *B. suis* (biovars 1, 2 and 3) responsible for swine brucellosis.

Despite their respective host preferences, *B. abortus* and *B. suis* have also been isolated from a great variety of wildlife species, such as bison (*Bison bison*), red deer (*Cervus elaphus*), feral swine and wild boar (*Sus scrofa*), red fox (*Vulpes vulpes*), European brown hare (*Lepus europeaus*), African buffalo (*Syncerus caffer*), reindeer (*Rangifer tarandus tarandus*) and caribou (*Rangifer tarandus groenlandicus*), and wildlife has thus to be considered as a potential reservoir for brucellosis in livestock. *Brucella melitensis* is rarely reported in wildlife.

Brucella ovis and *B. canis* are responsible for ram epididymitis and canine brucellosis, respectively, and are not

Infectious Diseases of Wild Mammals and Birds in Europe, First Edition. Edited by Dolores Gavier-Widén, J. Paul Duff, and Anna Meredith.

reported in wildlife in Europe. For *B. neotomae,* only strains isolated from desert rats in Utah, USA, have been reported.

Since the first description of an abortion due to *Brucella* spp. in a captive dolphin in California in 1994[5], several reports have described the isolation and characterization of *Brucella* spp. from a wide variety of marine mammals such as seals, porpoises, dolphins and whales worldwide, excluding the Antarctic (reviewed in[6]). The overall characteristics of these marine mammal strains are different to those of any of the six 'classical' *Brucella* species, and since 2007 *B. ceti* and *B. pinnipedialis* (infecting preferentially cetaceans and pinnipeds, respectively) are recognized as new *Brucella* species[6].

Experimental studies and epidemiological evidence suggest that birds are very resistant to *Brucella* spp. infection. In fish, recent studies related to natural infections of Nile catfish demonstrated that *B. melitensis* biovar 3 could be cultured from visceral organs[7].

Brucella Abortus

Because brucellosis eradication efforts in the EU and in the USA focused on bovine brucellosis, the emphasis was put on the identification of a possible reservoir of *B. abortus* in wildlife. When brucellosis was prevalent in cattle, numerous surveys identified occasional seropositive results in wild ungulates, particularly cervids[8]. Under free-ranging conditions, the infection was considered to be self-limiting or as a spillover of the infection in cattle. For example, in 1995, *B. abortus* was isolated from 7/112 culled chamois (*Rupicapra rupicapra*), but brucellosis did not seem to be present in larger areas of the Western Italian Alps, where bovine brucellosis is absent[9].

Nowadays, in countries where the bovine brucellosis eradication programmes are nearly completed there are few known sustainable reservoirs of *B. abortus* in wild species, other than bison and elk (*Cervus canadensis*) in the American National Parks of the Greater Yellowstone Area (GYA) and in the Canadian Wood Buffalo National Park. In South Africa, the African buffalo is considered a reservoir of *B. abortus*. However, recent studies in feral pigs on the Atlantic coast of South Carolina, USA, challenge these thoughts. Indeed, *B. abortus* wild-type and the *B. abortus* S19 and RB51 vaccine strains have been isolated from feral pigs on a property where no cattle had been kept in the area frequented by the feral swine since at least 1970[10]. This is the first report of *B. abortus* in feral swine and of a wildlife reservoir of *B. abortus* outside the GYA in the USA. In Europe, the isolation of *B. abortus* in pigs or wild boars is not reported.

A recent survey in Spain highlighted the fact that wild ruminants were not a significant brucellosis reservoir for livestock. *Brucella abortus* biovar 1 was isolated from only a single red deer. Thus, these results suggest that in Europe, wild ruminants occasionally acquire infection from brucellosis transmitted from infected livestock, rather than being true reservoirs of infection for livestock[11]. The available epidemiological information suggests that wild ruminants are not able to sustain *B. abortus* infection without introduction from infected cattle. Given the progress in the eradication programme of bovine brucellosis in the EU member states, it is unlikely that *B. abortus* will become a threat to wildlife in Europe in the future.

Brucella Melitensis

The known ecological range of *B. melitensis* in wildlife is more restricted than those of *B. abortus* and *B. suis*, although it is still a major problem in domestic small ruminants in southern European states and an important veterinary public health issue. Spillover from infected small ruminants has been documented in a few wildlife species, such as chamois and ibex (*Capra ibex*) in the French and Italian Alps[12,13] Recently *B. melitensis* has been isolated from one Iberian wild goat in Spain[11]. These reports highlight the fact that *B. melitensis* infection in European wildlife is nowadays anecdotal and always linked to the domesticated small ruminant reservoir. It will most likely become less important in the future, given the eradication of small ruminant brucellosis in Northern and Central Europe and the progress in the *B. melitensis* eradication programmes, in some Mediterranean EU member states. However, important foci of *B. melitensis* infections in small ruminants persist in the Balkans, putting wild ruminants of this region potentially at risk.

Brucella Suis

Brucella suis (biovars 1, 2, 3) is still widely distributed in the world. *Brucella suis* biovar 3 has only been isolated in Southeast Asia and the Americas. The taxonomical relevance of *B. suis* biovar 3, or at least the significance of its reference strain is questioned, as there is no single field strain of *B. suis* that matched both the microbiological and

genetic profiles of the biovar 3 reference strain[14]. Generally, the prevalence of brucellosis in domestic pigs is low, with the exception of Southeast Asia and South America.

Brucella suis infection is mainly restricted to feral pigs in the USA and Australia (*B. suis* biovar 1) and wild boars and European brown hares in Europe (*B. suis* biovar 2), where *B. suis* has been eradicated in the domestic pig population for decades.

Brucella suis biovar 2 very rarely causes disease in humans; when this does occur it is usually associated with immunodeficiency. In Croatia, during the period 1980–2003, *B. suis* biovar 1 was isolated from wild boars, pigs, brown hares and horses[15]. This is an important veterinary public health issue, because *B. suis* biovar 1 is a known zoonotic agent. However, to date, no human *B. suis* biovar 1 infection has been reported in Croatia[16]. In 1993, *B. suis* biovar 1 was isolated from a butcher handling imported feral pig meat in Belgium, where the last *B. suis* biovar 1 infection had been reported in a pig farmer in 1983[17].

Rangiferine brucellosis (i.e. brucellosis in reindeer and caribou) is caused by *B. suis* biovar 4 throughout the Arctic region, Siberia, Canada and Alaska and constitutes a serious zoonosis. *Brucella suis* biovar 4 may also infect moose and occasionally different carnivore species. *Brucella suis* biovar 5, isolated in rodents in Eastern Europe and for which very few strains are known, is most probably misnamed.

Brucella Suis *Biovar 2 In Eurasian Wild Boar*

Brucellosis in wild boar is widely distributed throughout Europe. During the 1990s, the number of wild boar reached a historical peak in the southeastern part of Belgium, with an estimated population of more than 10,000 individuals (45 animals per 1,000 ha of forest). This tendency was also noticed in neighbouring France, Germany and Luxemburg. Some of these animals were raised in enclosures before being released for hunting. Fortunately, this practice is no longer allowed but may have contributed to the establishment of an enzootic *B. suis* 2 infection in wild boar in Europe. *Brucella suis* biovar 2 is a sustainable infection and is maintained in wild boar independently of domestic pigs.

In France and Germany, *B. suis* biovar 2 infections have been reported in outdoor-rearing pig farms during the last decade, and wild boars appear to be the most likely source of infection.

In the Iberian Peninsula, *B. suis* biovar 2-infected wild boars are considered to be an important threat for the Iberian pig population reared in outdoor breeding systems. *Brucella suis* biovar 2 infection in wild boars could be of major concern should brucellosis control programmes in domestic pigs be implemented in the EU[11].

Brucella Suis *Biovar 2 In European Brown Hare*

Information regarding brown hare populations (and hence *B. suis* biovar 2 infection) is still very fragmented in Europe. In recent years, isolation of *B. suis* biovar 2 infection has been reported in central Europe and Spain. The occurrence of *B. suis* biovar 2 or anti-*Brucella* antibodies in free-ranging hares may depend on the number of close contacts with infected wild boars or domestic pigs. Indeed, an increasing seroprevalence of brucellosis in wild boars preceding an epizootic of hare brucellosis was observed in the Czech Republic[18].

Molecular studies revealed that the *B. suis* biovar 2 haplotype found in hares was not found in pigs or wild boars in Spain[11] but was similar to the one described in domestic pigs from France and Croatia and also in wild boar from France, Italy and Switzerland[15]. This raises important questions on the translocation of infected hares. However, hares may not contribute to the dissemination of brucellosis to the extent of wild boar (solitary, adult male boar may range very long distances if disturbed or when there is a food shortage), as hares have a non-migratory way of life and occupy small home ranges, which effectively reduces the interface with other wildlife.

In Denmark, during 1929–1999, 10 clinical outbreaks of *B. suis* biovar 2 infection in domestic pigs were recorded, and epidemiological evidence linked them to hares. *Brucella suis* biovar 2 infections in cattle have also been reported in Denmark[19] and the source of contamination is believed to have been hares, as there is no established population of free-range wild boar in Denmark.

Brucella Suis *Biovar 4 In Caribou, Reindeer and Moose*

Rangiferine brucellosis is enzootic in Siberia, Canada and Alaska in caribou and reindeer, and *B. suis* biovar 4 was isolated from both species in the 1960s. Human cases have been restricted to herders of diseased reindeer or caribou. It has been experimentally demonstrated that cattle exposed to *B. suis* biovar 4-infected reindeer can become

infected[20]. In Canada, a natural *B. suis* 4 infection was confirmed in moose for the first time in 1993[21]. In 1999, a serological study concluded that brucellosis was not present in reindeer in Finnmark, northern Norway[22].

Brucella Ceti and *B. Pinnipedialis*

Marine mammal brucellosis was first reported in 1994 from stranded harbour seals (*Phoca vitulina*), harbour porpoises (*Phocoena phocoena*) and common dolphins (*Delphinus delphis*) on the Scottish coast[23] and from an aborted fetus of a captive bottlenose dolphin (*Tursios truncatus*) in California[5]. In the 1990s, strains of *Brucella* spp., biologically and genetically different from the six classical *Brucella* species, were isolated from cetaceans and pinnipeds inhabiting seas and oceans of Europe and North America or kept in captivity. Anti-*Brucella* antibodies have also been detected in serum samples from several species of marine mammals from the northern and southern hemispheres. It is noteworthy that no *Brucella* spp. has been isolated from marine mammals in the Antarctic waters, although anti-*Brucella* antibodies have been detected in some marine mammals[24].

The isolation of *Brucella* spp. from an aborted bottlenose dolphin fetus indicated that marine mammal *Brucella* spp. may cause abortion[5]. If *Brucella* spp. infection leads to reproductive disorders in marine mammals, then brucellosis may play an important role in the population dynamics of these species[25]. The polar bear (*Ursus maritimus*) is the apex predator in the arctic marine food chain, and in the Svalbard area ringed seals (*Phoca hispida*), bearded seals (*Erignathus barbatus*) and harp seals are the main prey. Anti-*Brucella* antibodies were found in ringed seals and harp seals in the Svalbard area[26]. A seroprevalence of 5.4% of anti-*Brucella* antibodies was found in plasma samples from 297 polar bears from Svalbard and the Barents Sea[27]. To date, there is no indication of disease caused by *Brucella* spp. in the polar bear population at Svalbard. Therefore, the potential impacts of *Brucella* spp. exposure on individuals or the population is unknown.

An experimental inoculation of three pregnant cattle with a *Brucella* isolate from a Pacific harbour seal resulted in two of the animals aborting. This study indicated that marine mammal *Brucella* spp. is capable of producing seroconversion and abortion in cattle but is less pathogenic in that species than *B. abortus*[28]. Another experimental investigation demonstrated colonization, limited establishment of infection, and low pathogenicity of the three marine-mammal *Brucella* strains for sheep, and limited potential to transmit infection in sheep[29]. A marine mammal strain isolated from a human patient was not able to establish an infection in piglets[30].

Brucella Microti

A systemic disease occurred in a wild population of the common vole (*Microtus arvalis*) in South Moravia (Czech Republic) during the years 1999–2003. A novel species within the genus *Brucella* was isolated from these voles and given the name *Brucella microti*[7,31]. Long-term survival of *B. microti* in soil was shown – thus, soil might act as a reservoir of infection. *Brucella microti* was also isolated from the mandibular lymph nodes of red foxes hunted in the region of Gmünd, Lower Austria. *Brucella microti* is thus prevalent in a larger geographic area covering the region of South Moravia and parts of Lower Austria, and foxes could have become infected by ingestion of infected common voles[32]. No human or livestock infection with *B. microti* has been reported to date.

TRANSMISSION

When a heifer or a cow aborts, large numbers of organisms are excreted. The abortion is associated with the excretion of 10^{12}–10^{13} bacteria. Taking into account that a quantity of 15×10^6 *Brucella* deposited on the conjunctiva of heifers results in infection of 95% of them, the quantity of bacteria excreted in the course of a brucellosis-induced abortion could theoretically infect 60,000 to 600,000 gestating females, which in turn could infect other animals. This is called the 'multiplication relay of the infection'[33]. Assuming that this situation may also prevail in wildlife, the potential for infection is extremely high.

However, the prevalence of brucellosis in some wildlife species is very low and thus, besides classical factors considered to be important in transmission (host susceptibility, shedding, survival in the environment, etc.), behaviour of individuals and the interaction between wildlife and livestock at the interface may actually be the most important drivers for transmission. For instance, the reclusive calving behaviour of elk will minimize risk of *B. abortus* transmission to other animals and, in free-ranging settings, elk may be looked upon as a dead-end host. By contrast, where winter feeding is practised, the risk of infection increases dramatically by increasing density and enhancing the risk of exposure to an infectious abortion near the

feeding ground, resulting in feed contamination. In the GYA, winter feeding changed the behaviour and density of free-ranging elk, probably converting what in nature is a dead-end host to a maintenance host of *B. abortus*[8].

As far as marine mammal brucellosis is concerned, the fact that *B. melitensis* is able to multiply in fish warrants further studies in order to assess whether fish can be hosts for *B. ceti* and *B. pinnipedialis* and play a role in the epidemiology of the disease.

PATHOGENESIS, PATHOLOGY AND IMMUNITY

From studies performed in livestock, the infectious routes are mainly oral (e.g. licking of aborted fetuses or fetal membranes, ingestion of contaminated feedstuffs) via the conjunctival mucosa, and venereal: up to 10^4–10^5 colony-forming units (CFU) of *B. suis* biovar 1 per ml semen has been reported in pigs. In cattle, brucellosis can also be transmitted in utero or to the newborn calf immediately after birth.

Our knowledge of the pathogenesis of brucellosis is based on *in vivo* studies, performed mainly in mice and cattle as well as *in vitro* studies in murine macrophages and human macrophage cell lines.

Brucella spp. enters the organism mainly through oral, nasopharynx, conjunctival and genital mucosae but also through cutaneous lesions. Bacteria rapidly drain to lymph nodes and can persist there for a long time. If *Brucella* spp. are not eliminated at this stage, bacteraemia (which is usually transient in cattle but more persistent in pigs) and colonization of a broad range of tissues takes place. *Brucella* spp. are frequently isolated from lymphoid tissues associated with the mammary glands and reproductive organs but can also be found in other organs (e.g. bones, joints, nervous tissue). Abortion is associated with the extensive replication of *Brucella* spp. within the chorioallantoic trophoblasts of the placenta. This massive intracellular replication ruptures the infected trophoblasts and permits direct access to the fetus. The loss of placental integrity and fetal infection lead to abortion or to premature birth of a weak and infected calf.

Brucella spp. have a predilection for the gravid uterus, udder, testicle and accessory male sex glands, lymph nodes, joint capsules and bursae. At necropsy, purulent lesions and granulomas may be seen. In the cow, *B. abortus* localizes initially in lymph nodes, infects the gravid uterus during bacteraemia, and multiplies to enormous numbers in chorioallantoic trophoblasts. Retained placenta and residual infection of the uterus are often indicators of the disease. Characteristic placental lesions may vary in severity and are not pathognomonic. Acute or chronic placentitis is sometimes seen. Placentae may show degenerative and necrotic changes in the chorioallantoic trophoblasts at the edges of the cotyledons. The cotyledons may be normal or necrotic. In affected cotyledons, the trophoblastic epithelium lining the chorionic villi is necrotic. In some villi, epithelial necrosis and infiltration of mononuclear cells and neutrophils are observed. Bacterial colonies are found in the necrotic areas of villi, surrounded by necrotic cell debris and neutrophils. In infected mammary glands, diffuse inflammation has been observed and can lead to atrophy of the glandular tissue and fibrosis when the disease progresses.

In males, the predilection sites for infection are the reproductive organs and the associated lymph nodes. Seminal vesiculitis and/or orchitis and infection of the accessory sex glands are commonly observed. During the acute phase of infection the semen contains large numbers of *Brucella* spp., but as the infection becomes more chronic the number of excreted *Brucella* spp. decreases.

Fetuses infected with *Brucella* spp. may show pneumonia, serohaemorrhagic exudates in body cavities and lesions in the muscles. Other lesions such as abnormal abomasal content, fibrinous pleuritis, vasculitis and meningitis occur less frequently. Fetal bacteraemia occurs after replication of *Brucella* spp. in trophoblasts. Subsequently, fetal viscera and placental cotyledons become heavily infected with *Brucella* spp. Histological lesions observed in aborted fetuses are not specific enough to be able to incriminate *B. abortus* as the cause of abortion. In chronic cases, uni- and bilateral hygromas, mainly localized at the carpal joint, are observed in more than 60% of chronically infected animals.

Specific pathologies have been described in different wildlife species. In chamois and ibex, these include blindness and neurological signs as well as thick-walled carpal joints and enlargement of the testicle characterized by necrosis and fibrosis[12,13]. In wild boar one of the most striking features is that a very high percentage of infected animals can be detected in all categories of age by bacteriology, often in the absence of gross lesions[17]. There is no definitive explanation for this. In the brown hare, the infection is either latent or involves the development of granulomatous nodules or abscesses in the testes, liver,

spleen, lung and other tissues[18]. In reindeer when clinical signs are present, abortions and metritis are seen in females and orchitis in males. In both sexes, abscesses in joints and often bursitis and lameness are observed[34]. In moose, carpal pathology and osteomyelitis of subjacent bone was observed[21]. In common vole (*Microtus arvalis*) acute infections were characterized by oedema of extremities, occasionally with colliquating abscesses, arthritis, lymphadenitis, perforations of the skin resulting from colliquated abscesses, orchitis and peritoneal granulomas[31]. In marine mammals it is important to note that pathology induced by *Brucella* spp. is different in cetaceans compared with seals. As a general rule, no gross pathology has been associated with *B. pinnipedialis* infections in seals, whereas different acute and chronic pathological changes have been associated with *B. ceti* infection both in Odontoceti and Mysticeti. A range of *Brucella* spp. associated pathology has been found in cetaceans, which includes sub-blubber abscessation, hepatic and splenic necrosis, macrophage infiltration in liver and spleen, possible abortion, epididymitis, spinal discospondylitis, meningitis, lymphadenitis and mastitis. There is currently no information on the levels of abortion in seals or cetaceans[6]. A chronic, non-suppurative meningoencephalitis was found in three young striped dolphins (*Stenella coeruleoalba*)[35].

IMMUNITY

The first role of the innate immune system in *Brucella* infection is to reduce the initial number of bacteria. Activation of the classical pathway mediated by IgM and low concentrations of IgG has been considered to be the dominant bactericidal mechanism of serum against *B. abortus* during the early stage of infection. Neutrophils are probably the first immune-associated cells in human infection to encounter *Brucella* spp. Rapid phagocytosis of virulent and attenuated *Brucella* strains by neutrophils occurs only following opsonization with normal serum, but the role of these cells in controlling the infection is questionable, as survival of *Brucella* in neutrophils during early infection has been observed[36]. The macrophage is the main cellular host of *Brucella* spp. The bactericidal functions of these cells are mainly mediated by reactive oxygen intermediates (ROI). Reactive nitrogen intermediates (RNI) also play a limited role in the killing of *Brucella* in macrophages[37]. Both ROI and RNI are induced by interferon gamma (IFN-γ) and tumour necrosis factor alpha (TNF-α). Antigen processing and recognition is a key feature of antibacterial immune responses to intracellular bacteria. In contrast to other pathogens, which are primarily controlled by conventional major histocompatibility complex (MHC) II- and MHC I-restricted CD4+ or CD8+ T cells, respectively, unconventional T cells participate additionally in antibacterial protection. Both natural killer (NK) cells and $\gamma\delta$ T-cells play a role in the control of *Brucella* infection by killing *Brucella* infected cells and activating macrophages via activating-cytokine production[38].

The innate response against lipopolysaccharide (LPS) typically involves the release of a range of pro-inflammatory mediators, which locally promote inflammation. *Brucella abortus* induces interleukin (IL)-12 produced by human monocytes via the CD14 LPS preceptor[39]. Additionally, the ability of *Brucella* spp. to elicit IL-12 secretion will drive Th0 cells to differentiate into Th1 effector and memory cells.

Passive transfer of serum containing anti-LPS antibodies protects mice against virulent *B. abortus* challenge. Passive transfer of monoclonal antibodies directed against outer membrane proteins induced only a limited protective effect. Opsonization, probably coupled with enhancement of intracellular killing, is regarded as the principal protective role of antibody against *Brucella* infection.

Immunity against *Brucella* spp. requires cell-mediated immunity. In mice, antigen-presenting cells stimulated by *B. abortus* produce IL-12 that will induce a Th1 response mediated by CD4+ T cells. This Th1 response will stimulate CD8+ T cells to induce their direct cytotoxicity activity against *Brucella* infected cells, and to produce IFN-γ which will enhance the bactericidal functions of macrophages. The development of a Th1 response, under the control of TNF-α, IFN-γ and IL-12, promotes the clearance of the infection.

CLINICAL SIGNS

Brucellosis is characterized by reproductive disorders: i) abortion, retention of placenta, metritis, subclinical mastitis, infertility; ii) orchitis or epididymitis with frequent sterility; and iii) articular and peri-articular hygromas, seen in chronic infections. Abortion is the prime sign for acute brucellosis, and it often occurs during the second half of gestation. In 75 to 90% of the cases, infected cows will only abort once. A number of heifers, born from *Brucella*-infected dams, are latent carriers and will only be detected serologically at their first calving[40].

DIAGNOSIS

Laboratory diagnosis includes indirect (antibody detection) tests that can be applied to milk or blood as well as direct tests (classical bacteriology, polymerase chain reaction (PCR)-based methods). Isolation of *Brucella* spp. (or *Brucella* spp. DNA detection by PCR) allows certainty of diagnosis. Biotyping provides valuable epidemiological information, which allows tracing of infection sources in countries where several biotypes are co-circulating. However, when one particular biovar is the overwhelming isolated strain, classical typing techniques are of no help. In this context, new fingerprinting methods such as multiple locus variable (number of tandem repeat) analysis (MLVA) and multilocus sequence analysis (MLSA), have gained wider acceptance and will, in coming years, be used as routine typing and fingerprinting methods for molecular epidemiological purposes[41].

Several techniques are available to identify *Brucella* spp. The Stamp staining method is still often used, and even if this technique is not specific (other abortive agents such as *Chlamydophila abortus*, or *Coxiella burnetii* are also stained), it provides valuable information for the analysis of abortive material. Bacterial isolation is nevertheless always preferable and even required for the typing of the strain.

CULTURE

For the definitive diagnosis of brucellosis, the choice of samples depends on the observed clinical signs. In the case of clinical brucellosis, valid samples include aborted fetuses (stomach, spleen and lung), fetal membranes, vaginal secretions, colostrum, milk, sperm or fluid collected from arthritic lesions or fluid-filled joint bursae (hygroma). At *post mortem* examination, in order to confirm acute or chronic brucellosis, the preferred tissues for sampling are the genital and oropharyngeal lymph nodes, the spleen, the mammary gland and associated lymph nodes. For the isolation of *Brucella* spp., the most commonly used medium is Farrell's medium, which contains antibiotics to inhibit the growth of other bacteria present in clinical samples. Some *Brucella* species need CO_2 for growth. For liquid samples (milk or blood), sensitivity is increased by the use of a biphasic medium such as the Castaneda medium. Growth may appear after 2–3 days, but cultures can only be considered negative after 10–15 days of incubation.

Biotyping of *Brucella* spp. is performed by using different tests, the most important being agglutination tests, the dependence on CO_2 for growth, production of H_2S, and growth in the presence of specific factors. These techniques need standardization and experience, and they are performed in reference laboratories.

MOLECULAR IDENTIFICATION

Several PCR-based methods have been developed, and the best validated are those that use the detection of specific sequences of *Brucella* spp. These techniques were mostly validated on human samples but have become more widely applied in veterinary medicine. Relative high cost and the lack of standardization have limited their application to specific problems such as wildlife studies or rapid identification of incidents in uninfected areas. As a general rule, these techniques show a lower diagnostic sensitivity than culture; however, their specificity is close to 100%.

MOLECULAR TYPING

For the typing of *Brucella* spp., the AMOS PCR is often used[42]. This PCR and derived PCR allow the discrimination between *Brucella* species and between vaccine and wild-type strains but do not allow discrimination between biovars of a given *Brucella* species. A newly developed multiplex PCR assay (Bruce-ladder) can identify and differentiate for the first time all of the *Brucella* species and the vaccine strains in the same test[43]. These new techniques are valuable tools for the identification and characterization of *B. suis*, *B. ceti* and *B. pinnipedialis*, which are now the most important *Brucella* species found in European wildlife.

BRUCELLA SUIS BIOVARS

The taxonomic position of *B. suis* within the genus *Brucella* is subject to an ongoing debate. A number of genetic observations supported by independent studies have demonstrated that, with the exception of *B. suis* biovar 5 (probably misnamed), all *B. suis* and *B. canis* strains form a consistent group. A drawback persists for the distinction of *B. suis* biovar 4 from *B. canis*, but a single nucleotide polymorphism (SNP) in the *omp25* gene unique to *B. canis* can be included in a typing scheme[44].

BRUCELLA CETI *AND* B. PINNIPEDIALIS

A marker specific for the marine mammal strains was identified when amplification of the gene encoding the immunodominant BP26 protein revealed a larger than expected PCR product, reflecting the insertion of an *IS*711 element downstream of the gene[45]. A PCR based around bp26 has become a well-used test for differentiation of *Brucella* spp. associated with marine mammals from classical species associated with terrestrial mammals. Eventually, two new *Brucella* species labelled (with corrected etymology) as *B. ceti* for isolates from cetaceans and *B. pinnipedialis* for isolates from pinnipeds were validly published[2]. Further, MLSA studies suggested that *Brucella* strains from marine mammals corresponded to a cluster of five sequence types distinct from all previously described *Brucella* species from terrestrial mammals[44]. Recently the largest study to date examined 294 isolates from 173 marine mammals by MLVA[46]. More than 100 genotypes were identified and divided into five clusters that related to previous MLSA findings.

Brucellosis serology is usually performed using the same antigens as in domestic ruminant serology because the *Brucella* immunodominant antigens are associated with the surface 'smooth' lipopolysaccharide (LPS) and are to a large extent shared by all the naturally occurring biovars of *B. abortus*, *B. melitensis*, *B. suis*, *B. microti*, *B. ceti*, *B. pinnipedialis* and *B. inopinata*. It is important to note that *B. ovis* and *B. canis* present on their surface a 'rough' LPS, which is not antigenically related to smooth LPS. This means that infections by these two *Brucella* species cannot be detected by serological tests detecting anti-smooth LPS antibodies. Tests detecting rough LPS *Brucella* infections will not be discussed further, as to date neither *B. ovis* nor *B. canis* have been reported in wildlife in Europe.

It is virtually impossible to determine which species of *Brucella* induce anti-smooth LPS antibodies in the host. Some assays, such as indirect enzyme-linked immunosorbent assay (ELISA), rely on species-specific reagents that are not commercially available. This limitation of the lack of polyclonal or monoclonal antibodies to many wildlife species immunoglobulins can be partly overcome by the use of either Protein A or Protein G conjugates[47]. Other techniques such as competitive ELISA or the fluorescent polarization assay (FPA) do not rely on species-specific reagents and have been useful in marine mammals[47].

It is not appropriate to propose a single serological test as a reference test to assist veterinarians in the diagnosis of wildlife brucellosis. The ecology of wildlife brucellosis has become more and more important in order to understand serological results. In a first screening programme, a parallel interpretation of the first line tests should be chosen to improve the specificity of the detection of anti-*Brucella* antibodies[27]. The Rose Bengal plate test and/or the newly developed ELISA and the FPA should first be used. Cross-reactive bacteria such as *Yersinia enterocolitica* O:9 induce serological cross-reactions in the brucellosis serological tests that are almost indistinguishable from true brucellosis serological reactions[48].

The 'gold standard' in brucellosis remains the isolation of *Brucella* strains. If brucellosis is suspected in an animal or a wildlife population following positive serological results, attempts to isolate the organism are mandatory and thus should always be performed.

MANAGEMENT, CONTROL AND REGULATIONS

Preventive control and management measures should aim to decrease interfaces between livestock, wildlife and humans so that the disease risk in both livestock and free-ranging/captive wildlife is reduced and the zoonotic potential minimized.

If brucellosis occurs in a herd or flock, national and international veterinary regulations impose restrictions on animal movements and trade, which result in huge economic losses.

There is no vaccine currently available and registered for wildlife. In bison, elk and reindeer, the cattle S19 and RB51 vaccine strains have not shown satisfactory results in terms of safety and efficacy. Moreover, the isolation of these vaccine strains in feral pigs highlights that before any prospective use of a brucellosis vaccine in wildlife, the release of such vaccines has to be fully investigated, particularly in non-target species[10].

The control of brucellosis in wildlife relies mainly, if not exclusively, on good management practices. As dramatically shown in the GYA, elk (considered to be dead-end hosts when ranging freely) are becoming maintenance hosts for *B. abortus* when winter feeding is practised[8]. As a general rule, management practices enhancing the wildlife/livestock interface should not be implemented.

In Europe, brucellosis in terrestrial wildlife is nowadays restricted to *B. suis* biovar 2 infections in wild boars and hares. Given the ecology and the geographical distribution

of wild boar in Europe, *B. suis* biovar 2 infection in wild boar could become of major concern should brucellosis control programmes in domestic pigs be implemented in the EU.

PUBLIC HEALTH CONCERN

Brucellosis is an old disease with low mortality in humans (less than 2% of untreated cases). Yet human brucellosis remains the commonest zoonotic disease worldwide, with more than 500,000 new cases annually[16]. It is associated with substantial residual disability and is an important cause of travel-associated morbidity. The occurrence of the disease in humans is largely dependent on the occurrence of brucellosis in an animal reservoir, including wildlife. Today, the disease in humans is mainly occupational (abattoir, animal industry, hunters and health workers). Symptoms such as undulant fever, tiredness, night sweats, headaches and chills may continue for as long as 3 months before the illness becomes so severe and debilitating as to require medical attention.

In contrast to *B. suis* biovars 1, 3 and 4, *B. suis* biovar 2 has rarely been isolated from humans, and its zoonotic role is questioned. Zoonotic concern regarding marine mammal strains was initially raised following the recovery of a cetacean strain of *Brucella* from a laboratory worker in the UK who had seroconverted after suffering from headaches, lassitude and severe sinusitis[49]. In April 2003, the first report of community-acquired human infections with marine mammal-associated *Brucella* spp. was published. The authors described the identification of these strains in two patients with neurobrucellosis and intracerebral granulomas. It became apparent that all three reported cases of natural human infection associated with *Brucella* spp. from marine mammals[50,51] were associated with strain type 27 (ST27)[52]. Unfortunately the natural host of ST27 (first isolated from a captive dolphin in the USA) has not been identified, although there is molecular evidence of the presence of this genotype in minke whales (*Balaneoptera acurostrata*) from the Pacific Ocean[53].

In Norway and Greenland, there is a long tradition of consumption of meat from harp seals, hooded seals and minke whales – all species to be found infected with *Brucella* spp. In spite of this, brucellosis has not been reported in humans at risk (whale- and seal-hunters, veterinarians, other marine mammal meat handlers or consumers). Marine mammal *Brucella* were tested for their ability to infect human and murine macrophage cells. The study showed that some *B. ceti* and *B. pinnipedialis* isolates were virulent in these models of infection, whereas other isolates were not. In fact, all the *B. pinnipedialis* isolated from hooded seals did not show any ability to infect human and murine macrophage cells[46], which may be an explanation for the absence of human infection with hooded seal *B. pinnipedialis*.

SIGNIFICANCE AND IMPLICATIONS FOR ANIMAL HEALTH

A very important issue related to brucellosis in terrestrial wildlife is the distinction between a spillover of infection from livestock into wildlife, compared with a sustainable infection in wildlife. In the latter case, the concern of the livestock industry is to prevent the reintroduction of the infection in livestock, particularly in regions or states that are 'officially brucellosis-free' because of the costs linked to pre-movement testing. The development of the game farming industry has contributed to the re-emergence of brucellosis as an international concern for both livestock and wildlife, by increasing the density of potentially infected game species and the introduction of artificial feeding[8].

In Europe, given the progress of the national bovine brucellosis eradication programmes, it is unlikely that *B. abortus* will establish itself in free-ranging wild ungulates. The same can probably be said for *B. melitensis*, although foci of infection in small ruminants remain important in some southern European states and in the Balkans.

Although brucellosis has been eradicated in the domestic pig population for decades in Europe, *B. suis* biovar 2 infection in wild boar (which has established itself as a sustainable infection in almost all European wild boar populations) could become of major concern for outdoor reared pigs.

Translocation of infected hares in the absence of brucellosis regulations may contribute to its dissemination in Europe. Currently, there are many taxonomical issues related to marine mammal *Brucella* isolates that may lead to the description of new species and/or biovars in the near future. Transmission routes in the marine environment are unknown, and the role of fish as reservoirs has to date not been investigated.

The recent discovery of *B. microti* and *B. inopinata* poses new challenges on our understanding of brucellosis at the wildlife/livestock/human interface.

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CHAPTER

25

ANTHRAX

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Anthrax (also known as Charbon, Milzbrand) is an infectious disease caused by the bacterium *Bacillus anthracis.* Anthrax has clinico-pathological features similar to haemorrhagic fevers, with hyperacute or acute clinical signs and usually a fatal outcome.

AETIOLOGY

Bacillus anthracis belongs to the class *Bacilli*, order *Bacillales,* family *Bacillaceae,* genus *Bacillus. Bacillus anthracis* is a Gram-positive, aerobic, immobile, capsulated bacterium and forms spores, which are 1–1.5 µm wide and 5–6 µm in length. In tissue the bacterium is found singly, as clusters or as short chains, whereas from colonies, *B. anthracis* cells with square ends are arranged in long chains that gives them a particular look, similar to 'bamboo canes'. Outside the body in environmental temperatures between 14 and 42°C (optimum between 21 and 37°C) *B. anthracis* will sporulate. The spores are oval, and are released after lysis of the bacterium. Vegetative forms are inactivated within 30 minutes at 60–65°C. The spores are sensitive to destruction with 2–3% formaldehyde solutions at 40°C for 20 minutes, 0.25% at 60°C for 6 hours or 4% after contact of at least 2 hours, and are destroyed by 5% phenol and mercury chloride and 1% solutions of caustic soda and potash.

EPIDEMIOLOGY

Anthrax is a disease known to humans since ancient times, and the first reports appear in the Bible (Exodus, Chapters 7 to 9), as the characteristics of the fifth and sixth plagues are identifiable with anthrax. Between the late 19th and early 20th centuries anthrax was one of the infectious diseases identified with major mortality among domestic and wild animals. Probably the most serious incident occurred in 1923 in South Africa, where in one year it killed between 30,000 and 60,000 animals. The disease has lost its importance, now being of more sporadic occurrence in Europe. It is now an uncommon disease in much of Western Europe, Northern America and Australia. One exception is endemic foci in wild fauna in the African national parks[1]. In Europe, the major enzootic areas are Greece, Spain, Turkey, Albania, France and Southern Italy (Table 25.1).

Herbivores are particularly susceptible to anthrax infection, whereas omnivores and carnivores are moderately resistant but still succumb. Among herbivores, in addition to domestic species including donkeys and mules, wild

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TABLE 25.1 Anthrax outbreaks in wild animals in the last 15 years in Europe.

Country	Year	Animal species	Number of outbreaks	Number of deaths
Italy	1998	*Cervus elaphus*	1	4
Russia	1999	*Rangifer tarandus*	1	1
Ukraine	1999	*Rangifer tarandus*	1	1
Hungary	2002	*Capreolus capreolus*	1	1
Italy	2004	*Cervus elaphus*	2	8

Data from the World Organisation for Animal Health (OIE) and Promed

species are also susceptible – in particular, zebras and species of wild ruminants such as cervids (*Odocoileus virginianus, Cervus canadensis, Alces alces, Cervus elaphus, Rangifer tarandus, Capreolus capreolus)*, gnu (*Connochaetes taurinus, Connochaetes gnou),* buffalo (*Syncerus caffer, Bubalus bubalis, Bison bison)*, gazelles (*Gazella* spp., *Eudorcas* spp., *Nanger* spp.), antelopes (several genera) and kudu (*Tragelaphus imberbis, Tragelaphus strepsiceros*). Several anthrax outbreaks that occurred in Southern Italy showed that red deer are highly susceptible to infection with *B. anthracis* and that the mortality rate in these deer could be even higher than that observed in domestic animals[2]. Pigs and scavengers are more resistant to the disease than herbivores, and they must ingest a large number of bacilli bacteria to become infected and develop disease. Generally birds are resistant, but outbreaks have been seen in wild and commercial flocks of ostriches; their body temperature is lower than other avians, and this is considered to be the reason for their susceptibility.

Anthrax is not transmitted from infected individuals to healthy animals but by the ingestion of spores dispersed into the environment. *Bacillus anthracis* is known for its rapid proliferation and dissemination in receptive hosts, but little is known about its ability to grow and reproduce outside the animal. It is clear that in the external environment, where conditions are less favourable to the survival of vegetative forms, *B. anthracis* spores are one of the most advanced type of resistant life forms known in nature and can persist in soil for many years. Severe outbreaks of anthrax develop mainly during the dry months that follow a prolonged period of rain in the spring. These climatic aspects suggest that water plays an important role in the ecology of the bacterium. Rainwater, having washed the ground surface, tends to stagnate in the lower-lying areas, concentrating spores there. This sequence of events encourages the adhesion and distribution of spores on soil humus, so the chances to infect herbivores increase[3]. It is widely believed that the vegetative forms of *B. anthracis* sporulate when exposed to oxygen. Under these conditions it is assumed that in an intact carcass, putrefactive processes should destroy virtually all bacteria in a period of time ranging from 48 to 72 hours. However, rarely in nature are carcasses of dead animals left undisturbed by scavengers (Figure 25.1). Carnivores and scavengers are considered to be less susceptible to the disease than herbivores, and the vegetative cells do not survive transit through their more acidic stomach. However, by scavenging older carcasses containing spores, they may spread viable spores in their faeces. In rural areas, avian scavengers such as ravens (*Corvus corax*) and vultures can contaminate pastures or small bodies of water far from the original outbreak by spreading spores of anthrax in their faeces. Ticks collected from terminally ill animals have been found to contain *B. anthracis*[4], but they do not seem to play a significant role in the transmission of the disease, as inter-host transference of adult ticks is most unlikely. Laboratory studies have shown that stable flies (*Stomoxys calcitrans*) and the mosquitoes *Aedes aegypti* and *A. taeniorhyncus* are able to transmit the infection[5]. The role of the tabanid fly (*Haematobia irritans*) in the spread of the disease was confirmed in two old scientific papers[6,7]. Latterly tabanid flies are seen to be of greater importance[8]. Although non-biting flies can act as carriers of *B. anthracis* spores, they generally do not play an important role in the epidemiology of the disease for grazing animals, but are an important source of infection for browsers. Recently it was demonstrated that *Musca domestica* infected by feeding on anthrax-infected rabbit carcass can eliminate anthrax spores in their faeces and vomitus. It was also observed that anthrax spores were able to germinate and replicate in the gut content of insects, confirming the role of insects in spreading anthrax infection[9].

PATHOGENESIS, PATHOLOGY AND IMMUNITY

Bacillus anthracis expresses its pathogenic action mainly through the capsule and the production of a toxic complex consisting of three proteins, protective antigen (PA), lethal factor (LF) and oedema factor (EF). The toxic factors result from two plasmids: pXO1(182 kb), carrying the genes encoding for EF, LF and PA and pXO2 (96 kb),

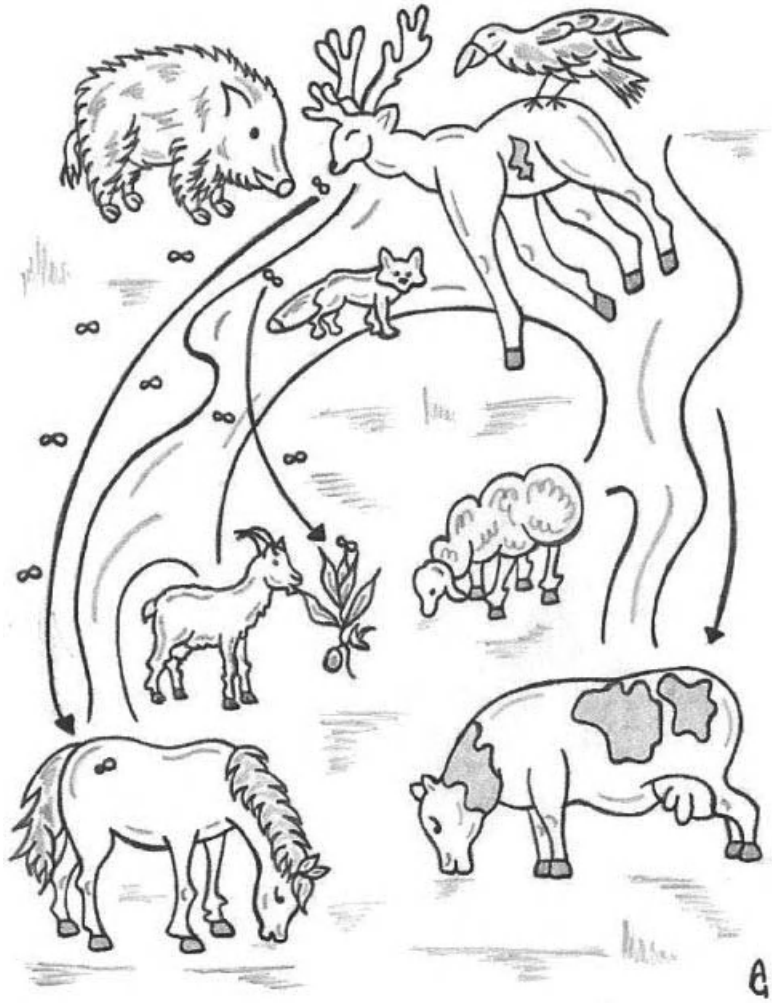

FIGURE 25.1 Anthrax cycle in rural areas of Europe. This cycle involves wild animals and domestic animals. Wild animals die from anthrax and the carcass is opened by scavengers. This permits the sporulation of *Bacillus anthracis*. Spores are disseminated in the environment by carnivores, wild boar and scavenger birds. Blowflies infect directly receptive animals (horses and cattle). Contaminated water could contribute to the increase of the contamination level of pasture. Rainwater, having washed away the surrounding ground, tends to stagnate in low-lying parts, favouring the concentration of spores. Flies could contribute to environmental contamination. This sequence of events encourages the adhesion and distribution of spores on soil humus, increasing the chances of infecting herbivores.

carrying the genes encoding for the biosynthesis of the capsule. The PA binds to cell-surface receptors and plays a fundamental role in pathogenesis. It has no direct toxic action but acts as a 'mover' of the other two toxic proteins into cells. The capsule, a linear polymer of γ-D-glutamic acid, is considered the other major virulence factor of *B. anthracis*. The capsule contributes to pathogenicity by enabling the bacteria to resist phagocytosis by macrophages and thus evade the host immune defences and promote septicaemia.

The most frequent method of penetration by spores is via the digestive system after the ingestion of spore-contaminated feed, forages and water. The portals of entry are micro-wounds that can be found in the mucous membranes of the mouth, pharynx and along the entire gastrointestinal tract. The infection can also occur through skin abrasions or skin lesions that may be caused by haematophagous insects (e.g. biting flies) acting as passive carriers or biological vectors. The severity of the disease depends on the sensitivity of the host, on the size of the infectious dose and on the route of penetration. It is considered that the spores of *B. anthracis* are carried by macrophages from the initial site of entry to the draining lymph nodes. The spores germinate, giving rise to

vegetative forms that are capable of producing the main virulence factors: the toxins and capsule. These enter the bloodstream, where they continue to rapidly multiply. The pathogenicity of *B. anthracis* depends on the quality of the capsular coat and the amounts of toxins produced, and on the susceptibility of the host species.

The principal lesions in septicaemic anthrax are widespread oedema, haemorrhage and necrosis. The nature and the extent of the lesions may vary and are dependent on the route of infection, host susceptibility and the virulence of the bacterium. Thus, in some carcasses, only lesions consistent with septicaemia are evident, whereas in others, localized necrotizing lesions are found.

In ruminants, in which the course of the disease is usually acute or hyperacute, the most consistent changes include: evidence of rapid *post mortem* decomposition of the carcass, with marked bloating soon after death; incomplete development of rigor mortis; oozing of blood or blood-stained fluid from the natural openings such as the nose, mouth and anus; dark-red, poorly clotted blood; petechiae and ecchymoses throughout the body; extensive pulmonary oedema; excessive amounts of blood-tinged serous fluids in the peritoneal, pleural and pericardial cavities; and oedema and haemorrhage in individual lymph nodes. As suggested by the names 'splenic disease' and 'miltsiekte', severe splenomegaly is considered by many to be the most characteristic gross change, but it is not a consistent feature and its absence does not exclude anthrax. Most wild ruminants that die from anthrax manifest a vasogenic brain oedema coupled with the presence in the larger blood vessels of poorly formed and disintegrating *post mortem* blood clots, containing numerous encapsulated bacteria. Severe inflammatory oedema of the soft tissues of the head, tongue and throat, stomach and intestines are characteristic features of anthrax in carnivores. *Bacillus anthracis* septicaemia and bacteraemia are consistent features at death. Innate host resistance to infection by *B. anthracis* appears to be dependent on the inhibition of the initial multiplication stage of infection.

CLINICAL SIGNS AND TREATMENT

The first indication of anthrax is finding dead animals. Herbivores such as cattle, sheep, goats and most wild ruminants mainly manifest peracute and acute clinical signs. The course of the peracute clinical disease is usually less than 2 hours and the acute form less than 72 hours. The majority of animals are found dead without having shown signs of illness. Terminally these animals usually show opisthotonos, with the forelegs rigidly extended. Blood-stained fluid sometimes exudes from the nostrils, mouth and anus (Figure 25.2) but is less common than many texts suggest, both in volume and frequency. Equids suffer from the acute form, with oedematous swelling of the body and sometimes colic. Typically, animals that have died of anthrax do not demonstrate rigor mortis.

FIGURE 25.2 Anthrax in red deer. In nature herbivores are particularly susceptible to anthrax infection, and omnivores and carnivores are moderately resistant but still succumb. Among herbivores, in addition to domestic species such as donkeys and mules, wild species are also susceptible – in particular wild ruminants such as deer. Rarely in nature are carcasses of dead animals left undisturbed by scavengers.

Carnivores, suids and poorly immunized animals usually show subacute to chronic signs, which may extend for more than 3 days before recovery or death. The most frequent signs are oedematous swelling of the face, throat, neck and/or ventral parts of the body. The infection may remain localized or it may progress to a septicaemia, which is usually fatal.

In most herbivores anthrax has so rapid a course that it is not always possible to implement successful treatment. Exposed livestock possibly incubating disease may be treated with long-acting antibiotics, such as oxytetracycline, followed by vaccination with Sterne vaccine 7–10 days later.

DIAGNOSIS

Suspicion of anthrax arises from the observation of clinical signs, the pathological and epidemiological findings. The

ecology of the bacterium limits the distribution of the disease, such that it is almost always confined to well-defined territories in which sporadic outbreaks, usually involving a few animals, occur. The frequency of these outbreaks tends to increase during dry summers that follow very wet springs[1].

LABORATORY DIAGNOSIS

When taking a sample from a suspected anthrax case, one needs to take precautions to prevent human infection, and environmental contamination. The optimum sample is a cotton swab dipped in blood. Putrefaction quickly destroys vegetative *B. anthracis*, which can therefore be difficult to isolate from carcasses just 48 hours after the death, especially in hot weather.

Microscopic Test

A preliminary examination of an unstained fresh blood smear will highlight the presence of stick forms or typical 'bamboo canes'. The organisms are immobile and well capsulated. The slide may be fixed and stained with Gram stain (*B. anthracis* is violet in colour), Giemsa stain (the bacilli are purple and the capsule a characteristic red mauve), MacFadyean stain (pink capsule) or Löffler stain, which uses methylene blue (bacterial bodies stain blue and the capsule reddish).

Cultural Test

Bacillus anthracis grows well in ordinary medium under aerobic or microaerophilic conditions, at temperatures between 12 and 44°C, but optimal growth occurs around 37°C and at a pH of 7.0 to 7.4. On nutrient agar it forms white colonies 3–4 mm in diameter with a rough surface, called 'glass beads' and with irregular margins with medusa head appearance at low magnification. On blood agar it does not cause haemolysis. It is normally sensitive to penicillin. Animal samples for culture include blood, exudates and organs. When suspecting the presence of spores in the material, such as in wool, hair, leather or environmental samples, it is necessary to first incubate the material at 72°C for 30 minutes to destroy contaminating bacteria, yeasts and moulds. It is recommended to use a semi-selective medium to isolate *B. anthracis*; for this, blood-containing media such as TSPB agar are preferable to the often-used PLET or a Knisley agar.

Polymerase chain reaction (PCR) is the method of choice as a parallel diagnostic test, whether performed directly on clinical samples after non-selective enrichment of mixed cultures, or as a confirmation test for suspect colonies. To identify virulent *B. anthracis* strains, and for the differentiation of non-virulent strains, the presence of both of the plasmids pXO1 (toxins) and pXO2 (capsule formation) must be confirmed.

Molecular Characterization

Bacillus anthracis represents one of the most genetically homogeneous bacteria, with strains sharing more than 99% of homologous nucleotide sequences. The high-resolution typing assay *par excellence* is that of the multiple-locus variable number tandem repeat analysis (MLVA), as it seeks to identify specific genomic regions known as variable number tandem repeats (VNTR). This technique, initially with 8 VNTR, was able to identify 89 genotypes from 400 isolates from around the world, whereas the 15 VNTR assay increased this to 221 genotypes with 1033 isolates. This method has now been increased up to 25 loci, which allows an excellent organism discrimination with this high genetic homology.

MANAGEMENT, CONTROL AND REGULATIONS

VACCINES

The veterinary vaccines currently used to control anthrax are composed of spores from attenuated strains of *B. anthracis*. They are classified into two categories:

- live attenuated vaccines, capsulated and without toxins, cap^+/tox^- (e.g. Pasteur vaccine)
- live attenuated vaccines, not capsulated with toxins cap^-/tox^+ (e.g. Sterne and STI vaccines).

The Pasteur vaccine is characterized by a plasmid pattern $pXO1^-/pXO2^+$. The Pasteur vaccine type 1 is not pathogenic for guinea pigs but is pathogenic for mice. The Pasteur vaccine type 2 is pathogenic for both guinea pigs and mice, but is not pathogenic for rabbits. Pasteur vaccines should not produce toxic factors and were used in the past, when they routinely produced a 3% mortality. Now only a few countries use this vaccine.

Sterne vaccine was produced for the first time in 1939[10]. It is very protective. The attenuation is due to the loss of plasmid pXO2, encoding the capsule synthesis; the spores germinate in the vaccinated animals. Non-encapsulated vegetative forms multiply, produce toxic factors and are then neutralized by phagocytes. This allows synthesis of enough toxins to stimulate the protective immunity, but insufficient to damage the host. The animal vaccines that use Sterne strain $34F_2$ are formulated with approximately 10^7 spores per ml suspended in 0.5 ml 50% glycerine-saline. The protective effect of a single dose of strain $34F_2$ vaccine is expected to last about 1 year, and an annual booster is recommended for livestock in endemic areas.

Anthrax control and eradication programmes involve targeted livestock vaccination, farmer awareness and prompt reporting of unexpected deaths, and the correct management of infected carcasses. Incineration *in situ* is the best method to reduce the contamination level of soil. It should be considered a global responsibility to implement anthrax control and eradication programmes, rather than restricting the control to emergency situations.

Vaccination of Wildlife

Administering injectable vaccines to wild animals requires their immobilization or vaccination by darting. Preliminary studies on oral vaccines indicate that they may be protective, but it is difficult to get wild herbivores to take up baits. The recent demonstration of the efficacy of plant-expressed *Bacillus anthracis* protective antigen will open up a new area of research in the mass vaccination of domestic and wild animals that live in high-risk areas for anthrax[11].

PUBLIC HEALTH CONCERN

Humans are usually resistant to acquiring infection, but when infected may show three different clinical forms: cutaneous, respiratory and intestinal.

The cutaneous form begins with the classic malignant pustule most often localized on the face, neck, arms, hands or legs. This is usually seen in high-risk occupations such as farmers, butchers, tanners, wool carders, shearers and veterinarians. At the point of entry of the bacterium, usually a pre-existing scratch, there is a skin redness, which turns into a papule. Characteristically this lesion is not painful. The surrounding area appears hyperaemic and oedematous. The papule develops into vesicles that spontaneously, or from scratching, break, and eventually it is covered with a black eschar.

Cutaneous anthrax is easily treatable with antibiotics, but if a pustule is neglected it may evolve into a fatal septicaemia. Untreated, approximately 10% of cutaneous cases result in death.

Intestinal anthrax results from the consumption of contaminated meat. The symptoms include nausea, loss of appetite, vomiting and fever followed by abdominal pain, vomiting of blood, severe diarrhoea, lesions and soreness in the throat, difficulty swallowing, and marked swelling of the neck and regional lymph glands. Intestinal anthrax results in death in 25 to 60% of cases[12]. The intestinal form is less frequent and occurs in developing countries or in areas where there is limited food safety hygiene.

The respiratory or pulmonary form of anthrax is the major cause of atypical haemorrhagic pneumonia, starting with flu-like symptoms and characterized by fever, muscle pains, coughing, red nose and bloody sputum. Untreated cases are fatal. Respiratory function is compromised by mediastinal expansion, large pleural effusions, and haematogenous and lymphatic vessel spread of *B. anthracis* into the lungs with consequent pneumonia. The central nervous system and intestines manifest similar haematogenous spread, vasculitis, haemorrhages and oedema[13].

SIGNIFICANCE AND IMPLICATIONS FOR ANIMAL HEALTH

In Italy, the European country in which anthrax has been most active recently, three main outbreaks involving domestic animals and humans during the last 14 years developed in areas inside the natural national parks. This epidemiological aspect results from a lack of removal of the carcasses of dead animals in wild areas, whereas in agricultural areas humans interrupt the cycle of *B. anthracis* by removing carcasses quickly. Thus wild areas could be considered the natural habitat of *B. anthracis* and the place of spore production. We can define 'rural area' as the zone located between agricultural and wild areas, where human activity is usually limited to the use of pastures. It represents the contact point between the wild and the agricultural worlds, and the habitat in which domestic and wild animals share the same areas. The proximity to the sources of production of anthrax spores, in the wild area, produces a level of contamination of soil, favouring the

sequence of events that gives rise to the disease in domestic animals in this area.

In disease-endemic areas, susceptible wild animals could represent a potential amplification factor for *B. anthracis* spores and increase the probability of outbreaks in domestic animals and in humans. There is a need to evaluate the safety and efficacy of *B. anthracis* vaccines in wild animals, especially deer, and investigate the inclusion of wild ruminants in the anthrax prophylaxis programmes.

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CHAPTER

26

CHLAMYDIACEAE INFECTIONS

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INTRODUCTION

Members of the family *Chlamydiaceae* cause multisyndromic disease in a wide range of species of mammals and birds, both wild and domesticated. These are, within the genus *Chlamydophila,* the species *Chlamydophila abortus*, *Chlamydophila caviae*, *Chlamydophila felis*, *Chlamydophila pecorum*, *Chlamydophila psittaci*, *Chlamydophila pneumoniae,* and within the genus *Chlamydia,* the species *Chlamydia muridarum* and *Chlamydia suis.* All members of the family *Chlamydiaceae* are Gram-negative obligate intracellular bacteria. Similar to most viruses but unlike most bacteria, the members of this family replicate only within host cells. The severity and type of disease caused by chlamydia in mammals and birds depends on the chlamydial species and strain (and its species of origin) together with the susceptibility of the host species.

Two different developmental stages can be identified during the cycle of infection and replication of chlamydiae. The 'elementary body' (EB) is a small, electron-dense, spherical body of 0.2–0.6 µm in diameter, and is adapted to survive in the external environment. It is the infectious form of the organism, which binds on receptors of susceptible target cells and is internalized by endocytosis and/or phagocytosis. Chlamydia inhibit the fusion of the endosome and the lysosome; hence the whole life cycle of chlamydiae occurs within the endosome. Within the host cell endosome the EB transforms into the 'reticulate body' (RB). RB (up to 1.5 µm in diameter) are metabolically active and pass through multiple rounds of binary fission. Some days after infection of the host cell, RB transform back into metabolically inactive, infectious EB that are released through the endosome, and host cell rupture or the inclusion is extruded by reverse endocytosis. Intermediary forms between EB and RB exist. Many chlamydiae co-exist in a subclinical state within specific vertebrates. Chlamydial endosymbionts (e.g. *Neochlamydia*, *Parachlamydia*, *Waddlia*) have been detected in free-living amoebae, suggesting that these hosts provide a vehicle and natural reservoir. Their role as source of infection in humans and animals is under discussion.

In production agriculture, diseases due to *C. psittaci*, *C. abortus*, *C. pecorum* and *C. suis* are of main relevance. *Chlamydophila psittaci* is the zoonotic agent of psittacosis/ornithosis and affects principally birds. *Chlamydophila abortus* primarily infects ruminants, principally sheep and goats and in some European countries is the most significant cause of infectious abortion in these species (e.g. epizootic/enzootic abortion in sheep). Strains of *C. pecorum* cause a number of disease syndromes in ruminants, koalas (*Phascolarctos cinereus*) and pigs. *Chlamydophila suis* causes respiratory disease, enteritis and conjunctivitis in swine.

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Diseases caused by chlamydia also occur in reptiles, amphibians and fish. Chlamydial infection of mammals and birds may result in systemic disease, pneumonia, abortion, rhinitis, conjunctivitis, arthritis, enteritis, or no apparent disease.

PSITTACOSIS/ORNITHOSIS

Avian chlamydiosis (psittacosis or ornithosis) is a contagious, often systemic disease, caused by the bacterium *Chlamydophila psittaci* (previously *Chlamydia psittaci)*. The organism causes a variety of clinical syndromes in a wide range of domesticated and wild animals and birds. Ornithosis refers to disease in, or transmitted from, domestic and wild birds, as opposed to disease in psittacines.

AETIOLOGY

Chlamydophila psittaci is an obligate intracellular bacterium that uses the host cell for energy. It occurs in the cytoplasm of eukaryote cells, forming membrane-bound cytoplasmic inclusions (endosomes).

EPIDEMIOLOGY

GEOGRAPHICAL DISTRIBUTION AND HOSTS

Chlamydophila psittaci primarily infects birds and has been demonstrated in about 465 species of 30 different orders. The disease has a worldwide distribution in wild bird species and may be more prevalent in gregarious and colonial nesting species. It is considered that many species have their own strains of the bacterium and that most species are probably susceptible to natural infection. Human infection was recorded frequently in the 20th century, with psittacines probably the commonest identified source of this infection. Disease occurrences in humans associated with poultry (ducks, geese and turkeys) are infrequent but may be severe. Modern diagnostic methods, in particular polymerase chain reaction (PCR), are extending the known host species range in wild birds and revealing high prevalences of infection within species. Recovery from clinical disease in wild birds may be followed by persistence of infection, with highly virulent strains being carried and extensively shed in the absence of apparent clinical signs in the hosts themselves. *Chlamydophila psittaci* comprises six avian serovars, and all should be considered transmissible to humans and potentially capable of causing disease.

Serovar A is endemic among psittacine birds and has caused sporadic disease in mammals, including humans, and tortoises. Serovar B is usually associated with pigeons but has also been isolated from turkeys. Additionally, it has been identified as the cause of abortion in a dairy herd[1]. Serovar C has primarily been isolated from waterfowl, and Serovar D mainly from turkeys. The host range of Serovar E includes pigeons, ratites, ducks, turkeys and occasionally humans; avian strains only occasionally jump to mammalian hosts.

Serovar F has been recovered from psittacines and turkeys. Two mammalian isolates (WC and M56) originated from epizootics in cattle (WC) and muskrats (*Ondatra zibethicus*; M56), respectively[2].

European studies on *C. psittaci* in feral pigeons (*Columba livia* var. *domestica*) conducted from 1983 to 2007 in Bosnia and Herzegovina, Bulgaria, Croatia, France, Germany, Italy, Spain, Slovenia and Switzerland revealed seroprevalences ranging from 19.4 to 95.6%[3]. Studies performed in the UK using complement fixation test (CFT) serology in archived blood samples from family groups of wild and domesticated birds showed high titres only in pigeons, collared doves (*Streptopelia decaocto*) and psittacines. Of 1,549 pigeons tested, 47.3% were seropositive for *C. psittaci*, whereas wild collared doves (n = 37) showed 51% seropositivity[4]. This study incidentally showed lower titres and prevalences in domesticated poultry, whereas in wild ducks the seroprevalence was 23% and in game birds 29%. In Scotland, *Chlamydophila psittaci* was demonstrated in gulls (*Larus* spp.), blue tits (*Parus caeruleus*), crows (*Corvus frugilegus*), chaffinches (*Fringilla coelebs*) and European robins (*Erithacus rubecula*), with respiratory disease, airsacculitis and hepatitis[5]. Surveys conducted on 20 species of free-living raptors in the Berlin and Brandenburg area, Eastern Germany revealed antibodies to *C. psittaci* in 267/422 (63%) of the sera tested. *Chlamydophila psittaci* was also detected in 74% of spleen and lung samples from buzzards (*Buteo* spp., *Pernis apivorus*), hawks (*Accipiter* spp.), falcons (*Falco tinnunculus* and *F. peregrinus*), kites (*Milvus* spp.), white-tailed sea eagles (*Haliaeetus albicilla*) and owls (*Tyto alba*, *Strix aluco*) tested by PCR but *C. psittaci* infection could not be correlated with gross lesions found in the birds[6]. In psittacines the prevalence rate ranges from 16 to 81% of samples collected in various studies, and a mortality rate of more than 50% has been reported. *Psittacidae* are major

reservoirs of chlamydiae, especially in captive populations. An incident in which multiple deaths occurred in a garden over several months in a range of passerine species led to the conclusion that more than one infectious agent was involved[5]. In other incidents, the clinical significance of chlamydial infection detected in tissues sampled at necropsy, e.g. in blue tit nestlings and a gull (*Laridae*), was unclear[5].

Although the role of some chlamydial species in birds, domestic animals and humans is well established, only sparse data are available on the situation in wild mammals. A German study on wild boar (*Sus scrofa*) from Thuringia provides a subtle hint for a possible *C. psittaci* reservoir. *C. psittaci*-DNA was detected in 57% of all animals examined. More than 80% of females were PCR-positive, compared to 43% of male boar. *Chlamydophila psittaci* DNA was amplified most frequently from lung, but also from pulmonary lymph node, intestine and uterus[7]. In clinically healthy alpine ibex (*Capra ibex ibex*) from Switzerland, 31% were found to be antibody-positive for *C. psittaci*[8]. A mass die-off among snowshoe hares (*Lepus americanus*) and muskrats was attributed to *C. psittaci* infection in Canada[9]. Investigations into the normal conjunctival bacterial flora in the North American opossum (*Didelphis virginiana*) and raccoon (*Procyon lotor*) revealed *Chlamydia*-positive PCR results in 18% of opossum and 30% of raccoon samples investigated[10].

HOST FACTORS

A trend from lower seroprevalences in young birds to higher rates in older individuals has been statistically confirmed[11]. A slightly higher seroprevalence in common buzzards (*Buteo buteo*) was attributed to different hunting and feeding behaviour (broad spectrum of prey, including carrion)[11].

EPIDEMIOLOGICAL ROLE OF WILD ANIMALS

Avian strains of *C. psittaci* relatively rarely jump to mammalian hosts, and mammal-to-mammal transmission of these strains is infrequent.

TRANSMISSION

Chlamydiae may be present in the conjunctiva, upper respiratory, urogenital and intestinal tracts of mammals and birds, indicating that transmission may be direct or indirect, via aerosol, contact with excretions and the venereal route. Faecal shedding occurs intermittently and can be activated through stress caused by nutritional deficiencies, prolonged transport, overcrowding, breeding, egg laying, treatment or handling. Periods of excretion during natural infection may vary depending on strain virulence, infection dose and host immune status. In ruminants, shedding of chlamydiae is highest at the time of parturition/abortion. Although transmission is possible by several routes, it primarily occurs by the respiratory route. Ingestion may not be so important, and vertical transmission and transmission by ectoparasites are probably relatively unusual occurrences.

PATHOGENESIS, PATHOLOGY AND IMMUNITY

Chlamydial infections usually produce well-balanced host–parasite relationships. Pathogenesis and the interaction between chlamydiae and the host are poorly understood. Infections of natural hosts usually cause relatively mild disease characterized by chronic, low-level persistent infections stimulating a relatively poor immune response in the host. However, if latently infected hosts are stressed, for example by factors such as those listed earlier, larger numbers of chlamydiae are excreted and clinical disease may result. It was previously considered that avian chlamydial strains were similar, that strains differed in virulence and that the host cleared the pathogen following infection. Now it is believed that strains are highly host-specific and that disease in natural hosts is mild with long recovery periods or persistent infections. Strains that infect other hosts may produce clinical, occasionally severe, disease; however, persistence of infection is not a feature in secondary hosts[12]. The intestinal tract is probably the natural habitat for chlamydiae; however, the organisms multiply in cells of the reticulo-endothelial system, epitheliae of the pharynx, conjunctivae, genital and intestinal tracts, in cells of the placenta and fetus, and in synoviocytes.

Extrapolating from work with poultry, aerosol infections colonize air sacs after several hours, progressing to infection of lung and air sacs. At 48 hours post-infection, blood, spleen, liver and kidney are infected, and this then frequently progresses to infection of muscle, pericardium and bone marrow. Nasal glands, providing moisture for the nasal mucus, and possibly responsible for aerosolized excretions, are infected for prolonged periods. Cells in

TABLE 26.1 Characteristics of *Chlamydiaceae* infections (modified according to Everett, 2000)[1].

	Chlamydophila						Chlamydia	
	psittaci	abortus	pecorum	pneumoniae	felis	caviae	suis	muridarum
Host(s)	Birds, mammals, humans	Ruminants, wild boar, other mammals, humans, reptiles	Ruminants, koala, pigs	Humans, koala, horses, reptiles, amphibians	Domestic cat, reptiles	Guinea pig	Domestic pigs, wild boar	Mice, hamsters
Route of entry	Pharynx, eye, genital tract	Oral, genital	Oral	Pharynx, eye	Pharynx, eye, genital tract	Pharynx, eye, urogenital tract	Pharynx	Pharynx, genital tract
Infected tissues	Conjunctiva, lung, genital, liver, spleen, brain, intestine	Placenta, spleen, fetal liver, intestine	Conjunctiva, bladder, joints, intestine, brain, prostate	Lung, brain, arteries, joints	Conjunctiva, lung, genital, brain	Conjunctiva, lung, genital, bladder	Conjunctiva, lung, intestine	Genital, lung, liver, kidney, spleen, intestine
Typical disease presentation	Respiratory, enteritis	Abortion	Respiratory, conjunctivitis, encephalomyelitis, polyarthritis	Respiratory	Respiratory, conjunctivitis	Conjunctivitis	Respiratory, conjunctivitis, enteritis	Respiratory

these tissues develop inclusions filled with RB. There is increasing evidence that the host's immune response in persistently infected animals significantly contributes to the tissue damage seen[13]. Characteristics of *Chlamydiaceae* infections are given in Tables 26.1 and 26.2.

The range of pathological lesions include exudative serositis, pneumonia, enteritis, splenomegaly, hepatomegaly, endocarditis, bronchopneumonia, airsacculitis and encephalitis[16]; however, these lesions are considered to be non-specific. Common features are necrotizing lesions in spleen, liver, pericardium and the respiratory tract. Severity of lesions varies with the virulence of strain, the susceptibility of the host, route of infection and the presence of concurrent disease. Concurrent disease, such as metabolic bone disease, trichomonosis and helminthosis was described in three species of wild columbiforms with chlamydiosis[5]. Pericarditis, pneumonia, perihepatitis, peritonitis and airsacculitis may be found in combination and are lesions characteristic of overwhelming systemic infections. In natural hosts infected by an endemic strain of chlamydia, the lungs rarely show lesions; however, airsacculitis is frequently present.

Microscopic examination confirms the appearance of gross lesions; however, there is a greater appreciation of the variation in the severity of these lesions (Table 26.2). Multi-focal splenic and hepatic necrosis is seen. Splenic lymphocytes are depleted and replaced by swollen macrophages. At the periphery of the necrotic lesions, basophilic inclusion bodies can be seen in the cytoplasm of hepatocytes, capillary epithelia and bile duct cells[12]. Inclusion bodies are present in the cytoplasm of cells within serosal exudates. The organisms may be found in tissues without histopathological lesions in sites such as intestinal epithelium and pancreas. Fibrino-purulent airsacculitis is usually present, sometimes with fibrinous pericarditis. In the lungs there may be a severe inflammatory response with destruction of tissue and occlusion of air spaces with cell debris and fibrinous exudates.

Induced immunity following infection includes systemic, mucosal, humoral and cellular responses and an IgA response to infection in epithelial cells.

CLINICAL SIGNS AND TREATMENT

Chlamydial infections can produce localized conjunctivitis, dyspnoea, pneumonia and airsacculitis, enteritis, diarrhoea, encephalitis, polyarthritis, anorexia, emaciation

TABLE 26.2 Incidents of chlamydial (chlamydophilosis – *Chlamydophila psittaci*) infection recorded by wildlife disease surveillance schemes in the UK 1996–2010.

Year	Number of incidents, species and mortality	Location	Clinical signs	Pathology	Notes	Reference
2010	Immature feral pigeon (*Columba livia*) × 1 Immature jackdaw (*Corvus monedula*) × 1	Scotland Scotland	Unable to fly, thin body condition Moribund, lethargic, thin body condition, vent feathers soiled with faeces	Fibrinous pericarditis, perihepatitis, airsacculitis, enteritis Intestines distended with green semi-solid content	Clinical avian chlamydiosis (chlamydophilosis) Clinical avian chlamydiosis	14 14
2009	Dunnock (*Prunella modularis*) × 3 Robin (*Erithacus rubecula*) × 1 Great tit (*Parus major*) × 3	Southern England	Not given	Not given	Clinical avian chlamydiiosis	14
2008	Immature chaffinch (*Fringella coelebs*) × 1 Robin × 1		Not given	Large plaque on air sac, fibro-necrotizing airsacculitis Hepatosplenomegaly, area of hepatic necrosis, airsacculitis	Clinical avian chlamydiosis Immunohistochemistry showed association between *Chlamydophila* and lesions	14
2006–2007	No reports					
2005	Feral pigeon × 3	Scotland	Not given	Not given	Clinical avian chlamydiosis	14
2004	Robin × 3 over 3 weeks Collared dove (*Streptopelia decaocto*) × 1 Collared dove (*Streptopelia decaocto*) × 1	East England Scotland Scotland	Found dead Not given Not given	Histopathology – typical lesions Not given Not given	Small outbreak of clinical avian chlamydiosis	14 14 14
2003	Feral pigeon × 1 Rook (*Corvus frugilegus*) × 1	Scotland Scotland	Not given Not given	Not given Airsacculitis	Bird with concurrent inclusion body hepatitis Bird with an undescribed chronic respiratory syndrome not caused by chlamydiosis	14 15
2002	Blue tit (*Parus caeruleus*) × 1	Scotland	Not given	Not given	Significance of chlamydophilal infection not known	15
2001	Immature rook × 2	Scotland		Airsacculitis, pneumonia pericarditis	Concurrent *Mycoplasma gallisepticum* infection; clinical significance of *Chlamydia* uncertain	15
2000	No reports					
1999	Immature feral pigeons, several birds	Southern England	Not given	Not given	Concurrent salmonellosis	15
1996–1998	No reports					

Diagnosis prior to 2003 by pathology (histopathology), detection of typical bacteria in stained smear preparations and exclusion of other pathogens (by culture); after 2003, diagnosis in addition with PCR. Although limited data, this was from national surveillance, and the sporadic nature of small outbreaks, clinico-pathological findings, concurrent disease and disease in young birds are reflected in these data.

and generalized disease[9,16]. Usually only one of these clinical manifestations will be seen in a given bird or animal under particular environmental or physiological conditions. In both mammals and birds, the severity of disease may vary from clinically inapparent infections to severe systemic infections. Depending on the *C. psittaci* strain and the avian host, pericarditis, airsacculitis, pneumonia, lateral nasal gland adenitis, peritonitis, hepatitis and splenitis can develop but may be difficult to assess clinically. Generalized infections result in fever, anorexia, lethargy, diarrhoea and occasionally shock and death. Chronic infections often develop in psittacine species and pigeons, with poor body condition and sometimes the loss of the ability to fly reported. Non-specific clinical signs including lethargy, anorexia, ruffled feathers, discharges and diarrhoea; weight loss in chronic cases also occurs. Diarrhoea is frequently present and may be green in colour, mucoid and profuse, giving rise to staining of the vent feathers. All these findings are non-specific and non-diagnostic in themselves. See Table 26.2 for reported clinical signs.

Treatment of birds is usually by using tetracyclines – in particular, doxycycline.

DIAGNOSIS

Diagnosis of chlamydial infections can be difficult; the clinical signs and gross pathological lesions are not sufficiently specific to allow diagnosis, and PCR, serology, antigen detection or demonstration of chlamydia are required for diagnosis. Serology is based on enzyme-linked immunosorbent assay (ELISA), micro-immunofluorescence test and CFT used for antibody detection with variable specificity and sensitivity. Detection of chlamydial antigens in tissues of dead animals, oculonasal and cloacal swabs, and faeces can be performed using ELISA or immunofluorescence staining techniques. Direct microscopy can be useful as a screening test, in which detection of the typical intracytoplasmic inclusions in tissue cells and cells in exudative discharges can support suspicions of disease. Cytology is performed on stained smears, e.g. with Giménez, Stamp or Macchiavello. PCR technology is improving detection sensitivity of the organism in clinical material (faeces and bronchial exudates) and more particularly in cases of subclinical infection, with results already indicating higher prevalences of infection that previously indicated with other diagnostic tests. PCR and microarray genotyping make species identification of chlamydiae a routine process. Extracellular culture has never been achieved; however, culture is possible in cell culture, with embryonated chicken eggs, but is difficult and requires biosafety level 3 laboratories. CFT serology has been used historically for general surveys on archived avian blood samples, including those from wild birds; however, the test has limitations, seen with non-specific reactions and the choice of a reliable cut-off value. Published references should describe these limitations (for example[4]). Detailed guidelines for the diagnosis of avian chlamydiosis are available in the World Organisation of Animal Health (OIE) *Manual of Diagnostic Tests and Vaccines for Terrestrial Animals*[17].

MANAGEMENT, CONTROL AND REGULATIONS

Animals with chlamydiosis should be handled in such a way that infection is not transmitted to humans or other animals. Known infected captive birds or animals should be managed in isolation. Strict sanitation measures and disinfection protocols are recommended, and occasionally it may be preferable to euthanize infected birds rather than risk human health. Columbiform species, including feral pigeons (*Columba livia*) and psittacines, in view of the high zoonotic potential of their respective chlamydial serotypes and high prevalences of infections, should be necropsied in biosecure cabinets. Prevention of chlamydiosis in captive displays should be based on quarantine, testing of newly introduced animals and regular group monitoring, for example by pooled faeces PCR testing.

Psittacosis in humans and psittacines is a notifiable disease in many European countries, Australia and the USA, and specific national requirements laid down by epizootic disease legislation together with legislation for the importation of animals must be adhered to. The high prevalence of chlamydiae in free-ranging animals suggests that wildlife hosts, particularly ungulates and feral columbiformes, should be considered in the control of disease caused by this pathogen[18].

PUBLIC HEALTH CONCERN

There is little secondary infection between humans. The zoonotic potential of *Chlamydiaceae* has to be considered

when handling or working with animals suspected of having chlamydial infections. Cleaning cages and enclosures as well as *post mortem* investigations should be carried out using appropriate personal protective equipment (PPE), or in biosecure cabinets. Recently, game was identified as a potential reservoir of *Chlamydophila* and a possible source of human infection. Hunters and people who handle game should be aware of the infection risk, and appropriate hygiene is recommended in order to minimize the risk of disease transmission to humans. Although feral pigeons are usually subclinically infected, in contrast collared doves more frequently develop clinical disease and therefore have a specific but largely unassessed potential to infect humans[19]. An outbreak of chlamydiosis occurred in women of the Faroe Islands through preparing subclinically infected fulmar (*Fulmarus glacialis*) for human consumption. Several authors[5] point out that members of the public should be made aware of the zoonotic risks from handling the carcases of wild birds, including garden birds, both from the chlamydial risks and from risks from salmonella and other zoonotic agents. This advice was extended to stressing the importance that strict management measures for personal hygiene and disease prevention were adopted in wildlife rehabilitation centres to minimize the spread of chlamydiae between species, including humans. The debate as to which wild species of birds pose consistent and real health risks to humans and domesticated species has not been answered. The role of pigeons as a source of disease in humans is still the subject of scientific debate. The columbiform family has a high prevalence of chlamydial infection; however, some authors consider they are of lesser concern[12], although there may be increased occupational risks (for example in steeplejacks). Other authors consider feral pigeons to be a significant source of infection for humans. The apparent higher levels of clinical disease in collared doves is also relevant. This latter species may be susceptible as a result of the significant increase in its geographical range in the past 100 years; however, there are very few reports of disease in humans acquired from this species. A lack of data has prevented a quantitative risk assessment, but in this context the development of PCR chlamydial testing for the first time may allow an opportunity to accurately assess prevalence of infection in respective wild species, and in purported avian populations suspected to be the source of human disease.

SIGNIFICANCE AND IMPLICATIONS FOR ANIMAL HEALTH

The effect of chlamydial infection on wild bird populations has yet to be assessed. Clearly outbreaks of disease with relatively high morbidity and mortality have been described in passerines, particularly garden passerines and corvids, and in members of the pigeon family. The nature of the disease in these species often appears to be chronic, and birds found dead and submitted for examination are few compared with the estimates of birds seen ill. It is therefore likely that sick birds die in areas unseen, or are removed by predators. Mortality in these reported outbreaks is probably underestimated, and increasingly there appear to be associations with concurrent disease. For example, a corvid respiratory disease syndrome has been noted in the UK for nearly a decade, consisting of airsacculitis and pericarditis, but only from a small number of individuals has chlamydia been detected. In this syndrome *Pasteurella multocida* is considered to be aetiologically important and chlamydial infection considered to be incidental. The frequency of clinical disease in collared doves has suggested that this species may be the source of infection for other wild birds – for example, robins (*Erithacsu rubecula*)[19]. In a large serological study it was found that housed chickens were seronegative, but low and consistent titres were found in poultry raised outdoors, suggesting that infection in outdoor poultry originated from exposure to wild birds[4].

OTHER CHLAMYDIAE

Chlamydophila abortus strains colonize the placenta and are primarily associated with cases of abortion and weak neonates, particularly in sheep and goats. The agent is endemic among ruminants (goats, sheep, cattle) worldwide and has also been associated with cases of abortion in a horse, a rabbit, guinea pigs, mice and swine. *Chlamydophila abortus* is a zoonotic pathogen and may cause abortion in women. Recently, *C. abortus* has been associated with gammopathy in the protein electrophoresis pattern in Spanish common (*F. tinnunculus*) and lesser kestrel (*F. naumanni*) nestlings. A concurrent *Chlamydophila* outbreak among sheep led to the suggestion of cross-infection between livestock, insects and a kestrel in the same area[20]. *Chlamydophila abortus* was also detected

in pulmonary lymph nodes, uterus and placenta of free-ranging wild boar in Germany[7]. High prevalence of antibodies against *C. abortus* and *Chlamydiaceae* was shown in wild ungulates from Spain, including wild boar, red deer (*Cervus elaphus*), roe deer (*Capreolus capreolus*), fallow deer (*Dama dama*), mouflon (*Ovis aries musimon*), Barbary sheep (*Ammotragus lervia*), southern chamois (*Rupicapra pyrenaica*) and Iberian ibex (*Capra pyrenaica*)[18]. A case of abortion caused by *C. abortus* in a springbok (*Antidorcas marsupialis*) has been reported from Paris Zoo[21]. Infections due to *C. abortus* in ruminants may result in fever as high as 41°C on day 1 or 2, which may last for 3–5 days. In sheep, abortions usually occur during the last month of gestation. In cattle, abortions occur in the last trimester and are usually sporadic. Abortion rates may vary between 1 and 5% in chronic infected herds and 30% in flocks with recently introduced *C. abortus*. Infection occurs via inhalation or ingestion and first affects the tonsil, from which it spreads via the bloodstream to other body sites. Placental infections establish between days 60 and 90 of gestation, with pathological lesions first seen after 90 days, and include loss of chorionic epithelial cells, fibrino-purulent arteritis, necrosis and sloughing of the endometrial epithelium. Infection of the fetus occurs secondary to placentitis, but the mechanism by which *C. abortus* migrates from the maternal side of the placenta to the fetus is still uncertain. Inflammation and necrotic foci are found in most fetal organs and tissues. Infected fetuses usually reveal enlarged popliteal and mesenteric lymph nodes having a demarcated cortex with follicles and germinal centres[22]. The commercially available IDEXX Chlamydiosis Total Ab Test is an indirect ELISA for the detection of antibodies against *C. abortus* in serum and plasma of ruminants. The guidelines for the diagnosis of enzootic abortion in ewes are given in the OIE *Manual of Diagnostic Tests and Vaccines for Terrestrial Animals*[15,23].

Chlamydophila pecorum strains are serologically and pathogenically diverse. Isolation is from mammals only: ruminants, koalas and swine. In koalas, reproductive disease, infertility, and urinary tract disease have been described. In other mammals, the agent causes abortion, conjunctivitis, encephalomyelitis, enteritis, pneumonia and polyarthritis[1]. *Chlamydophila pecorum* may produce diarrhoea in ruminants after oral infection. Polyarthritis/polyserositis due to *C. pecorum* is characterized by subcutaneous oedema and fluid-filled synovial sacs. Swollen joints contain a greyish-yellow turbid synovial fluid. Histopathological lesions are primarily inflammatory changes in the synovium, tendon sheaths and subsynovial tissues. Syndromes associated with chlamydial infections in the koala include keratoconjunctivitis with serous ocular discharge and mild blepharospasm, cystitis, increased frequency of urination and dysuria, and infertility[13].

Chlamydophila pneumoniae infects humans, koalas and horses, primarily causing respiratory disease. Koala isolates are most often recovered from ocular and urogenital sites, where they appear less pathogenic[1]. Chlamydiosis in the koala is the most intensively studied chlamydial infection of wild mammals worldwide, and the koala biovar of *C. pneumoniae* has been found in all Australian free-ranging koala populations that have been investigated.

Chlamydophila felis is endemic among domestic cats worldwide. The infection is characterized by severe conjunctivitis, blepharospasm, hyperaemia, chemosis and serous to mucopurulent ocular discharge, often concurrent with mild respiratory signs, slight nasal discharge and sneezing. *Chlamydophila felis* may also colonize the gastrointestinal and reproductive tract. Transmission of *C. felis* is by direct contact with infected secretions and droplet infection. Clinical signs develop within 4 days and gradually subside after 30 days post-infection, but long-term persistency has been suggested. Zoonotic infection of humans has been described[1].

Chlamydophila caviae primarily infects the mucosal epithelium, is non-invasive and is markedly species-specific for guinea pigs (*Cavia cobaya*)[1]. The natural site of infection is the conjunctiva, but *C. caviae* also infects the genital tract of guinea pigs.

Chlamydia suis strains have only been isolated from swine. In experimental studies *C. suis* caused subclinical intestinal infections in young weanling pigs, with intestinal lesions but without diarrhoea. *Chlamydia suis* has also been associated with conjunctivitis, pneumonia and a high proportion of apparently subclinical infections[1]. *Chlamydia suis* was detected in lung and pulmonary lymph nodes of free-ranging wild boar in Germany[7].

Chlamydia muridarum has been isolated from mice and hamsters. The strain from hamsters is not known to cause disease, but pneumonia has been seen in mice[1].The two latter species were recently detected by PCR in blood samples from Spanish common and lesser kestrels nestlings but have never been recorded in birds before[20].

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CHAPTER

27

BORRELIA INFECTIONS

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INTRODUCTION

The *Borrelia* are long, highly motile, helical bacteria, 10–30 µm long and 0.2–0.5 µm wide. They stain only weakly with Gram stain, and are usually visualized by dark-field microscopy. All the *Borreliae* are obligate parasites, labile in the environment and transmitted by arthropod vectors. Species that have been cultivated *in vitro* grow slowly at 30–35°C under microaerophilic conditions, and have complex nutritional requirements.

Borreliae are unique among bacteria in that their genome is comprised of a linear chromosome and a variable number of both linear and circular plasmids. Despite the atypical form of the DNA, most genes encoded on their chromosome are commonly found in other bacterial genomes. The genes encoded on the plasmid component of the genome, by contrast, appear to be unique to the genus *Borrelia* and show great variation between species and among strains[1].

Classification, identification and phylogeny of *Borrelia* are complex. Before molecular biology, species designation and taxonomy were based on the morphology of the isolate, which vector the spirochaete was found in or which host animal the isolate was infective for. It is hence often impossible to elucidate to which 'modern species' older scientific reports refer.

Today, the genus *Borrelia* consists of at least 33 different species, of which 15–16 are currently regarded as part of the Lyme borreliosis spirochaetes group (LBS), whereas the remaining may be divided into a cluster of Old World relapsing fever spirochaetes (ORFS), a cluster of New World relapsing fever spirochaetes (NRFS) and a recently described monophyletic group of reptile-associated *Borrelia* (Table 27.1). The LBS are transmitted principally by hard ticks in the *Ixodes* group, whereas the RFS are transmitted principally by soft body ticks in the genus *Ornithodoros*. Future data may, however, challenge current concepts and divisions, and there seem to be at least one intermediate group of borrelial spirochaetes. *Borrelia miyamotoi* sensu lato, a species that genetically bears many similarities to NRFS, is for example isolated from both *Ixodes persulcatus* and *I. ricinus* in Asia and Europe[12], and the closely related NRFS *B. lonestari* and *B. theileri* in North America are transmitted by ixodid ticks.

LYME BORRELIOSIS

Lyme borreliosis (LB) is a rapidly emerging tick-borne, complex multisystemic disorder caused by *Borrelia burgdorferi* sensu lato (sl) complex, here also referred to as Lyme borreliosis spirochaetes (LBS). In Europe, this

Infectious Diseases of Wild Mammals and Birds in Europe, First Edition. Edited by Dolores Gavier-Widén, J. Paul Duff, and Anna Meredith.

TABLE 27.1 Recognized and proposed species in the genus *Borrelia* in 2010.

Group	Species	Important (suspected) vector(s)	Typical reservoir host(s)	Disease in Europe	Distribution
Lyme borreliosis spirochetes (*B. burgdorferi* sensu lato)	***B. burgdorferi* ss**	*I. ricinus, I. scapularis*	Rodents, birds	Human LB (arthritis)	Europe, North America
	B. afzelii	*I. ricinus, I. persulcatus, I. hexagonus?*	Rodents	Human LB (erythema migrans, borrelial lymphocytoma, acrodermatitis chronica atrophicans)	Eurasia
	B. garinii	*I . ricinus, I. persulcatus, I. uriae*	Passerines, pheasants, seabirds	Human LB	Eurasia, circumpolar
	'B. bavariaensis'[a]	*I. ricinus*	Rodents	Human LB (neuroborreliosis)	Europe
	B. valaisiana	*I. ricinus, I. columnae*	Birds	Human LB?	Eurasia
	B. lusitaniae	*I. ricinus*	Lizards, birds, hedgehogs	Human LB (cutaneous)?	Southern and Central Europe, North Africa
	B. spielmanii	*I. ricinus*	Dormice (Gliridae)	Human LB?	Europe
	'B. bissettii'	*I. spinipalpis, I. pacificus, I. scapularis, I. ricinus*[b]	Wood mice, other rodents, lagomorphs	Human LB?	Central Europe[b], North America
	'B. andersonii'	*I. dentatus*	Cottontail rabbits (*Sylvilagus* spp.)	Not known	North America
	'B. californiensis'	*I. jellisonii, I. spinipalpis, I. pacificus*	Kangaroo rat (*Dipodomys californicus*), mule deer (*Odocoileus hemionus*)	Not known	California
	'Borrelia kurtenbachii'	*I. scapularis*	*Microtus pennsylvanicus*, other species?	Not known	Illinois, New York, Nova Scotia
	'B. carolinensis'	*I. minor*	Cotton mouse, Easter wood rat	Not known	Southeastern USA
	'B. americana'	*I. pacificus, I. minor*	Rodents, birds	Not known	USA
	B. tanukii	*I. tanuki*	Rodents, racoon dogs?	Not known	Japan
	B. turdi	*I. turdus*	Birds	Not known	Japan
	B. japonica	*I. ovatus*	Shrews and rodents	Not known	Japan
	B. sinica	*I. ovatus*	Chinese white-bellied rat (*Niviventer confucianus* (and other rodents?))	Not known	Central China, Nepal
	'B. yangtze'	*I. granulatus, I. nipponensis, Haemaphysalis longicornis*	Rodents	Not known	East Asia
Old World relapsing fever spirochetes	*B. recurrentis*	*Pediculus humanus*	Humans	Human relapsing fever	Ethiopia, Sudan, Somalia
	B. duttoni	*Ornithodoros moubata moubata*	Humans (pigs, chickens?)	Human relapsing fever, abortion, neonatal loss	East and Central Africa, Madagascar
	B. crocidurae[c]	*O. sonrai*	Rodents, insectivores	Human relapsing fever (mild)	Sahel, West and North Africa, Middle East(?)
	B. hispanica	*O. erraticus*	Rodents	Human relapsing fever, neurological	Morocco, Algeria, Tunisia, Southern Europe
	B. graingeri[*]	*O. graingeri*			
	B. baltazardii[*]			Human relapsing fever?	Iran
	B. persica[d]	*O. tholozani*		Human relapsing fever	Middle East, Central Asia
	B. caucasica[*]	*O. asperus (verrucosus)*	Rodents?	Human relapsing fever	Caucasus, Iraq
	B. latyschewi[*]	*O. tartakovskyi*	Rodents? Reptiles?	Human relapsing fever (mild)	Central Asia, Iran
	B. harveyi[*]		Monkeys?		
	B. tillae[*]	*O. zumpti*	Rats	Unknown	South Africa

New World Relapsing Fever Spirochetes	***B. anserina***	*Argas* spp.	Birds	Avian borreliosis in domestic fowl	Worldwide in tropical and subtropical areas
	B. turicatae	*O. turicata*	Rodents	Human relapsing fever	Southwestern USA
	B. parkeri	*O. parkeri*	Ground squirrels, prairie dogs	Human relapsing fever	Western USA
	B. hermsii	*O. hermsi*	Chipmunks, tree squirrels, birds	Human relapsing fever, disease in wild birds?	Western USA and Canada
	B. coriaceae	*O. coriaceus*	Mule deer, black-tailed deer (*Odocoileus hemionus*)	Unknown[e]	Western USA
	B. mazzottii[*]	*O. talaje*[f]			Central America
	B. venezuelensis	*O. rudis*[f]		Human relapsing fever (severe)	Central and South America
	B. brasiliensis[*]	*O. brasiliensis*			
	B. dugesii[*]	*O. dugesi*[f]			Central and South America
Intermediate group	***B. miyamotoi* sensu lato**	*Ixodes persulcatus, I. ricinus, I. scapularis*	Rodents	Unknown	Japan, Sweden, France, Germany, USA
	B. theileri	*Rhipicephalus (Boophilus)* spp. and *Rhipicephalus* spp.		Bovine borreliosis	North America, Southern Africa, Australia
	'B. lonestari'[g]	*Amblyomma americanum*	White-tailed deer (*Odocoileus virginianus*), turkeys?	Master's disease = southern tick-associated rash illness (STARI)	North America
Possible 'new' species	*'B. mvumii'*	*O. porcinus*	Pigs?	Human relapsing fever?	East Africa
	'B. davisii'	*Dermacentor variabilis?*	White-footed mouse (*Peromyscus leucopusk*)	Not known	North America
	Candidatus B. texasensis	*D. variabilis*?	Not known	Not known	North America
	'B. johnsonii'	*O. kelleyi*[f]	Bats?	Not known	North America
'New' group	*B. turcica*	*Hyalomna aegyptium*	Tortoises	Not known	Turkey

Compiled from Barbour et al. (1986)[3], Felsenfeld (1965)[2], Goubau (1984)[5], Larsson (2007)[4], Postic (2005)[6] and Stanek & Reiter (2011)[7].

Species names in quote marks are not listed in the 'List of Prokaryotic names with Standing in the Nomenclature'[8]

Species written in bold type are, or may become, present in Europe.

[*]Species listed in the 'List of Prokaryotic names with Standing in the Nomenclature'[8], but where no recent references describing their properties have been retrieved. The taxonomic standing of these 'old' species is an area that needs to be addressed.

[a]Proposed species name for *B. garinii* Osp A serotype 4

[b]A single *I. ricinus* found in Slovakia was reactive with probes specific for *B. bisettii* in 2003 and samples from patients in Slovakia and Czech Republic have been positive for this species[9]

[c]*B. crocidurae* seem to be a heterogenous species and include strains that formerly were regarded as distinct species, i.e. *B. merionesi, B. diplodilli* and *B. microti* and other less defined species

[d]*B. persica* seem to be a heterogenous species; '*B. uzbekhistanica*', '*B. sogdiana*', '*B. babylonensis*', '*B. turkmenica*' and several less defined species from Northern India may be grouped together in this binome

[e]*B. coriaceae* was suspected to be the cause of epizootic bovine abortion, but the aetiological agent appears to be from the Deltaproteobacteria[10]

[f]Some researchers prefer to assign these species to the genus *Carios*, while others still define them as *Ornithodoros* spp.

[g]Armstrong and others (1996)[11], seem to describe a very similar species found in *Amblyomma americanum* and propose the name '*B. barbouri*'

complex includes at least seven genospecies (Table 27.1). LB is also named Lyme disease, Lyme arthritis, Lyme carditis, Borreliosis, Erythema migrans, Bannwarth's syndrome, Garin-Bujadoux syndrome, Acrodermatitis chronica atrophicans, Borrelial lymphocytoma, Lymphadenosis benigna cutis, chronic lymphoycytic meningoradiculoneuritis and tick-borne meningoradiculoneuritis.

AETIOLOGY

Borrelia burgdorferi sl is an obligate parasite whose life cycle is maintained by hard ticks in the genus *Ixodes* and a large spectrum of mammalian, avian and reptilian hosts (see [3] for a review)[13]. The spirochaete does not survive for long periods outside a vector or a host.

Borrelia burgdorferi sl has a unique genome consisting of one linear chromosome and the largest number of plasmids of any characterized bacterium, comprising approximately 40% of the genome of the *B. burgdorferi* type strain. Only 8% of the genes on the plasmids have homologues outside *Borrelia*, and these similarities are not known to be bacterial virulence genes, suggesting that the plasmid genes may encode specific functions unique to this genus and that *B. burgdorferi* interacts with its hosts in basically different ways than other known bacterial pathogens[14]. At least one of the plasmids has been demonstrated to be essential under all growth conditions tested, and loss of others correlates with loss or reduction of infectivity in laboratory animals. Correspondingly, many of the genes necessary for evading the immune system and causing disease are probably plasmid-encoded. The plasmids seem to be in a state of rapid evolution, and their number, size and gene order vary substantially among strains and between species. Importantly, horizontal gene transfer between strains of *Borrelia* does occur[14].

Each genospecies of *B. burgdorferi* sl may to a certain extent be characterized by its vectors, host spectrum, organ affinity and geographical distribution. Different genospecies are associated with distinct clinical manifestations of Lyme borreliosis in humans. Accurate typing of genospecies relies heavily on molecular methods (see Wang, 1999 or Bergström et al., 2002 for a review)[15][16]. Serotyping of European and North American strains, using monoclonal antibodies against two outer surface proteins, OspA and OspC, has allowed identification of several serotypes[17]. Other methods of classification require cultivation of the spirochaetes, as ribotyping, pulsed-field gel electrophoresis (PFGE) and the current classical 'gold standard' methodology in taxonomy: DNA–DNA reassociation analysis[18]. The application of multilocus sequence analysis (MLSA) and similar techniques to taxonomy are expected to identify and discriminate more ecologically meaningful entities within the *B. burgdorferi* complex than we have today.

EPIDEMIOLOGY

GEOGRAPHICAL DISTRIBUTION AND HOSTS

The known distribution of LB in Europe is closely tied to the presence of ticks of the *Ixodes ricinus* complex, i.e. *I. ricinus* in most of Europe and both *I. ricinus* and *I. persulcatus* in some locations in Finland, the Baltic states and parts of Russia. *Ixodes ricinus* typically has a restricted distribution range in sites with a Mediterranean component, such as Greece, Italy, Spain, Portugal, France and northern Africa, but is more common in Continental Europe, the UK and southern/central Sweden and Finland. North of that, and in Norway, the tick is typically restricted to the coastline, but may be found in some isolated locations as far north as 68–70°N[19]. Within its distribution range, *I. ricinus* is most prevalent in areas where it can find protection from desiccation. This tick needs a relative humidity above 80% to survive and is found in areas with a wet and cool climate or where the microhabitat provide sufficient humidity (i.e. low vegetation, leaf litter, thick moss, grass or heather). This may typically be deciduous and mixed forest, but also other habitats where sufficient relative humidity allows tick survival. The tick population density generally decreases with altitude[20].

The prevalence of different genospecies of *B. burgdorferi* sl (and probably also the different genotypes within each species) seem to vary greatly between regions. *Borrelia afzelii* and *B. garinii* are the most common species in most of Europe, followed by *B. valaisiana* and *B. burgdorferi* ss. *Borrelia lusitaniae* is rare or absent in most regions, but prevalent in south-western Europe[21] and is the sole species of LBS in southern Portugal and North Africa. *Borrelia spielmaniii* and *B. bissettii* also seem to be found only in low prevalences and have a limited geographical distribution, and the most recently proposed species, 'B. bavariensis' seems to have a highly focal distribution[22]. The differences in prevalence of the different genospecies could

probably be explained by the prevalence of different reservoir hosts.

In addition to the borrelia cycle associated with *I. ricinus*, *I. uriae* transmit *B. garinii* among seabirds[23], whereas *I. hexagonus* transmit several LBS among hedgehogs, mustelids, foxes and other medium-sized animals[24]. Both these ticks are nidicolous, meaning that they live in the nest or burrow of their main host, and only occasionally bite humans and domestic animals. These semi-independent cycles of transmission may hence constitute 'silent foci' of *B. burgdorferi* in areas where *I. ricinus* is absent, meaning that LBS are present in the environment, even when human cases are rarely diagnosed. This implies that LBS infection in wildlife may be found further north, at higher latitude and perhaps even in dryer areas than commonly thought.

HOST FACTORS

Ixodes ricinus and *I. persulcatus* feed on virtually any mammal, bird or reptile, but only some of these species acquire systemic infection with LBS, which is determined to a large extent by the host's innate immune response. In ticks feeding on host species resistant to a strain of LBS, the complement pathways will activate C3b, which binds to the surface of the bacteria, inducing formation of the terminal complement complex and hence lysis of the spirochaetes already in the midgut of the tick[25]. However, some strains express complement regulatory-acquiring surface proteins (CRASP) on their surface that specifically bind complement-control proteins of a given animal species. This mechanism is proposed to prevent lysis. Typically, *B. garinii* and *B. valaisiana* will be lysed in the midgut of a tick feeding on rodents, whereas *B. afzelii* will manage to survive exposure to rodent complement. In ticks feeding on a thrush, pheasant or seabird, however, *B. garinii* and *B. valaisiana* will survive and *B. afzelii* will get killed, whereas exposure to cervid blood seems to induce lysis of all genospecies.

When an infection is established, the acquired immune response may be decisive for the outcome. Young animals and immunocompromised individuals have a greater risk of widespread bacterial dissemination. Experimental infection of neonates, for example, causes arthritis and carditis not seen in adults of the same species[26].

Tick infestation loads (and thereby borrelia infection risk) may be linked to characteristics of both the host species and the individual of a species. Some species have the ability to mount an efficient immune response against *Ixodes* ticks, thereby presumably preventing borrelial infection. This may be the case for voles, but not for mice[27]. Similar mechanisms may be at play in birds, where ticks feeding on a bird species such as the dunnock (*Prunella modularis*) have been found to be starving and having very low prevalence of infection compared with ticks on other ground-feeding birds[28]. Other species may be able to mechanically remove a significant proportion of the ticks from their skin[29].

Among rodents, males often have higher tick loads than females. This could be explained by differences in the level of testosterone, affecting both innate and acquired resistance to tick feeding, but also by sexual differences in spacing behaviour and size[27]. Space use and tick load may also be related to the individual behaviour of the animal[30].

ENVIRONMENTAL FACTORS

Borrelia burgdorferi sl is maintained in transmission cycles between *Ixodid* ticks and a wide variety of vertebrate hosts. Isolation or DNA detection of *B. burgdorferi* sl has also been described from several other arthropods, including mosquitoes, tabanid flies and fleas, but so far the *Ixodidae* seem to be the principal group of hosts. In Europe, the most important vector, at least for human LB, is *I. ricinus*. However, natural infections by *B. burgdorferi* sl have been recorded in 25 other ixodid tick species, and vectorial competence has been confirmed experimentally for 12 of these species[16].

The environment for *B. burgdorferi* sl consists of ticks and vertebrate hosts. The biotic and abiotic factors that are decisive for the tick population, the vertebrate host population and the interaction between them determine the fate of the population of LBS. To understand the ecology of these bacteria, one has to understand the ecology of ticks (see [17] for a thorough review)[27]. The *I. ricinus* tick has a complex life cycle, including four developmental stages: the egg and the three instars – the larva, the nymph and the adult (Figure 27.1). All three instars rely on blood as their only food source, and blood-feeding initiates development to the next life stage or, for the adults, reproductive activity. The life cycle of a European *I. ricinus* may take from 3 to 6 years to complete. Most of this time, the tick survives sheltered in the vegetation litter of the forest floor. In its periods of activity, it searches for a host by *questing*, i.e. sitting and waiting for an animal to pass by, to which it can cling and feed on. The feeding phases,

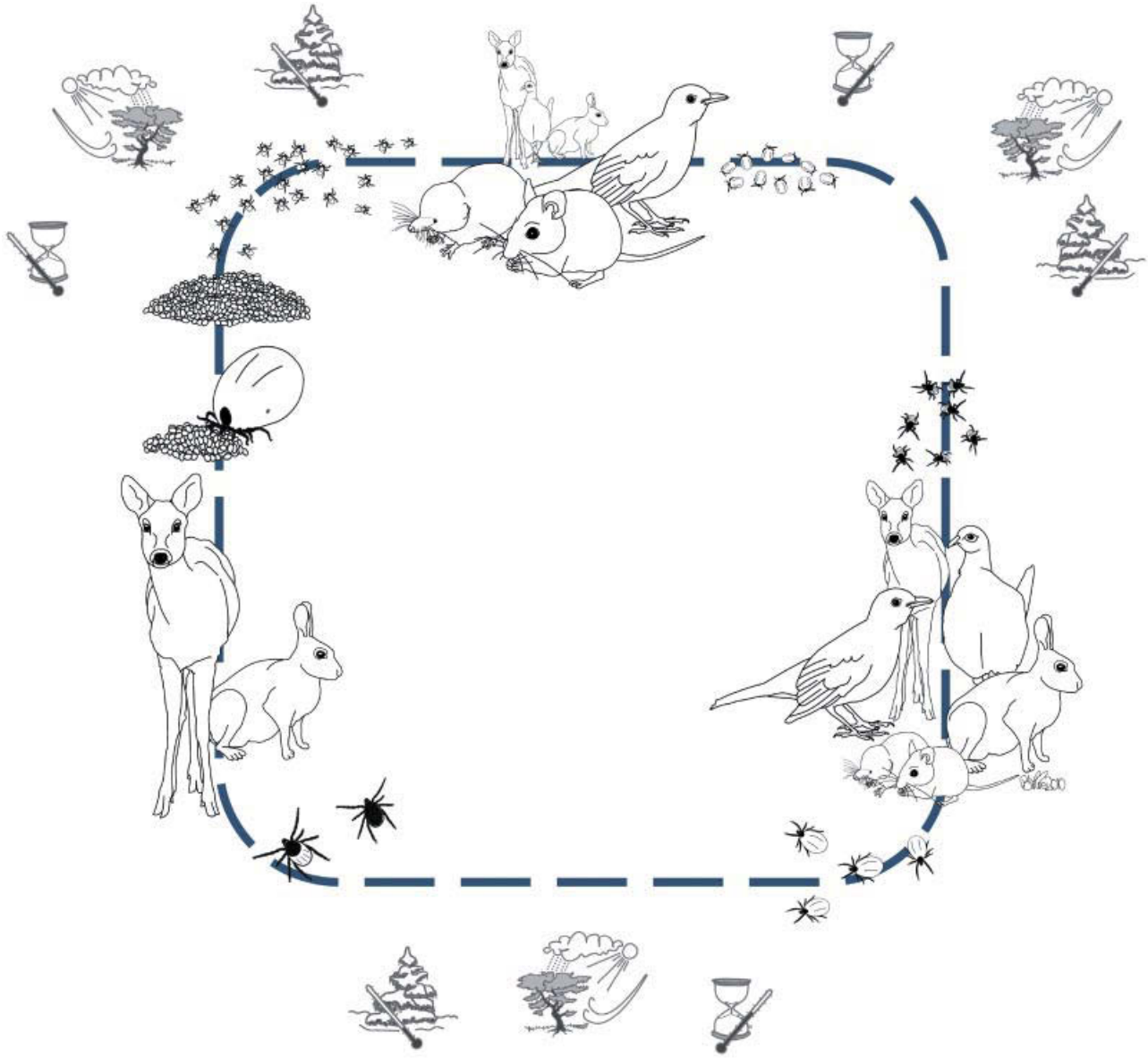

FIGURE 27.1 The life cycle of *I. ricinus* tick and factors that determine tick population density. The infection pressure of *Borrelia burgdorferi* s.l. on a given species, in a given locality, depends on a complex range of factors: firstly, on the abundance of ticks and the degree to which they feed on hosts competent to transmit the spirochaete to that species – the tick population density will depend on the suitability of the microclimate (illustrated by the *tree, cloud, sun and wind* above), i.e. to which degree the environment is humid enough and sufficiently protected against desiccation; the length of the growth season (illustrated by the *hourglass* and *thermometer*), i.e. how long the temperature is high enough to allow both development from one stage to another and host-seeking activity; protection against cold-stress during winter (illustrated by the *snow-covered spruce and thermometer*); and a high density of suitable hosts (illustrated by the various *host animals* above).

consequently, only occupy a minor proportion of the life of a tick.

The survival of the ticks, and the tick population density, may roughly be said to depend on five factors (Figure 27.1):

1. The presence of a suitable microclimate: ticks are very susceptible to desiccation caused by low relative humidity, sun and wind. Frequent rain and mist, or vegetation that shields from sun and wind, creates a humid microclimate that increases survival.

2. Long enough growth season to permit successful host seeking and development: little or no development takes place between 7 and 10°C, whereas the rate of development seems to increase with temperatures above that. Host-seeking activity by the tick larvae does not occur below a threshold temperature of 7–10°C, and for nymphs and adults does not occur below 5–7°C. Consequently, high temperatures over long periods are beneficial for the ticks.
3. Relatively mild winter or protection against very cold temperatures: on the one hand, temperatures below −10°C seem to severely diminish the tick population density, whereas deep snow cover, on the other hand, may provide effective insulation and ensure survival.
4. Presence of sufficient numbers of hosts to facilitate succession from the larval and nymphal stages: *I. ricinus* is an opportunistic parasite that clings to whatever animal passes by, but as larvae quest (sit and wait for a host) on lower vegetation than nymphs, a larger proportion of this instar feed on small mammals and ground-feeding birds. The population density of these animals will be vital, not only to allow development into the next instar, but also to minimize the questing time and thereby the risk of desiccation and energy depletion.
5. Presence of sufficient numbers of a large animal hosts, facilitating production of high numbers of eggs by the adult female tick.

FACTORS THAT AFFECT PREVALENCE AMONG TICKS

Although tick population density is a major determinant of LBS infection pressure, one should not expect a strict linear relationship between tick abundance and incidence of borrelia infection in hosts. The transmission cycles for *B. burgdorferi* sl, having a long-living systemic infection, are so robust that more or less wherever populations of competent ticks and hosts exist, the pathogen transmission cycle will be maintained. However, as transovarial transmission is rare, and different hosts have different ability to transmit the bacteria, the prevalence of LBS among the ticks may vary with temporal and spatial variations in host composition.

Furthermore, recent studies on *I. scapularis* and its hosts in North America suggest that abiotic factors such as daylength and a climate-related degree of temporal synchrony between larval and nymphal questing behavior may be an important determinant both for the overall prevalence of *B. burgdorferi* sl in ticks and the geographical variation in prevalence of different strains[31]. This model suggests that more or less seasonal synchrony of the immature instars will affect the efficiency with which ticks acquire infection from infected hosts, the duration of host infectivity relative to the timing of tick activity and the capacity for co-feeding transmission. Consequently, abiotic factors such as daylength and climate may be important determinants for the prevalence of LBS in ticks and hosts, the prevalence of different strains, and the pathogenicity of these strains.

EPIDEMIOLOGICAL ROLE OF THE AFFECTED SPECIES

To be a reservoir host of LBS, a vertebrate must: i) harbour vector ticks; ii) be susceptible for infection; iii) allow multiplication, development and persistence of the spirochaetes; and iv) allow transmission back to subsequently feeding ticks without diminishing the ticks' chances of successful development and feeding in their next life stage[16]. Different host species show different degrees of susceptibility for infection with different genospecies (or even strains) of LBS and consequently also varying degree of competence to support the transmission of LBS. The overall prevalence of infected ticks and the proportional prevalence of the different strains of *B. burgdorferi* sl in a given location may be a reflection of the proportional abundance of tick host species in that area, the relative proportion of the tick population that feeds on each host species and the degree of transmission competence each of these has for the actual strains.

Birds have a critical role as reservoirs of LBS. In terrestrial ecosystems especially, the species that forage on the ground in forest habitats are frequently infested with *I. ricinus* larvae and nymphs. Some of these species are also competent reservoirs for *B. garinii* and *B. valaisiana,* though the full avian reservoir range remains to be defined. Among the relatively few species investigated, thrushes (*Turdus* spp.) and the ring-necked pheasant (*Phasianus colchicus*) seem to play key roles in the maintenance of the terrestrial cycle of these two genospecies[28,32]. It has also been suggested that passerine birds function as reservoirs of *B. lusitaniae*[33]. In addition to the role of some species as competent reservoirs for *Borrelia*, migratory birds are important for dispersal of infected ticks to new areas, where local reservoir hosts can contribute to the establishment of new foci for the

transported borrelial strain. The function of the bird may theoretically be that of a mechanical vector, but it has been shown that migratory stress can reactivate latent borrelial infection[34]. Migration may hence be associated with a temporally increased reservoir competence, facilitating long-distance dispersal of borrelial spirochaetes.

In marine environments, a wide variety of colonial seabirds seem to constitute competent reservoirs for *B. garinii*. Here, the main vector is the tick *I. uriae*. Closely related *B. garinii* sequences in ticks from localities in both polar regions point at a trans-hemispheric dispersal of this genospecies by seabirds, most likely by reactivation of latent infections[32]. *Ixodes uriae* seem to form distinct races associated with one, or a few, closely related seabird host species (e.g. razorbills, guillemots, puffins, kittiwakes, fulmars) who tend to use different substrates within the breeding colonies, leading to the maintenance of several semi-independent disease cycles[35]. Correspondingly, *B. garinii* strains isolated from *I. uriae* show great diversity[23]. The terrestrial and marine transmission cycles of *B. garinii* are not totally separated, as some exchange of strains between them seem to occur. The marine cycles may hence serve as donors of new strains into the terrestrial ecosystems. Interestingly, recent studies have also detected DNA sequences grouping with *B. burgdorferi* ss and *B. lusitaniae* in arctic birds, suggesting that transmission cycles of genospecies other than *B. garinii* also may be maintained by *I. uriae* and marine birds[35].

Rodents such as mice, voles and rats are thought to constitute important reservoir hosts for genospecies like *B. afzelii*, *B. burgdorferi* ss and 'B. bavariensis'[36]. The mice in the *Apodemus* genus appear to remain persistently infectious for ticks once infected. Although infection is rarely detected by isolation from the animals, they feed a substantial proportion of the larvae, supplying the ecosystem with many infected nymphs. Conversely, in the bank vole (*Clethrionomys glareolus*), although infection results in high levels of spirochaetes in blood, the vole may not function as an important reservoir, as this species seems to mount an immune response against the ticks that diminishes their probability of successful feeding and moulting and thereby reduces the trans-stadial transmission of *Borrelia*.

Edible dormice (*Glis glis*), hazel dormice (*Muscardinus avellanarius*) and garden dormice (*Eliomys quercinus*) appear to constitute the key reservoir hosts for *B. spielmanii*[37], explaining the limited distribution of this genospecies.

Squirrels (*Sciurus* spp.) are also important reservoir hosts, as they are often heavily infested with subadult ticks, and these feeding ticks have a high prevalence of borrelial infection, mainly *B. burgdorferi* ss and *B. afzelii*[36].

There is a dearth of knowledge on the role of different species of shrews (Soricidae) in perpetuation of LBS in Europe. Based on studies in a location in eastern USA, Brisson and others (2008)[38] suggested that shrews are key reservoirs of *B. burgdorferi* ss. Evidence for an important role of shrews, especially the common shrew (*Sorex araneus*) has also been found in Sweden[39].

Hedgehogs also have some degree of reservoir capacity for several genospecies of LBS. This host may in addition function as a bridge between the two enzootic terrestrial cycles mentioned above, as hedgehogs are often infested by both *I. ricinus* and *I. hexagonus*.

Hares (*Lepus timidus* and *L. europaeus*) may play an important role in borrelial ecology, as these hosts are able to function both as maintenance hosts for all instars of ticks and have some degree of transmission competence, at least for *B. burgdorferi* ss. Hares may hence support enzootic cycles of borrelia in the absence of other mammalian species[40]. The role of rabbits (*Oryctolagus cuniculus*) is more controversial.

The role of cervids is also much debated. It is often stated that the increase in deer populations in Europe (and North America) is the main reason for the increase in human cases of Lyme borreliosis, as cervids are important as management hosts for the adult ticks. Despite this, studies on roe deer (*Capreolus capreolus*), fallow deer (*Dama dama*), white-tailed deer (*Odocoileus virginianus*), red deer (*Cervus elaphus*) and moose (*Alces alces*) suggest that these species are incompetent reservoir hosts for *B. burgdorferi*[36]. It has been suggested that although cervids increase the tick population, they serve as *zooprophylactic hosts*, serving to drive down the LBS infection rate in ticks. The degree of zooprophylaxis would depend on the relative proportion of the ticks fed by deer compared with competent reservoir species. So far, models suggest that zooprophylaxis only occurs at unrealistically high densities of deer[41]. On the other hand, studies in which host and borrelial DNA are isolated from ticks show that infected ticks may have fed on cervids[42]. Together with studies describing dermal infection with several genospecies of *B. burgdorferi* sl in cervids[43], this may suggest that co-feeding infection (see below) may occur in European cervids in the same manner as it does in Japanese sika deer (*Cervus nippon*) and sheep[44,45].

Little is known about the role of other large mammals such as badgers (*Meles meles*), other mustelids, foxes (*Vulpes*

spp.), wild boar (*Sus scrofa*) and bovids in the perpetuation of LBS. The presence of borrelial DNA from many of these species, generally not considered as reservoir hosts, may indicate that they to some degree could serve as reservoirs, perhaps through co-feeding transmission[42].

Reptiles have been regarded as refractory for LBS infection. However, several species of European wall lizards (Lacertidae) have now been identified as key reservoir hosts of *B. lusitaniae*.

TRANSMISSION

Transmission of *B. burgdorferi* sl. between ticks occurs mainly from infected nymphal ticks of one cohort to naïve larval ticks of another via reservoir hosts. There are few adult ticks compared with nymphs, and they tend to feed on incompetent hosts, rendering them only a minor role in the perpetuation of LBS. Likewise, the role and importance of vertical transmission seems to be minor. However, because of the high fecundity of female ticks, the contribution of vertical transmission to perpetuation of borrelia may be important in some systems.

Infection of vertebrate hosts relies entirely on transmission from ticks. There is no evidence for horizontal or vertical transmission from vertebrate to vertebrate.

Infection occurs when a fraction of the spirochaetes residing in the midgut of the tick responds to the arrival of blood by invading the salivary glands through the haemocoel. The spirochaetes follow the saliva into the feeding pool, i.e. the small, persisting haemorrhages the tick sucks blood from, multiply and invade the surrounding dermis. From the dermis they travel through the bloodstream, and finally disseminate to skin and other target organs such as joints, nervous tissue, heart and arterial walls. The ability and efficiency of tissue invasion may vary between genospecies[13]. It is hypothesized that spirochaetes persist in the extracellular matrix of these organs, remaining available for transmission to a subsequently feeding tick[46]. When new ticks start to feed it is believed that the spirochaetes migrate towards the feeding pool, probably directed by chemotactic components of the tick saliva.

CO-FEEDING

Transmission of LBS between infected and uninfected ticks does not necessarily rely on systemic infection in the vertebrate host. Infection may be acquired by *direct co-feeding* by uninfected ticks that are feeding simultaneously with infected ticks, even if there is some physical separation between the transmitting and acquiring ticks in the absence of spirochaetemia. In addition, uninfected ticks may acquire infection by *indirect co-feeding* from a localized site at which infected ticks have previously fed. Although the contribution of such non-systemic transmission may be negligible or masked in systems that are not host-limited, this mechanism may be important for LBS persistence in areas where reservoir hosts are not reliably abundant, as co-feeding transmission probably can occur in non-competent hosts.

PATHOGENESIS, PATHOLOGY AND IMMUNITY

The amount of LBS traveling through the salivary glands and invading the dermis is regarded to be relatively low, although exact knowledge of the size of the inoculum does not exist[26]. The protective and immune-modulating effects of the tick's saliva seem to be important for the survival of the spirochaetes. In addition, the LBS down-regulate immunogenic surface proteins, coat their surface with proteins showing great antigenic variation, induce secretion of substances that modulate immune defences and secrete soluble antigens that tie up the antibodies of the host; all of these together are mechanisms to escape and diminish the specific immune response of the host.

Once established in the feeding pool, the LBS multiply and start to spread outwards and downwards in the surrounding dermis with the help of their motility, moving through the extracellular matrix (ECM) with a speed up to 4 μm/s and having a tendency to localize in perivascular connective tissue[47]. The migration triggers local inflammatory responses by macrophages and dendritic cells and, as the acquired immune response comes into play, also specific T cells, resulting in the classic lesion of erythema migrans (EM). Concurrently, the spirochaetes invade blood vessels and are disseminated through the body of their host.

After the host's adaptive immune response becomes effective, the LBS disappear from the bloodstream and disseminate to various locations – typically the joints, tendons, fasciae, heart, muscle, urinary bladder, dermal and neural tissues. Here, they 'hide' in connective tissue with the help of multiple surface proteins that mediate

binding to collagens and other components of the ECM. Some spirochaetes may also be found intracellularly in certain cells. These mechanisms are thought to help the LBS to escape from immune responses and thereby facilitate long-lasting persistence and possibly protection against antibiotics[46,48].

In addition to the spreading through the circulation, some authors suggest that LBS also disseminate by migration through connective tissue, i.e. along blood-vessel walls and perineural sheaths.

The inflammatory manifestations associated with LB result from the host's innate and adaptive immune responses to the spirochaetes, as LBS do not have the ability to produce toxigenic molecules. The pathogenesis of the infection is linked to the genetic background of the vertebrate host and to the genotype of *B. burgdorferi* sl.[16].

Neurological complications in LB often result from inflammation of blood vessels supplying nerve roots and peripheral nerves in combination with the effect of the invasion of brain parenchyma[26].

Acute Lyme arthritis in mice and humans results from the borrelia-induced infiltration of mononuclear cells into the synovial tissue and release of inflammatory cells and mediators to the joint cavity. It may manifest as a rather uncomplicated inflammatory arthritis without erosive damage in cartilage or synovium. In some cases the arthritis persists for longer periods, bearing many similarities to rheumatoid arthritis.

Lesions of LB in wild animals may be expected to share many similarities with human lesions. However, chronic skin manifestations have to our knowledge not been reported in any non-primate species. EM in humans and rabbits is an expanding, annular, erythematous skin lesion with central clearing, histologically characterized by mild superficial perivascular infiltration of the dermal interstitium, mainly by T lymphocytes. The human borrelial lymphocytoma consists of dense dermal infiltrates of B lymphocytes and some plasma cells centred around vessels, sweat glands and nerves. The appearance may resemble a cutaneous lymphoma. In human achrodermatitis chronica atrophicans the epidermis is frequently thinned and there is oedema and a perivascular lympho-/plasmacytic infiltrate in the superficial dermis, often accompanied by dilated blood vessels.

In humans with meningoradiculoneuritis, lymphocytic involvement of the leptomeninges, ganglia and afferent and efferent rootlets is present and a focal microglial reaction may be evident. In experimentally infected dogs multifocal non-suppurative meningitis, encephalitis, perineuritis and periarteritis have been found.

Histopathologic findings in Lyme carditis in primates and mice include interstitial lympho-/plasmacytic endocarditits, myocarditis and pericarditis. The conducting system may reveal localized oedema and mild lymphocytic infiltrations of sinoatrial and atrioventricular nodes. Skeletal myositis with infiltration of connective tissue with mononuclear leucocytes is found in several animal models of LB.

In joints with Lyme arthritis, non-specific synovitis with lymphocytic/plasmacytic infiltration, often in pseudofollicular structures, is typically found. Exudation of neutrophilic granulocytes and fibrin into synovial cavities often occurs. In more chronic cases the synovial villi become hypertrophic, but the disease seldom progresses to pannus formation and erosion of cartilage.

LBS may be visualized in histological sections by Giemsa, carbol-fuchsin or silver staining methods, or immunohistochemical or *in situ* hybridization techniques.

Adaptive immunity has a critical role in the control and resolution of disease. The response develops rather slowly (3–6 weeks in humans) and is directed against an increasingly diverse array of proteins as the infection progresses. LBS disappear from the circulation when the specific antibody response comes into play. The protective immunity is, however, strain-specific, and spirochaetes can persist despite high titres of circulating antibodies when dissemination has occurred. Relapses of disease may also occur in spite of seemingly good protection. IgG may also contribute to disease pathogenesis by increasing the severity of inflammation. A variety of innate and adaptive T-cell subsets also participate in the immune response to *B. burgdorferi*, although their roles remain poorly defined.

CLINICAL SIGNS AND TREATMENT

The clinical syndrome of Lyme disease is best described in humans[26,49]. Clinically silent infections are common, mild manifestations are the general rule and severe disease is comparatively rare. The frequency of individual manifestations varies depending on the age and physiological status of the patient and the infecting genospecies.

The disease is traditionally staged in three stages, but a complete presentation of the disease is unusual.

Early, localized disease is characterized by the spreading of a painless, but sometimes itching erythema (EM) around the feeding site in a ring-like pattern. EM may be accompanied by malaise, fever, musculoskeletal pain, headache and fatigue and is most often caused by *B. afzelii* (70–90%), less frequently by *B. garinii* (10–20%), rarely by *B. burgdorferi* s.s. and only exceptionally by other species. Only a minor proportion (<10%) of clinical cases develop generalized disease. Multiple lesions may occur and are interpreted as a consequence of hematogeneous dissemination from the primary skin lesion.

Generalized or disseminated disease occurs weeks to months after the tick bite. Classic manifestations are Lyme neuroborreliosis, borrelial lymphocytoma, Lyme carditis and Lyme arthritis. Chronic manifestations typically include achrodermatitis chronica atrophicans (ACA), chronic arthritis and rare cases of chronic neuroborreliosis (see above).

Experimental disease in susceptible animals manifests many of the signs seen in human LB (see Barthold and others, 2010, for a review)[26]. Domestic dogs, for example, develop classical signs of LB, though EM is inconsistently observed. In addition, some dogs develop fatal kidney disease.

The rabbit is one of the few species that actually develops lesions of EM similar to humans, but seem to mount a very effective immune response that in most cases clears the infection before dissemination.

Inoculation of many rodents, among them some strains of laboratory mice (*Mus musculus*), hamsters (*Mesocricetus auratus*) and American white-footed mice (*Peromyscus leucopus*) results in disseminated, persistent infection, but without lesions. Infection of different strains of laboratory rats (*Rattus norvegicus*) and mice share many features in common with acute disseminated human LB. In such mice strains arthritis, carditis, vasculitis, myositis and peripheral neuritis may develop, but this species does not develop classical EM or neuroborreliosis.

Spontaneous disease has been described in domestic dogs, horses, cattle and sheep. The most prominent clinical sign seem to be lameness and mild fever, although neurological signs are reported in some studies.

There are very few reports of LB in wild species. High prevalence of *Borrelia* antibodies were found in reindeer herds that experienced musculoskeletal problems, suggesting that *Borrelia* could be a contributing factor[50]. Two hedgehogs showed disease attributable to borrelial infection. One of these animals developed EM, anorexia, ataxia, stiff gait and joint effusion, and subsequently died[51]. Disseminated infection with *B. afzelii* was described in a red fox on Hokkaido and mild inflammatory changes in liver, kidney and eye were attributed to the infection[52].

Recommended treatment against Lyme borreliosis in dogs and the most common forms of disease in adult humans is doxycycline. Antibiotic therapy has no impact on post-Lyme syndrome. Some evidence suggests that *B. burgdorferi* may persist in some dogs following antibiotic treatment. Some researchers argue that this is a consequence of medication-induced formation of resistant cysts that have the potential to revert to produce viable spirochaetes when favourable conditions return[53].

DIAGNOSIS

Diagnosis of LB is difficult even in humans, and laboratory confirmation of a borrelial infection is needed for all manifestations of Lyme borreliosis, with the exception of typical skin lesions. Taking into account the limited knowledge in this field and the apparent commensality between natural hosts and LBS, seropositivity and even presence of spirochaetes in blood and tissues does not warrant a diagnosis of LB if no other association between clinical signs and/or pathological lesions and infection can be confirmed.

Samples from Animals

Cultivation is the 'gold standard' of diagnosis of *B. burgdorferi*. In culture, the LBS are microaerophilic and slow-growing. They grow best in liquid media at 30–35°C and have complex nutritional requirements. The medium in common use is a modified BSK (Barbour, Stoenner and Kelly). However, as the numbers of viable bacteria in tissues and blood may be very low and the organism is very fastidious, negative results do not exclude infection. The sensitivity is very variable, but may be satisfactory for samples from skin, for example ear biopsies or needle aspiration from skin in areas where ticks collect. The sensitivity is very low for fluids such as blood, joint fluid or cerebrospinal fluid (CSF).

Genus- and species-specific polymerase chain reaction (PCR) methods can be used to detect borrelial DNA of both viable and non-viable organisms and is now a standard approach for direct detection of infection in ticks,

hosts and clinical specimens. Given that the number of spirochaetes in infected tissues or body fluids is generally very low, appropriate, controlled procedures for sample collection, transport and DNA preparation from clinical samples are critical for yielding reliable and consistent PCR results. Quantification of the borrelial burden in host tissues can be determined by either competitive PCR or real-time quantitative PCR[26].

In both cultivation and PCR it is important to realize that mixed infections occur, and that different strains have different tissue tropism. Negative results may not be regarded as conclusive, and positive isolation of one species in a sample may not exclude the presence of others.

Serology is often used on human samples, usually as a two-step procedure, starting with enzyme-linked immunosorbent assay (ELISA) followed by an immunoblot of positive specimens. These methods have many challenges, owing due to the antigenic complexity of European LBS and their variable expression of antigens. In wild animals, results of serologic tests not validated for the host species should be interpreted with caution. Serology is most appropriate when used to evaluate exposure at the population level. The seroprevalence should be expected to be low in non-competent hosts, as sensitivity of antibody detection in early, localized disease in humans is only between 20 and 50%[17].

In human cases of neuroborreliosis, an index based on the ratio between borrelia-specific antibodies in CSF and serum relative to the ratio between total amount of antibodies in CSF and serum, is used as a diagnostic criterion.

Xenodiagnosis is the method of choice for evaluating host infectivity to feeding ticks. The fact that a non-fed, non-infected tick larva or nymph acquires borrelial infection when feeding on a given host species and still has viable borrelia in its midgut in the next developmental stage is regarded as a proof of this host's reservoir competence. However, this method is labour-intensive and requires long-term handling and restraint of animals. A more practical method for assessment of reservoir capacity may be to compare the prevalence of infection among larvae that have fed on the host with that of questing larvae in the same area.

Detection in Ticks

In addition to cultivation and PCR, viable and motile *Borrelia* may be visualized directly by using dark-field microscopy on suspension of the midgut of ticks.

Identification of Host Blood Meal Source

Different techniques have recently been developed for identification of host blood meal source in ticks. Combined with isolation of borrelial DNA from the same ticks, this provides an interesting tool for analysis of transmission dynamics and host associations.

MANAGEMENT, CONTROL AND REGULATIONS

Management and control of LB may be directed against the vectors or the pathogen itself (see [54] and [55] for reviews). In humans, public education; personal protection measures such as wearing protective clothing, checking one's body daily for ticks (as early removal reduces the risk of infection), avoiding tick-infested areas, and applying tick repellent is used to diminish risks of infection. There is no vaccine against Lyme borreliosis currently available in Europe. Research into new vaccines is continuing, and at least one re-engineered OspA vaccine is currently being tested. Use of such a vaccine in Europe is complicated by the fact that there is great sequence diversity in OspA among the strains circulating in Europe.

Individual protection of livestock and domestic animals is commonly achieved by use of topical acaricides. However, effective and long-lasting measures against *I. ricinus* and *Borrelia* sp. have to be directed against the natural habitats and the hosts of the ticks.

One major approach will be to manipulate tick habitats in order to expose the free-living ticks to low relative humidity and soil moisture by mechanical clearing of vegetation, use of herbicides, intensive grazing by livestock, removal of leaf litter and controlled burns.

Another principal approach would be to protect natural hosts against ticks and/or borrelia. Treatment of hosts such as cervids with acaricides in automatic-application devices should be expected to cause a decline in the tick population by reducing the reproductive success of adult ticks. Experience from trials aimed at reducing the populations *I. scapularis* on white-tailed deer in USA point to this approach as safe, efficacious, economical and environmentally friendly[56]. Application of acaricides to small mammalian hosts through a baited box has the potential not only to impact tick abundance, but also to disrupt the transmission cycle of some borrelial genospecies. A future approach may be to vaccinate wild hosts against ticks, for

example by targeting constituents of tick saliva, or against *B. burgdorferi*. To make this feasible, an orally administered vaccine has to be developed.

A third approach would be to lower the populations of hosts, either by fencing or hunting. As deer feed such a large proportion of adult ticks, reduction of deer population is advocated to reduce reproductive success of *I. ricinus*. It remains unclear, however, how far a deer population must be reduced to impact the transmission dynamics of LB. It is important to emphasize that the number of questing ticks will increase in the first years after deer population reduction, as fewer deer remove adult ticks from the environment. Also, removal of a large, presumably reservoir-incompetent, host may cause more subadult ticks to feed on reservoir-competent hosts. If there is some kind of ecological competition between deer and a host-transmitting pathogenic LBS, a reduction in deer numbers may potentially cause an increase in risk of human disease. Management, habitat manipulation or management to reduce or possibly exclude small mammals, birds and/or lizards to control ticks and *Borrelia* has not been well studied, but in theory would be a logical approach in some circumstances.

There are currently no effective and widely applicable biological control methods available for the control of tick populations. Entomopathogenic fungi (*Beauveria* spp., *Verticillum* spp., *Metarhizium* spp.), nematodes (Mermithidae, Heterorhabditidae, Steinernematidae) and parasitoid wasps (*Ixodiphagus* spp.) seem to be the most promising candidates. (See Hartelt and others, 2008[57], for a recent review). Bacteria (i.e. *Bacillus thuringensis*) have also been proposed as potential agents. Chemical control by area-wide use of pesticides has been shown to provide relatively consistent, highly effective short-term suppression of ticks. However, public acceptance of area applications of synthetic chemicals for tick control appears relatively low. An alternative approach would be to use tick pheromones and related chemicals. These potential tools could theoretically be applied to disrupt tick behaviour or reproduction without deleterious consequences for the environment, for example by attracting ticks to a toxicant or a baited decoy system.

PUBLIC HEALTH CONCERN

Lyme disease is the most common vector-borne zoonosis in temperate regions of the northern hemisphere and has significant medical and economic impacts. Four of the LBS (*B. burgdorferi* ss, *B. garinii, B. afzelii, B. spielmanii*) are associated with human disease, and evidence now suggests that three others may also be pathogenic under certain circumstances (*B. valaisiana*, *B. lusitaniae* and *B. bisettii*). In addition, the newly proposed '*B. bavariensis*' seems to represent a recently evolved, hyperinvasive and aggressive genotype, which is suggested to be at the brink of an epizootic spread across Eurasia[58]. In Europe, the nymphs of *I. ricinus* are the main vector for *B. burgdorferi*. The risk of human infection is a product of the degree of contact between ticks and humans, prevalence of ticks and prevalence of the different genotypes of *B. burgdorferi* in ticks in a given location, and the pathogenicity of these genotypes.

SIGNIFICANCE AND IMPLICATIONS FOR ANIMAL HEALTH

It is generally considered that *B. burgdorferi* establishes persistent infections with minimal harm to its natural hosts. However, aberrant hosts, such as humans and domestic animals, may develop clinical disease as a deviation from the norm.

However, very little research on potential effects of borrelial infection in wild animals has been performed. It is improbable that infection with *B. burgdorferi* is a common cause of acute mortality in wild species, but it is possible that natural hosts develop non-fatal disease that escapes detection. Very few natural host species have so far been analysed for pathogenesis in experimental studies, and in the field it is even more difficult to detect disease caused by *B. burgdorferi*.

The possibility should not therefore be dismissed that borrelial infection may affect the life history traits of wild animals. Subclinical infections could plausibly result in loss of vigour with subsequent decrease in survival and reproduction. In particular, neonatal animals, having immature innate and acquired immune responses that favour development of disease, and animals that suffer from other disorders affecting the immune responses (i.e. anaplasmosis), may be at risk.

Genotypes of *B. burgdorferi* sl other than the four most important pathogens for humans probably have reservoir host–vector cycles that rarely if ever involve human infections and may, for this reason, have escaped attention. Much work remains to elucidate all possible aspects of borrelial infection in wild birds, mammals and reptiles.

RELAPSING FEVER SPIROCHAETES

Relapsing fever (synonym: typhinia) is an acute infectious disease caused by different *Borrelia* species, and is characterized by febrile episodes separated by afebrile intervals. The term relapsing fever (RF) has traditionally been used in connection with human disease (human relapsing fever (HRF). Disease caused by RF spirochaetes in animals is often designated spirochaetosis. Details on RF and the causative spirochaetes can be found in the reviews by Felsenfeld (1965)[2], Barbour & Hayes (1986)[3], Larsson (2007)[4] and Barbour & Guo, (2010)[26].

The severity of HRF ranges from subclinical to fatal. The main clinical sign is recurrent fever, which coincides with large numbers of spirochaetes in the blood (spirochaetaemia). The characteristic relapsing phenomenon is associated with the presence of multiphasic antigenic variation. As an immune response develops to the predominant antigenic strain, shifting of the outer surface proteins of the spirochaete produces variant strains that multiply and cause a recrudescing infection[26].

Infections caused by the RF spirochaetes are vector-borne, primarily by the soft-body ticks in the genus *Ornithodoros* (Family Argasidae), and are referred to as tick-borne relapsing fever (TBRF). An exception is *Borrelia recurrentis*, which is transmitted by the human body louse (*Pediculus humanus humanus*) and causes louse-borne relapsing fever (LBRF). The louse is strictly specific to humans with no animal reservoir for *B. recurrentis*. Large outbreaks of LBRF have occurred in Africa and Eurasia throughout the past century, but at present, the disease is primarily found in Ethiopia and adjacent areas.

Most TBRF are zoonoses. The TBRF persists in endemic foci around the world, but it is particularly a burden for humans in several African countries. In Table 27.1, the different TBRF *Borrelia* species with their vector, typical host(s) and geographical distribution are presented. The responsible *Borrelia* species is closely associated with its tick vector and many of them share parallel nomenclature. Although most TBRF spirochaetes are transmitted by *Ornithodoros* ticks, *B. anserina*, causing avian spirochaetosis in domestic fowl, is transmitted by several *Argas* ticks. *Ornithodoros* ticks are usually nocturnal feeders and feed for short periods of time (around 30 minutes), and inoculation of the spirochaetes in the host happens within minutes. The ticks are sedentary and inhabit sheltered environments close to their hosts, such as rodent burrows, bird nests, caves and human-made shelters[2].

Typical reservoir hosts for the TBRF spirochaetes are various wild rodents, but they also include insectivores, birds and other mammals (Table 27.1)[2]. Mice are reservoirs for several *Borrelia* spp. in nature, and the house mouse (*Mus musculus*) is the most commonly used species in animal experiments on RF. Mice develop a disease that is similar to HRF[4]. Details on the susceptibility of and pathology in various experimental animals used in RF studies can be found in the review by Felsenfeld (1965)[2] and Barthold et al. (2010)[26]. The pathogenesis and interactions between the TBRF spirochaetes and their host (both ticks and vertebrates) have been reviewed in depth by Barbour & Hayes[3] (1986) and Barbour & Guo (2010)[26].

RELAPSING FEVER IN EUROPE

TBRF borreliosis are considered to be emerging diseases. The estimated distribution of the disease in Europe is along the southern parts of the Mediterranean countries[59]. In this region, three zoonotic TBRF are present with a sporadic occurrence: *B. hispanica* with the vector *Ornithodoros erraticus erraticus* is found in the Mediterranean region (Portugal, Spain, Cyprus, Greece and North Africa). The natural hosts of *B. hispanica* are wild rodents. In Portugal and Spain, the vector has adapted to bite domestic pigs and to live in their environment. This adaptation has possibly made humans more prone to be bitten by the vector, and HRF have been diagnosed sporadically in Spain during the 20th century. Disease caused by *B. hispanica* is among the less severe HRF.

A new non-culturable RF *Borrelia* species was found in southern Spain in 1996[60]. It was isolated from three patients with RF and from the *B. hispanica* vector *O. erraticus erraticus*. Molecular analyses have shown that the spirochaete is related to, but outside, the monophyletic group of *B. hispanica*, *B. crocidurae* and *B. duttoni*. The reservoir is not known, but rodents are suggested.

Borrelia crocidurae is present in Turkey, the Sahel region, and West and North Africa. It is transmitted by the tick *O. erraticus sonrai*. Typical hosts for the tick are rodents (mainly rats) and insectivores. HRF caused by *B. crocidurae* is generally benign, but neurological complications may occur.

Close to Europe's boundaries in the south-east, three other TBRF borreliosis are present, namely: *B. persica* (vector: *O. tholozani*)[61]; *B. caucasica* (vector: *O. asperus*,

former O. *verrucosus*); and *B. latyschewii* (vector: *O. tartakovskyi*) (Table 27.1).

RELAPSING FEVER SPIROCHAETE-ASSOCIATED DISEASE IN BIRDS AND MAMMALS

AVIAN SPIROCHAETOSIS

Avian (fowl) spirochaetosis (avian borreliosis) caused by *B. anserina* is an acute septicaemic disease that occurs among domestic poultry worldwide in areas where fowl ticks in the genus *Argas* reside. The disease is most commonly seen in tropical and subtropical regions[32]. *Borrelia anserina* is considered an unusual member of the RF spirochaete group, as it has more than one vector and causes a disease that is not characterized by relapsing fever. The latter seem to be due to the fact that *B. anserina* does not appear to undergo antigenic variation[62]. Various *Argas* ticks both serve as the primary vector and reservoir of the spirochaete, which is transmitted trans-stadially and also transovarially in some tick species. Adult ticks can survive for up to 3–4 years without feeding and carry *B. anserina* longer than a year. The *Argas* ticks live in poultry houses or under tree bark. Mosquitoes and mites can also transmit the infection, and other routes of transmission that do not involve vectors are possible.

Gallinaceous birds (turkeys, chickens, pheasants) are the primary hosts of *B. anserina*, but natural infections also occur in domestic geese and ducks. Free-ranging flocks that are extensively reared are most likely to be infected. Additionally, the disease has been reported in canaries and the African grey parrot (*Psittacus erithacus*)[32,63]. The disease has not, to date, been diagnosed in free-living wild bird species. Strains of variable virulence exist and the disease may vary in severity. In birds infected with virulent strains, the disease is clinically characterized by elevated body temperature, depression, anorexia and green diarrhoea. Later in the course, birds show paresis or paralysis and become anaemic[63]. The predominant gross lesion is an enlarged and mottled spleen, often in combination with hepatomegaly and swollen kidneys. Usually the intestinal contents are green and mucoid. Small haemorrhages may be seen, especially in the liver and at the junction between the proventriculus and ventriculus. Infrequently a fibrinous pericarditis is present. The most characteristic histopathological findings are erythrophagocytosis and hemosiderosis in the spleen and liver[63]. Spirochaetes can be demonstrated in tissue sections, which are stained by silver impregnation methods. *Borrelia anserina* is not known to cause relapsing fever in humans.

Disease in Wildlife Caused by other RF Spirochaetes

The role of wild animals as reservoirs for *Borrelia* spp. is well established, but little is known regarding how these infections might affect them. Disease in wildlife infected with RF spirochaetes appears rare, and there are only a few reports of fatal spirochaetosis in free-ranging wild animals.

The first (and so far only) report of a RF-related spirochaetosis in a wild bird is from the USA. An acute septicemic spirochaetosis was diagnosed in an adult northern spotted owl (*Strix occidentalis caurina*) that was found dead in Washington in 1994[64]. Gross findings included normal body condition, enlarged liver and spleen, and serofibrinous polyserositis. Microscopic pathology revealed infiltration of inflammatory cells and mild multifocal necrosis in liver and spleen, and acute inflammation in the choroid plexus of the brain. Long, spiral-shaped bacteria were found in silver-stained microscopic sections of several organs, and by PCR these were identified as a *Borrelia* species, most closely related to *B. hermsii*. This agent causes RF in humans in the western USA and Canada (Table 27.1) and was endemic in the area where the owl was found. The authors suggested that the most likely source of infection for the owl was directly transmission by predation or contact with secretions or excreta from an infected animal/reservoir species. In 2008, a spirochaete identical to the owl isolate was detected in the blood of a pine squirrel (*Tamiasciurus hudsonicus*) that was captured in an area in Montana, USA, where humans had contracted RF[65]. This finding of the same *B. hermsii* strain in a mammal and a bird separated by more than 500 km showed a broader geographic distribution and host range for the bacteria than previously envisaged.

In Europe, the only report of fatal RF spirochaetosis is in a bat in the UK[66]. In 2008, a juvenile *Pipistrellus* species was found alive but died after a few days of rehabilitation. The major *post mortem* findings were anaemia, excess amount of fluid in the thoracic cavity, and enlarged liver, spleen and adrenal glands. Histopathological examinations showed multifocal necrosis and infiltration by macrophages in the liver, inflammation of the lungs, and extramedullary hematopoiesis in the spleen. Long, undulating bacteria

were found in Warthin–Starry-stained microscopic sections of the liver, lung and spleen and in blood vessels. By PCR these were identified as a novel spirochaete, most closely related to *B. recurrentis*, *B. duttonii* and *B. crociduraе,* which are associated with HRF in Africa and Asia (Table 27.1). The novel spirochaete was not related to a *Borrelia* isolate that has been identified in the bat tick *O.* (*Carios*) *kelleyi* in the USA[67]. An *Argas vespertilionis* larval tick was found on the dead bat, and this might have been the source of the infection, but PCR was not performed on the tick. This tick species is widely distributed in various bat species in Europe, southern Asia and North Africa.

The reports referred to above, identify RF spirochaetes as pathogens for wildlife, both among birds and mammals. Such infections might be under-diagnosed in wild animals.

The most rapid diagnostic test is the identification of the spirochaetes in Giemsa-stained blood smears obtained during a febrile period. Thick blood smears can improve the sensitivity, but the morphology of the spirochaete is better conserved in thin smears. Spirochaetes may also be seen by examining wet preparations of 10-fold diluted blood by phase contrast or dark field microscopy. An enrichment method based on centrifugation raises the sensitivity of microscopy detection[4]. Molecular methods (PCR) are the most sensitive diagnostic tool and are used with increasing frequency[61].

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CHAPTER

28

RICKETTSIALES INFECTIONS

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Rickettsiosis, anaplasmosis and ehrlichiosis are diseases of public health and veterinary importance that are acquired via tick bite. Numerous species of European wildlife play a key role in the epidemiology of all these infections, primarily as reservoir hosts. However, it remains unclear whether any of these diseases are an important threat to wildlife health.

AETIOLOGY

Rickettsia, Anaplasma and *Ehrlichia* species are obligate intracellular bacteria that are transmitted by arthropods, primarily ticks. Infections caused by members of all three genera have been detected in numerous species of European wildlife, but these infections are subclinical, reflecting the probable role of these animals as reservoir hosts. *Rickettsia* species are well-established human pathogens, whereas *Anaplasma* species have long been recognized as pathogens of veterinary significance.

The taxonomic order Rickettsiales comprises of several genera of bacteria, three of which are recognized as parasites or pathogens of European mammals. These three genera are *Rickettsia, Anaplasma* and *Ehrlichia*. The genus *Rickettsia* currently contains 27 valid species, although numerous other partially characterized strains have also been described. Species within the genus are classically delineated into two groups, the 'spotted fever group' (SFG) and the 'typhus group' (TG). This delineation, although originally merely based on the clinical presentation of human infections, reflects phylogenetic divergence within the genus. The majority of *Rickettsia* species belong to the SFG, although this group now includes a number of species that have not been associated with human disease. Of these species, at least 11 are known to be extant in Europe, including *R. aeschlimannii, R. akari, R. conorii, R. felis, R. helvetica, R. hoogstraalii, R. massiliae, R. raoultii, R. sibirica* and *R. slovaca*. The TG comprises of only two species, *R. prowazekii*, which causes epidemic, or louse-borne typhus, and *R. typhi*, the agent of flea-borne, or murine, typhus. Only the second of these species is associated with non-human (wildlife) hosts. The genus *Rickettsia* also includes one species, *R. bellii* that has diverged from both the SFG and the TG. This species together with some of the SFG are considered to be endosymbiotes of ticks, and 'rickettsia-like' endosymbiotes are considered to be extremely common in many species of arthropod. The genus *Anaplasma* contains seven valid species. Of these, there is evidence that five, namely *A. centrale, A. marginale, A. ovis, A. phagocytophilum* and *A. platys*, are encountered in European wildlife. The genus *Ehrlichia* contains five species, of which only two, *E. canis* and *E. muris* are found in Europe.

All members of the order Rickettsiales are obligate intracellular bacteria, and all members of the genera *Rickettsia,*

Infectious Diseases of Wild Mammals and Birds in Europe, First Edition. Edited by Dolores Gavier-Widén, J. Paul Duff, and Anna Meredith.

Anaplasma and *Ehrlichia* are transmitted by arthropods. The vast majority of *Rickettsia* species and all *Anaplasma* and *Ehrlichia* species are transmitted by ticks. The remaining *Rickettsia* species are transmitted by fleas, lice or mites.

EPIDEMIOLOGY

GEOGRAPHICAL DISTRIBUTION AND HOSTS

Rickettsia species are widely distributed across Europe. Their distribution is determined by that of the arthropods to which they are adapted. Thus, for example, among the SFG rickettsiae, *R. aeschlimannii* is restricted to southern Europe as a result of its association with the Mediterranean tick, *Hyalomma marginatum*, whereas *R. helvetica* is encountered across most of the continent, as it is associated with the widely distributed species *Ixodes ricinus*. However, the distribution of some *Rickettsia* species is extended, as they appear to be associated with more than one tick species; for example, *R. raoultii* DNA has been amplified from *Dermacentor reticulatus* and *I. ricinus* ticks. *Rickettsia felis* is widely distributed across much of the world because of its association with cat fleas (*Ctenocephalides felis*). *Rickettsia akari* also possesses a remarkably wide distribution owing to its association with *Liponyssoides sanguineus* mites, which are common ectoparasites of house mice (*Mus musculus*). The TG rickettsial species *R. typhi* also has a near-global distribution as it is (most commonly) associated with the rat flea *Xenopsylla cheopis*. In principle, the distributions of *Anaplasma* and *Ehrlichia* species are determined not just by the distributions of their tick vectors, but also the mammalian reservoir hosts they exploit. However, in practice, as members of both genera appear to exploit either numerous different reservoir hosts or a limited number of hosts that have a broad geographical range, their distribution is perhaps most influenced by the distribution of their biological vectors.

Anaplasma phagocytophilum is widely distributed across Europe as it exploits, primarily, several *Ixodes* species as vectors, and has been encountered in most countries within the continent. *Anaplasma platys* is associated with the brown dog tick (*Rhipicephalus sanguineus*), which is also widely distributed across Europe, particularly in the warmer countries bordering the Mediterranean. *Anaplasma ovis* is thought to be primarily transmitted by *Rhipicephalus bursa*, which in Europe is also restricted to the Mediterranean region. Reports of the presence of *A. centrale* in Europe are, to date, limited to Southern Italy (including Sicily). *Anaplasma marginale* has been encountered in many parts of southern Europe, including the Iberian Peninsula and Italy, and, occasionally further north, in Austria, Hungary and Switzerland. Among the *Ehrlichia* species, *E. canis*, like *A. platys*, is transmitted by *Rh. sanguineus* so is widely distributed, but far more common in the southern half of the continent. Little is known about the European distribution of *E. muris*; it has been reported in France and Slovakia, suggesting a broad geographical range. This suggestion is further supported by the likely role of the widely distributed *Ixodes* spp. in its transmission.

Of the five *Anaplasma* species encountered in European wildlife, *A. phagocytophilum* has been the most frequently reported. Evidence of infection has been reported in small mammals, including the bank vole (*Myodes glareolus*), rodent species in the genera *Mus, Apodemus* and *Microtus*, and shrews of the genera *Crocidura* and *Sorex*. Larger mammals, in particular deer, are also recognized as hosts for *A. phagocytophilum*. Infections have been reported in roe deer (*Capreolous capreolus*), red deer (*Cervus elaphus*), fallow deer (*Dama dama*) and sika deer (*Cervus nippon*), and in other ungulates including wild boar (*Sus scrofa*), bison (*Bison bonasus*) and mouflon (*Ovis orientalis*). Recently, infection has also been reported in a brown bear (*Ursus arctos*) in Slovakia. *Anaplasma phagocytophilum* infections have been reported in raccoons (*Procyon lotor*) in their native North America, so it is likely that animals introduced into Europe are also susceptible. Elsewhere in the world, infections have been found in other groups of mammals, including leporids and members of the Sciuridae family, but surveys of these animals in Europe have yet to be reported. Infections are also common in livestock, including cattle, sheep and horses, as well as companion animals. Although *A. phagocytophilum* is clearly able to exploit a wide range of terrestrial mammals, probably as reservoir hosts, there is, as yet, no indication of infections in marine mammals. Some evidence that birds and reptiles may also harbour the bacterium has been forthcoming, with a demonstration of *A. phagocytophilum* DNA in the blood of several bird species common to central Spain and in the blood of snakes and lizards in Northern California, USA.

The other *Anaplasma* species appear to have a more limited host range; *A. platys* is primarily associated with dogs, although infections in wild-living canids have not been described. *Anaplasma centrale* and *A. marginale* are

most frequently associated with domestic cattle; however, wild ruminants are thought to also play a role in the epizootiology of bovine anaplasmosis in some regions. Although the role of specific wild ruminant species has been studied in greatest depth in North America, some work has also been carried out in Europe. *Anaplasma marginale* DNA was detected in the blood of red deer in a region of Spain where infection is common in cattle. *Anaplasma ovis* infections are primarily reported in domesticated sheep and goats; however, the bacterium has also been detected in the blood of roe deer. *Anaplasma ovis* has been detected in reindeer (*Rangifer tarandus*) in East Asia, but infections in European populations have yet to appear.

Ehrlichia canis is a pathogen of domestic dogs, and is transmitted, like *R. conorii* and *A. platys*, by *Rh. sanguineus*. As yet, there is no evidence for infections in wild-living European canids; a survey of over 1500 red foxes (*Vulpes vulpes*) in Switzerland did not detect antibodies to *E. canis* in any animals, however, *E. canis* is not enzootic in the Swiss domestic canine population, and *Rh. sanguineus* infestation is rare. Conversely, a survey in Israel, where *E. canis* is enzootic in dogs, revealed 36% seropositivity among red foxes, suggesting that these hosts may serve as reservoirs for *E. canis* in regions of southern Europe where *Rh. sanguineus* populations are largest.

Ehrlichia muris was discovered to be infecting red-backed voles (*Eothenomys kageus*) in Japan, but isolates were also subsequently obtained from several other small rodent species in the same country. Evidence for the presence of *E. muris* in Europe was first obtained from a survey of yellow-necked mice (*Apodemus flavicollis*) inhabiting central Slovakia in 2007. DNA from *E. muris*, or at least *E. muris*-like organisms, has been detected in questing *I. ricinus* ticks in Slovakia and France.

ENVIRONMENTAL FACTORS

The distribution of *Rickettsia, Anaplasma* and *Ehrlichia* species is clearly restricted to that of their reservoir hosts and their competent vectors. The range of both is determined by environmental factors such as habitat, altitude, temperature and rainfall. Considerable effort has gone into understanding the environmental determinants of the distribution of ticks, and the temperature and humidity sensitivity of these arthropods has been quantified across Europe. As ticks serve as a reservoir as well as a vector for many *Rickettsia, Anaplasma* and *Ehrlichia* species, their ability to survive during periods when questing is not possible (primarily through winter) is of the utmost importance for the natural maintenance of the bacteria. Recently, work has shown that in infected ticks *A. phagocytophilum* induces the expression of a gene encoding a tick antifreeze glycoprotein, which is important for tick survival in a cold environment; hence it appears that *A. phagocytophilum* is enhancing its own persistence over winter by increasing the cold tolerance and survival of its vector.

EPIDEMIOLOGICAL ROLE OF WILD ANIMALS

Mammalian reservoir hosts have been identified for *Anaplasma* and *Ehrlichia* species, and for TG *Rickettsia* species, but the role of vertebrates in the natural maintenance of SFG *Rickettsia* species is less well known. The role of *Rickettsia, Anaplasma* and *Ehrlichia* species in wildlife disease is also uncertain. Infections caused by *Anaplasma* species have been frequently encountered in wildlife, but in general these surveys have been carried out on apparently healthy animals. Indeed, maintaining the health/fitness of a reservoir host is likely to better facilitate the transmission of *Anaplasma, Ehrlichia* and TG *Rickettsia* species to their arthropod vectors. Nonetheless, although reports of clinical disease in European wildlife are yet to be published, clear indication of the pathogenic potential of all three taxa can be drawn from studies of infections in domesticated animals and humans. *Anaplasma* and *Ehrlichia* species are recognized worldwide as pathogens of livestock and humans, and both SFG and TG rickettsiae are of public health importance. For *Anaplasma* and *Ehrlichia* species (and the TG member, *R. prowazekii*), the hosts in which disease is most often encountered are considered as reservoirs in which subclinical and/or chronic infections are the norm; thus it is reasonable to conclude that other, wild-living, reservoirs for these bacteria may also, on occasion, develop disease.

As discussed above, the role of wildlife in the natural maintenance of SFG *Rickettsia* species remains unclear. Despite the prevalence of SFG *Rickettsia* species in various European tick populations being relatively high (often over 10%), the prevalence of infections in the wildlife species that serve as major hosts for these ticks appears to be low. For example, in a recent survey in Poland, whereas 12.5% of *I. ricinus* ticks collected from 44 deer were found to contain *R. helvetica*, no evidence of infection was found in any of the deer themselves. Similarly, no evidence of infection was found in rodents or birds collected in the

same region. Evidence for *R. helvetica* infection has been found in a roe deer in Slovakia, although again, this was only one of over 100 animals surveyed. However, very recently, a survey of woodland mammals in southern Germany revealed serological evidence of exposure to SFG rickettsiae in almost 30% of individuals tested and polymerase chain reaction (PCR)-based evidence of infection in 8% of animals. DNA from two species, *R. helvetica* and *R. felis*, was detected in extracts prepared from the ears of these animals, thereby providing the first empirical support for a role for species such as *Myodes glareolus, Microtus agrestis* and *Apodemus flavicollis* as reservoir hosts for SFG rickettsiae.

Serological evidence of exposure to rickettsiae in European wildlife populations is also lacking, although strong support for host exposure to SFG group *Rickettsia* species can be drawn from studies on dogs, which are the primary host for *Rh. sanguineus* ticks, the vectors of *R. conorii*. In regions where *R. conorii* is endemic, the seroprevalence of *R. conorii* antibodies in dogs has been found to be high, and seasonal variation in seroprevalence correlates well with the active period of *Rh. sanguineus*. Early reports of isolation of *R. akari* (associated with peridomestic rodent-infesting *Lyponyssoides sanguineus* mites) from house mice in the USA have not been followed up in Europe, although it is widely considered that these rodents do play a direct role in the transmission of the bacterium. *Rickettsia felis*, most commonly associated with cat fleas, has been detected in numerous other blood-feeding arthropods, suggesting that they are being exposed to the bacterium even if they do not have a major role in its transmission. The role of mammals in the maintenance of TG rickettsiae appears to be clearer, as it is generally accepted that TG members exploit mammals as reservoir hosts. *Rickettsia typhi* is associated with wild rodents, in particular *Rattus* species, and anti-*R. typhi* antibodies have been observed in rats and other rodents in several European surveys. Thus, in summary, mammals are undoubtedly exposed to *Rickettsia* species through contact with blood-feeding arthropods. However, the frequency with which mammals become infected with SFG members, the kinetics of these infections, and their impact on host wellbeing, remains uncertain.

It is not entirely clear which of the mammalian species discussed above as being susceptible to *Anaplasma* or *Ehrlichia* infections are important as reservoir hosts in the natural maintenance of the bacteria, and which are merely accidental hosts. Small mammals, deer and livestock are thought to act as reservoir hosts for *A. phagocytophilum*, on the basis of either longitudinal studies of natural populations or experimental infections. Interestingly, in parts of Europe at least, *A. phagocytophilum* strains present in small mammals are genotypically distinct from those present in deer, suggesting that these two hosts form part of distinct, enzootic cycles. It appears likely that transmission of *A. phagocytophilum* in the enzootic cycle involving deer relies on *I. ricinus*, whereas transmission in the cycle involving small mammals relies on *Ixodes trianguliceps*, suggesting that subpopulations of *A. phagocytophilum* have evolved to exploit, exclusively, different vectors. Livestock are considered reservoir hosts for *A. centrale, A. marginale* and *A. ovis*, but the importance of wildlife in this role remains untested. Likewise, dogs are reservoir hosts for *A. platys* and *E. canis*, but wildlife species that are also parasitized by *Rh. sanguineus* may be able to fulfil this role too. It can only be assumed that small mammals act as reservoirs for *E. muris*, but, given that this species remains virtually unstudied, clarification of this role remains some way off.

TRANSMISSION

Rickettsia, *Anaplasma* and *Ehrlichia* species can be maintained in populations of arthropods by trans-stadial transmission, but only *Rickettsia* species can be transmitted transovarially. *Anaplasma* and *Ehrlichia* species are passed from infected vectors to non-infected vectors via the blood of mammalian hosts. Although this transmission route may also be employed by *Rickettsia* species, its relative importance in the natural maintenance of these bacteria is not known. Transmission between vectors may also occur via co-feeding, a process that involves an infected arthropod transmitting microorganisms in saliva or by regurgitation to an uninfected arthropod feeding nearby. Several *Rickettsia* species use co-feeding as a transmission strategy. For those *Rickettsia* species associated with fleas, *R. typhi* and *R. felis*, it has also been proposed that bacteria may be naturally shed in faecal material and that this material may be infectious either to susceptible hosts or flea larvae. Indeed, for *R. typhi*, this has long been considered the primary mode of transmission. However, recent experimental work has demonstrated that transmission via flea bite, at least between rats, also occurs. Interestingly, experiments have also revealed that some *Rickettsia* species can be transmitted by aerosol. Although the importance of this route in nature is unknown, its existence has led to concerns being raised that *Rickettsia* species could effectively

be used in bioterrorism or biological warfare. There is also increasing evidence that mechanical transmission, via arthropod mouthparts or other vehicles, have a role in the transmission of *Anaplasma* species. This mode of transmission may explain why *A. marginale* infections are encountered in places where tick vectors are apparently absent.

The mechanisms by which *Rickettsia*, *Anaplasma* and *Ehrlichia* species exploit their arthropod reservoirs/vectors have been explored to some degree. *Rickettsia* species can be acquired by arthropods by one of two routes – either via the midgut from infected blood meal or transovarially. Infection via the first of these routes occurs by rickettsial invasion of the tick midgut epithelial cells, followed by dissemination of the bacteria throughout the rest of the tick's body. Replication of rickettsiae occurs in salivary glands and ovaries, thereby facilitating transmission by feeding or to eggs. For *Anaplasma* and *Ehrlichia* species, the midgut is the first site of infection, as bacteria are acquired with ingestion of the blood meal into the midgut lumen. From here, bacteria enter the midgut epithelial cells and begin to replicate within membrane-bound vacuoles. Subsequently, bacteria migrate to and invade the salivary glands, and following this invasion, bacteria again begin to replicate in salivary gland acinar cells. This second round of replication is probably dependent on resumption of tick feeding on a mammalian host, and is followed by transmission via the saliva.

PATHOGENESIS, PATHOLOGY AND IMMUNITY

Anaplasma, Ehrlichia and some *Rickettsia* species are known to exploit mammals as reservoir hosts, and they do so using strategies that best facilitate their transmission to arthropods. For members of all three genera, these strategies appear not to provoke pathology but rather to involve a far more subtle manipulation of the host. Studies on wildlife are limited; however, it appears that *Rickettsia* species, on entering a susceptible host, are likely to be sequestered via lymphatic vessels to regional lymph nodes and then spread to various organs via the bloodstream. *Rickettsia* species have a tropism for endothelial cells and infection is thought to be characterized by disseminated vascular endothelial cell infections in all organs. For *Anaplasma* and *Ehrlichia* species, the early stages of infection are likely to be similar to those described for *Rickettsia* species. However, different *Ehrlichia* and *Anaplasma* species possess markedly different strategies of host interaction, primarily by targeting different types of host cells to subvert. *Anaplasma phagocytophilum* associates with neutrophils and probably endothelial cells, whereas *A. marginale* parasitizes erythrocytes. *Ehrlichia canis* and *E. muris* infect monocytic cells.

Unlike *Rickettsia* species, *Anaplasma* and *Ehrlichia* species are unable to synthesize lipopolysaccharide or peptidoglycan. Both of these molecules are important pathogen/microbe-associated molecular patterns, recognized by cells of the host innate immune system, so their absence is likely to help immune evasion by invading *Anaplasma* and *Ehrlichia* species. In the absence of a conventional envelope, the integrity of the outer membrane of these species is maintained by incorporating cholesterol from the host cell. Endocytosis leads to cell entry and the creation of an intracellular inclusion, but this inclusion does not progress along the endocytic membrane transport pathway, and thus is not subjected to lysosomal fusion. *Anaplasma phagocytophilum* remain within the inclusion and begin to multiply (these inclusions appear as classical 'morulae' in stained blood smears). Morulae eventually become swollen, and bacteria disperse when they are liberated from infected cells.

Little is known about the importance of specific bacterial proteins for intracellular survival, but one group of proteins that are likely to play a key role in granulocyte subversion are those that comprise a 'type four secretion system' that exports infection-mediating 'effector' proteins, including an ankyrin-repeat-domain-containing protein known as AnkA, directly into the host cell cytoplasm. The function of this, or other effector proteins, may underlie the ability of *A. phagocytophilum* to inhibit neutrophil apotosis, thereby prolonging the life span of its niche within a host. Another key mechanism by which *A. phagocytophilum* appears to prolong infection is through antigenic variation. The surface protein P44 has been found to be immunodominant, but it is now recognized that each bacterium has the ability to express one of about 100 different *p44* pseudogenes in its genome, thereby creating antigenic diversity within an infecting *A. phagocytophilum* population. Waves of sequentially expressed *p44* loci have been documented in experimentally infected animals, and it is thought that the subsidence of waves follows specific host immunological response to them. This feature of *A. phagocytophilum* has led to it being dubbed a 'microbial chameleon', exploiting its ability to change its surface coating to facilitate its persistence within a reservoir host.

Anaplasma marginale, despite its tropism for erythrocytes rather than neutrophils, shares some virulence traits with *A. phagocytophilum*. It too possesses a repertoire of *p44* pseudogenes, which are expressed in a similar, concerted, way. Unlike *A. phagocytophilum*, *A. marginale* has the capacity to generate genetic mosaics comprising fragments of different *p44* pseudogenes, a process that allows the generation of a vast array of antigenic variation within the P44 protein. Erythrocytes are the only known site of infection of *A. marginale*. The bacteria reside within membrane-bound inclusions (also called initial bodies), each containing up to eight bacteria. Up to 70% of erythrocytes may be parasitized during the early stages of overt infection, but hosts can also maintain far less intense infections subclinically for weeks or months, or possibly indefinitely. During overt infection at least, infected erythrocytes are phagocytosed by bovine reticulo-endothelial cells, resulting in development of anaemia. *Anaplasma centrale* and *A. ovis* also have a tropism for erythrocytes. *Anaplasma platys* has a tropism for blood platelets, and forms characteristic membrane-bound morulae within these cells. However, as with other *Anaplasma* species, how interaction between parasite and host is mediated remains to be defined.

Ehrlichia canis is a parasite of monocytic cells and, like *A. phagocytophilum*, within their target cell *E. canis* resides within membrane-bound inclusions called morulae. However, very little is known about the molecular mechanisms that mediate bacterial interaction with monocytic cells. Genomic analysis has revealed that *E. canis*, and other *Ehrlichia* species, possess a locus containing numerous copies of genes that encode for immunodominant 28 kDa outer membrane proteins. Expression studies suggest that some of these genes are preferentially expressed in macrophages, but their role remains elusive.

Despite the benign nature of infections in reservoir host species, *Rickettsia, Anaplasma* and *Ehrlichia* species have the capacity to provoke overt disease, primarily in 'accidental' hosts (see animal and public health concern sections below). No reports of the pathology associated with infections due to *Rickettsia, Anaplasma* or *Ehrlichia* species in European wildlife have been published; thus our knowledge of the pathology of these infections is drawn solely from observations in humans, companion animals or livestock, which, for most species, are accidental hosts. Many of the clinical signs associated with infections due to *Rickettsia* species result from damage to the vascular endotheliature and host response to this damage. The pathological outcomes of endothelial cell parasitism include, classically, an increase in vascular permeability leading to haemorrhage, thrombocytopenia and disseminated intravascular coagulation, but also vascular inflammation, oedema, increased association between leucocytes and the endothelium and the release of pro-inflammatory cytokines. The pathology associated with *A. phagocytophilum* infections includes splenic lymphoid depletion or hyperplasia. Lymph nodes may exhibit benign histiocytosis with lymphopenia or mild paracortical hyperplasia. Mild lymphohistiocytic perivascular infiltrates are typically observed throughout many tissues. *Anaplasma platys* infections can provoke generalized lymph node enlargement, and, histologically, lymph node and splenic lesions consist of lymphoid hyperplasia and plasmacytosis. The reported gross pathology associated with *E. canis* infections includes petechial and ecchymotic haemorrhages on the serosal and muscosal surfaces of most organs, in conjunction with generalized lymphadenopathy, splenomegaly and hepatomegaly. One of the most characteristic histopathologic findings is a perivascular plasma cell infiltrate in many tissues.

Infections by *Rickettsia, Anaplasma* and *Ehrlichia* species provoke an immune response in both reservoir hosts and accidentally infected hosts. Natural killer cell-derived interferon gamma and tumour necrosis factor are fundamental to the innate immune counter of infections through the activation of endothelial cells and macrophages, which are the major target cells for rickettsiae. In addition, as rickettsiae are inoculated through the dermis, resident dendritic cells (DC) play an important role in innate and acquired immunity by effectively presenting rickettsial antigens to T cells. T cells act against *Rickettsia* spp. in various ways, including the cytotoxic killing of infected target cells and cooperating with B cells in antibody production, which is an important defence against reinfection.

CLINICAL SIGNS AND TREATMENT

Although to date no reports of overt disease in European wildlife definitely attributable to *Anaplasma, Ehrlichia* or *Rickettsia* species have been published, the threat of these bacteria as veterinary pathogens is established through recognition of their role in companion animal and livestock morbidity. *Anaplasma phagocytophilum* has long been recognized as the agent of tick-borne fever (TBF) and as a contributor to tick-borne pyaemia ('stiffness') in sheep, in

northern Europe. TBF presents as fever, depression and loss of appetite. However, as TBF provokes severe leucopenia and compromises the function of peripheral blood neutrophils, secondary infections, particularly those caused by *Staphylococcus* species, are common. These infections frequently present as tick-borne pyaemia, affecting young (<12-week-old) lambs, and are characterized by crippling lameness and paralysis. The disease causes significant economic loss through debilitation and death of lambs. Other clinical complications include abortion and impaired spermatogenesis. TBF-like symptoms have also been recorded in *A. phagocytophilum*-infected dogs. *Anaplasma marginale* is also a long-recognized pathogen of livestock, causing bovine anaplasmosis, or 'gall sickness'. The disease is characterized as a progressive anaemia. Some acutely infected animals can lose condition and succumb very rapidly, whereas in others, disease progression is far less dramatic. Clinical signs include, fever, inappetence, loss of coordination, progressing to breathlessness and a high pulse rate. The urine may be brown, and mucous membranes appear pale and then yellow. Milk production falls and pregnant cows may abort. *Anaplasma ovis* causes a similar syndrome in sheep and goats. *Anaplasma platys* has long been recognized as a cause of canine cyclic thrombocytopenia. Most commonly, this syndrome presents as a fever, but some cases may also develop more severe clinical signs, including lethargy, appetite/weight loss, pale mucous membranes, petechial haemorrhages of the skin and oral mucosa, and purulent nasal discharges. *Ehrlichia canis* causes monocytic ehrlichiosis in dogs, characterized by fever, anorexia and myalgia, progressing to lymphadenomegaly, polyarthritis and neuromuscular signs such as seizures or ataxia. Infections also often lead to bleeding disorders, particularly nosebleeds. Although disease caused by SFG rickettsiae is frequently encountered in dogs in North America (primarily *R. rickettsii*, the agent of Rocky Mountain spotted fever), the veterinary relevance of this group of bacteria in Europe is far less clear. Perhaps the most credible report to date is that detailing the presence of *R. conorii* DNA in the blood of dogs in Sicily presenting with an ephemeral syndrome presenting as fever, anorexia and lethargy.

The effectiveness of tetracyclines for the treatment of *Rickettsia, Anaplasma* and *Ehrlichia* infections is well documented. Although the clinical efficacy of tetracyclines during the acute phase of infection is often very rapid, long durations for treatment are generally recommended to ensure eradication of bacteria within their intracellular niches. Hence, for example, the recommended regimen for canine monocytic ehrlichiosis is, typically, 10 mg/kg doxycycline daily for up to 15 days.

DIAGNOSIS

Clinical diagnosis of *Rickettsia, Anaplasma* and *Ehrlichia* infections is not straightforward – hence laboratory confirmation is desirable. As obligate intracellular bacteria, *Rickettsia, Anaplasma* and *Ehrlichia* species are difficult to isolate, culture is not used for routine diagnosis. Co-cultivation with eukaryotic cells, as used in virology, is feasible for all *Rickettsia, Anaplasma* and *Ehrlichia* species, and in reference laboratories, this approach has proven a relatively sensitive means of diagnosis. However, it requires specialized facilities and expertise and is time-consuming. Hence, simpler and more rapid diagnostic approaches, including blood smear examination, serology and PCR are far more widely used. *Anaplasma* and *Ehrlichia* species are all haemoparasites and can be observed by microscopic examination of stained blood smears. Giemsa or Wright stains are the most widely used staining techniques. However, the sensitivity of this approach is regularly questioned: although it seems useful for the detection of *A. phagocytophilum* morulae in the granulocytes of sheep or *A. platys* in canine thrombocytes, it is not considered a reliable means of diagnosing canine monocytic ehrlichiosis (caused by *E. canis*). Blood smear examination is routinely used to detect *A. marginale, A. ovis* and *A. centrale* infections in livestock – in particular in developing countries, where other diagnostic resources are lacking. Blood smear examination is not of any use in diagnosing infections due to *Rickettsia* species. For these infections, PCR and serology are the mainstay of laboratory diagnostics. Numerous PCR assays have been described of varying complexity, and have been applied to DNA extracts prepared from various clinical samples, including whole blood, buffy coat, eschar biopsy or even arthropods removed from a suspected case. PCR-based methods have also been described for *Anaplasma* and *Ehrlichia* species and are widely used. Serology is also useful for all pathogens. Various assay formats and antigens have been used to capture antibodies, and these have been shown to have varying degrees of specificity. Cross-reactivity between members of the same genus and even other pathogens can provoke misleading results, but this problem can often be resolved with cross-absorption studies and western blotting. Ideally, a four-fold rise in antibody titre can be

observed in paired sera, but for most patients diagnosis is based on a 'significant' single antibody titre. For most *Rickettsia, Anaplasma* and *Ehrlichia* species, these assays are now well evaluated, and are commercially available. Serology is also widely used to determine exposure rates in epidemiological studies of susceptible populations, often in tandem with PCR-based methods.

MANAGEMENT, CONTROL AND REGULATIONS

Prophylaxis for veterinary infections caused by *Rickettsia*, *Anaplasma* and *Ehrlichia* species is primarily attempted through control of haematophagous arthropods. Livestock-inhabiting areas where ticks are endemic are routinely treated with organophosphorus compounds or synthetic pyrethroids by plunge dipping or by using 'pour-ons'. Anti-tick and flea products for companion animals are also widely available. In the USA, trials are ongoing to explore the efficacy of reducing the risk of tick-borne disease in highly endemic locales through deployment of a self-application device that treats white-tailed deer with acaricide.

Although vaccines against various *Rickettsia* species were developed for human use during the last century, their efficacy and practicality is questionable, and there has been little or no recent development of them. Thus no vaccines are currently licensed in Europe for either medical or veterinary use against any *Rickettsia, Anaplasma* or *Ehrlichia* species.

For *A. marginale*, vaccination has been an economical and effective way to partially control bovine anaplasmosis worldwide. Both live and killed vaccines, derived from bacteria recovered from infected bovine erythrocytes, have been found to induce protective immunity that counters clinical disease, although neither type prevents cattle from becoming persistently infected with *A. marginale*. As mentioned above, *A. centrale*, by virtue of its antigenic similarity and relative avirulence, has also been deployed as a vaccine against *A. marginale*. Development of more sophisticated vaccines with the ability to prevent infections has been hindered by the antigenic diversity of the species. However, even if efficacious vaccines were available for all wildlife-associated *Rickettsia, Anaplasma* and *Ehrlichia* species, the delivery of these vaccines to wildlife reservoirs would present an enormous challenge.

PUBLIC HEALTH CONCERN

Several of the *Rickettsia* species encountered in Europe, together with *A. phagocytophilum*, have been associated with human infections. Cases of *R. aeschlimannii* have been reported in North Africa, where the bacterium is more prevalent than in Europe. *Rickettsia akari*, the causative agent of rickettsialpox, and *R. conorii*, the agent of Mediterranean spotted fever, are both well-established human pathogens. *Rickettsia felis* is an emerging pathogen; human cases are being reported with increasing frequency across Europe and elsewhere in the world. *Rickettsia helvetica* has recently been associated with a case of meningitis in Sweden. *Rickettsia massiliae* infections have been reported in Italy and southern France, *R. raoultii* and *R. slovaca* are associated with tick-borne lymphadenopathy, another emerging human infection that is probably present across much of Europe. Finally, about a dozen human cases of *R. sibirica* infection have also appeared, to date, in the literature. The one TG species present in Europe, *R. typhi*, is also a well-established human pathogen, causing murine typhus. Typically, the clinical symptoms of SFG rickettsioses include fever, headache, muscle pain, rash, local lymphadenopathy and a characteristic inoculation eschar (sometimes referred to as a 'tâche noire') at the site of the arthropod bite.

Anaplasma phagocytophilum infection in humans, referred to as human granulocytic anaplasmosis, is currently far more frequently recognized in the USA than Europe, where the incidence is second only to that of *B. burgdorferi* infection. However, human infections have been reported in various parts of Europe, and in several countries serological surveys have suggested significant levels of exposure to the bacterium. Human granulocytic anaplasmosis typically presents non-specifically, with fever, headache, myalgia, malaise, thrombocytopenia and leucopenia among the most common symptoms.

SIGNIFICANCE AND IMPLICATIONS FOR ANIMAL HEALTH

Several *Rickettsia* and *Anaplasma* species, as well as *E. canis*, have veterinary significance, being associated with infections in livestock and/or companion animals. *Rickettsia conorii* infections have been occasionally reported in dogs, and evidence that such infections may be more common

than currently recognized. The seroprevalence of anti-*Rickettsia* antibodies in southern European dogs is remarkably high. *Anaplasma phagocytophilum* is a significant pathogen of sheep and cattle in *I. ricinus*-infested upland regions of North-western Europe. It is associated with tick-borne fever and tick pyaemia, perhaps affecting 1% of the national flock. *A. phagocytophilum*-induced disease, akin to that described for humans, has also been reported in other livestock and companion animals, demonstrating the 'generalist' character of the species. Other *Anaplasma* species are far more host-specific: *A. marginale* causes bovine anaplasmosis, which is encountered in tropical and subtropical areas throughout the world and is a major constraint to cattle production in many countries; *A. ovis* provokes a similar disease, but in sheep and goats rather than cattle; *A. centrale* is also a pathogen of cattle, but provokes only mild disease; *A. platys* is responsible for infectious cyclic thrombocytopenia in dogs, which has been described throughout the world. *Ehrlichia canis* is the agent of canine monocytic ehrlichiosis, a potentially life-threatening infection.

References for further reading are shown below[1–7].

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CHAPTER

29

MYCOPLASMA INFECTIONS

ROBIN A.J. NICHOLAS AND MARCO GIACOMETTI

INTRODUCTION

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Mycoplasmas are members of the class *Mollicutes*, which comprises all the wall-less bacteria. They are characterized by their small genome size (0.58–0.22 Mbp), a low G+C content (23–40 mol %) of the genome and a permanent lack of a cell wall. Over 200 species have so far been described and have been isolated from plant and both invertebrate and vertebrate animal hosts. Many more remain to be characterized. By far the majority are non-pathogenic, living on mucosal surfaces, where they remain reliant on the host for their nutritional requirements, being totally devoid of genes for biosynthetic processes. Some, however, can be invasive and cause serious diseases such as contagious bovine and caprine pleuropneumonia and atypical pneumonia in humans, one of the most common community acquired pneumonias worldwide. Generally the features of mycoplasma diseases in animals include respiratory signs, often accompanied by arthritis, mastitis and eye infections; infertility, abortions and nervous signs may also be seen.

Mollicutes inhabit most animal wildlife species, including insects, fish, amphibians, reptiles, birds and mammals. New species are being detected frequently in many animals such as desert tortoises, snakes, mink, crocodiles, birds of prey and sea mammals, where they are probably of little consequence under normal situations. However, mycoplasmas are notorious for exploiting stressful situations, including those caused by weather fluctuations, overcrowding and, probably linked to these, hormonal disturbances. It is likely that the majority of reported outbreaks of mycoplasmosis in wildlife species are associated with the unnatural conditions of captivity or semi-captivity found in zoos, wildlife parks and farms where animals are intensively reared. The conditions under which laboratory animals such as rabbits, rats and mice are bred is also conducive to horizontal spread of mycoplasmas like *M. pulmonis*. This has implications both for the health of the laboratory animal, in which high morbidity and low fertility may be seen, and to the results of the experiments in which these animals are being used. In these circumstances, normally non-pathogenic strains can cause disease because of a depressed host immune system. More commonly, however, pathogenic species in one host species may have the opportunity to infect a different host species by close contact, a situation that would be unlikely to occur in the wild. Recent outbreaks of what was thought to be a highly host-specific disease, contagious caprine pleuropneumonia, in various gazelle species in a private collection on an island in the United Arab Emirates was linked to the

Infectious Diseases of Wild Mammals and Birds in Europe, First Edition. Edited by Dolores Gavier-Widén, J. Paul Duff, and Anna Meredith.

introduction of goats[1]. Even in the wild, food shortages brought about by seasonal changes may force wildlife to feed closer to domesticated species, as has been seen with the spread of infectious keratoconjunctivitis from sheep to the wild small ruminant species described in this chapter. The mycoplasma flora of captive animals may not be representative of animals in the wild but may have implications in the event of escape or reintroduction to the wild, a distinct possibility in many zoos and wildlife parks.

MYCOPLASMAS INFECTION OF WILD SMALL RUMINANTS

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Small ruminants are affected by several important mycoplasma diseases. Contagious caprine pleuropneumonia (CCPP), caused by *Mycoplasma capricolum* subsp. *capripneumoniae*, has not been seen in Europe for nearly 100 years but was detected in Thrace in 2005 threatening the borders of Greece and Bulgaria[2]. CCPP has now been shown to be capable of infecting wildlife, so surveillance should be extended to susceptible species in which acute respiratory disease accompanied by high mortality is reported.

Of most relevance to Europe is the World Organisation for Animal Health (OIE)-listed disease contagious agalactia. This disease can be caused by four different mycoplasmas, which are, in descending order of importance: *M. agalactiae*, *M. mycoides* subsp. *capri* (formerly *M. mycoides* subsp. *mycoides* LC), *M. capricolum* subsp. *capricolum* and *M. putrefaciens*. During a survey of over 300 free-ranging Spanish Ibex (*Capra pyrenaica hispanica*), the majority of which had been hunted or captured live in the mountains of Andalucia, single or mixed cultures of mycoplasmas were detected in the ears and eyes of 18% of animals[3]. The most common species was *M. agalactiae*, which was detected in nearly half of the positive cases, frequently with the commensal *M. arginini*. The respiratory pathogen, *M. mycoides* subsp. *capri*, was detected less frequently. Interestingly, *M conjunctivae* was not detected in any ibex, although the authors pointed out that no clinical signs or lesions were seen in the population studied; it is also possible that the techniques used may have not detected the highly fastidious *M. conjunctivae*. The high prevalence of *M. agalactiae* in this wild population is almost certainly a result of the interaction with domestic small ruminant flocks in which contagious agalactia is endemic and will seriously compromise any attempts to eradicate the disease from the region.

In other parts of the European uplands, *M. mycoides* subsp. *capri* has been isolated from the eyes of adult male Alpine ibex (*Capra ibex*) originating from the Valle d'Aosta Region in Northern Italy. The animals suffered bilateral ocular discharge with diffuse inflammation, severe corneal involvement of the left eye and mild corneal opacity of the right eye. Histological examination revealed a keratoconjunctivitis characterized by lymphocytic and plasmacellular infiltration[4]. Highly sensitive molecular tests confirmed the absence of *M conjunctivae* from these samples.

INFECTIOUS KERATOCONJUNCTIVITIS IN CAPRINAE

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Infectious keratoconjunctivitis (IKC) is a highly contagious infection of the eye characterized by inflammation of the conjunctiva and cornea and is common in some wild Caprinae species as well as in domestic sheep and goats. The major single cause of IKC in chamois, ibex and in other Caprinae species, including domestic sheep and goats, is *Mycoplasma conjunctivae*[5]. The term 'infectious keratoconjunctivitis' is used to describe a clinical condition that may be caused by more than one pathogen.

AETIOLOGY

The aetiology of IKC in chamois and other wild Caprinae species is not fully understood. Several pathogens have been suspected to be aetiological agents of IKC. In domestic sheep however, pathogenicity has been demonstrated for two agents that have been isolated from the eyes: *Chlamydophila psittaci* and *M. conjunctivae*. The pathogenicity of *M. conjunctivae* has been demonstrated in domestic sheep, domestic goats, Alpine ibex (*Capra i. ibex*) and European mouflon (*Ovis orientalis musimon*)[5]. This organism has also been implicated as an aetiological agent of IKC in Alpine chamois[6] and Pyrenean chamois

(*Rupicapra p. pyrenaica*)[7]. In the eastern Swiss Alps, the occurrence of *Chlamydiaceae* in Alpine chamois is low[8]. It is now generally accepted that *M. conjunctivae* is the major single cause of IKC in domestic and wild Caprinae species. An association has been demonstrated between the mycoplasmal load in the eyes and the severity of clinical signs[9]. Phylogenetically, *M. conjunctivae* belongs to the *Mycoplasma neurolyticum* cluster of the *hominis* group, and is closely related to *M. bovoculi* and *M. ovipneumoniae*.

Using a molecular method (DNA sequence determination of a variable domain within the adhesin gene *lppS*), more than 40 different strains of *M. conjunctivae* have recently been subtyped and identified. It has been shown that Australian strains from sheep introduced to Croatia in 1995 belong to a distinct cluster that is different from all other isolates which originate from European Alpine countries[10].

EPIDEMIOLOGY

The first outbreak of IKC in wildlife was reported in Alpine chamois in the Austrian Alps in 1916[11]. Since then, numerous outbreaks have been described in wild Caprinae species in other European countries, including Switzerland, Italy, France, Slovenia, Spain, Andorra and Cyprus[5]. IKC is frequently observed in Alpine chamois and in Alpine ibex, but it has also been described in other wild Caprinae species, such as Pyrenean chamois, European mouflon and Cyprian mouflon (*Ovis orientalis ophion*).

In individual chamois and ibex, spontaneous recovery is the most prevalent course of IKC. At the population level, mortality is usually low (<5%). In particular situations, however, mortality can reach 30%. For example, high mortality was seen in chamois in the Gran Paradiso (Italy) and Vanoise (France) National Parks in 1981–1983, in the Aosta Valley (Italy) in 1998, in the Simmental-Gruyère-Region (Switzerland) in 1997–1999, and in Grisons (Switzerland) in 2005–2007. Severe outbreaks have repeatedly been reported also in Alpine ibex, sometimes simultaneously with sympatric chamois[5,12].

Population recovery in the post-epidemic period is either rapid or occurs within a time lag of 5 to 6 years, depending on the mortality rate observed.

The characteristics of the infectious agent may be an important factor influencing the course of the disease, and therefore mortality, in affected herds. Variation in pathogenicity may be due to differences in adhesion of the mycoplasma variable membrane lipoprotein, LppS, which has been identified as an adhesin. It has been proposed that differences in mortality may be due to the strain of *M. conjunctivae* involved, and/or to host factors (e.g. genotype, health condition, overcrowding), to secondary infections or to environmental predisposing factors (e.g. ultraviolet irradiation)[6].

In Alpine chamois, death associated with IKC is most often recorded in females and juveniles. Only a small number of affected adult males die, possibly reflecting a lower percentage of this sex/age-class within chamois populations. Furthermore, a lower probability for infected adult males could be caused by sexual segregation during most of the year[5,13].

Outbreaks in domestic caprinae associated with severe signs are usually observed when the agent is introduced into naive herds by infected animals from other flocks.

In chamois, IKC epidemics can progress rapidly largely because of the highly contagious nature of IKC. When changing from winter to summer range, individually marked Pyrenean chamois were recently shown to move distances up to 5 km within a few days, with Alpine ibex exceeding 10 km.

In some smaller locations, IKC cases are generally observed over a short period only (up to 2 years). In large mountain ranges, however, outbreaks may spread along the mountain chains for dozens of kilometres and persist for several years longer with hundreds of deaths before the epidemics stop.

Intraspecific transmission of *M. conjunctivae* occurs throughout the year. However, IKC losses in chamois are more frequent during summer and autumn. This coincides with the presence of domestic sheep grazing extensively on summer mountain pastures[5].

In the domestic sheep population, the infection is endemic and self-maintained worldwide. In Switzerland, at least one positive sheep was detected in 89% of the herds, and in positive herds that were tested, up to 57% of the individual animals tested positive[14]. The mixing of sheep during grazing, shows and markets, the introduction of new animals to a flock and the presence of (susceptible) lambs during much of the year maintains *M. conjunctivae* infection within sheep populations.

The long-term maintenance of *M. conjunctivae* infection in chamois populations is not completely elucidated.

Seroprevalence in chamois subpopulations with no outbreaks within 2 years of sampling was low compared with subpopulations experiencing ongoing outbreaks. After an outbreak in Simmental-Gruyères in 1997 to 1999, the seroprevalence dropped down to zero after 2 years. This may be the result of the limited contact of chamois from different herds, the strict seasonal birth period and the high mortality. Persistence of infection in individual animals does not exceed 3 to 6 months, as seen in domestic sheep[5]. However, detection of *M. conjunctivae* in eyes without clinical signs has been reported in domestic and wild Caprinae individual animals and is not always associated with apparent outbreaks[9].

TRANSMISSION

IKC is highly contagious within herds, with *M. conjunctivae* being excreted in ocular effusions, via aerosol and possibly by eye-frequenting insects[5]. Mycoplasmas generally do not survive long in the environment, and therefore transmission must occur quickly through direct contact or vectors. Transmission of *M. conjunctivae* between the same host species (e.g. between sheep and sheep, or chamois and chamois) does not require vectors and occurs predominantly or exclusively by contact.

Mountainous habitats are shared by domestic and wild animals grazing on the same pastures, and short-distance encounters between free-ranging individuals of different Caprinae species, domestic and wild species, are not uncommon events in the Alps[9,15]. In these circumstances, transmission of *M. conjunctivae* between domestic and wild Caprinae species is likely to be via aerosol infection or by eye-frequenting insects rather than direct contact. It is assumed that the probability of interspecific transmission increases as distance between animals decreases. Flies are likely to play a central role in interspecific transmission of *M. conjunctivae* on alpine meadows. Excessive lacrimation is an attractive source of proteins, salt and water for insects, and flies' feeding habits are not species-specific, particularly when hosts graze in close proximity. Four genera of Muscidae were identified as potential vectors of *M. conjunctivae*: *Hydrotaea*, *Musca*, *Morellia* and *Polietes*[16]. It is probable that flies play a role as accessory vectors, but their role might be of primary importance in the sporadically occurring interspecific transmission of *M. conjunctivae* on alpine meadows.

FIGURE 29.1 Infectious keratoconjunctivitis in an adult male Alpine ibex (*Capra i. ibex*): the cornea shows oedema with sero-mucous ocular effusion; blindness is reversible. Photograph: Luca Pellicioli.

PATHOGENESIS, PATHOLOGY AND IMMUNITY

The period of incubation after experimental infection with *M. conjunctivae* is short (2–4 days)[17]. In early stages, IKC presents as a unilateral or bilateral conjunctivitis associated with serous or mucous effusions. Conjunctivitis can persist for some weeks without any sign of keratitis, but the disease may progress to more severe forms. Mild keratitis is characterized by either oedema (Figure 29.1) or by perilimbic neovascularization, which can occur without preceding corneal oedema in the vertex area. Corneal ulceration may occur in severe cases, occasionally leading to corneal perforation. Ruptured corneas show anterior synechiae and melanin deposits forming staphyloma. The stages are progressive, but healing may commence at any time, except for eyes with ruptured corneas[18].

IKC caused by *M. conjunctivae* is a specific ocular disease. The absence of brain lesions in blind ibex and chamois presenting with circling movements suggests that in wild Caprinae affected by IKC changes in behaviour are generally not a consequence of cerebral lesions but are probably the result of disorientation and stress in blind animals[13].

In Caprinae, the immune response to *M. conjunctivae* infection is frequently effective. In experimental infections, specific antibodies as detected by immunoblot

analysis appear 2–4 weeks post infection[19]. Immunoblot analysis has revealed the major specific immunogenic proteins of *M. conjunctivae* of naturally and experimentally infected chamois, ibex and domestic sheep. Antibodies to the 175, 73, 68, 60 and 33 kDa antigens appear to be specific to *M. conjunctivae*[19]. However, little is known about the protective effect of acquired immunity, and it may be insufficient to resist natural infections.

CLINICAL SIGNS

In individual animals, clinical signs last from 2 weeks to some months. Although IKC usually provokes mild clinical signs in domestic Caprinae, it can have fatal consequences in wildlife. In wild Caprinae, the disease may progress to bilateral corneal rupture. Blind chamois and ibex inhabiting particularly treacherous steep rocky terrain may fall from cliffs, present abnormal behaviour such as disorientation and circling movements, and die of starvation. Occasionally, mortality can reach 30% and have a marked impact on demography. In domestic sheep and goats, signs are generally moderate but occasionally severe in adult animals, and usually mild in lambs.

After the disappearance of clinical signs, animals can act as healthy carriers of *M. conjunctivae*. The length of the period is not completely elucidated, but it might be limited to up to 6 months, as shown in domestic sheep[20]. Detection of *M. conjunctivae* in eyes without clinical signs is common and has been reported in domestic and wild Caprinae individuals[9].

DIAGNOSIS

Diagnosis of *M. conjunctivae* infections can be done with isolation of the organism by culture and subsequent identification by immunological methods. For culture, eye swabs are dipped into Transwab® transport medium and processed within 24 hours of collection. Culture is performed on standard mycoplasma broth medium enriched with 20% horse serum, 2.5% yeast extract and 1% glucose[21]. Culture of *M. conjunctivae* requires specialized technical expertise.

A polymerase chain reaction (PCR) assay was developed for rapid direct detection of *M. conjunctivae* in clinical material. Conjunctival swabs are dipped in tubes without transport medium and stored at −18°C until analysis. Nested PCR is based on unique sequences of the *rrs* genes (16S ribosomal ribonucleic acid (rRNA)) of *M. conjunctivae* and show a higher sensitivity than culture. Currently, the method of choice for diagnostic purposes is a TaqMan real-time PCR based on the lipoprotein gene *lppS* (EMBL/GenBank accession number AJ318939), which provides a fast and a very sensitive detection of *M. conjunctivae*[22].

An indirect enzyme-linked immunosorbent assay (ELISA) method based on the Tween 20 extracted fraction of *M. conjunctivae* strain HRC/581T has been developed to detect *M. conjunctivae* antibodies in sera of domestic sheep and Alpine chamois[5].

MANAGEMENT AND CONTROL

In wild Caprinae, spontaneous recovery is the most frequent course of the disease. Therefore, shooting of all animals affected with IKC, regardless of the severity of clinical signs, is an inappropriate measure for disease control[20]. However, individuals presenting with irreversible ocular lesions (corneal perforation) or being in poor general condition and/or injured should be shot by experts for humane reasons.

Treatment of affected free-ranging chamois or ibex should not be considered, because the prognosis in individuals is not known in advance; animals presenting with mild ocular lesions may show self-healing, and irreversible ocular lesions will not respond to treatment. Other control measures should include minimization of unnecessary human interference (e.g. cross-country-walks and uncontrolled hunting) in affected areas to avoid putting animals to flight (which could lead to falls and injury or death). Furthermore, long-distance movements of infected animals can disseminate the disease, potentially introducing it to new areas.

Prevention of IKC in wild Caprinae should focus on preventing the spillover of *M. conjunctivae* from domestic sheep. Thus, salt-lick use on mountain pastures should be controlled to reduce the frequency of encounters and physical contact between individuals of different Caprinae species. Diseased domestic sheep and goats should be reported and not be moved into habitats frequented by susceptible wild Caprinae.

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MYCOPLASMAS OF BIRDS

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FIGURE 29.2 Pheasant showing severe eye lesions caused by *Mycoplasma gallisepticum*.

Mycoplasma infections produce significant disease in the poultry industry, particularly in battery farms, where they can rapidly disseminate through birds housed at high densities, leading to huge losses. The ability of some, such as *M. gallisepticum* and *M. synoviae,* to spread vertically through the egg makes control particularly difficult. Even in free-range flocks, mycoplasmas can be a problem because of the close contact at roosting, although losses are generally not so high. The situation in the wild is not as clear, but generally typical mycoplasma diseases such as sinusitis, conjunctivitis and chronic respiratory disease are only seen sporadically. However, where wild species such as pheasants and partridges are intensively bred to meet the growing demand for hunting, they suffer the mycoplasma diseases seen in high-density flocks typical of the poultry industry. This intensification, seen in Europe over the last 20 years, has led to adult birds that are susceptible to a range of infectious and non-infectious diseases, and mortality rates of 5–10% or higher are commonly seen. The largest causes of death and morbidity in game birds are sinusitis and conjunctivitis (Figure 29.2), which are most often associated with mycoplasma infection. The sourcing of eggs from flocks free of *M. gallisepticum* has led to an improvement in this sector over recent years, although losses are still seen from time to time.

Many mycoplasmas have been isolated from pheasants, including *M. pullorum, M. iners* and *M gallinarum*, but they are not considered major pathogens; their main effect seems to be in obscuring the detection of the more fastidious proven pathogens such as *M. gallisepticum* and *M. synoviae*. However, the development of sensitive and specific molecular tests that are capable of detecting these mycoplasmas directly in clinical material, often with other species, has improved the accuracy of diagnosis.

Racing pigeons, which are often kept in overcrowded lofts throughout Europe, suffer respiratory disease. This has been linked, although not definitively, with mycoplasmas such as *M. columborale, M. columbinum* and *M. columbinasale.* In an outbreak of both ocular and respiratory disease affecting 10 and 20% of show and racing pigeons respectively, *M. columborale* was the main organism isolated from a cage of over 300 birds in Sicily that had been confined for over 2 months[23]; a gradual improvement was seen in the birds following treatment with tylosin. These mycoplasmas have also been detected in apparently healthy feral pigeons, suggesting they may be part of the natural flora of these birds.

In 1994 a new disease called 'house finch conjunctivitis' spread across the eastern USA and soon became ubiquitous in the finch population. The disease caused unilateral and bilateral conjunctival and periorbital swelling, which was often complicated with nasal discharge and mucopurulent drainage, impairing both vision and respiration. Following initial reports of the disease in the house finch (*Carpodacus mexicanus*), American goldfinches (*Carduelis tristis*), blue jays (*Cyanocitta cristata*) and house sparrows (*Passer domesticus*)[24] have also become infected, indicating a wider host range than previously thought. No such epidemic has yet been seen in Europe, although conjunctivitis is seen sporadically in a range of bird species, including

birds of prey, but rarely involving *M. gallisepticum*. Other *Mycoplasma* species have been reported in wild birds, including *Mycoplasma sturni*, which has been associated with conjunctivitis in the European starling (*Sturnus vulgaris*)[25]; these European outbreaks were clinically very similar to those seen in American finches. These observations offer further evidence that mycoplasma-associated conjunctivitis is a potentially important emerging infection affecting the welfare of both wild free-living and captive birds. This infection may have ecological effects as a consequence of altered population densities of affected species. The 60% fall in the eastern house finch population was attributed to the arrival of *M. gallisepticum,* probably from contact with infected domestic poultry[26].

Mycoplasma sturni was the predominant *Mycoplasma* species found during a study to determine the causes of mortality in wild birds in Britain. It was detected in oropharyngeal or conjunctival swabs taken from blackbirds (*Turdus merula*), rooks (*Corvus frugilegus*), carrion crows (*Corvus corone*), magpies (*Pica pica*) and starlings not required as given earlier. Birds that were infected with *M. sturni* were suffering from a range of infectious and non-infectious conditions, although none that would be considered typical mycoplasma diseases, so its significance remains unknown. More recently *M. sturni* was detected from some of a large population of rooks which had died, or were dying, from acute respiratory disease in the UK, although it is likely that the main cause of disease was *Pasteurella multocida*[27].

Although reported many times in birds of prey, the role of mycoplasmas as pathogens is still unclear. *Mycoplasma* spp. were detected by culture and a genus-specific PCR in healthy, free-ranging raptor nestlings and birds of prey from rehabilitation centres, sampled during a routine ringing programme[28]. These included *Mycoplasma buteonis*, *M. falconis* and *M. gypis*. Unidentified mycoplasmas were also isolated from western marsh harriers (*Circus aeroginosus*), a Eurasian hobby (*Falco subbuteo*) and a barn owl (*Tyto alba*). Given the lack of clinical signs or pathology, it was likely that mycoplasmas in these raptors were commensals rather than pathogens. However, in an earlier study many of the same strains were isolated from birds of prey held at a sanctuary that showed respiratory disease, suggesting that the mycoplasma may cause or contribute to the cause of disease under the stress of captivity[29].

The isolation and characterization of four mycoplasmas found in the upper respiratory tract of four sick Eurasian griffon vultures (*Gyps fulvus*) that were housed in a Sicilian rehabilitation centre has been reported[30]. These included *Mycoplasma gallinarum*, an unidentified mycoplasma highly similar to *Mycoplasma glycophilum*, and two unidentified mycoplasmas with similarities to *Mycoplasma falconis* and *Mycoplasma gateae,* a mycoplasma normally associated with cats. No other pathogens were detected. The significance of large numbers of as yet unidentified mycoplasmas in great white pelicans (*Pelecanus onocrotalus*), a threatened European breeding species, is also unknown, but birds appeared to be healthy.

A mycoplasma closely related to *M. gallisepticum* was identified from the lung and liver of different captive Humboldt penguins (*Spheniscus humboltdi*) in a zoo in the UK[31]. It was speculated that the most likely source of this infection was ducks and other wild or semi-wild species in adjoining enclosures. Later a new species, *M. sphenisci,* was isolated from choanal discharge from a jackass penguin (*Sphenus demersus*), which may represent a unique species found in the penguin. Interestingly, although *M. sphenisci* was believed to be pathogenic in the jackass penguins, king penguins (*Aptenodytes patagonicus*) which were close by showed no clinical signs. A similar situation arose in the UK, where *M. gallispecticum* and *M. gallisepticum*-like strains were isolated from captive Humboldt penguins exhibiting high mortality. King penguins co-housed with the Humboldts were found to have *M. lipofaciens* and *M. spenisci* but showed no clinical signs. It was thought that *M. gallisepticum* may be an important cause of morbidity and mortality in Humboldt penguins, an endangered species, although it is not clear how they would become infected in the wild.

It is common to isolate unidentifiable mycoplasmas from birds, and many of these will remain unidentified for many years owing to the time-consuming nature of the characterization process. Although the use of 16S rDNA sequencing has greatly improved the screening process, much biochemical, serological, morphological and genetic analysis is still required for describing new species.

MYCOPLASMA INFECTIONS OF AQUATIC MAMMALS

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In 1988, more than18,000 harbour seals (*Phoca vitulina*) in the North and Baltic Seas died of an acute disease char-

acterized by pneumonia, polyarthritis, nervous signs, abortions, diarrhoea and skin lesions[32]. Although influenza and influenza-like viruses were implicated as primary causes, the isolation of over 100 strains of mycoplasmas, many from diseased animals, coupled with the mycoplasma disease-like clinical signs, strongly suggested their contributory role in the disease process. Two new species were characterized and named: *M. phocacerebrale* (later respelled *M. phocicerebrale*) and *M. phocarhinis* (later *M. phocirhinis*). A few years earlier, a mycoplasma later characterized as *M. phocidae*[33] was isolated during an outbreak of pneumonia in harbour seals off the coast of New England, USA from which an influenza virus was also detected. The ability of these mycoplasmas to cause disease in mice and induce cytopathic effects in organ cultures provided some evidence to link these mycoplasmas with disease. Moreover, *M. phocicerebrale* has also been strongly associated with severe lesions following biting by harbour and grey (*Halichoerus grypus*) seals in the wild and in captivity, which has led to infected wounds (Figure 29.3), sometimes requiring the amputation of flippers. This link was convincingly shown by the isolation of mycoplasmas from both the teeth and bite wounds of captured seals[34]. It is not surprising then that *M. phocicerebrale* has also been detected in a human condition known as 'seal bite finger', a common injury seen in sealers, trainers and other occupations associated with the seal industry. This probably represents one of the few examples of zoonosis involving a mycoplasma.

There are fewer reports of mycoplasmas in other sea mammals. One brief report described the isolation of two serologically different mycoplasmas from the lungs of harbour porpoises (*Phocoena phocoena*)[35]. More recently *M. phocicerebrale* and novel *Mycoplasma* species were isolated from harbour porpoises and a Sowerby's beaked whale (*Mesoplodon bidens*) that had died following strandings on the Scottish coast; unusually one novel strain was isolated from the kidney of the whale[36]. Many of these cetaceans had pneumonic lesions, suggesting a pathogenic role for mycoplasmas.

FIGURE 29.3 Grey seal (*Halichoerus grypus*) showing severe necrotic lesions of the flipper caused by a bite wound from which *Mycoplasma phocicerebrale* was isolated.

OTHER MYCOPLASMA INFECTIONS

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Porcine infectious anaemia is caused by the formerly classified eperythrozoon *Mycoplasma suis*. It is a well-known disease mainly affecting pigs that occurs worldwide, particularly in China, where it has a high prevalence. This organism may have zoonotic potential. The DNA of this uncultivable haemotrophic organism was recently detected in 10% of a small wild boar (*Sus scofa*) population in Germany[37]. The study also showed that there were two genetically distinct groups representing strains similar to those found in the USA and Europe and those found in China.

Commercial rearing of rabbits often leads to respiratory and digestive diseases, which may be stress related. In a study in Spain, mycoplasmas were detected in nearly half of all cases of commercial rabbit pneumonia, although other pathogens were also detected, including *Bordetella bronchiseptica* and *Pasteurella* spp., although in fewer numbers[38].

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CHAPTER

30

ESCHERICHIA INFECTIONS

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Escherichia coli inhabits the large intestine and distal ileum of most vertebrate species. The majority of the strains are of low virulence and may cause opportunistic infections, whereas certain strains are of high pathogenicity and may cause a variety of intestinal, septicaemic and extra-intestinal diseases, which have been most intensively studied in food-producing animals.

AETIOLOGY

Escherichia species share the common characteristics of the family *Enterobacteriaceae*. They are Gram-negative, medium sized (0.4–0.6 × 2–3 μm) facultative anaerobic rods, catalase-positive, oxidase-negative, capable of fermenting glucose and nitrate reductase-positive. *Escherichia coli* is the major (10^7 to 10^9 organisms/gram faeces) facultative anaerobic organism of the normal intestinal flora of most animal species. *Escherichia albertii* is a recently described member of the *Enterobacteriaceae* previously regarded as a strain of *Hafnia alvei*[1].

Serologic classification of *E. coli*, in particular of the O antigen of the lipopolysaccharide and the H antigen of the flagellum, allows discrimination between pathogenic and non-pathogenic strains. In addition, the capsular antigen (K) is also used for classification. Seven pathovars of intestinal *E. coli* currently exist, of which enterotoxigenic *E. coli* (ETEC) is the most common cause of diarrhoea in livestock. Shiga toxin-producing *E. coli* (STEC), also known as verotoxin-producing *E. coli* (VTEC), are associated with diarrhoea and dysentery in neonates and young ruminants and oedema disease in swine, as well as cutaneous and renal glomerular vasculopathy in dogs. STEC strains causing distinct clinical signs in humans are known as enterohaemorrhagic *E. coli* (EHEC), of which the serotype O157:H7 is the most commonly implicated in human haemorrhagic enteritis. Enteropathogenic *E. coli* (EPEC) cause diarrhoea in several animal species, most importantly in rabbits, pigs and dogs. Pathogenic intestinal *E. coli* further include enteroinvasive (EIEC), enteroaggregative (EAEC), diffuse adherent (DAEC) and necrotoxic (NTEC) strains. The latter are also involved in septicaemia and urinary tract infections. The group of extra-intestinal *E. coli* (EXPEC) comprise bacteria, among them avian pathogenic (APEC) and uropathogenic (UPEC) *E. coli*, involved in a variety of infections including septicaemia, infections of the respiratory and urinary tract, metritis and mastitis[2]. *Escherichia albertii* has been associated with diarrhoea in humans but was also found in birds, causing subclinical infections and fatal enteritis[3].

EPIDEMIOLOGY

Escherichia coli inhabit the lower small and large intestine of all mammals. Carnivores usually carry larger amounts

Infectious Diseases of Wild Mammals and Birds in Europe, First Edition. Edited by Dolores Gavier-Widén, J. Paul Duff, and Anna Meredith.

of this bacterium compared with omnivores and herbivores. The organism is shed with faeces and can survive for weeks and months in faecal particles and dust. Extraintestinal disease is most often caused by *E. coli* belonging to the normal microbial flora of the animal, whereas enteric disease is usually caused by pathogenic *E. coli* contracted from an exogenous source. Infection occurs via the oral route. Vectors (e.g. the flies *Stomoxys calcitrans* and *Musca domestica*) may also transmit *E. coli*.

Wildlife may play a role as carriers and transmitters of Shiga toxin-producing *E. coli* O157:H7 and non-O157 in nature. High rates (51.8%) of STEC/EHEC-positive faecal samples from clinically normal roe deer (*Capreolus capreolus*) and red deer (*Cervus elaphus*) have been found in a German study[4]. In Spain, STEC were detected in 24.7% of red deer, 5% of roe deer, 33.3% of fallow deer (*Dama dama*), 36.4% of mouflon (*Ovis musimon*) and 8.5% of wild boar (*Sus scrofa*) samples investigated[5,6]. Wild brown rats (*Rattus norvegicus*) tested VTEC-positive in a Danish study[7]. Wild rabbits (*Oryctolagus cuniculus*) may also act as vectors for *E. coli* O157 and non-O157-VTEC[8]. VTEC were also found in wild boar in Sweden[9], and in gulls (*Larus* spp.), lapwings (*Vanellus vanellus*), crows (*Corvus corone corone*) and jackdaws (*Corvus monedula*) in the UK[10]. A high proportion of feral pigeons (*Columba livia*) were found to harbour STEC in Italy[11]. EPEC were isolated from faecal samples of Antarctic fur seal (*Arctocephalus gazella*) pups from the South Shetland Islands[12]. Similar strains found in wild mammals, birds and livestock indicated transmission between domestic animals and wildlife or a common environmental source[7,13].

In Scotland, *E. coli* O86:K61 was associated with disease and high mortality in wild birds in household gardens where supplementary food was provided. Chaffinches (*Fringilla coelebs*), greenfinches (*Carduelis chloris*) and Eurasian siskins (*Carduelis spinus*) were most frequently affected[14]. *Escherichia coli* O86 was also isolated from an emaciated dead blackbird (*Turdus merula*) (Colville, 2010, cited in[15]. Isolates from dead finches, previously identified as *E. coli* O86:K61, were subsequently shown to actually be *E. albertii*[3]. This organism was also found to be the probable cause of death in several wild birds from USA, Canada and Australia[3]. In a study in Poland it was found that *E. coli* O86 could contribute to nestling mortality in wild sparrows (*Passer* spp.)[16]. *Escherichia coli* 2 (non lactose-fermenting) was found in a blue tit (*Parus minor*) that died of colibacillosis[15]. Pure isolates of *E. coli* 1 were grown from liver, spleen and lung of a bittern (*Botaurus stellaris*) that revealed airsacculitis, pneumonia, pericarditis, hepato- and splenomegaly. Polygranulomatosis (Hjärre's disease) caused by *E. coli* O8:H9 and O48:H8 was reported in a free-living common buzzard (*Buteo buteo*) from Germany[17]. VTEC were isolated from several internal organs of a greenfinch in the UK[18]. *Escherichia coli* also accounted for septicaemia in common buzzards, bats[19], hedgehog (*Erinaceus europaeus*)[18], European brown hare (*Lepus europaeus*)[18], cirl bunting (*Emberiza cirlus*) and red kite (*Milvus milvus*) in the UK (Vaughan 2010, cited in[15]. The bacterium was cultured from a pulmonary abscess in a red squirrel (*Sciurus vulgaris*) in the UK[15] and was associated with enteritis in roe deer in the UK[18].

PATHOGENESIS AND PATHOLOGY

The vast majority of *E. coli* that belong to the normal intestinal flora are non-pathogenic. Predisposing factors such as insufficient passive immunity in neonates, stress due to weaning and recent change in feed, a heavy grain diet and poor hygiene contribute to rapid growth and transmission of the pathogenic strains and onset of disease[20]. Pathogenicity is associated with virulence genes encoded by plasmids, bacteriophages or pathogenicity islands (PAI), including genes for enterotoxins, fimbriae or pili (plasmid-encoded), phage-encoded Shiga toxin genes, and PAI-encoded genes in EPEC and EHEC as well as UPEC[2]. The main virulence factors of pathogenic *E. coli* are summarized in Table 30.1. Receptors for *E. coli*-adhesins are expressed for only the first week of life in calves and for the first 6 weeks of life in piglets[20]. The relevance of *E. coli* as a pathogen in wild mammals is less clear. *Escherichia albertii* isolates from birds possessed virulence genes for intimin (*eae*) and cytolethal distending toxin (*cdtB*), suggesting that *E. albertii* is pathogenic to birds. However, the determinants of pathogenicity in birds remain to be clarified[3].

In domestic animals, *E. coli* is a frequent cause of mastitis in cattle, pyometra and cystitis in dogs and cats, and oedema disease in swine. In poultry, respiratory or digestive exposure to large numbers of *E. coli* causes colisepticaemia or colibacillosis, respectively – diseases of high economic impact. Young animals develop acute septicaemia or airsacculitis and fibrinopurulent polyserositis. Low colostral uptake predisposes neonates of some species to septicaemia. Few reports describe disease caused by *E. coli* in wild animals in Europe.

TABLE 30.1 Virulence factors of pathogenic *E. coli* and *E. albertii* (modified according to Amnise 2008[21]; Gyles & Fairbrother, 2004[2]; Oaks et al., 2010[3]).

Organism	Virulence factors	Disease in animals
ETEC	Enterotoxins STI, STII (heat stable), LTI and LTII (heat labile), fimbriae, AIDA[a], EAST[b] 1	Neonatal diarrhoea, diarrhoea in young animals
STEC	Shiga toxin (Stx1 and/or Stx2), intimin (*eae*), LEE[c], fimbriae, alpha haemolysin, extracellular serine protease	Diarrhoea, dysentery, oedema disease, cutaneous and renal glomerular vasculopathy; zoonotic potential
EHEC	Haemolysin (Hly_{EHEC})	Of no significance
EPEC	LEE, intimin, bundle forming pili	Diarrhoea
EIEC	Ipa[d], *Shigella*-enterotoxin 2	Of no significance
EAEC	EAST-1, plasmid encoded protein, fimbriae	Occurrence and relevance unknown
DAEC	Fimbriae, AIDA-1, intimin	Occurrence and relevance unknown
NTEC	Cytonecrotic factor (CNF1, CNF2), cytolethal distending toxin (CDT), fimbria, afimbrial adhesin	Enteritis, septicaemia, urinary tract infection
APEC	Eerobactin, fimbriae, temperature-sensitive haemagglutinin	Septicaemia, cellulitis
UPEC	Eerobactin, CNF1, fimbriae, alpha haemolysin, iron-sequestering systems	Urogenital tract infection, pyometra
E. albertii	Intimin, CDT	Diarrhoea

[a]AIDA – adhesin involved in diffuse adherence
[b]EAST – heat stable enteroaggregative enterotoxin of EAEC
[c]LEE – locus for enterocyte effacement
[d]Ipa – Invasion plasmid associated proteins

Caseonecrotic, granulomatous lesions caused by *E. coli* infection are called coligranulomas and are often found in wild birds. Preferred locations are air sacs, lungs, serosas, gastrointestinal tract and liver. Histopathologically the granulomas show central areas of caseous necrosis surrounded by a zone of cellular infiltration with macrophages, lymphocytes and abundant heterophils. A fibrous capsule surrounds the granulomas. This type of lesion, and additionally Gram-negative rods phagocytosed by giant cells, were described in a common buzzard (*Buteo buteo*)[17]. *Escherichia albertii*-associated gross lesions in a gyrfalcon (*Falco rusticulos*) and redpolls (*Carduelis flammea*) were inconsistent, but several redpolls had darkened intestines distended with excessive yellow to green digesta. Histologic findings in redpoll finches were consistent with acute, severe, fibrinous and necrotizing proventriculitis, multifocal heterophilic enteritis and small-crypt abscessation. Classic attaching-and-effacing lesions typically associated with *eae*-positive *Escherichia* were not detected. In the gyrfalcon, evidence of septicaemia was found[3].

CLINICAL SIGNS

Clinical signs of infection vary according to the type of *E. coli* involved and include watery diarrhoea, dehydration, emaciation and death due to endotoxic shock. Reports about clinical signs specifically in wild animals are sparse. The case of *E. coli* (O8:H9, O48:H8) infection in a free-ranging buzzard presented with poor body condition, wet ruffled feathers and several external granulomatous lesions[17].

DIAGNOSIS

Escherichia coli and *E. albertii* grow on routine diagnostic media. Differentiation of *E. coli* isolates is important to distinguish pathogenic from non-pathogenic types. Pathogenic *E. coli* are quite often haemolytic, and most of the animal strains are strongly lactose-fermentings. A presumptive identification is made from growth characteristics and/or biochemical tests. Serotyping is a well-established and a valuable method based on differences in cell wall polysaccharides (O antigens), capsular polysaccharides (K antigens), flagellar proteins (H antigens) and fimbriae (F antigens). Presently there are more than 170 O antigens, 80 K antigens and 56 H antigens known. In ETEC of animal origin, F-antigen determination is of importance. Particular media (e.g. E medium, minca agar) are required for sufficient expression of fimbriae. Commercial test kits such as latex agglutination tests or specific antisera are available for the detection of F antigens. An enzyme-linked immunosorbent assay (ELISA) measuring *E. coli* K99 antigen is also available commercially. Demonstration of enterotoxins can be performed by ELISA and enzyme immunoassay (EIA)[20]. Polymerase chain reaction (PCR) and other procedures, including pulsed-field gel electrophoresis (PFGE), random amplified polymorphic DNA

(RAPD) analysis, amplified fragment length polymorphism (AFLP) and ribotyping, are also often used to determine virulence factors in *Escherichia* spp. and to further characterize isolates within a distinct serotype[2].

MANAGEMENT, CONTROL AND REGULATIONS

During the last two decades, VTEC O157:H7 has risen in importance as a public-health risk worldwide. It is considered that up to one-third of domestic ruminants are healthy, subclinical carriers of O157:H7 and are the main reservoirs for human infections. Diagnostic methods have been developed to selectively detect O157:H7 in human clinical cases and food. For the latter, a validated International Standard detection method (EN ISO 16654:2001) is available. According to the World Organisation for Animal Health (OIE), emphasis should be given to the isolation and identification of *E. coli* O157 and other VTEC from carrier animals[22]. On a mandatory basis, EU member states annually report their data on detection of VTEC/STEC in food and animals to the European Commission and the European Food Safety Authority based on Directive 2003/99/EC. In Germany, verocytotoxigenic *E. coli*-associated disease in domestic animals must be reported to the appropriate federal state authority. Currently there is no comprehensive surveillance for *E. coli* and *E. albertii* in wild animals, or recommendations for *E.coli* control in these species.

PUBLIC HEALTH CONCERN

Infection with O157:H7 is zoonotic, and the infection is most often contracted from consumption of contaminated food. Cattle are the main reservoir, but small ruminants and pigs are also known to carry *E. coli* O157:H7. Game (roe deer, red deer, fallow deer) may be carriers and a potential source of human pathogenic STEC and EHEC strains. In Germany, 51.8% of wildlife ruminants carried STEC, and similar rates were reported from Spanish deer. Non-O157 STEC carriage rates in Belgian and German deer meat varied from 10% to 22%. Hunters and people who handle game should be aware of the contamination risk. Proper hygiene is recommended in order to minimize the risk of EHEC and STEC food-borne infection in humans[4,13]. O157 infections in humans have also been associated with visits to wildlife parks, farms and petting zoos. In England an outbreak of *E. coli* O157 affected ten children and two adults who visited an animal collection open to the public; four were hospitalized but all recovered. Microbiological investigations following a meeting of an outbreak control team (OCT) identified the organism in 17 of 29 (59%) wild rabbit faecal samples from around the site, suggesting that contact with wild rabbit faeces can be a risk factor for acquiring human infection[23]. Guidance for owners and employees of petting zoos has been written with general advice on pathogens likely to be present in the petting zoo environment and steps to be taken to minimize the risk of human infection (e.g. the Australian 'Petting Zoo Infection Control Guideline', and the Dutch 'Hygiene Code for Petting Zoos')[24]. There are also recent guidelines for UK zoos[24]. Whether *E. albertii* can be transmitted from animals to humans is unknown; however, a zoonotic potential has been suggested[3].

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CHAPTER

31

SALMONELLA INFECTIONS

ALESSANDRA GAFFURI AND J. PAUL HOLMES

INTRODUCTION

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Salmonellosis is an infectious disease caused by bacteria belonging to the genus *Salmonella*. *Salmonella* are Gram-negative, non-spore-forming rods that are aerobic or facultatively anaerobic; most species are motile. *Salmonella* are not very resistant to physical or chemical agents and are destroyed by heat, during common cooking procedures, pasteurization and ordinary disinfectants. The genus belongs to the Enterobacteria family and its nomenclature and classification have been changed and restructured multiple times. Currently the genus *Salmonella* includes two species: *S. enterica* and *S. bongori*. *Salmonella enterica* is divided into six subspecies, which are identified by a Roman numeral and by a name; each of these subspecies includes different serotypes, classified on the basis of the somatic and flagellar antigens. The subspecies are: *S. enterica* subsp. *enterica* (I), *S. enterica* subsp. *salamae* (II), *S. enterica* subsp. *arizonae* (IIIa), *S. enterica* subsp. *diarizonae* (IIIb), *S. enterica* subsp. *houtenae* (IV) and *S. enterica* subsp. *indica* (VI). *Salmonella enterica* subsp. *enterica* contains most of the salmonellae that are pathogenic for warm-blooded animals, whereas the others usually affect cold-blooded animals and contaminate the environment. *Salmonella* can be found worldwide both in animals and humans, associated with a range of conditions from subclinical carriage in the intestine to generalized septicaemic infection and death.

Salmonellosis is an important zoonosis in the EU; infections in humans are mostly caused by food contaminated with *Salmonella* spp. In addition, environmental contamination can be an important source of infection for humans and animals, and in particular for wildlife. Contamination of the natural environment is frequently as a result of human activities, such as livestock farming and waste disposal.

Wildlife can play an important role in the epidemiology of *Salmonella* infection, and can act as an indicator of environmental contamination and as a maintenance host. Ingestion of contaminated water and food is the most likely route of infection for wildlife; for example, raptors and carnivores consuming infected carcasses can become infected themselves and spread *Salmonella* in their habitat, often as subclinical carriers. Salmonellosis in wildlife can be a health threat for humans and domestic animals;

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surveillance programmes in wildlife can be useful to improve our understanding of the epidemiology of *Salmonella* infection and contribute to the multidisciplinary approach to a global problem.

SALMONELLA INFECTIONS IN WILD BIRDS

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Salmonellosis in wild birds, or avian paratyphoid, is an infectious disease caused by bacteria belonging to the genus *Salmonella,* which, following ingestion, can cause septicaemia and death or subclinical infection.

EPIDEMIOLOGY

Salmonella infection in wild birds has been widely reported throughout Europe, including Norway, Spain, Sweden, Germany, the UK, Czech Republic, Croatia, Switzerland and Denmark[1]. Descriptions of outbreaks of salmonellosis causing mortality, particularly in passerines, have been published from several countries, including Norway, the UK and Sweden.

Reports of infection are recorded in a wide variety of wild bird species in Europe. Many of these species may be simply carrying the organism in their intestine without showing any signs of clinical disease, but a few species are particularly susceptible to disease. Studies have reported rates of *Salmonella* detection from: i) surveys of live birds that have been caught and cloacal swabs or faeces cultured; or ii) results of salmonella isolation from dead birds that have died from systemic salmonellosis. Although there are over 2,300 serovars of *Salmonella*, only a small number typically cause severe systemic disease, and these serotypes are often associated with only a few wild species. *Salmonella* Typhimurium is the predominate serovar associated with mortality in wild birds.

As the infection is acquired orally, the feeding ecology of the bird species is a key factor that influences the potential of species to become infected. Birds that share their environment with human activities, e.g. in sewage works and refuse dumps, are more likely to carry *Salmonella*. Gulls (*Larus* spp.) in particular are associated with *Salmonella* carriage in these situations. Surveys in gulls illustrate varying prevalences of *Salmonella* in faeces or faecal swabs, e.g. 9.2% of 2,985 herring gulls (*Larus argentatus*) at a refuse tip in the Clyde, Scotland, and 2.7% of 1,047 black-headed gulls (*Larus ridibundus*) in Central Park, Malmö, Sweden. In these surveys the gulls appeared healthy and the range of serotypes reflected the local environmental contamination. Gulls have also occasionally been reported to have clinical salmonellosis[2].

Waterbirds that feed on vegetable matter appear to have a lower prevalence. However, several reports of infection in a range of waterbirds, including teal (*Anas crecca*), tufted duck (*Aythya fuligula*), pochard (*Aythya ferina*), mute swans (*Cygnus olor*) and Canada geese (*Branta canadensis*), have been associated with water contaminated with sewage.

Raptors may acquire *Salmonella* through the consumption of infected prey or carrion, and 4.19% of 310 raptor faecal samples in a survey in Spain were positive for *Salmonella*. Sampling of raptor faeces can reveal a variety of different serotypes reflecting a range of different prey sources.

Several species of the Passerine family are recognized as particularly susceptible to *Salmonella* infection and disease. Outbreaks of disease in birds that visit garden feeding stations have been recorded for many years. There are geographical differences in the species most commonly affected. In the UK, greenfinches (*Carduelis chloris*), house sparrows (*Passer domesticus*) and chaffinches (*Fringilla coelebs*) are the species most frequently affected. In one survey between 1997 and 2000 in Scotland, of 97 dead birds at garden feeding stations with salmonellosis, there were 89 greenfinches, 4 house sparrows, 1 goldfinch (*Carduelis carduelis*) and 1 great tit (*Parus major*)[3]. This is in contrast to Norway, where between 1999 and 2000, the bullfinch (*Pyrrhula pyrrhula*) was the commonest species affected, followed by the siskin (*Carduelis spinus*), common redpoll (*Carduelis flammea*) and greenfinch[4].

Other species can become infected in these situations, including great tit, blue tit (*Parus caeruleus*), starling (*Sturnus vulgaris*), hawfinch (*Coccothraustes coccothraustes*) and robin (*Erithacus rubecula*), but disease in these is much less frequently recorded. Tits, for example, often collect food from hanging feeders and fly to a distant perch to eat it, whereas finches may stay at the feeder for longer or feed on the ground below a feeder, resulting in potentially prolonged exposure to contaminated material, often over extended periods of time. Little is known about species

differences in resistance, but it appears that different strains of *S.* Typhimurium are associated with different groups of birds, with definitive types (DT) 56/variant and DT 40 frequently isolated from finches and sparrows, whereas DT 41 and 95 are frequent in gulls and DT 99 in feral pigeons (*Columba livia*).

Differences in mortality may be due to the strain of *Salmonella* present, the species susceptibility to the circulating organism, the local occurrence of the species (redpolls have a circumpolar distribution), the density of the population visiting the garden, the intensity of provisioning and the level of environmental contamination with *Salmonella*.

Experimental infections in chickens have shown that older birds are less susceptible to the lethal effects of S*almonella*. Age-related effects in wild birds are less well understood. Age-related differences in S*almonella* carriage have been described in herring gulls (*Larus argentatus*) in England, and black-headed gulls (*Larus ridibundus*) in Sweden with higher carriage rates in first-year birds and juveniles. In the herring gulls, this was thought to be because of age-related feeding behaviour, with the juveniles feeding at sewage outflows. Several mortality studies report no sex predispositions, but an increased mortality rate in male greenfinches has been described.

Salmonellosis in passerines occurs mostly in the winter months. In a survey of wild birds submitted to a Scottish laboratory[2] between 1995 and 2003, most cases in greenfinches and chaffinches were diagnosed between January and March, and in house sparrows between October and March. Reports from Norway display a similar pattern, with a marked seasonality in passerines but no apparent seasonality in a range of other species affected. Most isolations were made between January and April, with a distinct peak in February and March, affecting mostly bullfinches, greenfinches, siskins and redpolls. A similar seasonality has been reported in Sweden and Germany and elsewhere in the UK.

This apparent seasonality may be biased because in the winter months the birds tend to congregate at feeding stations, and this is the time of year when most effort is put into observations by the public. Information is therefore sparse about the causes of death in the birds when they are more dispersed in the summer months.

Infected birds contaminate the environment through excretion of *Salmonella* in the faeces. In passerines this is important where large numbers of birds congregate at bird feeding stations. Healthy carriers are thought to be the major source of fatal infections and 2% of cloacal swabs from 1,990 healthy passerines were positive for *Salmonella* in a Norwegian survey. The primary carrier species are the same as the species commonly affected. A variety of species can become infected, but disease predominates in the species with a high carriage rate. Infected birds themselves can be the source of infection for other animals, either through being eaten by predators such as cats (*Felis domesticus*), foxes (*Vulpes vulpes*) and raptors, or through contamination of the domesticated animal (e.g. farm) and human environment.

Other groups of birds may acquire *Salmonella* through exposure to an environment not contaminated directly by bird faeces but from other sources such as human sewage. Disease is less common and infection may be of short duration, suggesting that birds infected in this way have a limited role in perpetuating the infection in their group.

Transmission is by the faecal-oral route, and the source will depend upon the food that the bird is eating and the level of environmental contamination.

In passerines, healthy carriers are considered to be the major source of infection for other wild birds. Disease occurs at feeding stations, where contamination of the feeding environment may facilitate transmission between birds. Regular sampling of faeces from bird tables and underneath feeders indicates that *S.* Typhimurium can survive long term in the environment at some sites and that the bacteria can survive for weeks in the environment.

It is significant that there has been a large increase in the feeding of birds in gardens since the middle of last century. In the UK it has been estimated that 60,000 tons of peanuts and seeds were sold for wild birds in 2003, with 67% of households regularly feeding birds.

It has also been found in one study that the number of a certain species of bird (house sparrows) may be more important, in relation to the presence of environmental contamination, than the total number of birds visiting the feeding station[5].

PATHOGENESIS, PATHOLOGY AND IMMUNITY

The route of infection is by oral ingestion. The infectious dose for wild birds is not known but is likely to vary due to species susceptibility and serotype of *Salmonella*. Oral doses of 10^9 *S. Typhimurium* have been found to be lethal for 20% of 3-day-old broiler chicks.

Research into the pathogenesis of *Salmonella* infection and disease in experimental animals and chickens has revealed the key features of the establishment of infection and development of disease. The critical stages are entry into the intestinal tract, colonization and invasion of the intestinal mucosa, dissemination throughout the body to a variety of organ sites and survival and replication in host tissue, principally macrophages.

After oral ingestion and colonization of the intestinal tract, the success of the adherence of the organisms to the intestinal epithelial cells and subsequent invasion is dependent upon a number of virulence factors. Several genes have been identified that are thought to be associated with virulence. The information for these virulence factors is often encoded in clusters of genes on chromosomes known as salmonella pathogenicity islands. Some virulence genes may also be located on plasmids (which are transmissible extrachromsomal DNA elements) that have been associated with bacterial pathogenicity. Knowledge of these attributes allows for detailed study of isolates for epidemiological investigations[6].

Although in chickens the epithelial cells of the caecae and ileo-caecal junctions are often sites of invasion by *Salmonella,* it has been suggested that, because of the severe lesions in the crop and oesophagus seen in passerines, these may be the predilection sites for bacterial invasion in this group of birds (Figure 31.1).

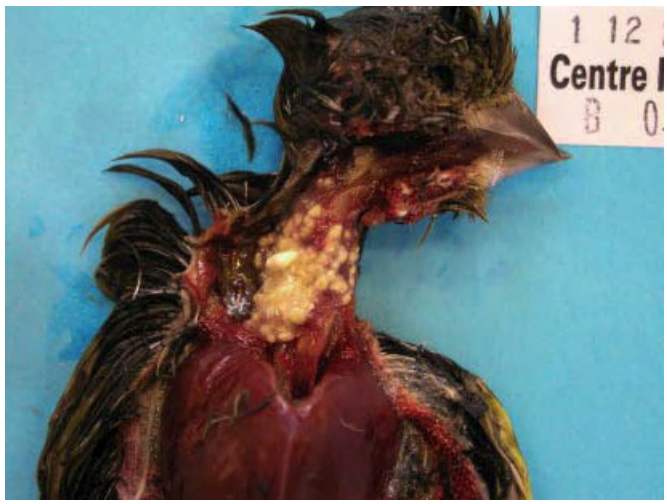

FIGURE 31.1 Necrotic lesions in the crop of a greenfinch due to *Salmonella* Typhimurium (Crown copyright 2010).

Once invasion has occurred, the salmonella organisms are able to survive and replicate within the macrophages, where they are relatively sheltered from attack by the bird's immune defences. Although the exact mechanism of *Salmonella* invasion is not fully understood, it is thought that the bacteria are carried by cells of the reticulo-endothelial system to systemic sites.

Disease associated with salmonellosis occurs principally in passerines infected with *S.* Typhimurium, and several large surveys of birds found dead give a good indication of the main gross lesions. The predominant lesions are found in the crop and oesophagus with multiple, often coalescing, yellow nodules on the mucosa (Figure 31.1). Other organs can also be affected. There are species differences in the sites and extent of the lesions, and regional variations in the species affected, which may be due to the sero or phage type present or varying abundance of the target species.

In Norway, gross findings have been described in 94 small passerines that had died with septicaemic salmonellosis at 87 private feeding sites[4]. Eighty-four per cent had poor body condition, 78% necrosis of the crop/oesophagus and 73% enlarged spleen. Scattered foci of necrosis were present on the surface of the liver in 53% of cases, spleen 46%, proventriculus 13% and intestine 5.3%. Similar signs are reported elsewhere, but the species affected may be different. The necrotic lesions in the crop mucosa, which can be up to 4 mm in diameter, may be visible from the serosal surface and can protrude into the lumen, sometimes partially obstructing the oesophagus. Fragments of food material, e.g. peanut, can be adhered to the necrotic tissue. Peri-hepatitis may be present, and the enlargement of the spleen can be dramatic.

The gross lesions are not pathognomonic and must be differentiated from other causes of ingluvitis and septicaemia – for example, trichomoniosis.

Lesions in the other major orders of birds are less common, but purulent arthritis, peritonitis, pneumonia and focal liver necrosis are seen in affected feral pigeons, and purulent arthritis, pneumonia, peri-hepatitis and nephritis are seen in gulls.

Histopathological lesions in the crop and oesophagus consist of severe necrosis, which can affect the entire mucosa and submucosa and underlying *lamina muscularis*. Large necrotic masses are present surrounded by mixed inflammatory cells, heterophils and mononuclear inflammatory cells, and large amounts of Gram-negative bacteria. Lesions in other areas of the gastrointestinal tract can

be similar but less severe. Lesions in liver and spleen consist of severe necrosis with accumulations of inflammatory cells and occasional multinucleated giant cells. Tissues from some carcases can reveal microscopic findings of acute necrosis in organs that appear grossly normal, indicating that the infection can be acute or peracute.

Multiplication of the bacteria in the body leads to severe endotoxaemina. Salmonella have lipopolysaccharides in their cell walls that are potent endotoxins, which are released when the bacteria die. They result in a severe inflammatory response with activation of a variety of chemical mediators, which can result in septic shock. Although antibody production in chickens can be used as an aid to diagnosis, this has not been evaluated for diagnosis in wild birds.

Little is known about the recovery of wild birds from clinical salmonellosis, as most diagnosed cases are examined following death. In fatal cases the overwhelming infection, severe inflammatory response and septic shock, leads to irreversible multiple organ failure.

CLINICAL SIGNS AND TREATMENT

Sick birds are rarely seen, and it is therefore difficult to assess the length of clinical disease and progression of clinical signs. Where signs have been described they are typically from cases in passerines associated with garden feeding stations, where there is regular observation. Affected birds are often only seen for a few hours before death, but some may survive for over 24 hours. There is a rapid deterioration in the bird's condition, with a reluctance to fly when approached, eyes tending to close, disorientation, lethargy and ruffled or fluffed-up feathers, before it collapses and dies. Intense thirst and difficulty in swallowing may be seen, and affected birds frequently stay close to feeders or water baths, trying to feed until just before death. Blindness has been described, associated with replacement of the eyeball with inspissated pus.

The clinical signs are not specific for salmonellosis and could be seen with a variety of diseases of wild birds.

Salmonellosis in birds can be treated with antibiotics after appropriate antibiotic sensitivity testing. Treatment of birds in the wild is usually not undertaken, as it is not possible to target only the diseased birds and ensure that therapeutic doses are administered to the birds. Consumption of inappropriate doses can lead to selection of antibiotic-resistant bacteria. If diseased wild birds are caught, the disease has usually progressed to such an extent that treatment is not indicated and euthanasia is more appropriate. The zoonotic potential of *Salmonella* also means that treatment of individuals is generally not undertaken. However, severely ill birds presenting to a rehabilitation facility may undergo an intense period of supportive care in an attempt to save the bird. This would include fluid therapy, supportive nutrition and antimicrobial therapy. First-line agents commonly used for these cases include amoxicillin/clavulanate or enrofloxacin. This treatment is often instigated without a diagnosis.

DIAGNOSIS

Diagnosis requires isolation of the organism from birds with typical clinical signs and gross and or histopathological lesions. Bacterial cultures are made (in a biosafety level 2 laboratory) from affected organs, typically intestine, liver and spleen. A variety of bacteriological cultural techniques are used for detecting *Salmonella* in biological samples, depending on the source of the sample, the reason for the sampling and local diagnostic laboratory preferences. Most involve selective enrichment broths, and growth on selective and differential solid media. Samples in which the S*almonella* organisms may be in a desiccated state may need pre-enrichment in buffered peptone water. Enrichment media, such as selenite or tetrathionate broth, promotes the growth of *Salmonella* and inhibits other faecal flora. Enriched samples are subsequently plated on salmonella-selective media, such as xylose lysine desoxycholate (XLD) or brilliant green agar. Incubation is typically at 37°C for 24–48 hours. When suspect *Salmonella* colonies are present, serological and biochemical tests are used to confirm the presence of S*almonella* to the serogroup level. Serotypes are identified on the basis of their antigenic structure based upon their O (somatic) and H (flagellar) antigens detected by agglutination tests. They are classified according to the Kauffmann–White scheme.

There are several further methods used to identify differences in individual isolates that assist with epidemiological investigations[7]. Phage typing is a technique based upon the sensitivity of a particular isolate to a series of bacteriophages at appropriate dilutions. Different biovars of Typhimurium are susceptible to different series of bacteriophages, and it is possible to identify over 300 different definitive types (DT) after phage typing. This is generally carried out at reference laboratories.

Pulsed-field gel electrophoresis (PFGE) is a method used to separate fragments of DNA. These fragments from different isolates can be compared to reveal the genetic relationships between organisms. DNA-based typing methods, such as PGFE, can be highly discriminatory and useful for short-term outbreak investigations and long-term surveillance of *Salmonella* epidemiology. Antibiotic sensitivity profiling results are also used for comparison of isolates.

Screening of populations can be undertaken following live capture of birds and culturing of faecal or cloacal swabs or washings. Faecal samples can be collected from bird roosts or other flocking sites and culture for *Salmonella* can be also be undertaken from bird carcases found dead or culled.

Principal components analysis of biometric data has been used to assist in monitoring for salmonella in greenfinch, and a low fat and low body weight could be useful indictors of *Salmonella*-positive greenfinch.

MANAGEMENT, CONTROL AND REGULATIONS

Disease in wild birds largely occurs in association with garden feeding stations. Advice regarding minimizing disease risks associated with this provisioning is to clean and disinfect bird feeders (and water baths) and feeding sites regularly, use several feeding sites in any one garden and rotate between these feeding areas. These measures are intended to try to reduce the build-up of contaminated faeces in any one area. There are other factors to consider, including the design of feeders (easy to clean), the location of the feeders (avoiding overhanging branches) the frequency of supply of the food and the amount of food provided. In the event of an outbreak of disease, continuing to provide food may attract infected birds and spread infection, but conversely stopping food provision could encourage infected birds to disperse to other feeding stations. If naturally occurring food and water supplies are locally available it may be best to stop, or reduce, feeding in order to try to prevent large numbers of birds visiting potentially infected properties. The value of adopting these control measures has yet to be confirmed.

Wild birds should be prevented from having access to human food preparation areas, domestic animal feed stores and domestic animal housing.

There are no EU regulations covering *Salmonella* infection in wild birds, although there are several directives aimed at domestic poultry.

PUBLIC HEALTH CONCERN

The majority of *Salmonella* serovars are zoonotic or potentially zoonotic, and wild birds may be a source of infection for humans and domestic animals. In humans salmonellosis varies from a self-limiting gastroenteritis to septicaemia, and subclinical infection can also occur. The main route of infection is oral and therefore can occur as a result of eating or smoking with hands contaminated with *Salmonella* or from eating contaminated food.

Examples where this may occur include handling infected birds or their immediate environment, which is contaminated with faeces, handling contaminated garden bird feeding equipment, eating food that has been contaminated with bird faeces or from contact with other animals that have been infected by wild birds, e.g. domestic cats.

There are several well-documented outbreaks of disease in humans, which have strong epidemiological links to wild birds. In one outbreak in Norway, 349 people were confirmed infected with *S.* Typhimurium at the same time of year, January to April, as fatal salmonellosis in wild passerines. Detailed epidemiological investigations revealed links to wild birds, and the risks of infection in people included drinking untreated water, having direct contact with wild birds or their droppings and eating snow, sand or soil.

House sparrows have been implicated in an outbreak of *S.* Typhimurium PT (phage type) 160 gastroenteritis in people in a British hospital, associated with suspected faecal contamination in the kitchens.

Comparison, using PFGE, of 142 isolates from a variety of Norwegian wildlife concluded that passerines constitute an important source of infection for humans, but strains from gulls and pigeons were much less significant. Different conclusions were made following molecular studies on 32 *Salmonella* isolates from passerines in northern England[6]. Twenty-nine *S.* Typhimurium isolates were found to be closely related, and some appeared to be clonal, but all the isolates tested lacked the *sopE* gene. This gene has been associated with some human disease outbreaks due to *S.* Typhimurium and so it was considered that the 29 isolates may not have represented a large zoonotic risk.

SIGNIFICANCE AND IMPLICATIONS FOR ANIMAL HEALTH

Salmonella excreted by wild birds can cause disease, or infection, in farmed or pet animals and can result in the spread of antibiotic-resistant organisms, sometimes across country borders via migration. Wild and domestic mammals, particularly carnivores, can become infected through ingestion of infected birds.

As wild birds have considerable mobility, they have the potential to spread *Salmonella* widely to farmed animals. In a study in the Czech Republic, examinations on one farm found *S.* Typhimurium PT 104 in cloacal swabs from 7 out of 30 house sparrows and 1 serin (*Serinus serinus*) and in 21 out of 64 clinically ill calves.

Foxes and domestic cats are susceptible to S*almonella* infection when they eat infected bird carcasses. Fourteen out of 215 (6.5%) foxes shot in Norway were found to be carrying *Salmonella*, and nine of these isolates were *S.* Typhimurium, with the identical PFGE profile to that found causing outbreaks of mortality in passerines in the winter.

Enteric disease occurs in domestic cats associated with a history of eating dead wild birds or the isolation of wild bird strains, e.g. *S.* Typhimurium DT 40 or 56. These can then be a source of infection for humans.

The role of the disease in wild bird population dynamics is less clear. Local mortality at feeding stations can appear dramatic, with outbreaks lasting up to 4 months with multiple deaths. Although there is speculation that this could be a contributing factor to population declines, this has yet to be proven for salmonella infection.

SALMONELLA INFECTIONS IN WILD MAMMALS

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Salmonellosis, also known as paratyphoid fever, *Salmonella* gastroenteritis, and *Salmonella* septic syndrome, is a bacterial infection that causes a range of disease, from gastroenteritis and bacteraemia to a subclinical lifetime carrier state.

EPIDEMIOLOGY

Salmonellae are found throughout the world. *Salmonella enterica* subsp. *enterica* and its serogroups A, B, C1, C2, D and E, are commonly found in warm-blooded domestic and wild animals. Salmonellosis has been reported in wild boar (*Sus scrofa*) and in other wild mammals, such as red foxes (*Vulpes vulpes*), badgers (*Meles meles*), hedgehogs (*Erinaceus europaeus*), European brown hares (*Lepus europaeus*) and small rodents. It is seen less frequently in deer, which is probably the result of their feeding habits. Moreover, the absence of the gall bladder, which *Salmonella* can colonize, also seems to eliminate them as carriers. Environmental contamination by *Salmonella* may differ significantly from one area to another, and wild animals may reflect the *Salmonella* contamination level of their habitat. Consequently, distribution of the infection and of the various serotypes can be dissimilar within the same species in different locations. In wild boar *Salmonella* was cultured from rectal faecal samples from 22% of the animals in Northern Portugal[8] and from caecal content in 27% of the wild boar in Northern Italy[9]. In Spain two different serotypes were isolated from 7.5% of pooled liver, intestine and spleen samples from wild boar[10]. The most frequent serotypes isolated in these investigations belonged to the *S. enterica* subsp. e*nterica*, namely: *S.* Worthington, *S.* Coeln, *S.* Ball and *S.* Typhimurium. In this species serological surveys in Slovenia[11] and in Spain[12] showed 47% and 7% positivity, respectively.

Salmonella infection is also described in wild carnivores. From foxes and badgers in Northern Italy *S.* Typhimurium, *S.* Veneziana, *S.* Thompson and *S.* Heidelberg were isolated[13]. In Spain, *Salmonella* was detected in 7.1% of the red foxes and in 18.2% of the badgers investigated; the most frequently isolated serotypes were *S.* Enteritidis, *S.* Newport, *S.* Give and *S.* Umbilo[10]. In Norway, 6.5% of red foxes showed faecal carriage of *S.* Typhimurium, *S.* Hessarek and *S.* Kottbus[14].

In Cornwall, UK, a 14-year study on badgers demonstrated that 7.2% of animals were infected; the most common isolated serotypes were *S.* Agama, *S.* Indiana and *S.* Typhimurium[15].

Although salmonellosis has been reported in wild ruminants, it has seldom been detected in Europe. In Denmark, a roe deer (*Capreolus capreolus*) was found to be positive for *Salmonella*, but 575 animals from the same area were found to be negative[16]. Low prevalence is also reported in roe deer and red deer (*Cervus elaphus*) in Northern Italy[17,18]

TABLE 31.1 *Salmonella* isolations and seroprevalence in wild mammals.

Country	Wild boar		Foxes	Badgers	Hedgehogs	Roe deer	Red deer	Reindeer
	Isolates/tested animals	Seropositive/ tested animals	Isolates/tested animals	Isolates/tested animals	Isolates/tested animals	Isolates/tested animals	Isolates/tested animals	Seropositive/ tested animals
Denmark[16]					23/176	1/575		
Iceland[19]								2/59
Italy[9,13,17,18]	217/786		19/462	10/105		2/289	0/60	
Norway[21,16]			14/215		73/320			
Portugal[8]	17/77							
Slovenia[11]		85/178						
Spain[10,12]	3/40	5/78	1/14	2/44		0/18		
UK[15]				350/4881	17/78			

and in Spain[10]. In Iceland, a serosurvey performed on 59 wild reindeer (*Rangifer tarandus tarandus*) showed two positive samples[19].

Small mammals and rodents are considered a potential source of transmission or maintenance of *Salmonella*. These animals reflect the contamination of their habitat, so infection prevalence can vary significantly[20].

Hedgehog populations are considered high-level carriers of *Salmonella* in many countries. A study on the prevalence of *Salmonella* Typhimurium in Norwegian hedgehogs showed results varying from 0 to 40%, depending on the area sampled[21], whereas in Denmark and in England the hedgehog seems to be a reservoir for *S.* Enteriditis PT11[22].

The data from all of these studies are summarized in Table 31.1.

As *Salmonella* are probably able to adapt to multiple hosts, it is likely that most wild animals, including rodents, birds and reptiles, are susceptible to infection. The probability of infection is also influenced by environmental contamination and feeding habits. Adult animals generally have a more pronounced immune response and higher level of antibody production towards a variety of antigens and are therefore more resistant than young animals to *Salmonella* infection[18]*;* any factor that can lower the immunological response might increase the incidence and severity of infection[9].

Although animals are the major reservoir for *Salmonella*, environmental contamination caused by humans and livestock has become an increasing source of infection. Survival of the bacterium in the environment depends on climatic conditions; wet and shaded areas are more suitable than those exposed to the sun. *Salmonella* can survive for up to 4 months in pond water and in pasture soil and for more than 2 years in dried bovine manure and avian faeces. Wild avian species can be a source of infection and can also act as carriers and spread the organism in the environment, as well as carnivores, which may be infected with *Salmonella* following the ingestion of infected prey animals.

Salmonellosis occurs more frequently during the cold months of the year, i.e. November to April in Europe[10].

A wide range of wild animals can be infected, and it is possible that all species can be accidental hosts and can potentially be reservoirs as healthy carrier-shedders.

The reservoir for *Salmonella* is the intestinal tract of warm- and cold-blooded animals. As *Salmonella* can survive in the environment for a long time, infection may occur following oral ingestion of contaminated food or water. The most likely route of transmission is faecal-oral, but infection through the mucous membranes of the conjunctivae or upper respiratory tract is also possible.

PATHOGENESIS, PATHOLOGY AND IMMUNITY

The pathogenesis and evolution of the infection depend on the virulence of the serotype, the infectious dose and the status of the host. The virulence genes of *Salmonella* determine their ability to overcome host defences and the innate defences of the digestive system. In fact, the alimentary tract is a hostile habitat for *Salmonella* because of the acid barrier of the stomach and the intestinal bacterial flora. Following ingestion, *Salmonella* rapidly colonize the intestinal tract, invade the intestinal mucosa and multiply in the regional lymphoid tissue. From the intestinal phase the infection can develop to a systemic form as the pathogens spread from the gut-associated lymphoid tissue (GALT) into the circulatory system. The spleen and the

liver are the first organs involved in the septic form; in these organs the bacteria can survive and multiply within the macrophages. Fatal bacteraemia with endotoxaemia may occur a few days after inoculation. The resistance of the host to systemic infection or a low infectious dose can result in chronic subclinical carrier status, and these animals can potentially become shedders.

The lesions associated with salmonellosis differ depending on the disease form and the *Salmonella* serotype involved. The septicaemic form usually shows hyperaemia of the gastric mucosa, colitis, enlargement of mesenteric lymph nodes, liver and spleen and lung congestion. Focal liver necrosis is sometimes present. In the enteric form, enlarged and hyperaemic mesenteric lymph nodes and necrotic enteritis, colitis and button ulcers are observed. In some cases and particularly in subclinical carriers, infection is not associated with macroscopic or microscopic lesions.

A report of *Salmonella* Choleraesuis infection in free-ranging wild boar describes splenic hypertrophy, focal liver and kidney necrosis and haemorrhagic enteritis[21]. In farmed wild boar *Salmonella* Choleraesuis was described to cause ulcerative ileitis, typhlitis, colitis and petechial haemorrhage on serosal surfaces; some animals showed yellow foci in the liver and enlarged spleen[8]. In red deer with *Salmonella* infection, intestinal meteorism (gas in the digestive system), lung congestion and haemorrhagic petechiae on the serosa was described[11], and emaciation and haemorrhagic enteritis was reported in an infected red fox found dead[16].

The histological features of the septicaemic form are those typical of Gram-negative sepsis and are mainly associated with endothelial damage. In particular, hyperplasia of reticular splenic cells and lymph nodes, generalized swelling of endothelial cells and histiocytes and fibrinoid thrombi have been reported. The macroscopic necrotic liver foci are characterized histologically by acute coagulative hepatocellular necrosis and reactive granulomas when there is a longer course of development. In the spleen, small necrotic foci containing many *Salmonella* bacteria may be present. In the enteric form, there is necrosis of cryptal and surface enterocytes, involving also the *muscularis mucosa* and the submucosa. Necrosis of Peyer's patches or lymphoid atrophy are characteristic.

Once infected, the host develops a rapid immune response that can be influenced by the dose, the virulence and the antigenic characteristics of the bacteria, and by the age of the host. Very young and old animals may have a poor immunological response. In domesticated animals an antibody response can be detected 1 week after infection and can last for 10 weeks or longer. IgM are the first immunoglobulins produced, followed by IgG and IgA. IgG levels can persist for long periods, while IgM and IgA progressively decrease. The intensity of antibody production depends on the antigenic determinants; serological cross-reactivity may occur in hosts infected with different species of *Salmonella*[9].

CLINICAL SIGNS AND TREATMENT

Salmonella infection may be clinically not evident, as subclinical carrier status is common.

Clinical infection is seldom described in free-living animals and clinical signs may depend on animal species, host status (physiological, co-infection, stress) and serotype. The resulting wide range of visible signs depends on whether localization of the infection is enteric or septic. In the former case they are characterized by diarrhoea and weight loss, while in the latter by fever, lethargy, dyspnoea, diarrhoea, abortion and death. During a salmonellosis outbreak in farmed wild boar the most frequent clinical signs were progressive weight loss, anorexia, weakness, lethargy and death after about 5 days[8]. In red deer with peracute and acute disease, anorexia, depression and nervous clinical signs were described[11]. Experimentally infected foxes did not show any clinical signs[21].

Treatment, for example of wild animals such as hedgehogs brought to rehabilitation centres, is often not recommended because of the zoonotic implications, and strict hygiene precautions must be followed. If undertaken, treatment consists of supportive care (fluid therapy, antidiarrhoeal drugs) and appropriate antibiosis, preferably based on culture and sensitivity testing. Antibiotic treatment may, however, promote carrier status. Prognosis is usually grave, especially in cases of septicaemia, and severely affected animals should be euthanized.

DIAGNOSIS

Diagnosis is based on bacterial isolation, whereas serology is useful for population screening.

Cultures can be performed from samples collected during necropsy, and specimens should be taken where

visible lesions are present, particularly from the liver, spleen, mesenteric lymph nodes, small intestine and colon. In living animals faecal samples or rectal swabs are suitable for culture. Environmental specimens, such as water, soil or waste and sewage disposal, can also be cultured.

Direct isolation on non-selective (e.g. blood agar) and weakly selective media (Gassner, MacConkey agar) can be performed in the case of septicaemic and acute infection, but standard isolation methods are based on the use of pre-enrichment, enrichment and selective plating media. International standard methods (ISO 6579:2002 annex D) involve pre-enrichment in buffered peptone water, enrichment on modified semi-solid Rappaport–Vassiliadis (MSRV) and isolation on XLD and other selective plate media. Pre-enrichment media are useful for isolating *Salmonella* from environmental samples, manure, faeces and subclinical animals and for detecting *Salmonella* that has been damaged or stressed by inhibitory effects (heating, freezing or otherwise processing). Buffered peptone water or universal pre-enrichment broth is commonly used. Enrichment media are liquid or semi-solid agar media that enhance *Salmonella* growth and inhibit other bacteria. The most widely used media are: Muller–Kauffman broth, selenite F, selenite cystine, Rappaport-Vassiliadis broth, semi-solid Rappaport–Vassiliadis medium and semi-solid *Salmonella* medium. Selective plating media are inoculated with enrichment media and allow the inhibition of growth of other bacteria and differentiate *Salmonella* colonies through biochemical characteristics. Brilliant green agar, Rambach agar, XLD and Hectoen enteric agar are among the wide range of specific media available. Presumptive *Salmonella* colonies are identified by biochemical tests and serotyping. Other detection methods are immunomagnetic separation, enzyme-linked immunosorbent assay (ELISA) and nucleic acid recognition methods; these methods are used in human foodstuff hygiene testing and are not validated for animal and environmental sources[12].

There are a number of diagnostic tests for serological screening in livestock. They are used to identify infected populations, not infected individual animals, although they can identify carrier status, when an animal may excrete the bacteria intermittently but has persistent IgG concentrations[18]. Serological diagnosis may be affected by the sensitivity of the test and by the limited range of *Salmonella* serovars or serogroups the test can detect. Some non-invasive serovars cannot be detected by serological assays; moreover cross-reaction between serovars and other bacteria may occur[12]. Tube and microagglutination techniques, as well as competitive ELISA, can be used in all animal species, including wild mammals. Serological surveys have been performed on wild boar in Spain[12] using the tube agglutination test, whereas an ELISA commercial kit was used in Slovenia[11]. Wild reindeer in Iceland were tested with lipopolysaccharide-coated antigens from *S.* Typhimurium and *S.* Choleraesuis and a specific anti-reindeer immunoglobulin peroxidase conjugate[19].

MANAGEMENT, CONTROL AND REGULATIONS

Salmonellosis is widespread in livestock, especially in the pig and poultry industries; environmental contamination from livestock and human waste and sewage is the major risk for wildlife infection. Therefore, control and management strategies are adopted to decrease the prevalence of infection in intensive animal husbandry and eliminate environmental pollution. Particular attention should be paid to biosecurity measures and rodent control, as rodents are involved in maintaining the infection in farms and in the surroundings[10]. In the member states of the EU, a compulsory survey on the prevalence of *Salmonella* spp. in pigs and poultry is carried out to achieve the EU's objective to eradicate *Salmonella* and other food-borne zoonotic infections.

PUBLIC HEALTH CONCERN

Humans contract *Salmonella* mainly through the ingestion of contaminated food. As *Salmonella* may have a wildlife reservoir and also contaminate the environment, some outdoor activities may be responsible for human infection. Drinking contaminated water, eating snow and direct contact with wild birds and their faeces are all factors that increase the risk of infection. In some cases human outbreaks correlate to an infected wildlife population, as happened in Norway, where hedgehogs were probably responsible[21]. Another possible source is game meat. Hunters should be trained to avoid contaminating meat with intestinal content or faeces. A trained person should

examine the organs of the hunted animal to detect lesions consistent with diseases that may be dangerous for the consumer. The marketing and trade of game meat must observe EU legislation, in particular Reg. (EC) 853/2004 and Reg. (EC) 852/2004.

SIGNIFICANCE AND IMPLICATIONS FOR ANIMAL HEALTH

Mutual transmission of *Salmonella* between domestic and wild animals can occur; the most effective way to prevent spread of infection among different species populations is to eradicate the disease in livestock and adopt appropriate husbandry hygiene and management protocols. Safety measures should be taken on farms to control the presence of potential carriers such as birds, small mammals, foxes, badgers and hedgehogs. More attention should be paid to the health of domestic animals that graze on summer pastures to avoid pasture contamination. In addition, human and animal waste should be properly treated before disposal.

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CHAPTER

32

CAMPYLOBACTER INFECTIONS

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Campylobacter spp. are Gram-negative, thin, curved rods. Their cells (0.2–0.5 µm in width) form characteristic seagull-shaped and sometimes long spiral forms and are motile by a single flagellum. Most of the species are microaerophilic. *Campylobacter* were once placed in the genus *Vibrio*, and some of the diseases are still occasionally referred to as 'vibriosis'[1]. The following species have been isolated from wild animals: *Campylobacter coli* (synonyms *C. hyoilei*, *Vibrio coli*), *C. hyointestinalis*, *C. jejuni* (synonyms *C. fetus* subsp. *jejuni*), and *C. lari* (synonyms *C. laridis*).

The species of the genus *Campylobacter* are distributed worldwide among domestic and wild animals and birds, but in most cases they live as commensals on the mucosa of the oral cavity and intestinal tract. The most important domestic animal pathogens are *C. fetus* subsp. *fetus*, *C. fetus* subsp. *venerealis*, *C. jejuni* and possibly *C. hyointestinalis* and *C. mucosalis*. Many *Campylobacter* spp. are transmitted via the faecal-oral route, whereas *C. fetus* subsp. *venerealis* is transmitted by infected bulls or artificial insemination with contaminated semen. Wild birds and wild mammal populations are regarded as reservoirs of *Campylobacter*. In a large study, 1,794 birds, representing 107 species from 26 families, were examined. An overall *Campylobacter* prevalence of 21.6% was found, but it varied from 0 to 100%. Certain bird taxa had high prevalences (e.g. shorebirds, wagtails, pipits, starlings and thrushes), whereas others did not[2]. The prevalence of *Campylobacter* spp. was highly influenced by feeding habits and ecosystem, an observation that had been previously described in another study[3]. In this study 540 wild birds were examined and the overall prevalence was 28.6%. By contrast, an overall prevalence of 1.4% was reported from a study performed on wild bird samples from Northern England. The latter study revealed that wild birds carry livestock-associated strains of *C. jejuni*, but owing to the apparent absence of wild bird strains in livestock the authors suggested that the route of infection is predominantly from livestock to wild birds[4]. Others reported that different host species largely carry their own *Campylobacter* spp., indicating that cross-species transmission is rare[5,6]. Table 32.1 gives an overview of the occurrence of *Campylobacter* spp. in wildlife.

Campylobacter jejuni and *C. coli* can be found in large amounts in the faeces of food-producing and companion animals. Diarrhoea in dogs has been associated with the presence of *C. jejuni*, and mild disease clinical signs such as soft-to-watery faeces are seen in livestock. Extra-intestinal infections in livestock include abortion (sheep, goat, pigs) and mastitis (cattle). *Campylobacter hyointestinalis* was recovered from pigs with enteritis and *C. mucosalis* was identified in pigs suffering from proliferative enteritis. Although the mechanisms of pathogenesis in *C. jejuni* and *C. coli* infection have received substantial inves-

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TABLE 32.1 Reported detection of *Campylobacter* spp. in wild mammals and birds in Europe.

Host (common name)	Host (genus/species)	*C. coli*	*C. hyointestinalis*	*C. jejeuni*	*C. lari*	*Campylobacter* spp.	Geographic origin	References
Hare	*Lepus* sp.	c		c, d			Norway, Denmark, Sweden	7, 8
Rabbit	Not specified				c		UK	9
Wild boar	*Sus scrofa*	c		c		c	Sweden	8
Roe deer	*C. capreolus*		c	c		c		
Moose	*Alces alces*			c				
Red fox	*Vulpes vulpes*			d			Denmark	7
Seal	Not specified			d				
Badger	*Meles meles*			d	c		Denmark, UK	7, 9
Hedgehog	*Erinaceus* sp.			d			Denmark	7
Squirrel	not specified			d				
Red squirrel	*Sciurus vulgaris*			c			Italy	10
Canada goose	*Branta* (*B.*) *canadensis*						Sweden	8
Brent goose	*B. bernicla*					c	Sweden	2
Common eider	*Somateria mollissima*					c		
Northern pintail	*Anas acuta*					c		
Eurasian teal	*A. crecca*					c		
Mallard	*A. platythynchos*			c			Norway	11
Goldeneye	*Bucephala clangula*			c			Norway	3
Eurasian sparrow hawk	*Accipiter nisus*			d		c	Denmark, Sweden	7, 2
Common buzzard	*Buteo buteo*			d			Denmark	7
Pheasant	Not specified	c		c, d	c		Denmark	7
Common ringed plover	*Charadrius hiaticula*				c	c	Sweden	2
Grey plover	*Pluvialis squatarola*					c		
Common sandpiper	*Actitis hypoleucos*			c		c		
Ruddy turnstone	*Arenaria interpres*				c			
Dunlin	*Calidris* (*C.*) *alpina*	c		c	c	c		
Red knot	*C. canutus*				c			
Curlew sandpiper	*C. ferruginea*			c	c	c		
Little stint	*C. minuta*			c		c		
Temminck's stint	*C. temminckii*				c			
Common snipe	*Gallinago gallinago*					c		
Broad-billed sandpiper	*Limicola falcinellus*	c		c	c			
Jack snipe	*Lymnocryptes minimus*					c		
Ruff	*Philomachus pugnax*	c		c				
Eurasian woodcock	*Scolopax rusticola*					c		
Wood sandpiper	*Tringa* (*T.*) *glareola*			c		c		
Common greenshank	*T. nebularia*					c		
Feral pigeon	*Columba livia*			c			Norway	11
Gull	*Larus* spp.			c, d	c	c	Norway, Sweden, Northern Ireland, Denmark	3, 7, 12 8
Long-eared owl	*Asio otus*			c			Sweden	2
Ural owl	*Strix uralensis*			c			Norway	3
Redwing	*Turdus* (*T.*) *iliacus*			c			Sweden	2
Common blackbird	*T. merula*	c		c	c	c		
Song thrush	*T. philomelos*			c		c		
Fieldfare	*T. pilaris*			c				
Mistle thrush	*T. viscivorus*			c				

(*Continued*)

TABLE 32.1 (*Continued*)

Host (common name)	Host (genus/species)	*C. coli*	*C. hyointestinalis*	*C. jejeuni*	*C. lari*	*Campylobacter* spp.	Geographic origin	References
Common tern	*Sterna hirundo*	c		c			Norway	3
Eurasian reed warbler	*Acrocephalus scirpaceus*			c			Sweden	2
Goldcrest	*Regulus regulus*					c		
Great tit	*Parus major*					c		
Winter wren	*Troglodytes troglodytes*			c	c			
Western jackdaw	*Coloeus monedula*			c				
Carrion crow	*Corvus* (*C.*) *corone*	c		c		c	Norway	3
Hooded crow	*C. cornix*	c		c		c		
Rook	*C. frugilegus*			d			Denmark	7
Raven	*Corvus* sp.			d				
Magpie	*Pica* sp.			d				
Common starling	*Sturnus vulgaris*			c	c	c	Sweden	2
Meadow pipit	*Anthus pratensis*				c	c		
White wagtail	*Motacilla alba*	c		c				
Dunnock	*Prunella modularis*			c				
European greenfinch	*Carduelis chloris*	c						
Yellowhammer	*Emberiza* (*E.*) *citrinella*			c				
Ortolan bunting	*E. hortulana*	c						
Reed bunting	*E. schoeniclus*			c			Norway	3
Puffin	*Fratercula arctica*	c		c		c		

c – carrier status, no clinical disease reported
d – animal found dead or dying

tigation, owing to their role in human disease, little is known about the pathogenic mechanisms of most other *Campylobacter* species. *Campylobacter fetus* subsp. *fetus* spreads from the intestinal tract to secondary sites, including the placenta. Abortion in the third trimester of ovine gestation results from placentitis. Abortion may also result from *C. fetus* subsp. *venerealis* infection in cattle. The organism colonizes the reproductive tract in an ascending route following venereal infection moving from the vagina to cervix, uterus and oviducts. *Campylobacter jejuni* produces different adhesins and is able to survive in host macrophages. In *C. hyointestinalis* isolates from pigs with enteritis a cytotoxin has been suggested[13].

Clinical disease in free-ranging animals has not been described to date, but gross and microscopic lesions caused by *Campylobacter* are likely to be similar to those in domestic and captive wild animals.

Different specimens are required for the diagnosis of *Campylobacter* spp. from various clinical conditions. Transport medium is recommended for samples suspected for *C. fetus* infection. For the detection of *C. fetus* subsp. *venerealis*, cervical mucus from anoestrus cattle as well as preputial washings from bulls are required. Both subspecies of *C. fetus* as well as *C. jejuni* can be demonstrated in fetal abomasal contents in cases of bovine and ovine abortion. Direct microscopy of smears using dilute carbol fuchsin (DCF) or fluorescent antibody staining is most reliable for the detection of *C. fetus*. *Campylobacter jejuni* can be seen in wet mounts of faeces by phase contrast or dark field microscopy. Rectal swabs or faeces are used to isolate *C. jejuni* and other intestinal *Campylobacter*. Bile should be examined for the detection of *C. jejuni* in avian vibrionic hepatitis. Smears of mucosal scrapings stained after modified Ziehl–Neelsen stain or sections prepared for histopathology and silver stained are recommended for the presence of *C. mucosalis* and *C. hyointestinalis*. Isolation procedures vary and require several selective media depending on the *Campylobacter* species investigated and are therefore often limited to reference laboratories[1]. PCR is also used to identify *C. jejuni* and *coli* in bacterial cultures as well in faecal or milk samples.

Campylobacter spp. pathogenic for humans are widely distributed among wild animals, but the importance of wildlife as a source of infections in humans is unknown.

Incidents with an association between passerine birds, in particular small crows and blue tits (*Parus caeruleus*), and *Campylobacter* sp. contamination of milk for human consumption by pecking open the bottle tops appear now to be less frequently recorded[14]. *Campylobacter jejuni* sequence types isolated from geese correspond to those sampled from cases of human disease; hence, the possibility that such strains could cause human disease or waterborne outbreaks on rare occasions cannot be completely dismissed[6].

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CHAPTER

33

LEPTOSPIRA INFECTIONS

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Leptospirosis (also known as Weil's disease, canicola fever, canefield fever, mud fever, nanukayami fever, rat catcher's yellows, Fort Bragg fever and pretibial fever) is a disease of public health and veterinary importance that is encountered throughout the world. Leptospirosis can be a mild flu-like illness, or a severe disease leading to jaundice and kidney failure. Although infections are common in many species of European wildlife, the role of leptospirosis in wildlife morbidity remains unclear.

AETIOLOGY

Leptospira species are Gram-negative bacteria that exploit a wide range of mammals as reservoir hosts. Bacteria are shed from infected mammals in urine, and susceptible hosts usually acquire infection through contact with contaminated watercourses. In reservoir hosts, infections are usually chronic and asymptomatic, with bacteria typically colonizing the renal tubules. Pathology can result from these chronic infections, but acute disease is more common in non-reservoir hosts; leptospirosis is one of the most common zoonoses on the planet and is recognized as a significant threat to livestock worldwide.

Members of the genus *Leptospira* are Gram-negative, spirochete bacteria. Before 1989, the genus comprised of only two species: *Leptospira interrogans*, which embraced all disease-associated leptospires, and *Leptospira biflexa*, which contained saprophytic, environmental strains. *Leptospira interrogans* was subdivided into a large number of antigenically distinct serovars, many of which were identified as being associated with particular mammalian reservoir hosts, and therefore had ecological relevance. In 1989, taxonomic re-evaluation led to restructuring of the genus and the recognition of more species within it. Subsequently more new species have been proposed, such that today there are currently 17 validated species within the *Leptospira* genus, namely: *L. alexanderi, L. biflexa, L. borgpetersenii, L. broomii, L. fainei, L. inadai, L. interrogans, L. kirschneri, L. kmetyi, L. licerasiae, L. meyeri, L. noguchii, L. parva, L. santarosai, L. weilii, L. wolbachii* and *L. wolffii*, although several as yet unnamed genomospecies have also been proposed. Some strains that, before 1989, would have been classified as *L. interrogans* are now accommodated in other taxa; hence several different *Leptospira* species are now recognized as pathogens of veterinary and public health importance. However, the division between pathogenic and non-pathogenic leptospires is not as clear as it once was, as some of the more recently proposed species are considered to be of uncertain, or opportunistic, pathogenicity. These taxonomic changes have not precluded the use of the established serological classification

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of leptospires, and there are now as many as 300 serovars recognized. Similar serovars are brought together into serogroups, but, rather confusingly, many serogroups are found in more than one species of *Leptospira*. This incongruence remains the most significant obstacle in establishing a useful, contemporary classification within the genus.

Leptospira species possess a characteristic long, fine, helical-shaped appearance. They are motile by means of flagellae that lie within the periplasmic space of the bacterium rather than extending from its outer membrane. The outer membrane contains lipopolysaccharide, and it is the structural heterogeneity in the carbohydrate component of this molecule that underlies leptospiral serovar diversity.

EPIDEMIOLOGY

Leptospira species are encountered more or less throughout the world. Leptospirosis has been reported throughout Europe, from the Scandinavian countries in the north to those bordering the Mediterranean Sea in the south, and from Ireland in the west to Azerbaijan, Kazakhstan, Russia and Turkey in the east. It is now widely accepted that almost every mammal can serve as a reservoir host for leptospires; thus it is not surprising that many different European wildlife species have been implicated in this role. Among European insectivorous species, leptospires have been associated with hedgehogs (*Erinaceus europaeus* and *Erinaceus roumanicus*), shrews (including *Crocidura, Neomys* and *Sorex* species) and moles (*Talpa europaea*). Leptospiral infections have also been detected in numerous species of bat, including *Eptesicus serotinus, Myotis daubentonii, Nyctalus noctula* and *Pipistrellus pipistrellus*. Rodents represent a huge reservoir for leptospires, with many species having been implicated as reservoirs; evidence for the role of squirrels remains scant, although grey squirrels (*Sciurus carolinensis*), which are present in some parts of Europe as a result of introduction, have been shown to harbour leptospires in their native North America. Leptospiral infections have been found in almost all European surveys of woodland-inhabiting mouse and vole species. Small woodland murine and arvicoline rodents, such as wood mice (*Apodemus sylvaticus*) and other *Apodemus* species, and *Myodes* and *Microtus* species, are widely distributed across Europe and are considered among the most important reservoirs of some leptospiral serovars. Numerous surveys in several different countries have demonstrated the presence of infections in a significant proportion of local woodland rodent populations. Other studies have demonstrated the role of larger rodents, particularly those living in aquatic or semi-aquatic habitats, in the natural maintenance of leptospira. Muskrats (*Ondatra zibethicus*) and coypu (*Myocaster coypus*), both introduced species, are considered important reservoir hosts for leptospira. Infections in beavers (*Castor fiber*) are yet to be reported, but are likely. Rats (*Rattus norvegicus* and *Rattus rattus*) are widely considered the most important reservoir of zoonotic leptospires, and, as with woodland rodents, numerous surveys have confirmed the presence of infections in rat populations across Europe.

Lagomorphs are also carriers of leptospires, with infections reported in both rabbits (*Oryctolagus cuniculus*) and hares (*Lepus* spp.). Evidence for the infections in mustelids has been published; leptospires, or anti-leptospiral antibodies, have been detected in various mustelid species, including European and American mink (*Mustela lutreola* and *Mustela vison*), western polecats (*Mustela putorius*), pine martins (*Martes martes*), stone martins (*Martes foina*) and badgers (*Meles meles*). Infections have also been recorded in other carnivores, including genets (*Genetta genetta*), mongooses (*Herpestes ichneumon*), red and arctic foxes (*Vulpes vulpes* and *Alopex lagopus*), wolves (*Canis lupus*), lynx (*Lynx lynx*) and brown bears (*Ursus arctos*). Leptospires have been detected in raccoons (*Procyon lotor*) in their native North America.

Leptospiral infections have been reported in both wild-living and domesticated ungulates; indeed, in the latter group of animals, leptospirosis is a well-recognized disease of veterinary and economic significance. Among wild-living ungulates, evidence for infection has been reported for wild boar (*Sus scrofa*), various deer species (including *Cervus elaphus, Dama dama, Capreolus capreolus*), mouflon (*Ovis orientalis*) and bison (*Bison bonasus*). Serological evidence of infections in moose (*Alces alces*) in Canada has been reported.

Marine mammals are also susceptible to leptospirosis. A catastrophic outbreak of acute disease in captive common seals (*Phoca vitulina*) was reported in 2006 in a zoo in the Netherlands, where, interestingly, the seals shared the same water system as a colony of coypu. Disease in captive and rehabilitated seals has also been reported in the USA, as have infections in wild-living pinnipeds. Finally, leptospirosis has been reported in Europe's only endemic non-human primate, the Barbary ape (*Macaca sylvanus*). Although it has occasionally been proposed that birds, particularly waterfowl, may play a role in the natural

maintenance of leptospires, clear evidence for this remains elusive.

As outlined above, it is now recognized that different *Leptospira* species and serovars have evolved to exploit different mammals as reservoir hosts. For example, *L. borgpetersenii* serovar Hardjo and *L. interrogans* serovar Hardjo are most frequently associated with cattle, *L. interrogans* serovar Canicola with dogs, *L. interrogans* serovar Pomona with pigs, *L. interrogans* serovars Icterohaemorrhagiae and Copenhageni with rats, and *L. interrogans* serovar Bratislava with hedgehogs. The extent to which leptospiral species or serovars have adapted to specific hosts, and the mechanism that underlies such adaptation, are uncertain. For example, although rats are widely considered to be one of the most important reservoir hosts for zoonotic leptospiral strains, it remains uncertain as to whether this is primarily a reflection of the specific host adaptation of, say, *L. interrogans* serovar Icterohaemorrhagiae to rats or the result of other epidemiological factors, such as rat demographics, population dynamics and/or habitat preferences (specifically their proximity to humans). Often, in environments where leptospires are thought to be maintained by rats, other mammals tend to harbour the same serovar; however, the relevant contribution of these mammals as hosts in leptospiral enzootic cycles is yet to be quantified. Thus, they may be acting merely as incidental hosts, or may be fulfilling a more important role in the natural maintenance of the bacteria.

The ecology of leptospires is not fully understood, but the current general paradigm is that they exploit mammal species as reservoir hosts by establishing chronic infections in the renal tubules of the kidneys (often referred to as the 'carrier phase') that can persist for months or longer. From this niche, bacteria are shed in urine, contaminating the environment. Susceptible hosts thus acquire infection indirectly from infected animals by coming into contact with environmental bacteria. The existence of this environmental step in the natural cycle of leptospires means that their transmission is influenced not just by the population dynamics of their reservoir hosts but also by abiotic determinants such as climate and hydrology. The persistence of leptospires in the environment is, generally, favoured by 'warmth and wetness'; hence leptospirosis is more prevalent in the tropics and subtropics than the temperate regions of the world.

Leptospiral infection does not necessarily provoke disease. Indeed, it is likely that, for those mammal species that serve as reservoir hosts, chronic, sub-clinical infection is the norm. However, in accidental hosts, or immunocompromised individuals within a reservoir host population, infection can result in systemic, potentially life-threatening disease.

PATHOGENESIS, PATHOLOGY AND IMMUNITY

Acquisition of leptospiral infection occurs via mucosal surfaces and via cuts or trauma to the skin. The number of leptospires required to produce an infectious dose in nature is unknown, but estimates have been derived from experimental infections. These studies have shown that infectious dose is very much dependent on host, *Leptospira* species and serovar. For example, inoculation of hamsters with 10^8 of *L. interrogans* serovar Icterohaemorrhagiae resulted in 50% mortality, whereas inoculation of the same number of *L. interrogans* serovar Copenhageni resulted in the death of all animals. In the absence of specific antibodies, leptospires are able to multiply rapidly on entering the vasculature, from where they disseminate and further replicate in numerous sites around the body. In immunocompetent hosts, this systemic infection eventually provokes an antibody-mediated response that controls and clears the infection from most of the body, although bacteria colonizing the renal tubules often persist, perhaps as a result of somewhat immune-privileged nature of this site, and a chronic infection is established. In the absence of an effective humoral response, systemic infection can be augmented, resulting in profound disease. The pathology of systemic leptospirosis is determined by a combination of direct effects of leptospires, and leptospiral products on cells and tissues, and damage resulting from immune response to infection. For example, leptospiral disruption of blood-vessel function can provoke ischaemia and thus precipitate damage to the parenchyma of various organs, and leptospires also produce toxins that may also have direct detrimental effects on infected tissues. However, in keeping with other Gram-negative bacteria, leptospiral lipopolysaccharide (endotoxin) is a potent activator of the innate immune system, and hence a potentially damaging inflammatory response. However, the progression of disease is not solely dependent on host susceptibility; different serovars tend to be associated with different presentations. For example, in dogs, serovars Canicola and Bratislava are more often associated with renal dysfunction, whereas serovars Icterohaemorrhagiae and Pomona

provoke more hepatic disease. Although clinical disease is most frequently associated with acute-phase leptospiral infections, chronic infection is not without consequences. Interstitial nephritis and other renal lesions associated with chronic leptospirosis have been documented in a range of reservoir hosts, and it is thought these manifestations may progress to fibrosis and subsequent renal failure. Several studies have explored the means by which leptospires persist in renal tubules. This stage is mediated primarily by interaction between leptospires and tubule epithelium, with bacteria closely associated with epithelial cells and the extracellular matrix. Indeed, although the extent of infection markedly varies, from one or two tubules in some animals, to nearly all tubules in others, the intensity of infection is usually low, with most bacteria lying in contact with the epithelium, forming only a thin lining to the lumen.

Although a handful of reports of leptospire-associated pathology have emerged from North America in wildlife species whose range extends into Europe (for example, a survey of red foxes (*Vulpes vulpes*) in Canada revealed that severe haemorrhagic nephritis and interstitial nephritis were common), no European reports of leptospire-induced pathology in wildlife have appeared in the international literature. Thus, veterinary knowledge of the pathologic consequences of leptospiral infection is primarily based on observations of disease in dogs and livestock. In canine leptospirosis, external gross signs may include congested or icteric mucosa, with petechial or ecchymotic haemorrhaging. Internally, kidneys become enlarged and take on a pale, yellowish appearance. In severe cases, the renal capsule may adhere to the kidney surface and subcapsular haemorrhages are common. In less severe cases, white spotting may be seen in the renal cortex, particularly along the corticomedullary junction. In chronically infected, or recovered, individuals, the kidneys may be scarred or shrunken. With hepatic leptospirosis, the liver becomes enlarged, takes on a yellow colour and develops interlobular markings. Histological examination of infected kidneys reveals some variation, thought to be attributed to the specific behaviour of different serovars and individual immune responses. However, in general, renal lesions are relatively insubstantial, consisting of limited tubular necrosis and interstitial oedema. Chronic renal infections are histologically characterized by diffuse interstitial inflammation, particularly around the corticomedullary junction. This inflammation is primarily made up of plasma cells with fewer lymphocytes and macrophages. This pathology can progress to diffuse interstitial fibrosis with some multifocal lymphoplasmacytic inflammation in kidneys that have been long infected. Leptospires can be observed in tissues using silver staining or immunohistochemistry. In early-stage infections, bacteria appear, adhering to the luminal surface of renal tubule epithelial cells, but they are less apparent in chronic leptospirosis.

CLINICAL SIGNS AND TREATMENT

No clinical case reports of acute leptospirosis in European wildlife have been published in the international literature; thus veterinary knowledge of the spectrum of clinical presentations associated with leptospires is drawn from observations of infected dogs or livestock. The clinical signs of canine leptospirosis depend on the age and immune status of the animal and the identity of the infecting strain. Common signs associated with acute canine leptospirosis include pyrexia (39.5–40°C), shivers and myalgia, then subsequently vomiting, dehydration and peripheral vascular collapse, although numerous other non-specific manifestations have also been reported. Mucous membranes become congested and petechial and ecchymotic haemorrhages can appear. Progressive deterioration in renal function is reflected in oliguria or even anuria. Icterus is another common sign in canine leptospirosis, and has been observed in foxes as well as domesticated dogs. Intestinal and pulmonary manifestations can also develop as disease develops. As mentioned above, renal dysfunction is also a consequence of chronic leptospiral carriage. Leptospiral infections in livestock (primarily cattle and pigs) are primarily recognized in the form of reproductive disorders, which are manifestations of the chronic form of infection. However, acute leptospirosis, albeit usually self-limiting, is observed in young animals, presenting as fever (40.5–41°C), anorexia, dyspnoea from pulmonary congestion, icterus, haemoglobinuria and haemolytic anaemia. In older animals, reproductive disorders take the form of abortion, which is thought to occur several weeks or months after initial infection and is more common in animals nearing term. Alternatively, infected mothers may yield stillbirths, premature births or weakened infected offspring. An abortion storm in a breeding herd is often the first indication of leptospirosis infection in cattle, because the mild initial signs often pass unnoticed. Infertility may also be a presentation in endemically infected herds, possibly as a consequence of localization of infection in the uterus and

oviducts. In dairy herds, acute leptospirosis may result in widespread pyrexia and a sudden drop in milk production of up to 75%. The remaining milk can be thick, yellow and blood-tinged, with thick clots and a high somatic cell count. The udder is typically soft and flabby, which is unique for leptospirosis. Milk production can return to normal within 2 weeks, even in the absence of treatment, but cows may not recover to full production during that lactation cycle.

DIAGNOSIS AND TREATMENT

Diagnosis of leptospirosis relies on either demonstration of bacterial presence in infected tissues or quantification of specific antibodies. Demonstration of the presence of bacteria can be achieved using a variety of approaches, including direct microscopy, immunodetection, culture and methods based on the polymerase chain reaction (PCR). The simplest or most 'low-tech' means of confirming a leptospiral infection is by darkfield microscopic examination of clinical specimens, most commonly urine. Darkfield microscopy is employed as leptospires are not easily stained using standard bacterial stains. Wet-mount preparations allow the motility and flexing of leptospires to be observed, thereby facilitating discrimination of bacteria from thread-like debris in the sample. However, this approach is not particularly sensitive, and it has been suggested that an infection intensity of as many as 10^5 organisms/ml is required to be sure of observing bacteria. Thus, a negative result using darkfield microscopy should not curtail the use of other diagnostic approaches. Leptospira can also be observed histologically using Giemsa or silver stains. Again, the sensitivity of this approach is considered to be limited. Immunodetection, most commonly in the form of direct fluorescent antibody tests, is also used to detect leptospires in tissue imprints or body fluids. However, owing to the extreme serological diversity of leptospires, tests are usually serovar- or serogroup-specific. Other forms of immunodetection have also been developed – in particular, urinary antigen enzyme-linked immunosorbent assays (ELISA). The value of these assays appears to be good in terms of their specificity and sensitivity relative to other diagnostic approaches, ease of use and cost. However, again, the specificity of these ELISAs is limited to particular serovars or serogroups. PCR-based methods are also widely employed and can be designed to detect all members of the genus or to be specific to *Leptospira* species. DNA extracts for use in PCR can be prepared from fresh, frozen or fixed tissue suspected of being infected, and a range of PCR formats, including nested and real-time assays (the latter allowing quantification of infection intensity) have been described. Sequencing of PCR products can be used to facilitate identification of infecting *Leptospira* species, but not serogroup or serovar. Isolation of bacteria using specific culture techniques is also possible and in ideal circumstances is the preferred means of diagnosis. However, leptospires are notoriously fastidious organisms requiring specific media and prolonged incubation times. Isolation attempts are usually made on suspected infected internal tissues or, more commonly, on urine. Multiple urine samples should be tested because of the intermittent nature of leptospiral shedding. The most common medium used for isolation attempts is Ellinghausen-McCullough-Johnson-Harris (EMJH) medium. Leptospiral cultures are incubated under aerobic conditions at about 30°C for prolonged periods, typically at least 12 weeks, and often timescales of 30 weeks are advised. The presence of leptospires in these cultures is monitored by periodic darkfield observations.

Serological tests are perhaps the most widely used means of diagnosing leptospiral infections. The microscopic agglutination test (MAT) is considered the 'gold standard' of serological tests, and relies on the formation of bacterial aggregates when a dilution series of serum is mixed with suspensions of an appropriate serogroup. Although this approach has stood the test of time, it is considered labour-intensive, time-consuming and difficult to perform and control. Furthermore, its ability to accurately delineate serovars within serogroups has been frequently questioned. Diagnosis of infection may be based on a single titre, although the magnitude of this is dependent on circumstance. Demonstration of a four-fold rise in MAT titre is a reliable means of serologically confirming acute disease. Other serological test formats have also been developed, including a latex agglutination test and ELISA. For the diagnosis of infections caused by serovars associated with human and companion animal leptospirosis, numerous commercial serological test formats are also available, including easy-to-use dipstick-style tests. Assays based on slide agglutination or haemagglutination that incorporate broadly reactive antigens are useful, as they allow the detection of specific antibodies in a range of different host species.

Acute leptospirosis can be effectively treated with antibiotics, and, by inhibiting the multiplication of bacteria in the blood system, early antibiotic intervention can have

a significant impact on the progression of disease, curtailing the more profound complications of infection. It is therefore recommended that antibiotics be prescribed immediately on suspicion of leptospirosis. Penicillin and its derivatives are considered the drugs of choice for treatment of acute disease, although other classes of antibiotics have also proven to be effective in this role. Antibiotic prescription may also be successful in elimination of chronic infections.

MANAGEMENT, CONTROL AND REGULATIONS

Control of leptospirosis in livestock and companion animals is primarily achieved through vaccination. Vaccines are most often inactivated suspensions of one or more leptospiral serovars, determined by the animal species being vaccinated and geographic location. Although numerous experimental vaccines based on cellular extracts have been tested, commercial vaccines are, with few exceptions, whole cell products combined with suitable adjuvants. Although vaccination is effective against acute, clinical disease, it does not necessarily prevent chronic leptospiral carriage; thus the usefulness of vaccination in eradicating leptospirosis from, for example, a herd, flock, kennel or farm, is limited. Ideally, infected animals should be isolated. Control of leptospirosis caused by rodent-associated serovars can be addressed by reducing rodent numbers in the vicinity of livestock or companion animals. Furthermore, as leptospire transmission involves an aquatic, environmental stage, effective removal of shed urine and maintenance of animals in clean, dry conditions will reduce transmission rates.

The veterinary and zoonotic importance of leptospirosis is reflected in its inclusion, for a long period of time, as a 'listed' (formerly list B) disease with The World Organisation for Animal Health (OIE).

PUBLIC HEALTH CONCERN

In humans, the early phase of disease is typically characterized by a flu-like illness. Progression to systemic disease occurs in 5 to 15% of cases, with pulmonary haemorrhage, myocarditis and kidney and liver failure among the more common complications. The International Leptospirosis Society estimates that up to 500,000 cases of human leptospirosis occur annually worldwide. However, this number is probably an underestimate, as surveillance is poor in many areas where leptospirosis is likely to be most common. Most cases occur in the tropics, where 'warmth and wetness' favour the environmental persistence of leptospires. Exposure to leptospirosis is associated with contact with water, for example as a result of rice farming, but also during periods of heavy rainfall. Outbreaks of leptospirosis are very common among residents of tropical and subtropical urban (rodent-infested) slums during these periods when infrastructural shortcomings facilitate transmission of the disease. Human leptospirosis is also of public health significance in temperate climes, and is often acquired by recreational exposure occurring in water sports. There have been several recent high-profile outbreaks of leptospirosis associated with competitive outdoor events, such as triathlons or adventure races.

SIGNIFICANCE AND IMPLICATIONS FOR ANIMAL HEALTH

Canine leptospirosis is widely recognized across Europe and in many countries and has led to widespread vaccination of dogs against serovars Icterohaemorrhagiae and Canicola. Not all canine leptospirosis is caused by these serovars, and, increasingly, leptospires belonging to serovars Australis, Bratislava, Grippotyphosa or Sejroë are being encountered. Cats are also susceptible to leptospirosis, but the disease is considered to be a far less important threat to the wellbeing of cats than dogs. Numerous cases of equine leptospirosis have been reported, in a variety of clinical presentations, ranging from common renal and hepatic dysfunction to rarer forms such as uveitis and reproductive disorders. Serological evidence of leptospiral infection is common in horses, and the predominant serovars reported are Pomona, Bratislava, Icterohaemorrhagiae and Grippotyphosa. Among European production animals, leptospirosis is recognized most frequently in pigs and cattle. In pigs it is primarily a cause of reproductive disease, with strains belonging to serovars Bratislava and Pomona being most commonly associated with disease. In cattle, strains belonging to serovar Hardjo are commonly encountered, with infections being associated with a sudden drop in milk production, abortions, birth of weak calves or infertility.

Given the frequency of clinical leptospirosis in livestock and companion animals, it is reasonable to assume that cases do occur in wildlife but simply go undiagnosed. Our lack of awareness of such cases is probably associated

with the usually non-fatal nature of infections and difficulties associated with diagnosis. Improved diagnostic tests, including serology and PCR, may be useful for identifying endemic leptospiral infections in wild species and indicating their prevalences with a view to understanding epidemiology and natural cycles of infection.

Further reading about leptospirosis is recommended in the references given below[1–6].

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CHAPTER

34

COXIELLA BURNETII INFECTION

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Coxiella burnetii is the causal agent of Q fever, a disease affecting humans and animals. Infection with *C. burnetii* is usually subclinical but can produce acute and chronic disease in humans as well as acute disease in animals – typically abortion in farmed ruminants.

AETIOLOGY

Coxiella burnetii, previously known as *Rickettsia diaporica* or *R. burnetii*, is a small, obligate, intracellular, Gram-negative bacterium (0.2–0.4 μm wide and 0.4–1 μm long) that belongs to the family Coxiellaceae and has a genome that ranges from 1.5 to 2.4 Mb, depending on the strain. *Coxiella burnetii* is found in two different morphological forms in persistently infected cells: the 'small cell variant' form and the 'large cell variant' form. These forms correspond to different intracellular development stages of *C. burnetii*. *Coxiella burnetii* also displays antigenic variations owing to mutations in the lipopolysaccharide (LPS). The smooth form of LPS corresponds to the phase I and the rough form of LPS to the phase II antigenic variant of *C. burnetii*. The phase I variant is found in naturally infected hosts and is highly infectious. The phase II form has low infectivity and only occurs after serial passages in cell cultures or embryonated egg cultures[1].

EPIDEMIOLOGY

GEOGRAPHICAL DISTRIBUTION AND HOSTS IN EUROPE

Coxiella burnetii is a ubiquitous infectious agent reported in almost worldwide distribution, including infection in livestock in many European countries. It has been shown to circulate among European wildlife in Cyprus (24 local and migratory bird species), the Czech Republic (red deer (*Cervus elaphus*), roe deer (*Capreolus capreolus*), fallow deer (*Dama dama*), mouflon (*Ovis aries*) and Eurasian wild boar (*Sus scrofa*)), France (chamois (*Rupicapra rupicapra*)), Italy (fallow deer and lion (*Panthera leo*) in a safari park), Poland (European bison (*Bison bonasus*)), Portugal (waterbuck (*Cobus ellipsiprymnus*) and sable antelope (*Hippotragus niger niger*) from a zoo), Slovakia (mouflon, red deer, fallow deer and yellow-necked mouse (*Apodemus flavicollis*)), Spain (red deer, roe deer, mouflon, Eurasian wild boar, European hare (*Lepus europaeus*), wood mouse

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(*Apodemus sylvaticus*), house mouse (*Mus musculus*), griffon vulture (*Gyps fulvus*) and black kite (*Milvus migrans*)) and the UK (brown rat (*Rattus norvegicus*)).

HOST FACTORS

Virtually all life kingdoms, including mammals, birds and arthropods, are considered to be able to harbour *C. burnetii*[1]. It has been reported in domestic mammals, such as camel, horse, water buffalo, cattle, sheep, goat, swine, rabbit, guinea pig and mouse and domesticated birds such as chicken, pigeon, duck, goose or turkey[1]. In wildlife, *C. burnetii* infection has been reported in ruminants, suids, carnivores (including marine carnivores) and small mammals, as well as in several migratory and resident bird species and reptiles, such as tortoises and snakes. The information available on *C. burnetii* in wildlife is mainly about exposure to the pathogen, and little is known about its ecological drivers. In humans, incidence of Q fever tends to be higher in men than women, and this is thought to be caused by an increased risk of occupational exposure[1]. Risk of exposure to *C. burnetii* in sheep and goats seems to increase with age[2], but management may be a significant driver of this risk. Based on known *C. burnetii* epidemiological patterns in livestock, host population factors such as density, social behaviour or the structure of the ecological community can be expected to influence the risk of exposure of humans to *C. burnetii* from wildlife sources.

ENVIRONMENTAL FACTORS

Coxiella burnetii has been found in more than 40 tick species, and ticks are presumed to play a relevant role in the wild cycle of *C. burnetii*. However, the epidemiologic role of ticks is not sufficiently understood and is currently controversial.

Human outbreaks of Q fever appear to be linked to season, with higher incidence in spring and summer[1], probably in association with the seasonality of livestock reproduction rather than to any specific climatic conditions. Nonetheless, a higher prevalence was described in chamois after cold and snowy winters. This was attributed to the effect of hard climatic conditions on individual fitness[3] of chamois, as *C. burnetii* is highly resistant in the environment, and hence climatic conditions might not greatly impair the viability of infective forms.

Current knowledge is insufficient to define the epidemiological role of wildlife species in *C. burnetii* infection. Nonetheless, there are reports that suggest that wild mammals excrete *C. burnetii* in the same manner as domestic ruminants; these potentially infective tissues include products of abortion and stillbirth in wild ruminants[4] and rabbits[1] as well as infective placenta in the case of a Pacific harbour seal (*Phoca vitulina richardsi*). Seroprevalence in black-tailed deer (*Odocoileus hemionus columbianus*) in North America was found to peak in January, then decrease until April and increase again after the calving season in May, suggesting a higher excretion of *C. burnetii* around the birth season[5].

Transmission of *C. burnetii* from animal to animal and from animal to human occurs mainly by infective aerosols. Owing to the expected higher excretion associated with gestation and parturition, airborne transmission when animals abort or give birth could also be an epidemiological driver in wildlife.

PATHOGENESIS, PATHOLOGY AND IMMUNITY

Owing to the importance of airborne transmission of *C. burnetii*, the respiratory route of infection is considered the most important. Aerosol-transmitted infective forms of the organism have been found to infect humans with doses as low as just a single infective cell[1]. The digestive, vertical and sexual routes of transmission are also possible. *Coxiella burnetii* is also excreted in milk.

Experimentally it has been shown that *C. burnetii* is engulfed by local macrophages after it enters the body. Bacteria are thereafter disseminated to the rest of the body, and localize particularly in tissues of the reticuloendothelial system, especially the spleen and liver. *Coxiella burnetii* replicates well in the acidic conditions within the phagolysosomes, and after cell rupture new host cells are infected by the released bacteria. *Coxiella burnetii* is present in lungs, liver, spleen and blood after acute infection and it can be excreted, mainly around parturition, in faeces, vaginal secretion, semen, milk and urine[1].

In humans, where many cases of infection are asymptomatic, *C. burnetii* may cause atypical pneumonia, characterized by gross consolidation of the lungs, interstitial pneumonia and alveolar exudates, and granulomatous hepatitis[1]. In domestic ruminants, infected placentae may show intercotyledonary fibrous thickening and discolored

exudates; the myometrium and the stroma adjacent to the placentomal area may show a severe inflammatory response; trophoblast cells appear altered and foamy. Metritis may be observed in cattle. Similar pathological manifestations probably occur in wild animals. Chronic coxiellosis has not been reported in animals, although latent carriage of *C. burnetii* may occur in animals, as apparently happens in humans[1]. The cells, tissues and organs in which persistent *C. burnetii* infection occurs are currently not known.

Clearance of infection in pregnant goats observed after abortion was suggested to be caused by an effective immune response[6]. After infection, the host produces anti-phase I and II antibodies to neutralize *C. burnetii* infection. Serologic patterns differ between species; whereas sheep are able to mount an early immune humoral response (within 2 weeks), anti-phase II antibodies reach their peak in cows some weeks later. Infection induces gamma interferon (FN-γ) production that activates monocytes and macrophages, producing nitrogen and oxygen intermediates that lead to the intracellular killing of the pathogen. *In vitro* experiments have found that T-cell immunity is effective in the control of Q fever, although it cannot clear the infection and prevent chronic manifestations of the disease.

CLINICAL SIGNS

Clinical outcome of infection by *C. burnetii* is highly variable and depends primarily on the infection route, the infectious dose, the pathogen strain and host immune status[1]. In animals, disease caused by *C. burnetii* is rarely reported, and only reproductive manifestations of infection may be apparent. Experimental infection of chickens[7] and goats[6] showed no clinical outcome of infection, except for pregnant goats, in which late-term abortion occurred. Even though most cases are subclinical, both in animals and humans, acute onset of disease may be seen in humans (fever, atypical pneumonia, hepatitis, abortion) and animals (pneumonia, abortion, stillbirth, weak newborn animals, infertility, metritis, mastitis); and chronic disease (a chronic fatigue type syndrome, sometimes with endocarditis) has only been described in humans. The clinical outcome of infection by *C. burnetii* in wildlife consists of a few reports of abortion and stillbirth in zoo antelopes[4] and abortion in rabbits[1], and recently *Coxiella*-like organisms have been found to cause fatal disease in psittacines and toucans in North America[8].

DIAGNOSIS

Diagnosis of acute Q fever in cases of abortion or stillbirth requires detection of *C. burnetii*. This can be done through staining tissue samples, immunohistochemistry, direct isolation in cell cultures, embryonated eggs or laboratory animals, capture enzyme-linked immunosorbent assay (ELISA) or by polymerase chain reaction (PCR); however, examinations must be done in biosecure facilities. There are several serologic tests employed for demonstrating the existence of anti-*C. burnetii* circulating antibodies[9]. Detection of complement-fixing antibodies has been widely used to provide evidence of exposure to *C. burnetii* but indirect fluorescence assay (IFA) and ELISA have proven more sensitive and specific serologic methods. Distinct detection of anti-phase I and II antibodies also helps to distinguish recent infections from historic infections.

Analysis of anti-*C. burnetii* antibodies (especially by IFA or ELISA) is the quickest and cheapest diagnostic method for epidemiological studies in wild animals. Some tests developed for domestic animals can be used in wildlife, but there is a need to develop serological tests for a wide range of wildlife species. For live animals and ticks, PCR is a very sensitive and quick method to detect *C. burnetii* presence or excretion by shedders, but many infected animals do not shed bacteria, reducing its diagnostic power. PCR undertaken on target organs could also be a good screening method alone or in combination with serologic tests for diagnostic purposes in wildlife.

MANAGEMENT, CONTROL AND REGULATIONS

Owing to its ubiquitous nature, high environmental resistance, wide host range and low infective dose, control and eradication of *C. burnetii* from endemic natural foci is difficult, if not impossible. Mass vaccination with phase I microorganisms/antigens has proven to be effective in reducing shedding and incidence of abortion in livestock when performed over a significant period of time and in conjunction with testing and culling[10]. No vaccination control trials have been carried out in wildlife

PUBLIC HEALTH CONCERN

Q fever is considered an emergent or re-emergent disease. Recent massive outbreaks of Q fever in the Netherlands

have indicated the need to control *C. burnetii* infection in livestock and to stimulate more intense epidemiological investigations. Most human outbreaks arise from infection from livestock, but little is known about the role of wild animal species. Increased human/wildlife exposure rates, increasing wild ungulate populations across Europe and the particular characteristics of *C. burnetii*, as mentioned, make the need for research in wildlife of special relevance.

SIGNIFICANCE AND IMPLICATIONS FOR ANIMAL HEALTH

Coxiella burnetii has a significant impact in livestock because of the reproductive disorders it causes. As an example, 9% of ovine abortions in the Basque Country (Spain) were found to be caused by Q fever[11]. By contrast, recent surveys in the region have found low circulating *C. burnetii* antibodies in wild mammals and birds[12,13]. This difference in seroprevalence may indicate that wildlife is not important in the epidemiology of livestock infection in this region, which is considered to be hyperendemic for Q fever[2]. These results suggest that livestock are the more significant reservoir of *C. burnetii* rather than wildlife. The significance of wild species in the epidemiology of *C. burnettii* within and between human, domesticated and wild species is not known at present, and more research is required in this field.

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CHAPTER

35

LISTERIA INFECTIONS

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Listeriosis, also known as circling disease, is found worldwide in domestic animals, humans and, less frequently, in wildlife. Infection may be latent, or cause encephalitis, abortion, mastitis or systemic disease.

AETIOLOGY

The genus *Listeria* currently contains five species, although classification is under constant review. Two species, *L. monocytogenes* and *L. ivanovii*, are pathogenic, with *L. monocytogenes* being the more important of these. *Listeria monocytogenes* is a facultative intracellular Gram-positive aerobic or microaerophilic rod. Eleven serotypes have been recognized based on somatic and flagellar antigens. Cultivation in the laboratory is done on common media, such as trypticase agar or brain-heart infusion agar, where the bacteria grow in translucent colonies in 24–48 hours. When observing these colonies at a 45° angle, a characteristic blue-green sheen is appreciable on their surface. On blood agar, *L. monocytogenes* is haemolytic, but haemolysis occurs in a narrow area around the colonies, in contrast to *L. ivanovii*, which produces a wider zone of haemolysis than that seen with *L. monocytogenes*. *Listeria monocytogenes* is negative for oxidase and hydrogen sulphide and positive for catalase, and has characteristic 'tumbling' motility at room temperature (<25°C) owing to the production of peritrichous flagella that are absent or rare at 35–39°C.

EPIDEMIOLOGY

The natural habitat of *Listeria* spp. is in organic matter in the soil, such as decomposing plants, where they can live as saprophytes. *Listeria monocytogenes* can also be isolated from healthy animals, both domestic and wild species, and humans (between 6 and 24% of faeces from these animal hosts and humans are positive). *Listeria monocytogenes* is therefore a frequent component of the normal intestinal flora of animals – but only when it passes across the oral or intestinal mucosae does disease occur.

Listeria monocytogenes has been isolated from a wide range of hosts, including several mammalian families (artiodactyls, carnivores, lagomorphs, rodents, insectivores, primates) and several bird species, and also from invertebrate hosts (i.e. haematophagous arthropods). Listeriosis occurs particularly in domestic ruminants and has been described in more than 40 species of animal[(1)]. In wild mammals it has been found in fallow deer (*Dama dama*), moose (*Alces alces*), roe deer (*Capreolus capreolus*), wild boar (*Sus scrofa*), red deer (*Cervus elaphus*) , reindeer (*Rangifer tarandus*), red fox (*Vulpes vulpes*), European brown hare

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(*Lepus europaeus*), mountain hare (*Lepus timidus*), red squirrel (*Sciurus vulgaris*), capercaillie (*Tetrao urogallus*), willow partridge (*Lagopus lagopus*), white stork (*Ciconia ciconia*) and pheasant (*Phasanius colchicus*)[2–5].

The most frequent forms of disease caused by *L. monocytogenes* are encephalitis, septicaemia and abortion or stillbirth. Genital and encephalitic forms rarely occur simultaneously in the same animal or flock. *Listeria ivanovii* is recorded from as wide a host range as *L. monocytogenes*, but as a pathogen it is seen only in ruminants, where it occasionally causes abortion but does not cause central nervous system (CNS) infection[2–4].

Listeria spp. can grow in soil, water and faeces, and on vegetables, and is a facultative pathogen in vertebrate and invertebrate species. Bacteria from the environment are usually the source of infection for animals and exposure to this bacterium from environmental contamination is difficult to avoid. *Listeria* is able to survive and multiply in different environmental conditions and can grow at a pH range from 5 to 9 and at temperatures from 4 to 45°C. The bacteria can grow rapidly in silage that is not conserved correctly (pH higher than 5).

Listeria monocytogenes can be carried subclinically by a range of animal species and by humans, and the organism can be shed in faeces. In livestock, wildlife and human populations, the same *L. monocytogenes* strains may infect these different hosts, but it is also possible to find different strains of the bacterium in the environment and in water in the same area where positive animals are sampled[6].

Outbreaks of infection are usually linked to ingestion of highly contaminated feed, or to heavily contaminated environments, e.g. soil, which contaminate the fodder or water with high levels of the bacterium. Silage has been frequently incriminated as a source of *Listeria* for domestic animals, although the organism can be readily found in fodder, grass and plants in rural or in wild environments. Populations of *L. monocytogenes* are probably maintained in the environment by continuous faecal-oral cycling and enrichment in ruminant hosts[1]. Wild animals are thus continuously exposed to the bacterium, but only in rare circumstances does infection lead to disease.

PATHOGENESIS, PATHOLOGY AND IMMUNITY

Listeria monocytogenes is usually ingested; it crosses the intestinal mucosa and produces a bacteraemia. The liver is probably the first target organ. It can also cross the placenta and cause fetal infection. There is evidence that encephalitis is mainly due to bacteria that enter the oral mucosa, invade the trigeminal nerve and, moving along this nerve, enter the brain and thus cause inflammation. It has also been suggested that bacteria can also penetrate the tooth pulp and reach the brain via the trigeminal nerve. Less frequent routes of infection are through the conjunctiva and the nasal mucosa.

Listeria monocytogenes and *L. ivanovii* are intracellular pathogens that survive in macrophages, epithelial cells, hepatocytes and endothelial cells. After cellular uptake, probably by M cells of the intestinal mucosa, but also by crypt cells, *Listeria* escapes from the phagosome, multiplies in the intracytosol and spreads to adjacent cells. When the bacteria leave infected cells they are phagocytosed by macrophages and neutrophils; however, this can lead to systemic spread. Listerolysin, a cholesterol-binding cytolysin, is the factor that, in phagocytosis and cell invasion, regulates intracellular survival by facilitating the escape from the phagocytic vesicle. It is produced and secreted mostly (50-fold) by extracellular bacteria compared with bacteria that are in the host cell cytosol (cytoplasm). The cell-to-cell spread of the bacterium is favoured by phospholipases produced by *L. monocytogenes* that enable the organism to exit from the double membrane vesicles in which the bacterium is enveloped when passing from one cell to another. Intracellular organisms polymerize actin, forming a tail that facilitates motility and penetration of the organism into a new host cell. Macrophage activation prevents the intracellular multiplication of bacteria and increases the inactivation of ingested organisms.

In septicaemic forms, miliary foci of necrosis are usually visible in liver, spleen, lung, kidney and lymphoid tissues. Septicaemia may also result in uterine infection with abortion. Foci of necrosis are also visible in abortion material, in the cotyledons with a suppurative placentitis. The fetus has necrotic foci resembling those of septicaemic infection. The brain of infected ruminants may appear normal at gross inspection. Typical histopathologic lesions are meningoencephalomyelitis characteristically affecting the white and grey matter of the brainstem, which can extend rostrally to the diencephalon and caudally to the cervical spinal cord. Histologically there is early necrosis, with bacteria in the necrotic foci. Later there is a predominance of neutrophils and necrosis (microabscesses) and gitter (phagocytic) cells. These changes are accompanied by perivascular cuffing of mononuclear cells. There may

also be leptomeningitis, perineuritis, and ganglioneuritis (trigeminal ganglion).

Infection is more widespread than disease, and the onset of a strong cellular response renders the host resistant to the organism. The cellular response seems to decrease after some months in experimentally infected mice, but in nature animals are probably frequently challenged by the bacteria. The humoral response has a lesser role in protection. Vaccination with live attenuated organism is protective, while killed organisms do not generate a protective immunity[1].

CLINICAL SIGNS

Listeriosis can manifest as a CNS infection, as abortion, as a generalized septicaemia and as mastitis in dairy cattle. When the CNS is involved, adult ruminants show meningoencephalitis with depression and confusion, protrusion of the tongue, and salivation, blindness, head pressing, and paralysis of facial and throat muscles. The affected animal can be unstable on its legs and seeks support from objects such as fences; when it walks, the tendency is to move in a circle – hence the common name of circling disease. At the terminal stage animals remain immobile on the ground and tremors are frequently seen. Death occurs in 2 to 3 days after the onset of clinical signs. Listeria abortion usually takes place in late gestation. Septicaemic listeriosis is usually associated with very few clinical signs, as affected animals die without these being observed. In young ruminants and monogastric animals, signs of meningitis without brain involvement may be seen, whereas clinical listeriosis is relatively rare in non-ruminant species; however, listeriosis has been noted in pregnant female European brown hares.

DIAGNOSIS

For cultivation of *Listeria*, to increase the recovery from visceral organs, placenta and fetuses, the tissues can be mixed with a liquid nutrient medium and incubated at 4°C for 1 week or longer. Subcultures should be done at 1, 3, 4 and 12 weeks in blood agar from the original broth. Selective broths (e.g. Listeria Enrichment Broth or Fraser Broth) are more sensitive and specific than direct non-selective plating. There are also chromogenic agars available for presumptive identification of *L. monocytogenes*. Antibodies against *L. monocytogenes* can be tested by serological tests such as the agglutination test, but the sensitivity and specificity are poor, so serology is of limited diagnostic value[1]. Molecular methods to characterize isolates, including molecular serotyping, ribotyping, allelic typing of the *sigB* gene using polymerase chain reaction (PCR), and multilocus sequence analysis (MLSA), can also be used and have been described in clinical investigations in domestic ruminants.

MANAGEMENT, CONTROL AND REGULATIONS

Control of the bacteria in the environment is not feasible apart from good standards of hygiene that aim to reduce food and water contamination by organisms shed in the faeces. Outbreaks in wildlife are usually reported in captive or managed herds (e.g. foraging herds). *Listeria monocytogenes* sporadic infections with low mortality, less than 3%, are reported in the European brown hare[3]. Avoiding wild animals congregating at supplementary feeding sites, and preventing the provision of supplementary food on the ground, as well as avoiding access to foodstuffs left on pasture for livestock, are management actions that can be adopted to control this infection in wildlife. Considering that many livestock infections are due to poor-quality silage, use of such silage to feed wildlife should be avoided.

PUBLIC HEALTH CONCERN

Listeria monocytogenes is one of the most important foodborne zoonoses. Direct transmission to humans, even through contact with infected animal tissues, rarely occurs. The main risk for human health is through consumption of infected animal food, and infection from milk, patès, cheese or other food items contaminated by the bacterium. There are many reports of the occurrence of *Listeria*, mainly *L. monocytogenes*, in game carcasses, where prevalence of *L. monocytogenes* infection ranges from 0 to 12.5% of the wild game meats sampled. Wild boar appear to be the species with the highest prevalence, because up to 23.5% of meat from this species is contaminated[7]. This apparently high prevalence in wild boar meat could be the result of different hunting techniques (animals driven to guns instead of stalking or stand hunting), which tend to lead to more abdomen shots (resulting in meat contamination with *Listeria* spp. tracking from the digestive tract), or to the dressing of carcasses in the field. However, this

prevalence is not directly linked to primary carcass infection (i.e. systemic infection of the animal prior to slaughter), because meat infection is usually due to carcass dressing in the field and in the later processing stages (cold storage rooms or processing facilities), where environmental contamination with the bacteria can occur[7].

SIGNIFICANCE AND IMPLICATIONS FOR ANIMAL HEALTH

Although there are occasional reports of outbreaks of listeriosis in captive or managed herds, cases in free-ranging wild animals are sporadic. Therefore it seems that *Listeria* generally has a minor influence on wildlife populations. A report on white stork chicks indicates that there was no effect of *Listeria* spp. infection on the subsequent survival of the infected chicks[5].

The main implication of *L. monocytogenes* infection in domestic animals is in dairy cows, which develop subclinical mastitis, with shedding of *L. monocytogenes* in the milk. If this milk is not then pasteurized there is a potential for causing human infection from the consumption of the unpasteurized dairy products.

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CHAPTER

36

CLOSTRIDIUM SPECIES AND BOTULISM

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INTRODUCTION

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Clostridial bacteria are bacilli that are obligate anaerobes. They form spores to survive adverse environmental conditions, and generally stain Gram-positive. They are widely distributed in soil, aquatic environments, decaying organic matter and on mucosal surfaces or within digestive tracts of humans and animals. Many, including the species discussed here, produce toxins that are responsible for their pathogenicity. Although free-ranging wildlife undoubtedly are affected by numerous clostridial species, *Clostridium botulinum* is the most significant and widely reported.

BOTULISM

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Botulism, also known as limberneck, western duck sickness, alkali poisoning or duck disease, is intoxication by neurotoxin produced by *Clostridium botulinum*, which acts on the peripheral nervous system to cause progressive flaccid paralysis.

AETIOLOGY

Clostridium botulinum has been classified into seven different types (A–G) based on the antigenically distinct neurotoxin (A, B, C1, D, E, F and G) that each produces. Neurotoxin production occurs only in growing vegetative cells, but it is not released until cells die. *Clostridium botulinum* types C and D are unique, in that the genes encoding neurotoxin production are located on infectious bacteriophages. Loss of the bacteriophage results in loss of toxigenicity, but toxin-producing potential can be restored with reinfection of the bacterium by the bacteriophage. Only types C and E are significant for wildlife. Type C strains can also produce C2 and C3 toxins, which are not neurotoxic, and their roles in wildlife outbreaks are unknown. An alternate classification scheme based on physiological characteristics divides *C. botulinum* into four

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groups. Types C and E are placed in groups III and II, respectively.

EPIDEMIOLOGY

GEOGRAPHICAL DISTRIBUTION IN EUROPE

Although a well-known cause of wild bird mortality in North America since the beginning of the 20th century, botulism in wild birds was not documented in Europe until the 1960s. The first report of botulism caused by type C1 neurotoxin was in waterfowl in urban ponds in southern Sweden in 1963[1]. Since then, confirmed outbreaks of type C botulism in wild birds have occurred throughout Europe in Austria[2], Czech Republic[3], Denmark[4], France[5], Germany[6], Hungary[7], Ireland[8], Italy[9], the Netherlands[10], Norway[11], Serbia[12], Slovenia[13], Spain[13] and the UK[14]. Botulism in birds in Poland is virtually unknown, but a presumptive outbreak of type C botulism in mallards (*Anas platyrhynchos*) was described in which polymerase chain reaction (PCR) examination detected the type C toxin gene from a bacterial culture of intestine from one duck[15].

To date, the only reports of type E botulism in Europe occurred in Friesland province, in the Netherlands, in 1975[10] and in the Canche Estuary of northern France in 1996[16].

Surveys for spores of *C. botulinum* types C and E in environmental samples demonstrate that spores are widely, but variably, distributed throughout Europe. Although found in soil, they are most prevalent in aquatic sediment. Overall prevalence is generally low, but certain areas are heavily contaminated. Type E spores are especially prevalent in marine sediments (100%) and shores (84%) of the Baltic Sea, the Sound between Sweden and Denmark, the Kattegat and Skagerrak[17]. Heavy contamination can also be much more localized. For example, 60% of samples from the Norfolk Broads in Britain contained type E spores[18] compared with 2.7% of samples from aquatic mud collected throughout the UK[19]. High concentrations of spores have also been found at sites of previous avian botulism outbreaks (e.g. the Norfolk Broads in the UK[19], British landfill sites[20], the Guadalquivir Basin, Spain[21]). In the Netherlands, 71.6% of mud samples from areas with prior avian botulism had type C spores present[22]. However, high spore prevalence does not necessarily correlate with high incidence of botulism. There are no reports of type E botulism in birds from the heavily spore-contaminated Baltic, whereas only 1 out of 25 (4%) sediment samples from the site of the type E outbreak in Northern France contained type E spores[23].

HOST FACTORS

A list of 263 and 31 species known to be affected by type C and type E botulism, respectively, has been published[24]. Most bird species, with the possible exception of vultures and other carrion eaters that may have natural and/or acquired resistance, are susceptible to botulism. Age, sex and species composition of affected birds in an outbreak reflect local presence, abundance and feeding habits of birds. Because most cases of botulism occur following ingestion of preformed toxin, feeding habits are the most significant host factor. Depending on the source of toxin in a given outbreak, dabbling ducks that feed at the water's surface, diving ducks that feed on bottom sediment, shorebirds that feed in shoreline sediment and gulls that feed on carcasses and/or other decaying organic matter have all been particularly vulnerable to type C botulism. Type E botulism occurs in fish-eating birds and can also affect fish. Thousands of birds, primarily black-headed gulls and herring gulls (*Larus ridibundus* and *L. argentatus*), died in northern France, presumably after feeding on fish waste[16]. Loons, gulls, grebes and occasional waterfowl have suffered from type E botulism in North America. The limited outbreak in the Netherlands occurred in ducks, but the species was not specified. Presence of concurrent fish and bird mortality can indicate type E involvement.

In many incidents of type C botulism in Europe, like in the rest of the world, waterfowl (Anatidae) are the most common species affected, and outbreaks are thought to be perpetuated by the carcass–maggot cycle. However, a different presentation of avian botulism is prevalent in northern Europe. In the UK[14,25], Ireland[8] and Sweden[26], large outbreaks of type C botulism have been reported primarily in gull species (Laridae). In these outbreaks, numerous species of seabirds, particularly herring gulls (*Larus argentatus*), were affected. Epizootics took place along large stretches of coastline, on coastal islands and in estuaries. In the UK and for some gull deaths in Ireland, local landfill and refuse sites were thought to be the source of toxin[8,14], but in no case was the source of toxin definitively determined. Gulls were presumed to have consumed

toxin via their scavenging habits. In one incident in the UK, the deaths of 700 gulls were attributed to the spread of contaminated poultry manure onto fields where gulls fed. In Scotland during an outbreak, lesser black-backed gulls (*Larus fuscus*) were under-represented in the die-off, and one possible explanation was their more maritime feeding habits[25].

ENVIRONMENTAL FACTORS

Necessary environmental factors for a botulism outbreak include suitable environmental conditions and appropriate substrate for bacterial growth and toxin production.

Clostridium botulinum type C grows best at temperatures between 30 and 37°C and growth has not been demonstrated at temperatures below 10°C. Most outbreaks therefore occur during the summer and early autumn. In both the UK and the Netherlands, incidence of botulism outbreaks increased during unusually hot summers[10,14,27]. However, occasional winter and spring outbreaks have been documented in these countries and in the Czech Republic[28,29,36]. Internal temperature in a rotting duck carcass can be significantly higher than ambient temperature, resulting in a suitable microenvironment for toxin production[30], and type C toxin has been shown to persist through winter in submerged containers[28] and dead maggots[29]. Both of these factors make outbreaks in colder weather possible. Additionally, some of the Dutch outbreaks occurred near industrial activities that artificially warmed surrounding water[10]. By contrast, type E can grow and produce toxin at lower temperatures than type C. Optimal growth occurs between 25 and 30°C, and growth has been documented at temperatures as low as 3.3°C. The outbreaks in France in 1996 took place in February and November and outbreaks in North America often occur during cool autumn weather.

Clostridium botulinum requires an anaerobic environment and a high protein substrate for growth. Decaying invertebrate and vertebrate carcasses provide excellent conditions for this. Any environmental factor that results in increased numbers of carcasses and/or allows carcasses to persist can help trigger an outbreak if other necessary factors are present. Examples include sudden changes in water level or water quality, severe weather, chemical spills or pesticide use, limited scavenger access and improper disposal of animal waste.

EPIDEMIOLOGICAL ROLE OF THE AFFECTED SPECIES

Birds dying from botulism provide additional substrate for bacterial growth and can perpetuate an outbreak. Although botulism is an intoxication, expansion of an outbreak via contaminated carcasses can mimic spread of an infectious disease[31]. Carcasses also further contaminate the local environment with *C. botulinum* spores. A large proportion of healthy animals living in an environment contaminated with botulinum spores have spores within their gastrointestinal tracts or other tissues (e.g. 40.5% of animals[10]; 50% of mallards[32]). This is of no consequence until the animal dies, regardless of the cause. If conditions are favourable, spores can germinate within the carcass and potentially set off a botulism outbreak. Birds and other animals such as fish can act as mechanical carriers of spores, redistributing spores within the environment. Gulls in particular are thought to concentrate spores at sites that they are attracted to. This may help to explain higher spore prevalence in landfill and refuse sites versus the general environment in the UK[20]. Refuse tested before it was added to landfill sites did not contain detectable spores, further supporting the hypothesis that contamination takes place at the landfill.

TRANSMISSION

In most cases, botulism in wildlife occurs following the ingestion of preformed toxin (food poisoning). Transmission therefore occurs via contaminated food or water. Any decaying organic matter hosting a microenvironment conducive to *C. botulinum* growth and toxin production may be a potential toxin source. The best-studied scenario is the carcass–maggot cycle of type C botulism in which decomposing carcasses are the microenvironment for *C. botulinum* proliferation and toxin production, and maggots feeding on these carcasses become contaminated with toxin. Other birds are then exposed to botulinum toxin when they consume these toxic maggots, leading to potentially huge and explosive outbreaks. In one British outbreak, mallards had gorged on maggots shown to contain high levels of botulinum toxin[33]. In an outbreak in the Czech Republic necrophagous larvae and pupae of the dipterous flies *Lucilia sericata* and *Calliphora vomitoria* contained 80,000 mouse LD_{50}/g of botulinum toxin[29]. Based on experimentally derived toxic doses, a single toxic

larva contained enough toxin to kill a duck. They also detected type C toxin in water around carcasses, and from a ptychopterid larva, leeches (Hirudinidae) and sow-bugs (*Asellus aquaticus*) from within carcasses, but at a much lower concentration (8–800 mouse LD_{50}/g). In the Salton Sea in California, USA, tilapia fish (*Oreochromis mossambicus*) were implicated as the source of toxin in a type C outbreak in fish-eating birds, and toxin was detected in the gastrointestinal tracts of sick and dead fish[34]. In British and Swedish outbreaks of botulism in herring gulls (*Larus argentatus*), the scavenging habits of these birds were thought to bring them into contact with the toxin source. In the UK and Ireland, gulls were suspected of ingesting type C toxin at local landfill and refuse sites[8,20]. Type E botulism occurs following ingestion of toxin-containing fish or other marine products.

PATHOGENESIS, PATHOLOGY AND IMMUNITY

Botulinum toxin is the most toxic substance presently known[35] and botulism usually occurs following ingestion of preformed toxin. Oral toxic dose for type C botulism varies greatly between species. In birds, waterfowl are most susceptible, and scavengers and carrion eaters are most resistant. Experimentally, mouse minimum lethal dose per bird varied from 19,000 in teal (*Anas crecca*) and 64,000 in coots (*Fulica atra*) to 2,500,000 in crows (*Corvus corone*) and black-headed gulls (*Larus ridibundus*). The mouse LD_{50} for mallards (*Anas platyrhynchos*) was 320,000/bird[36].

In other animals (e.g. foals, poultry), toxico-infectious botulism has been reported. Under the right conditions, spores within the intestinal tract germinate, allowing toxin to be produced *in vivo* and absorbed. The other documented form of botulism occurs when a wound is contaminated with spores, and germination and toxin production occur under anaerobic conditions. The significance of these modes of acquisition for wildlife is unknown.

The toxin is absorbed from the gastrointestinal tract and travels via the blood and lymphatic systems to the site of action in the peripheral nervous system. Here, it inhibits acetylcholine release from peripheral cholinergic motor and autonomic nerve endings. Clinical signs in animals are caused by sustained blockage of acetylcholine release at the neuromuscular junction. Toxin does not cross the blood–brain barrier. Uptake of botulinum toxin is closely linked to nerve stimulation, i.e. stimulation facilitates intoxication of the nerve cell[37]. The botulinum toxin molecule is composed of a heavy chain and a light chain. A three-step mode of action has been proposed[35]:

1. the neurotoxin binds to the presynaptic cell membrane via the heavy chain
2. the neurotoxin is internalized into the presynaptic nerve cell via endocytosis and the light chain is translocated into the cell cytoplasm
3. the light chain acts as a zinc-endopeptidase to cleave proteins involved in synaptic vesicle docking and fusion to the plasma membrane, thereby blocking the release of acetylcholine at the neuromuscular junction.

This results in the characteristic flaccid paralysis. Each type of botulinum toxin targets a different site on one of three proteins, and type C neurotoxin has two sites of action on two different proteins.

There are no specific gross or microscopic pathologic changes. The acute onset of intoxication means that affected birds typically are in good nutritional condition. Because paralysis limits the ability to eat and drink, birds generally have empty gastrointestinal tracts and are dehydrated. Urates and bright green faeces can distend the cloaca and stain feathers around the vent of affected birds. Waterfowl often have leeches in the eyes, nares and oral cavity.

The binding of botulinum neurotoxin to the presynaptic membrane is irreversible and the resulting flaccid paralysis is progressive. If intoxication is severe, death results from paralysis of respiratory muscles. If birds are on the water when intoxicated, paralysis of neck muscles can cause death by drowning. In other cases, paralysis leads to death by exposure to the elements, dehydration, starvation or predation.

Duration of botulinum toxin action is not known in animals. However, in humans, time to complete recovery is typically 2–4 months at the neuromuscular junction, but can take more than a year in the autonomic nervous system[37].

Botulinum neurotoxin is so toxic that, for most species, the dose required to develop acquired immunity through natural exposure is higher than the lethal dose[38]. No neutralizing antibodies were found in mallards given multiple sublethal doses of botulinum toxin type C[36]. Naturally occurring antibodies in captive ducks suffering from botulism 2 weeks after onset of disease were detected, but

these disappeared by 5 weeks[39]. Immunity was short-lived and not protective against future exposure. However, in a study of free-living scavengers and carrion-eaters, 90% of turkey vultures (*Cathartes aura*), 42% of crows (*Corvus brachyrhynchos*), 25% of coyotes (*Canis latrans*) and 17% of Norway rats (*Rattus norvegicus*) sampled had naturally occurring antibodies to various types of *C. botulinum* toxin[38]. Similarly, although ring-billed gulls (*Larus delawarensis*) are susceptible to botulism type E, antibody production was experimentally induced in gulls fed lower levels of toxin[40]. Sublethal natural exposure in the wild may induce some degree of immunity. Vaccination of wild birds against botulism type C with commercial mink vaccine[41] improved survival in challenge studies, but in most cases vaccination of free-ranging birds is not warranted.

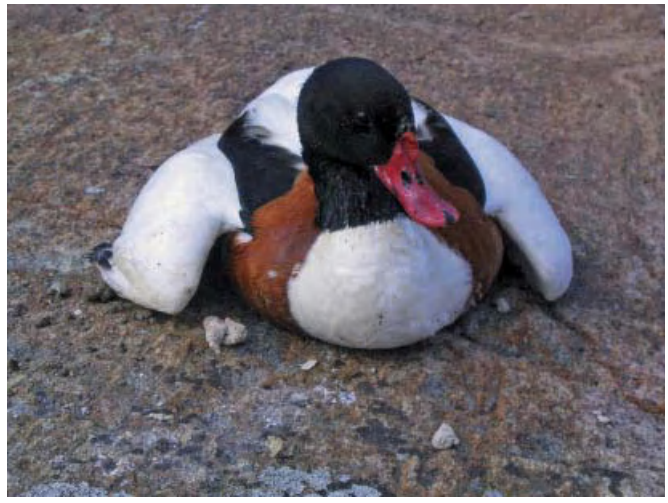

Figure 36.1 A common shelduck (*Tadorna tadorna*) moderately affected by botulism type C. Wings are dropped at the sides and the duck has lost the use of its legs, but it remains alert and responsive.

CLINICAL SIGNS AND TREATMENT

The hallmark sign of botulism is progressive, flaccid paralysis of skeletal muscles. The rate of onset and the severity of the clinical signs reflect the amount of toxin ingested. In early stages, birds have difficulty flying or can only fly short distances. Once flight capability is lost, birds can still often stand and walk, although gait may be impaired or uncoordinated. Eventually, they become recumbent, but may be able to propel themselves forward with their wings. While resting, wings can be dropped away from the body (Figure 36.1). In late stages, birds are prostrate and neck, head and respiratory muscles are affected. Inability to support the head results in 'limberneck', described in ducks and other species (Figure 36.2). Breathing becomes laboured, and the nictitating membrane may be prolapsed. Until birds reach the final stages, they generally are bright and alert, despite the paresis and paralysis.

Treatment of affected birds can be highly effective (75–90% recovery rate[24]). Mildly to moderately affected birds can be treated simply by providing food, water and shelter from the elements and predators. Antitoxin has been given in some cases, but toxin already bound to and taken up by nerve cells cannot be neutralized. Recovered birds have no protective immunity so must not be released back into the site of an ongoing outbreak. Vaccination of birds prior to release can help reduce re-intoxication. Treatment requires a large number of resources and may only be warranted in certain situations (e.g. if threatened or endangered species are involved).

Figure 36.2 A herring gull (*Larus argentatus*) severely affected by botulism type C. The bird is prostrate and the neck muscles are paralyzed (limberneck).

DIAGNOSIS

Definitive diagnosis of botulism is challenging and disease often diagnosed presumptively based on clinical signs coupled with the absence of gross and microscopic lesions and negative test results for other aetiologies. Toxin proliferation within a decaying carcass can occur *post mortem*, resulting in false positives, so the ideal sample

for confirmatory testing is serum from sick birds. Because the majority of toxin can be fixed at the site of action in the peripheral nervous system and result in false negatives, it is best to test a number of birds in a given outbreak. Serum can be frozen until tested, but toxin activity significantly decreases over time.

Botulinum toxin is a protein, so the ideal confirmatory test must detect the presence of biologically active proteins. Currently, the mouse bioassay is the only test available for type C botulism that meets these requirements. Functional *in vitro* assays for type E are under development. Protocols for the mouse test vary, but briefly, serum samples (0.5–1 ml) are injected into two groups of mice. One group receives protective antitoxin 30 minutes before serum injection. The test is considered positive if unprotected mice show clinical signs of botulism or die and protected mice remain unaffected throughout the test. Onset of clinical signs can be delayed if toxin concentrations within the sample are low, so mice are observed for 4 days. *Post mortem* examinations can be carried out on any dead mice to ensure that no other aetiologies (e.g. bacterial infection with *Pasteurella multocida*) were responsible.

For a variety of reasons, including the terminal use of animals, cost, and time needed to reach a diagnosis, numerous other diagnostic tests have been developed. Tests for human botulism have been reviewed and compared[35]. Other diagnostic tests used for wildlife include immunoassays (e.g. enzyme-linked immunosorbent assay (ELISA)) and molecular techniques (PCR). ELISA tests, although more rapid, are not as sensitive as the mouse test. An improved test, an antigen-capture ELISA for type C botulism that concentrates toxin present within a sample, has been developed[42]. At sample volumes less than 1 ml of serum, the mouse bioassay is still more sensitive. However, at these volumes, this ELISA is a good screening test if three or more animals from a given outbreak are tested. If sample volumes greater than 1 ml are used, this ELISA may be as sensitive or more sensitive than the mouse test. Unfortunately, this volume of serum often is difficult to obtain from birds in natural outbreaks.

PCR techniques have been developed to detect the botulinum neurotoxin gene, but these tests are best employed to detect the presence of spores in the environment. Caution should be used when interpreting toxin production potential from environmental samples using PCR, because this technique does not detect activity of the gene. Additionally, botulinum spores often are present in healthy animals, so detection of the toxin gene in samples from animals by PCR does not mean that the animal died from botulism. A PCR technique to detect the type C toxin gene in only active cells provides stronger evidence that the animal died from botulism[43].

A promising *in vitro* assay is the endopeptidase-mass spectrometry assay under development for botulism diagnosis in humans[44]. This functional assay takes advantage of the specific enzymatic activity of each botulinum toxin. Cleaved protein products are then detected with highly sensitive mass spectrometry, and toxin type is determined based on product size. This assay is designed to detect human botulism, i.e. only types A, B, E and F, and requires further refinement.

MANAGEMENT, CONTROL AND REGULATIONS

The management and control of botulism are difficult, but the most effective way to mitigate losses from botulism in wildlife is through prevention of outbreaks. Because decaying organic matter can serve as a substrate for *C. botulinum* growth and toxin production, environmental management should focus on minimizing die-offs of invertebrates and vertebrates that overwhelm local scavenger capacity. Suggested strategies include avoidance of sudden changes in water levels of managed wetlands, removal of physical hazards for birds, avoidance of practices resulting in fish kills, delay of hunting until cooler weather and promotion of scavenging activity[31]. Excluding susceptible birds from an affected area during an outbreak has been attempted. Removal of carcasses during an epizootic should, in theory, be an effective way to eliminate substrate for further bacterial growth and toxin production. However, the effectiveness of botulism control via carcass pick-up during an outbreak is questionable. In one study, on average only 32.1% of marked carcasses were detected, and larger species were over-represented in carcass collection[45]. Small, open, easily accessible environments may be more suitable to this type of control. Finally, landfill site management to limit attractiveness for birds may help to prevent botulism in gulls.

Botulism in humans, but not wildlife, is notifiable at the EU level (Decision no. 2119/98/EC of the EU Parliament and Council).

PUBLIC HEALTH CONCERN

Humans are susceptible to type E botulism, so care should be taken when handling tissues and samples from birds

that died from type E. Type C neurotoxin does not affect humans.

SIGNIFICANCE AND IMPLICATIONS FOR ANIMAL HEALTH

Botulism in wildlife has little significance for domestic species. One outbreak of botulism type C in cattle was linked to a waterfowl die-off in a wetland in Canada where cattle were grazing and drinking[46], but this is unusual. Conversely, botulism is one of the most significant diseases of migratory birds, especially waterfowl and shorebirds, worldwide. Huge die-offs of hundreds of thousands or even a million birds have been reported in North American waterbirds. In Europe, die-offs have not been as dramatic, but some outbreaks have claimed thousands of waterfowl and gulls. The largest reports include 60,600 and 70,000 birds in the Netherlands in 1976 and 1983, respectively[10], at least 10,000 gulls in France in 1996[16], 4,040 birds, primarily gulls, in the UK in 1975[14] and probably thousands of seabirds, primarily herring gulls, in Sweden from 2000 to 2004[26]. Additionally, botulism outbreaks may negatively impact threatened and endangered species. For example, avocets (*Recurvirostra avosetta*), a critically endangered species in the Czech Republic, were involved in a botulism outbreak there[47]. Other rare or endangered birds killed by botulism in the Czech Republic included spoonbills (*Platalea leucordia*), curlews (*Numenius arquata*), black-tailed godwits (*Limosa limosa*) and redshanks (*Tringa totanus*). Finally, although botulism can be the proximate cause of death of wildlife, the ultimate cause of the epizootic (e.g. the initiating conditions that caused the increased numbers of carcasses initially or the environmental change that facilitated *C. botulinum* germination and toxin production) may be of the most significance. Thus, changes in patterns and prevalence of botulism outbreaks may signal underlying environmental perturbation.

TYZZER'S DISEASE

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Tyzzer's disease, also named haemorrhagic disease of muskrats and Errington's disease, was first described as a fatal epizootic diarrhoeal disease in Japanese waltzing mice (*Mus musculus*). It is characterized by typical necrotic lesions in the liver with intracellular bacteria adjacent to the necrotic foci, and in epithelial cells of the intestine[48].

Clostridium piliforme (previously named *Bacillus piliformis*) is an obligate intracellular, pleomorphic, spore-forming bacterium. Its vegetative form (within the host) comprises Gram-negative slender rods, 0.5 µm wide and 8–10 µm long, which do not survive long outside a host. The spores are resistant to freezing and thawing and may remain stable and infective for years in the environment. Six serologic groups are identified among isolates of *C. piliforme*, and a single host species might be infected with strains of more than one group.

Tyzzer's disease is most commonly known as an acute disease of laboratory animals (mouse, rat, hamster, gerbil, rhesus monkey) and commercially bred rabbits but also affects horses (foals) and other domestic animals. A few cases have occurred in captive birds[49]. In colonies of laboratory animals mortality can be very high. Greatest loss occurs in weanlings, but adult individuals are also affected. Clinical disease appears in animals stressed by transport, overcrowding, poor sanitation or corticosteroid administration. Tyzzer's disease has been described in a variety of free-ranging and captive mammals. A case of Tyzzer's disease in a free-ranging Eurasian otter (*Lutra lutra*) occurred in a cub found weak on the Isle of Harris, Scotland[50].

Infectious spores are shed in the faeces and transmission is believed to be by ingestion of spores. Tyzzer's disease is an intestinal infection that spreads to the liver via the portal vein. The location of bacteria only within apparently healthy cells at the border of necrotic areas indicates a cell-to-cell spread by contact. *Clostridium piliforme* seems to have an affinity for epithelial and smooth muscle cells of the intestine, hepatic and myocardial cells[48]. Gross lesions vary among species, but enteritis is usually present. Most commonly, the caecum, colon, and terminal ileum are affected. Ulcerative haemorrhagic colitis and typhlitis and focal hepatic necrosis are typical findings in muskrats (*Ondatra zibethicus)*. Characteristic lesions in the liver appear as multiple 1–3 mm white foci. Lesions in the myocardium appear as streaks or white foci in some species[49]. Excess serous fluids in the pleural, pericardial and peritoneal cavities were striking features in a European otter[3] and also occur in other species. Splenomegaly has been described in some cases. Histologically, necrosis of intestinal epithelial cells, submucosal oedema and polymorphonuclear cell infiltration are seen. Bacilli are demonstrated in epithelial and muscle cells. Hepatic lesions vary from foci of acute coagulation

necrosis with only a few granulocytes to fibrotic scars surrounded by macrophages and occasional multinucleated giant cells. Bundles of long, slender bacilli are present in apparently healthy cells at the margins of necrotic foci. In chronic lesions, organisms may be absent[49].

In most species, Tyzzer's disease is an acute fatal disease. Diarrhoea, depression, anorexia, and a ruffled, uneven hair coat may be observed during the brief clinical course. Dysentery occurs in cottontail rabbits (*Sylvilagus floridianus*) and muskrats[49]. Bilateral corneal oedema has been reported in a Eurasian otter[50].

Diagnosis of Tyzzer's disease is usually by microscopic demonstration of the characteristic intracellular bacteria associated with compatible lesions in sections with silver staining, such as Warthin–Starry or Levaditi. Liver is routinely chosen for examination and intestinal tissue should also be examined. Serological diagnostic methods are developed for laboratory animals but have not been validated for wild animals. *Clostridium piliforme* does not grow in cell-free media but can be grown in embryonated eggs and several mammalian cell lines.

The acute course of disease and intracellular location of the organism make treatment difficult. Although antibiotic treatment with oxytetracyclins, penicillins, cephalosporins or erythromycin may be attempted to control outbreaks in laboratory animals, there are no suitable measures for the control and treatment of Tyzzer's disease in wild animals.

OTHER CLOSTRIDIAL DISEASES IN WILDLIFE

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Reports of disease in wildlife caused by other clostridial species are uncommon. Their toxins target different organ systems: enterotoxaemia (*C. perfringens*), muscle, superficial soft tissue and systemic toxaemia (*C. chauvoei*, *C. novyi*, *C. septicum*, *C. perfringens*), and the nervous system (*C. tetani*). Like *C. botulinum*, they grow and produce toxin under appropriate conditions, but this occurs *in vivo*. With the exception of enterotoxaemia caused by *C. perfringens*, disease tends to occur in individual wild animals rather than causing large epizootics. Although most reports of these diseases in wildlife come from areas outside of Europe, they are briefly summarized here because these clostridial species are ubiquitous.

CLOSTRIDIUM PERFRINGENS – NECROTIC ENTERITIS

Clostridium perfringens causes acute ulcerative, haemorrhagic and necrotizing enteritis in birds.

This bacterium is found in soil and in the gastrointestinal tracts of animals. Types A–E have been described based on the combination of exotoxins produced by the bacterium. In wildlife, types A and C are most significant. Type A produces only alpha toxin, and type C produces alpha and beta toxin.

This disease was first described in wild waterfowl in Florida, USA. In Europe, 155 ducks were reported to have died from *C. perfringens* enterotoxaemia in 1981 and 1982 in the lower Rhine region, Germany[51]. More recently, 30 whooper swans (*Cygnus cygnus*) in East Anglia, UK died in November 2003 after a dry summer and wintering birds had been supplemented with wheat[52]. A review of earlier records revealed six other suspected incidents involving 65 whooper swans and 14 mute swans (*Cygnus olor*). In five of six incidents, supplemental wheat had been provided. Several incidents were associated with environmental change such as temporary changes to local aquatic habitat. Incidents occurred between November and January. The largest epizootics (hundreds of birds) of necrotic enteritis recorded occurred during autumn months in migrating wild geese in western Canada[53]. Other free-ranging species affected in isolated, suspected outbreaks of necrotic enteritis include western bluebirds (*Sialia mexicana*) and a greater sage grouse (*Centrocercus urophasianus*) in western USA[54,55], crows (*Corvus macrorhynchos*) in Japan[56] and lorikeets (*Trichoglossus* spp.) in Australia[57]. Necrotizing enteritis associated with *C. perfringens* type A was the most common cause of death in captive capercaillies (*Tetrao urogallus*) in Norway[58].

Necrotic enteritis occurs when intestinal conditions favour proliferation and toxin production of *C. perfringens*. Disease has been associated with sudden dietary changes such as those encountered during migration, trypsin inhibitors in feed, gut stasis, stress and intestinal parasitism[53]. In migrating geese in western Canada, these authors hypothesized that a sudden shift in diet to carbohydrate-rich grains containing trypsin inhibitors allowed *C. perfringens* to colonize the intestine. Supplemental wheat

provided to swans in the UK coupled with environmental stressors may have helped to initiate disease.

Toxigenic *C. perfringens* colonize tips of intestinal villi, where they produce and secrete alpha and beta toxins. Alpha toxin is a lecithinase and causes necrosis, and beta toxin paralyses the intestine. Damage to the intestinal mucosa leads to necrosis and ulceration and further invasion of bacteria. Beta toxins are inactivated by proteases such as trypsin, so dietary trypsin inhibitors permit active toxin to persist in the intestine and exert its effect. Gross and microscopic changes are characterized by ulceration, haemorrhage and necrosis in the intestine. Watery, often blood-stained content and fibrin casts are frequently seen in intestines grossly. In geese from western Canada, whole grain and legumes were found in the upper digestive tract and in a few cases undigested grain was seen throughout the intestine[53]. Lesions tend to be more common in the mid and distal intestine, but can involve the entire intestine and even colon[52]. Birds generally are in good nutritional condition. Overgrowth of large Gram-positive rods is seen in the damaged intestine microscopically. Lesions start at the tips of villi, but may progress transmurally. Fibrin thrombi and infiltration by heterophils are also often seen. The liver may be haemorrhagic and friable or may show multifocal necrosis, as observed in 28% of affected captive capercaillies[58].

Birds typically are found dead, but clinical signs have occasionally been described; affected geese had reduced alertness and were unable to fly[53], swans were described as 'drowsy and wobbly'[52] and lorikeets showed general malaise and had diarrhoea[57].

Standard diagnostic techniques employ anaerobic bacterial culture followed by typing of *C. perfringens* using laboratory animal bioassays, immunoassays (e.g. immunofluorescence or ELISA) or molecular techniques (PCR). Because *C. perfringens* is a normal inhabitant of the gut and proliferates in decaying carcasses, it can be difficult to definitively diagnose enterotoxaemia. Characteristic gross and microscopic lesions and evidence of bacterial toxigenicity should accompany a positive culture before necrotic enteritis caused by *C. perfringens* is diagnosed as cause of death.

OTHER CLOSTRIDIA

Clostridial myonecrosis and malignant oedema (also called gas gangrene) from *C. chauvoei*, *C. novyi*, *C. septicum* and/or *C. perfringens* result from deep wounds contaminated with bacterial spores. In an appropriate localized anaerobic environment, spores germinate and produce toxin *in vivo* that is absorbed systemically. Clostridial myonecrosis has been reported in various captive and free-ranging mammals, but these incidents generally have been sequelae to injections or darting during capture and handling. Naturally occurring wounds from intraspecific aggression or accidental trauma could result in similar disease.

Tetanus is caused by a neurotoxin produced by *C. tetani*. Spores present in the environment gain access to the body via wounds and germinate if favourable anaerobic conditions exist. The neurotoxin produced acts on the central nervous system at presynaptic sites of spinal motor neurons. Blockage of the inhibitory mediators gamma-amino butyric acid and glycine cause uncontrolled muscle stimulation, resulting in localized or generalized hypertonia of skeletal muscles with accompanying spasms or contractions. Tetanus has been documented in a free-ranging grey squirrel (*Sciurius carolinensis*) in Canada with severe trauma to the foot from a leg-hold trap[59], and a presumptive case of tetanus was reported in a free-ranging hippopotamus (*Hippopotamus amphibius*) with skin lacerations in South Africa[60]. There are no published reports of tetanus in European wildlife.

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CHAPTER

37

OTHER BACTERIAL INFECTIONS

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FUSOBACTERIUM NECROPHORUM INFECTION

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Fusobacterium necrophorum infection occurs worldwide and has been associated with disease (necrobacillosis) in domestic mammals, especially ruminants, for more than a hundred years[1].

Fusobacterium necrophorum (Family Bacteroidaceae) is an obligately anaerobic, non-spore-forming, pleomorphic, Gram-negative bacterium that tends to form filaments[1,2]. The bacterium is a normal inhabitant of the mammalian alimentary tract and an opportunistic pathogen. Two major biotypes are recognized: Biotype A, *F. necrophorum* subspecies *necrophorum*, and biotype B, *F. necrophorum* subspecies *funduliforme*. *Fusobacterium n. funduliforme* is the predominant inhabitant of the alimentary tract, whereas *F. n. necrophorum* is the most pathogenic, and the type associated with classical necrobacillosis in animals.

Necrobacillosis has been reported in various wildlife species, mostly from continents other than Europe[3]. In the past, this disease was recognized as a serious health problem in semi-domesticated tundra reindeer (*Rangifer tarandus tarandus*) in northern Scandinavia and Russia[4]. Reports from European wildlife include free-living European bison (*Bison bonasus*) in Poland[5] and wild tundra reindeer in southern Norway[6].

Virulent strains of *F. n. necrophorum* may be present in the intestinal tract of animals and spread into the environment via faeces, causing infection in the feet, for example, with further spread via discharges from open lesions. The risk of disease and pathogen transmission is especially high in herd-forming species, or following other types of animal congregation. In North America, outbreaks of necrobacillosis in wild ruminants have been associated with concentrations of animals at water holes under drought conditions, and around feeding stations at times of food shortage[3]. The genital form of necrobacillosis seen in free-living male bison in Poland was suggested to be linked to heavy faecal contamination of their winter feeding sites[5].

The serious necrobacillosis problem that occurred previously in semi-domesticated tundra reindeer in northern Scandinavia was linked to the intensive herding practised at that time, with frequent gathering of animals in confined spaces[4]. This regular crowding in small, muddy and faeces-contaminated areas apparently functioned as a good environment for the bacterium and site of transmission between animals, with digital necrobacillosis as the main

Infectious Diseases of Wild Mammals and Birds in Europe, First Edition. Edited by Dolores Gavier-Widén, J. Paul Duff, and Anna Meredith.

clinical manifestation. Other clinical manifestations seen in these reindeer herds were oral, skin, genital, udder and eye infections; conditions that were considered secondary to digital necrobacillosis.

Outbreaks of digital necrobacillosis occurring recently in wild tundra reindeer in southern Norway were apparently associated with extended periods of precipitation and high air temperature during the summer[6]. Permanently damp pastures presumably improve the survival of the bacterium in the environment and predispose to digital skin infection. Skin that is damp for long periods is more vulnerable to abrasions, creating suitable entrance sites for the bacterium.

Fusobacterium necrophorum gains entrance through epithelial defects or small wounds in the skin or alimentary tract and proliferates in the subepithelial connective tissue. Growth is stimulated by the presence of facultatively anaerobic bacteria, e.g. *Actinomyces pyogenes* and *Staphylococcus aureus*, which provide lowered oxygen tension in the tissue. Pathogenicity is linked to the production of various toxins, with leucotoxin and endotoxin considered the major virulence factors[1,2]. Necrobacillosis lesions may occur in almost any part of the body but are most frequently recognized in the feet (digital necrobacillosis) and mouth (oral necrobacillosis).

Lesions produced are cellulitis and progressive peripheral coagulative necrosis linked to vasculitis, thrombosis and ischaemia. In digital necrobacillosis, gross lesions include swelling of the foot and cutaneous sinus tracts with sparse discharge of pus[6]. Subcutaneous tissues are inflamed and thickened with focal necrosis, and tendons, tendon sheaths, joints and periosteum of the digital bones are often affected. Occasionally, secondary necrotic foci may be found in the mouth, rumen, liver and lungs. Microscopically, digital lesions are characterized by deep skin ulcers with centrally located necrotic tissue, bordered by a zone of oedema and intense inflammation with granulation tissue and fibrosis. Bone lesions are characterized by necrosis, fibrosis and bone proliferation. Elongated filamentous bacteria can be seen at the periphery of the necrotic areas, even with standard haematoxylin and eosin stains.

In primary oral necrobacillosis, areas of necrosis may occur in various soft tissues of the mouth cavity, and may also affect the mandibular bones[7]. Oral necrobacillosis frequently leads to secondary foci of infection (necrosis) in the lower alimentary tract, especially the rumen, and in the liver.

The protective immunological response to *F. necrophorum* infection is weak, and no effective vaccine has been developed[2]. However, in bovine digital necrobacillosis naturally acquired immunity seems to inhibit reinfection by the same strain for at least 6 months[8]. Some protective acquired immunity also seems to occur after outbreaks among wild ungulates[6].

Clinical signs observed in digital necrobacillosis include swelling and lameness in one or several feet. The severity of signs varies from immobility, when several limbs are heavily affected, to the slight and transient signs caused by mild infections. Clinical signs of oral necrobacillosis include excess salivation, as well as problems in chewing and swallowing food. Oral infection also predisposes to aspiration of infectious materials and subsequent fusobacterial pneumonia. Spread of infection via the general circulation may terminate in septicaemia and rapid death.

Therapeutically, penicillins, tetracyclines and macrolides are efficient against *F. necrophorum* if administered at an early stage of infection[2].

A confirmatory diagnosis is dependent on isolation of *F. necrophorum* using anaerobic culture techniques. Even under optimal laboratory conditions, *F. necrophorum* can be difficult to isolate as a result of culture dominance by facultative anaerobic bacteria. In such cases, direct detection of the bacterium in tissues using fluorescence *in situ* hybridization is a useful diagnostic technique (Figure 37.1).

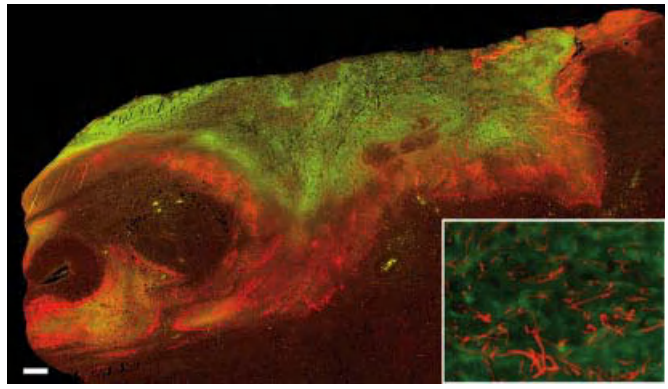

FIGURE 37.1 Digital necrobacillosis in wild reindeer. Fluorescence *in situ* hybridization of a necrotic skin lesion: multiple microcolonies of *F. necrophorum* (red fluorescence) invading the surrounding connective tissue (brown colour). Debris with only a few *F. necrophorum* is characterized by green autofluorescence. Insert: detail showing multiple filamentous *F. necrophorum* within the lesion. Scale bar = 400 µm. (From Handeland et al., 2010)[6].

Feeding of free-living and captive wildlife, in muddy and faecally contaminated areas should be avoided. *Fusobacterium necrophorum* is also associated with anaerobic infections in humans, especially in the oral cavity[2]. However, subspecies *necrophorum*, which causes classical necrobacillosis in animals, does not seem to be involved in human infection[9]. Therefore, the zoonotic potential of animal necrobacillosis can be regarded as negligible.

HELICOBACTER INFECTIONS

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Helicobacter infections are reported from mammals and birds, and mainly infect the stomach, intestine, liver and gallbladder. Their pathogenicity in wild animals is poorly understood.

Helicobacter organisms are small (0.5–1.0 μm in width, 2.5–4.0 μm in length), curved, microaerophilic Gram-negative rods, which are motile by several sheathed flagella. More than 40 candidate and definitive species of *Helicobacter* have been described, and all occur in the gastrointestinal tract of humans and animals worldwide. Genera closely related to *Helicobacter* are *Wolinella*, *Flexispira*, *Campylobacter* and *Arcobacter*.

Helicobacter spp. have been described in various captive wild, domestic, laboratory animal and pet species, but little information is available on the occurrence of *Helicobacter* and associated disease in wild animals. *Helicobacter hepaticus* and *H. bilis* are mouse pathogens frequently affecting laboratory mice colonies, where they colonize the liver and cause hepatitis. *Helicobacter hepaticus*-induced lesions may progress to hepatocellular tumours. *Helicobacter pylori* causes chronic gastritis and gastric cancer in humans. In free-living wild animals worldwide, *Helicobacter* spp. infection has been described in carnivores, marine mammals and birds. In European wildlife, *Helicobacter* and *Helicobacter*-like organisms (HLO) have been observed in free-ranging red foxes (*Vulpes vulpes*)[10–12] and lynx (*Lynx lynx*)[12]. The gastric mucosa from 17 of 25 (68%) lynx and from three of four (75%) red foxes was positive for *Helicobacter* spp. by polymerase chain reaction (PCR) in a Swedish study. Phylogenetic analysis grouped these *Helicobacter* into a cluster including '*Candidatus* H. heilmannii', *H. salomonis*, *H. felis* and *H. bizzozeronii*[12]. In Germany, 88% (44/50) of the red foxes investigated for HLO by immunohistochemistry (IHC) of the stomach were positive[10]. By electron microscopy, these HLO appeared similar to *Candidatus* H. heilmannii. Gastric HLO were also demonstrated in wild red foxes from Turkey. Histology and electron microscopy revealed HLO that resembled *H. felis*, *Candidatus* H. heilmannii, and *H. pylori*. These authors concluded that the gastric mucosa of free-ranging red foxes is commonly colonized by HLO, which possibly reflects the characteristic social and/or feeding behaviour of foxes (for example, by mutual grooming) or the potential commensal nature of *Helicobacter* in this animal species[11]. Wild barnacle goose (*Branta leucopis*) and Canada goose (*B. canadensis*) were found to carry *Helicobacter canadensis* without concurrent disease[13].

Pathogenesis and virulence factors in *H. pylori* have been studied intensively because of its pathogenic role in humans. In domestic animals, in particular pigs, gastric ulcers have been linked to *Helicobacter* spp. infection, but the exact role of the bacteria in the development of lesions is not clear. None of the lynx and red foxes investigated in the Swedish study showed macroscopic lesions in the stomach or liver[12]. Chronic gastritis characterized by focal lympho-plasmacytic cellular infiltration was reported in 11 of 44 HLO-positive red foxes[10]. HLO infection in Turkish red foxes was associated with chronic mild or moderate inflammatory changes but without any correlation between the presence or density of bacteria and gastric inflammation[11].

Most *Helicobacter* spp. are carried by animals and humans without causing apparent disease. Clinical signs in animals include vomiting, diarrhoea and weight loss, but have not been described in wildlife.

The isolation and identification of *Helicobacter* spp. is difficult and is not attempted in most veterinary diagnostic laboratories. Scrapings of the gastric mucosa might be examined microscopically for the presence of motile, helical-shaped organisms. Alternatively, the organisms may be visualized on histologic sections prepared with silver stain (a method non-specific for *Helicobacter* spp.) or by immunofluorescence or immunoperoxidase methods. Preparations may also be examined by electron microscopy. Most of the *Helicobacter* species considered as pathogenic produce urease, which can be tested for on biopsy specimens. Assays for the detection of antigen and antibodies are available. PCR requires tests for several housekeeping genes to identify *Helicobacter* on a species level[14].

The zoonotic potential of the *Helicobacter* species found in wild animals in Europe is not clear.

BARTONELLA INFECTIONS

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Infections caused by *Bartonella* species are now widely recognized as being of public health and veterinary importance. Among these, cat scratch disease, manifesting as a regional lymphadenopathy, is perhaps the most frequently encountered. Although *Bartonella* infections are very common among European wildlife species, the pathogenic threat they pose to these species remains unclear.

AETIOLOGY

Bartonella species are Gram-negative bacteria with a specific tropism for mammalian erythrocytes. They exploit a wide range of mammalian species, including humans, livestock, companion animals and wildlife, as reservoir hosts. In general, each *Bartonella* species is adapted to a specific mammal species, and in this host, infections tend to be chronic but asymptomatic. Several *Bartonella* species are pathogens of veterinary and public health significance, but disease usually results from 'accidental' infection of a non-reservoir species or when a reservoir host is immunocompromised.

There are currently 24 valid *Bartonella* taxa, although, as numerous other strains have been partially described, the true breadth of the genus is likely to be considerably wider than is presently recognized. The *Bartonella* genus was formally removed from the Rickettsiales in the mid 1990s, and more recently, its members have also been distinguished from mycoplasma-like *Haemobartonella* and *Eperythrozoon* species.

EPIDEMIOLOGY

GEOGRAPHICAL DISTRIBUTION AND HOSTS

Bartonella species are obligate parasites of mammals and arthropods. The range of mammals parasitized by bartonellae is broad, and includes humans, companion animals and livestock as well as wildlife. Bartonellae have been associated with all of the most common mammalian orders, including Artiodactyla, Chiroptera, Carnivora, Diprotodontia, Insectivora, Lagomorpha, Primates and Rodentia. Recent evidence suggests that even cetaceans carry bartonellae. Blood-feeding arthropods serve as vectors for bartonellae, with fleas, sandflies, lice and possibly ticks implicated in this role. The extent to which bartonellae interact with arthropods remains little studied, so it is feasible that they may also serve as reservoirs for the bacteria. Indeed, recent studies have identified novel bartonellae that appear to be specifically adapted to arthropods, possibly being maintained in them by vertical transmission.

Bartonellae have been encountered on all continents except Antarctica. In Europe, the medical significance of *B. henselae* has provoked a large number of surveys of domestic cats (the reservoir host) in many different countries. Surveys for bartonellae in other mammalian species, although less frequently reported, are nonetheless numerous. Bartonellae have been recognized by microscopic observation of blood smears drawn from various wildlife species for over 100 years. Contemporary surveys of wildlife for *Bartonella* species have encountered infections in many different species across Europe. Most surveys have focused on rodents, with infections reported in numerous countries from Ireland, the UK, France and Portugal in the west, to Russia in the east, and from Scandinavia in the north to countries bordering the Mediterranean in the south. Infections have been recorded in numerous rodent taxa, including, most commonly, mice of the genera *Apodemus* and *Mus*, and voles belonging to the genera *Myodes* and *Microtus*. Infections in other common and widespread rodents such as *Rattus norvegicus* and *Sciurus vulgaris* have also been reported. European shrews (Soricidiae) also serve as reservoir hosts to bartonella infections, as do other small mammals including bats (Chiroptera) and rabbits (Leporidae, Lagomorpha). Evidence for infections in mustelids has yet to be produced. There is also scant evidence for *Bartonella* infections in European large carnivores, although very few have been tested; infections have been described in such animals elsewhere in the world. Artiodactyla have been found to harbour bartonellae, including roe and red deer (*Capreolus capreolus* and *Cervus elaphus*). A very recent report suggests bartonella infections in harbour seals (*Phoca vitulina*).

The application of molecular methods, most commonly comparative DNA sequence analysis, has demonstrated

that, in general, *Bartonella* species are host-specific. Among the 24 valid *Bartonella* taxa, only a handful have been shown to exploit more than one mammal species as reservoir hosts; these *Bartonella* species (*B. doshiae*, *B. grahamii* and *B. taylorii*) are associated with rodents, and have been shown to be able to parasitize sympatric host species. Furthermore, a specific host may be parasitized by more than one *Bartonella* species; for example, felids appear to serve as reservoir hosts for *B. henselae*, *B. clarridgeiae* and possibly *B. koehlerae*.

ENVIRONMENTAL FACTORS

The epidemiology of bartonella infections in reservoir hosts is generally characterized by seasonality, with infection prevalence rising to a peak in late summer and early autumn before declining during winter and early spring. Different studies have reported varying infection prevalences, but, in general, a significant proportion of the population is infected. For example, in the UK, the prevalence of bartonella infections in field voles (*Microtus agrestis*) ranges between about 10% in spring to 50% in late summer.

HOST FACTORS

There is no clear evidence that host factors such as age or sex have a significant influence on susceptibility to infection, however, as would be expected, both host population density and the population dynamics of arthropod vectors are important determinants of bartonella transmission. For example, there is an inverse correlation between prevalence of *B. henselae* infections in domestic cats and latitude, which is thought to reflect the relatively small populations of cat fleas (*Ctenocephaledes felis*) in high latitude countries compared with the larger populations that are supported in warmer and wetter climates.

EPIDEMIOLOGICAL ROLE OF WILD ANIMALS

As obligate parasites of mammals, *Bartonella* species have evolved to exploit their reservoir hosts in a manner that best facilitates their transmission and natural persistence. The current paradigm for bartonellae is a strategy for host exploitation involving chronic, subclinical infections of the blood rather than overt disease or pathology, and such a strategy is clearly appropriate given their dependence on arthropods for transmission; blood-feeding ectoparasites are far more likely to be encountered by healthy, fit individuals.

TRANSMISSION

The route of acquisition of a bartonella infection remains a matter of debate, but the current paradigm is one in which bacteria are shed in the faeces of arthropods onto the skin of the host, then this contaminated material is inoculated through the skin as the host scratches itself to relieve the irritation of being bitten or infested. Direct transmission during blood feeding by an arthropod probably also occurs for some *Bartonella* species.

PATHOGENESIS, PATHOLOGY AND IMMUNITY

Once inside the host, bartonella are thought to migrate to a primary niche, possibly vascular endothelial cells or macrophages. From here the bacteria are released into the bloodstream, where they encounter and then invade erythrocytes. Inside the red cells, bartonellae initially replicate, but the number of intracellular bacteria soon stabilizes. The integrity of infected erythrocytes does not appear to be significantly affected, and they are thought to remain in circulation for as long as there are uninfected cells, thereby facilitating their uptake by haematophagous insects. Studies of both experimentally and naturally infected reservoir hosts have demonstrated that bartonella infections are chronic, lasting weeks, months or even years. Investigations into the molecular basis of infection have demonstrated the presence of specific factors such as adhesions and specialized secretion systems in most *Bartonella* species that mediate host interaction.

Infection of a reservoir host does not normally appear to provoke pathology. For example, the author has never observed any gross pathological signs associated with *Bartonella* infections during dissections of large numbers of field voles (*Microtus agrestis*), despite some animals having a very poor body condition and/or having other debilitating conditions or disease. However, usually mild pathologies associated with *Bartonella* infections have been described, albeit very rarely, in those companion animals that serve as reservoir hosts for bartonellae, and following experimental infections. Accidental infection of individuals that do not belong to reservoir host species appears far more likely to provoke pathology; *B. henselae* infections in

humans most commonly present as cat scratch disease, a syndrome characterized by lymphadenopathy.

The nature and scale of immune response to *Bartonella* infection by a reservoir host species is not fully understood. Hosts do mount a humoral response, but the effectiveness of this in curtailing infection is uncertain. Although some experimental work has suggested a key role for antibodies in the cessation of bacteraemia, natural infections appear to continue when high titres of circulating antibodies are detected; a capability that may, in part, be the result of occupation of erythrocytes, a site of complete immune privilege.

CLINICAL SIGNS AND TREATMENT

There are no reports of morbidity or mortality among wildlife species that are clearly linked to *Bartonella* infection. Indeed, given the high prevalence of infection in apparently healthy reservoir hosts, establishing a link between bartonellae and a specific clinical manifestation is very difficult. Nonetheless, some evidence that *Bartonella* infections can provoke disease in a minority of individuals among a reservoir host population has emerged from studies on *B. henselae*, which parasitizes cats; the presence of *B. henselae* DNA has been demonstrated in relevant tissues of animals with uveitis and endocarditis, and epidemiological surveys have suggested that seropositive cats are more likely to have kidney disease and urinary tract infections, stomatitis and lymphadenopathy. In experimentally infected cats, fever, lymphadenopathy, mild neurological signs and reproductive disorders have been reported. Treatment of infections relies on antibiotics, often prescribed for a prolonged period. A number of different regimens have been found to be effective.

DIAGNOSIS

The detection of bartonella infections in reservoir host species is relatively straightforward. The examination of Giemsa or Romanowski-stained blood smears under 1000× magnification reveals characteristic intra-erythrocytic rod-shaped bacteria (Figure 37.2). However, this approach is relatively insensitive, as infection intensity can be low (<1 in 10,000 erythrocytes infected). The isolation of bacteria is probably the most sensitive means of determining infection status, and is achieved by inoculating blood samples onto axenic media; several media have been shown to be suitable, most of which contain up to 10% whole blood. Some liquid media have also been described. Bartonellae can be recovered from fresh blood, or from stored whole blood or blood cell pellets. Often blood samples are frozen before the inoculation of media, as this is thought to enhance the recovery of bacteria. Given their fastidious and slow-growing nature, cultures of bartonellae are particularly susceptible to contamination. PCR-based detection methods have proven a popular alternative to culture for the demonstration of ongoing bartonella infections. Numerous different assays have been described, targeting various genetic loci and with either genus- or species-specificity. The identification of bartonellae to species level is usually achieved by comparative sequence analysis of PCR amplification products; again, various genetic loci have been used for this. *Bartonella* species are generally inert to most routine biochemical tests, so the differentiation of species using phenotypic methods is not possible. However, serological methods, using species-specific polyclonal or monoclonal antibodies, do offer an alternative approach when PCR is not possible. Testing for antibodies, most often using an immunofluorescence test format is also used in epidemiological studies, although use of this approach for some species of wildlife is compromised by an absence of appropriate conjugates.

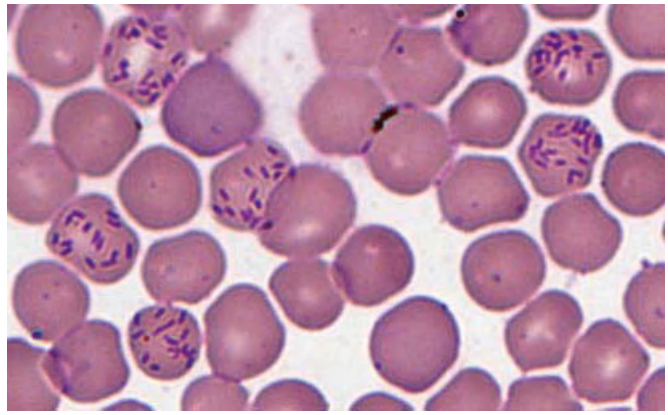

FIGURE 37.2 Diff-Quik (Romanowski)-stained smear of field vole blood demonstrating the presence of intra-erythrocytic bartonellae (note up to about 12 rod-shaped organisms inside four of the erythrocytes in this smear). Observed at ×1000 magnification.

PUBLIC HEALTH CONCERN

Bartonella species are established zoonotic pathogens; the feline-associated species *B. henselae* is associated with an

increasing spectrum of clinical syndromes in humans including, most commonly, cat scratch disease, but also endocarditis, ocular syndromes and vascular proliferative diseases such as bacillary angiomatosis and peliosis hepatis. Some of these presentations have also been encountered in companion animals. Reports of human disease caused by other *Bartonella* species have also been published, but these remain rare; it seems likely that the relative medical importance of specific *Bartonella* species is tempered by the frequency of contact between humans and its particular reservoir host species. The wildlife-associated species implicated in human disease include *B. grahamii*, which has been associated with neuroretinitis, *B. alsatica*, which exploits rabbits as its reservoir host, and has been associated with lymphadenitis and endocarditis, and *B. elizabethae*, which naturally parasitizes rats, and also been associated with endocarditis. Infection may occur via the bite of an infected arthropod, but, a more common route of transmission to humans appears to be via the scratch of a claw contaminated by infected arthropod faeces.

SIGNIFICANCE AND IMPLICATIONS IN ANIMAL HEALTH

Bartonella species are also associated with disease in livestock and companion animals. For example, the deer-associated species *B. schoenbuchensis* was isolated from a French cow with no clinical signs, and *B. bovis*, for which cattle are the reservoirs, has been implicated as a cause of endocarditis in cattle (again suggesting that serving as a reservoir host does not come without a cost). *B. henselae* is increasingly being recognized as a pathogen of dogs.

MANAGEMENT, CONTROL AND REGULATIONS

Control of infections is difficult, but is mediated primarily through the control of ectoparasite fauna. In domesticated animals at least, the regular application of insecticides/acaricides is considered the most practical means for infection control. Currently there are no national or international requirements to report *Bartonella* infections in Europe, and the diagnosis of *Bartonella* infections in livestock does not currently have any consequences for their management.

References for further reading are shown at the end of this chapter[15–17].

STAPHYLOCOCCUS INFECTIONS

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Staphylococci are commonly associated with suppurative pyogenic infections, abscess formation and skin infection. In veterinary medicine, three *Staphylococcus* species, *S. aureus*, *S. intermedius* and *S. hyicus*, are of major pathogenic importance. Meticillin-resistant *S. aureus* (MRSA) is of emerging concern in veterinary medicine and has been reported from a wide range of animals (e.g. pigs, horses, dogs, rabbits, cattle) both as a cause of infection and in healthy carriers. MRSA has been isolated from a free-ranging European hedgehog (*Erinaceus europaeus*) suffering from rhinitis[18].

Staphylococci are Gram-positive bacteria (0.5–1.5 μm in diameter), which occur singly, in pairs, tetrads, short chains of three to four cells, and in irregular clusters or 'bunches of grapes'. Most staphylococci are catalase-positive facultative anaerobes, oxidase-negative and non-motile. Forty species of the genus *Staphylococcus* have been identified, ten of which contain a subdivision with subspecies designation[19].

Staphylococcus infection may result in a variety of disease conditions, including pyogenic skin infections, abscess formation, mastitis, septicaemia and toxic-shock syndrome.

Staphylococci commonly colonize the skin, skin glands, and mucous membranes of a wide range of animal species and humans. In addition, they can be found in the intestinal, genitourinary, and upper respiratory tract. They occur worldwide and have evolved with their host species to some degree. Different animal species harbour a different staphylococcal flora; hence, from a veterinary perspective, distinguishing between host-associated groups of *Staphylococcus* strains can be useful. Although different *Staphylococcus* spp. have been described in association with disease in wild animals, most cases in wildlife are attributed to *S. aureus*. Staphylococci are facultative pathogenic organisms that require some injury to the skin or mucous membranes to become established in underlying tissues. Predisposing damage might result from arthropod bites, cuts and traumatic abrasions of the skin or deep wounds

from fighting. Many infections are endogenous, but transmission of the bacterium between individuals (e.g. adults and suckling young, littermates) occurs. Some examples of *Staphylococcus* spp. infections in free-ranging wild animals in Europe are summarized in Table 37.1.

Staphylococci can be divided into two groups: 'major' and 'minor' pathogenic species. Formerly, the old terms 'coagulase-positive' and 'coagulase-negative' were used to distinguish between pathogenic and less pathogenic species and coagulase-production still continues to be widely used as criterion for the identification of pathogenic staphylococci associated with acute infections. The major pathogenic staphylococci in veterinary medicine are mentioned above. The group of minor pathogenic species consists of e.g. *S. epidermidis*, *S. warneri*, *S. delphini*, *S. schleiferi* subsp. *coagulans*, *S. pseudintermedius*, *S. caprae*, *S. haemolyticus*, *S. scuiri*. Several potential virulence factors have been described as important for *Staphylococcus* infection. Most of these factors have been studied in *S. aureus* but have also been reported in *S. intermedius* and others. These factors can be divided into cell-associated components (Protein A, capsular polysaccharides, peptidoglycan, lipoteichoic acid, adhesins), exoenzymes (coagulase, lipase, hyaluronidase, hyaluronate lyase, proteases), and exotoxins (toxic-shock syndrome toxin (TSST-1), enterotoxins, epidermolytic toxins, haemolysins, leucocidin)[28].

Types of disease and pathologic changes in free-ranging and captive wildlife are given in Table 37.1. In hares (*Lepus* spp.), abscesses caused by *Staphylococcus* sp. have been described. They are most often located in the subcutaneous tissues, sometimes extending into muscles, but may be found in any organ or tissue. Staphylococci, mostly *S. warneri*, were found to be the cause of pyoderma in mucocutaneous dermatitis caused by poxvirus in mountain hares (*Lepus timidus*) in Finland[23]. *Staphylococcus aureus* and. *S. scuiri* are found regularly as a secondary cause of dermatitis and septicaemia associated with squirrelpox lesions in red squirrels (*Sciurus vulgaris*) in the UK[22].

Specimens for diagnosis may include exudates, pus, milk, skin swabs and affected tissues. Direct microscopy of Gram-stained smears may show Gram-positive cocci in the typical 'bunches of grapes' formation. Staphylococci can be grown easily on Columbia sheep blood agar containing 5% blood.

It is certain that food animals or pets might be a source of human *Staphylococcus* infection, but humans may also serve as cause of infection in wild animals. A study on the Spanish imperial eagle (*Aquila adalberti*) revealed human-associated *Staphylococcus* infection in nestlings handled for wildlife conservation purposes. Chicks handled without disposable gloves showed a higher infection rate (45%) compared with those handled with gloves (4%)[29]. It is strongly recommended that people handling wildlife (casualties) should implement proper hygiene measures to prevent mutual transmission of staphylococci and other zoonotic pathogens. In captivity, particularly in breeding facilities, strict hygiene measures should be implemented.

STREPTOCOCCUS INFECTIONS

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Streptococci are pyogenic bacteria of the family Streptococcaceae, genus *Streptococcus*, and are commonly associated with suppurative infections and abscess formation. Streptococci are Gram-positive cocci less than 2 μm in diameter that occur in pairs or chains of varying lengths. Most streptococci are facultatively anaerobic, catalase-negative, non-spore-forming and non-motile. Most of the streptococci of veterinary importance occur worldwide as commensals of the upper respiratory and lower urogenital tract. Pyogenic streptococci cause mastitis in cattle and other hosts, equine strangles, meningoencephalitis, arthritis and cervical lymphadenitis in swine, neonatal septicaemia in kittens and lymphadenitis in juvenile cats and laboratory rodents. Some of the streptococci of veterinary importance are *Streptococcus agalactiae*, *S. canis*, *S. dysgalactiae* (subsp. *dysgalactiae*, *equisimilis*), *S. equi* (subsp. *equi*, *ruminatorum*, *zooepidemicus*), *S. gallolyticus* subsp. *gallolyticus*, *S. halichoeri*, *S. iniae*, *S. marimammalium*, *S. phocae*, *S. pneumoniae*, and *S. suis*.

The broad host range and diversity of tissue tropism of veterinary pathogenic streptococci suggest multiple paths in the evolution of this bacterial genus. The genus comprises highly host-adapted pathogens (e.g. *S. equi* subsp. *equi*) uniquely fitted for invasion of specific host tissues (e.g. *S. agalactiae*) but also includes pathogens with almost no host adaptation (e.g. *S. equi* subsp. *zooepidemicus*, *S. dysgalactiae* subsp. *equisimilis*) and capable of causing disease only as opportunists concurrent with pre-existing tissue damage or impaired immune functions[30]. Streptococci are susceptible to desiccation and do not survive for

TABLE 37.1 Types of *Staphylococcus* disease and associated lesions in free-ranging animals in Europe.

Host	*Staphylococcus* species	Disease condition/carrier (C) status	Gross and microscopic lesions/abnormal blood test results	Geographic origin of the report	Reference
Red squirrel (*Sciurus vulgaris*)	*S. aureus*, ST49#, c+*	Scabs on nose, lips, digits; ischaemic necrosis of digits, swollen lip covered by dried exudate, inflamed swelling of the eyelid	Exudative, ulcerative and necrotic dermatitis, epidermal hyperplasia and hyperkeratosis	UK	20
	S. aureus, ST49#, c+	Respiratory distress	Inflamed nasopharyngeal and laryngeal mucosa with whitish, plaque-like lesions; tongue and nasopharynx with mucosal ulceration and necrosis; lungs congested with localized areas of consolidation and marginal emphysema; inhalation pneumonia secondary to laryngeal ulceration and necrosis	UK	21
Red squirrel	*S, aureus* *S. scuiri*	Bacterial infections secondary in most squirrel pox cases contributing to the dermatitis lesions; both *S. aureus* and *S. scuiri* may cause septicaemia in squirrel pox cases	Pyogranulomatous suppurative/exudative infections of the skin	UK	22
Mountain hare (*Lepus timidus*)	*S. aureus*, c+	Secondary bacterial infection in contagious muco-cutaneous dermatitis	Pyogranulomatous, suppurative infection of the skin	Finland	23
Chaffinch (*Fringilla coelebs*)	*S. aureus*, c+	Tassle-like foot lesions	Lesions (not specified) in multiple organs suggestive of *S. aureus* septicaemia	UK	24
Mountain hare	*S. xylosus*, c−; *S. warneri*, c−	Secondary bacterial infection in contagious mucocutaneous dermatitis	Pyogranulomatous, suppurative infection of the skin	Finland	23
European brown hare (*Lepus europaeus*)	*S.* species	Pyogenic skin infection, subcutaneous abscesses	Abscessating lesions in internal organs, septicaemia	Germany	25
Spanish ibex (*Capra pyrenaica hispanica*)	*S. aureus*, c+; *S. xylosus*, c−; *S. epidermidis*, c−	C (nares, conjunctiva, vagina)	–	Spain	26
European bison (*Bison bonasus bonasus*)	*S. aureus* c+, *S. epidermidis*, *S. capitis*, *S. chromogenes*, *S. hominis*, *S. lentus*, *S. sciuri*, *S. warneri*, *S. xylosus*, c−	C (normal vaginal microflora)	–	Poland	27
Bank vole (*Clethrionomys glareolus*), root vole (*Microtus oeconomus*), field mouse (*Apodemus agrarius*), common shrew (*Sorex araneus*), lesser shrew (*S. minutus*)	*S. stepanovicii* sp. nov., c−	C (skin, fur, intestinal tract)	–	Poland	19

*c+ – coagulase-positive; c− – coagulase-negative, C – carrier status, # – sequence type (ST) 49 according to the multilocus sequence typing (MLST) database

long periods outside the animal host. *Streptococcus* is transmitted by direct contact, mastitic milk, pus, nasal discharge, or feed or water directly contaminated by a shedding animal. The occurrence of *Streptococcus* spp. in free-ranging and captive wild animals is summarized in Table 37.2.

Virulence of the pathogenic streptococci is based on surface structures that directly or indirectly impede phagocytosis, for example due to binding of host plasma proteins or fibrinogen. The hyaluronic acid capsule and the antiphagocytic cell wall M-proteins are the best-known streptococcal virulence factors. Other factors include streptolysin S and O, a hyaluronidase, proteases, streptokinase, neuraminidases and leucocidal toxins[30].

Clinical signs of *Streptococcus* infection are non-specific. Types of disease and pathologic changes in free-ranging and captive wildlife are given in Table 37.2.

Specimens for isolation of the organism depend on the disease condition and may include nasal discharge, pus, mastitic milk and affected tissue. As streptococci are susceptible to desiccation, swabs should be placed in transport medium. Direct microscopy of Gram-stained smears may show Gram-positive cocci in the typical chain formation; pneumococci (*S. pneumoniae*) occur as pairs of bacteria. Streptococci can be grown on blood agar, selective blood agar and MacConkey agar. Inoculated plates are incubated aerobically at 37°C for 24–48 hours. Colony appearance, haemolysis, biochemical reactions and other

TABLE 37.2 Occurrence of *Streptococcus* in free-ranging wildlife in Europe.

Host(s)	*Streptococcus* species	Disease condition/carrier status (C)	Gross and microscopic lesions	Geographic origin of the report	Reference
Roe deer (*Capreolus capreolus*)	*S. gallolyticus* subsp. *gallolyticus*	Weakness	Subcutaneous oedema, multiple petechiae and ecchymotic haemorrhages incl. pulmonary artery, aortic valve, endocardium; cardiac vasculitis	Spain	31
Red fox (*Vulpes vulpes*)	*Streptococcus* sp., group G	Subcutaneous purulent lesions around bite wounds	Septicaemia, toxaemia secondary to penetrating bites	UK	32
Eurasian river otter (*Lutra lutra*)	*S. dysgalactiae*	Bite wounds, abscess, isolated from lung	Severe subcutaneous infection	UK	33, 34
Harbour seal (*Phoca vitulina*)	*S. equi* subsp. *zooepidemicus*	Secondary infection concurrent to phocine distemper outbreak; isolated from lung, liver, spleen, kidney, intestine, vaginal swab	Associated lesions not described	German North Sea	35
Caspian seal (*Phoca caspica*), harbour seal, grey seal (*Halichoerus grypus*)	*S. phocae*	Secondary infection concurrent to phocine distemper outbreak; isolated from lung, liver, spleen, heart, cerebrum	Associated lesions not described	Caspian Sea, German North and Baltic Sea	36, 37
Grey seal	*S. phocae*	Bite wound	Septic arthritis	UK	38
Grey seal, habour seal	*S. marimammalium*	Isolated from lung	Associated lesions not described	UK	39
Grey seal	*S. halichoeri*	C	Associated lesions not described	UK	40
Spanish ibex (*Capra pyrenaica hispanica*)	*S. equi* subsp. *zooepidemicus*	C; isolated from nasal and vaginal swabs	Associated lesions not described	Spain	26

characteristics (e.g. CAMP test) are used to identify *Streptococcus* species. The majority of pathogenic streptococci possess a dominant serologically active cell wall antigen used as the basis of the Lancefield grouping system for streptococcal identification. These group-specific antigens (A–H and K–V) are used in a variety of methods for serogrouping an unknown isolate. The pyogenic animal streptococci belong to groups B, C, D, E, G, L, U and V[30].

RHODOCOCCUS EQUI INFECTION

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Rhodococcus equi (synonym: *Corynebacterium equi*) in the family *Nocardiacea* is a Gram-positive pleomorphic coccobacillus or rod-shaped bacterium capable of fermenting glucose. It reduces nitrates, is catalase-positive and produces urease. It usually has a capsule and sometimes is weakly acid-fast. Pathogenicity of *R. equi* is attributed to the ability to persist and multiply in, and eventually destroy, alveolar macrophages. Virulence can be attributed to certain surface proteins (virulence associated protein (Vap) family) and plasmids. Foal virulent strains revealed VapA protein, whereas pig virulent strains express VapB. Phospholipase C and cholesterol oxidase ('*R. equi* factors') are also produced and probably play a role in pathogenesis. *Rhodococcus equi* resembles a soil organism and may be a commensal in the intestine of horses and other herbivores. It is an important cause of suppurative bronchopneumonia, arthritis and abscesses in foals, but it may also cause tuberculosis-like lesions in the lymph nodes of cattle and pigs, as well as granulomatous lesions in the liver of young goats. Infection in other animals is rare and most likely the result of immunosuppression[41].

Submaxillary lymph nodes of 482 wild boar (*Sus scrofa*) from Hungary were examined for the presence of *R. equi* by culture. Of these wild boars, 12.4% carried *R. equi*, which was comparable to the carrier rate detected among healthy backyard pigs in Hungary[42]. Characterization of 82 *R. equi* isolates obtained revealed that 21 isolates carried the *vapB* gene corresponding to intermediate virulence, whereas the remaining 62 strains were avirulent. The latter was not unexpected, as avirulent *R. equi* are widespread in soil. Lesions in wild boars were not described.

CORYNEBACTERIUM INFECTIONS

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Corynebacteria are pyogenic bacteria causing a variety of suppurative infections. The genus *Corynebacterium* has undergone considerable expansion in the past decade. The following species have been isolated from wild animals: *Corynebacterium aquilae*, *C. caspium*, *C. falsenii*, *C. felinum*, *C. jeikeium*, *C. renale* group, *C. sphenisci*, *C. spheniscorum* and *C. ulcerans*.

Corynebacteria are Gram-positive small pleomorphic rods of about 0.5 µm in width that occur in rod, coccoid, club and filamentous shapes. They are non-spore-forming, non-acid-fast, catalase-positive, oxidase-negative and usually facultatively anaerobic, and the animal pathogens are non-motile. In some *Corynebacterium* species virulence is attributed to toxins such as phospholipase D (PLD)[43].

Corynebacteria have been isolated from a variety of environments and clinical sources. They also occur as part of the indigenous flora of animals, but there is less information on the nature of these corynebacterial species, their host distribution[44] and virulence. Members of the *C. renale* group, *C. renale*, *C. cystitidis* and *C. pilosum*, belong to the normal flora of the urogenital tract of animals. All hydrolyse urea and possess pili, which mediate adherence to host epithelial cells of the urogenital tract. They are opportunistic pathogens, causing purulent cystitis and pyelonephritis sporadically, in particular in cattle. *Corynebacterium cystitidis* causes posthitis, preputial ulcerative dermatitis, and ulcerative vulvovaginitis in sheep and goats. *Corynebacterium pseudotuberculosis* causes caseous lymphadenitis in sheep and goats and abscesses in various domestic animals. *Corynebacterium ulcerans* causes mastitis in cattle and may infect humans consuming raw milk; expression of its toxic PLD is important in the pathogenesis of infection.

Only few data about *Corynebacterium* species associated with disease in wild mammals have been reported. *Corynebacterium ulcerans* was isolated from the lungs of two free-ranging otters (*Lutra lutra*) found dead in the UK. This was the first report of *C. ulcerans* in otters, but the agent was not regarded as the primary cause of death. However, further studies are necessary to determine the host range of *C. ulcerans*[45].

Members of the *C. renale* group were isolated from male European bison bulls suffering from balanoposthitis. *Corynebacterium caspium* has been associated with severe suppurative balanoposthitis and necrosis of the mucosa of the penis and prepuce in a Caspian seal (*Phoca caspica*) infected with canine distemper virus[36].

DERMATOPHILUS INFECTIONS

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Dermatophilosis, also named streptothricosis, lumpy wool disease and contagious dermatitis, is an acute, subacute or chronic exudative to proliferative skin disease affecting various domestic and wild animal species, as well as humans. Dermatophilosis is caused by infection with *Dermatophilus congolensis*.

Dermatophilus congolensis, is an aerobic, Gram-positive actinomycete bacterium with an unusual fungus-like life cycle and morphology. The agent is thought to be maintained in small foci of infection on a carrier animal or within scab particles in dust. Dormant immature zoospores that are resistant to desiccation and high temperature are generally transferred among hosts. When mature, these spores are motile by multiple flagella and are infective. The mature spores may initiate infection in damaged skin. In smears from lesions, *D. congolensis* is usually seen as filamentous and branching hyphae up to 0.3–0.6 µm long and 0.6–1.0 µm wide. These hyphae consist of coccoid bacterial cells arranged longitudinally, in a symmetrical chain leading to the unique morphology ('rolls of coins') of *D. congolensis*[46].

Dermatophilosis affects a variety of domestic and wild animal hosts, including terrestrial and aquatic mammals and reptiles. Birds are generally resistant to dermatophilosis. The disease has a worldwide distribution and is most prevalent in humid, tropical and subtropical regions but has also been described from countries with temperate climates[47]. In continental Europe, only a few cases of dermatophilosis in wildlife have been reported, including in Alpine chamois (*Rupicapra rupicapra*) from Switzerland[48], Austria and Italy[49], and roe deer (*Capreolus capreolus*) from Switzerland[50]. Seropositive reactors (>20%) have been detected in chamois from the French Alps, suggesting a circulation of the pathogen throughout the Alps[49].

The epidemiology of dermatophilosis is still uncertain. In tropical climates, the disease has a seasonal pattern, occurring during wet periods. Accordingly, the morbidity and mortality rate of the disease vary depending on the season[47]. Elsewhere, such patterns are less evident[51]. *Dermatophilus congolensis* is not highly pathogenic *per se*, and predisposing factors (e.g. rainfall, humidity, damp coat hair, cuts and traumatic abrasions of the skin, ectoparasites) are considered to favour infection, eventually leading to severe disease. Normal skin seems to be resistant to infection. *Dermatophilus* can be transmitted mechanically by contact with infected animals, via ectoparasites (ticks, flies, biting flies, lice, mites) and also through intradermal inoculation by contaminated thorn bushes[47]. More severe lesions of dermatophilosis associated with tick infestation (*Amblyomma variegatum*, *Ixodes ricinus*) have been described in cattle[47] and roe deer[50] but not in Alpine chamois.

Following entry to the epidermis, localized growth of *D. congolensis* induces erythema, hyperplasia of epidermal cells, exudation and formation of crusts. In chronic disease, the lesions spread widely, with extensive exudates and crusts, which become scabs. Affected areas are usually found on the back, tail, carpal and tarsal regions but might also appear on udder, scrotum, neck and head.

Histologically the lesions are mainly restricted to the upper layers of the epidermis. The *stratum corneum* is usually markedly thickened and the underlying dermis is superficially infiltrated with neutrophils and mononuclear inflammatory cells. Hyperkeratosis, hyperplasia of the basal layers of the epidermis, degeneration of epidermal cells as well as oedema, neutrophilic infiltration and microabscesses of the dermis have been described in Alpine chamois[49].

Clinical signs and lesions can vary in severity and the extent of the skin area affected, involving a small number of discrete spots, large confluent areas or virtually the entire skin. In Alpine chamois, clinical signs were exudative dermatitis and paintbrush lesions consisting of focal matting of hair[48] as well as proliferative dermatitis[49].

The unique clinical appearance of *D. congolensis* allows a strong presumptive diagnosis of dermatophilosis on the direct examination of stained smears. Microscopic investigation can be made of detached crusts if the material is moist. The smear can be stained by Gram, Giemsa or Wright's stain. Giemsa is a better stain to show the

characteristic morphology of the bacterium. Typical dark purple stained segmenting filaments and coccoid spores arranged in rouleaux form are seen. Skin biopsies and crusts can be processed routinely for histology, stained similarly and examined. Isolation and identification of the organism is required if the classical morphology is not seen by direct microscopy[51]. *Dermatophilus congolensis* is comparatively easy to culture, but Haalstra's method for primary isolation of *D. congolensis* should be used to overcome growth of contaminant organisms from scab material[46].

BRACHYSPIRA INFECTIONS IN BIRDS

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The first account of presumed intestinal spirochaetes in birds was published in 1910 and involved morphological descriptions of microorganisms from free-living red grouse (*Lagopus lagopus scoticus*)[52]. Today, five validated or proposed spirochaetal species assigned to the genus *Brachyspira* have been reported to cause enteric disease and production losses in pigs and/or poultry.

AETIOLOGY

Brachyspira organisms (trivial name brachyspires) are small (0.2–0.45 × 1.7–11 µm), helically coiled, highly motile, anaerobic but oxygen-tolerant spirochaetes that colonize the large intestines of some mammalian and avian species (Figure 37.3). They may also be isolated from faecally contaminated environments such as duck ponds. Ultrastructurally, brachyspires exhibit typical spirochaetal features, including subterminally attached, bipolar periplasmic flagella that reside in the space between a loosely attached lipid bilayered outer membrane and the protoplasmic cell cylinder. A wave-like or rotational motility centred around the cell axis is an important, albeit not universally present, spirochaetal feature. The spirochaetal design allows brachyspires to penetrate and move efficiently in intestinal mucin,

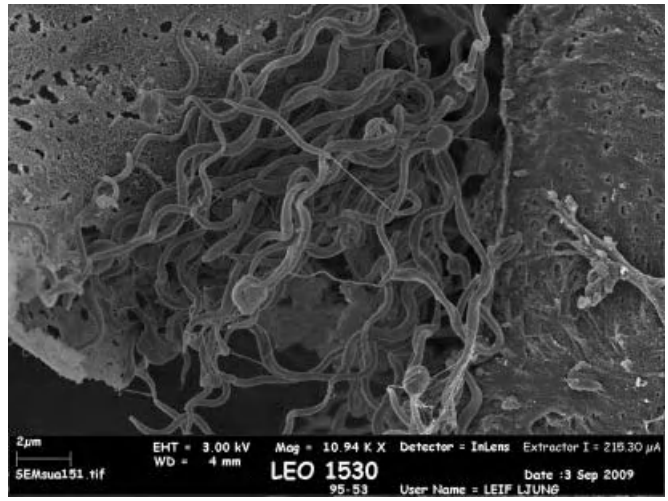

FIGURE 37.3 Scanning electron micrograph of '*B. suanatina*' cells. Image: DS Jansson and L Ljung.

which immobilizes many externally flagellated bacteria. Complete genome sequences of *B. hyodysenteriae*, *B. pilosicoli* and *B. murdochii* were recently published[53–55]. Genus *Brachyspira* is the sole member of the family Brachyspiraceae, which forms a distinct line of descent among the deeply branching genera of the monophyletic phylum Spirochaetes. Brachyspires have undergone repeated taxonomic amendments over the last 25 years, and they have previously been classified as belonging to genera *Vibrio*, *Treponema*, *Serpula* and *Serpulina*. Currently, the genus *Brachyspira* includes seven validated species, and nine species have been provisionally proposed (Table 37.3).

The diseases caused by brachyspires are:

- swine dysentery (pigs; *B. hyodysenteriae*)
- colonic spirochaetosis, synonyms porcine intestinal spirochaetosis, spirochaetal diarrhoea (pigs; *B. pilosicoli*)
- human intestinal spirochaetosis (*B. pilosicoli*, *B. aalborgi*)
- avian intestinal spirochaetosis (*B. alvinipulli*, *B. intermedia*, *B. pilosicoli* in chickens, *B. hyodysenteriae* in common rhea (*Rhea americana*)).

Brachyspira hyodysenteriae is the aetiologic agent of severe necrotizing typhlocolitis in pigs and common rheas, whereas *B. pilosicoli* (pigs and chickens) and *B. intermedia* and *B. alvinipulli* (chickens) cause non-specific colitis and/or typhlitis, diarrhoea and production losses. In other

TABLE 37.3 List of species assigned to genus *Brachyspira*.

Brachyspira species[a]	Published host range
B. aalborgi	Human, non-human primates
B. hyodysenteriae	Pig, rat, mouse, common rhea (*Rhea* spp.), mallard (*Anas platyrhynchos*), chicken, goose
B. innocens	Pig, dog, horse, chicken
B. pilosicoli	Pig, dog, horse, non-human primates, human, chicken, pheasant, grey partridge, feral waterbirds, common rhea
B. intermedia	Pig, chicken
B. murdochii	Pig, rat, chicken
B. alvinipulli	Chicken, domestic goose, red-breasted merganser (*Mergus serrator*), dog
'*B. canis*'	Dog
'*B. pulli*'	Chicken, dog
'*B. ibaraki*'	Human
'*B. christiani*'	Human
'*B. suanatina*'	Pig, mallard
'*B. corvi*'	Jackdaw (*Corvus monedula*), hooded crow (*C. corone cornix*), rook (*C. frugilegus*)
'*B. rattus*', '*B. muridarium*', '*B. muris*'	Rats and/or mice

[a]Species within quotation marks have not been fully validated and recognized

hosts, including free-living wild birds and humans, a disease association has not been unequivocally proven.

EPIDEMIOLOGY

Few published data are available regarding geographical distribution, species distribution and prevalence of brachyspires in free-living wild birds. In Sweden an overall prevalence of 78% from two free-living wild mallard (*Anas platyrhynchos*) populations was reported[56]. Some isolates were identified as *B. hyodysenteriae*, whereas others were identified as the closely related proposed species '*B. suanatina*'[57], or as *B. intermedia*, *B. pilosicoli*, '*B. pulli*', *B. alvinipulli* and closely related genotypes (D.S. Jansson, unpublished observations). More recently, also in Sweden, *Brachyspira* spp. were isolated from healthy passerine birds of the genera *Corvus*[58] and *Turdus*, and from healthy gruiform and charadriiform birds, i.e. Eurasian Coot (*Fulica atra*) in Sweden and a snowy sheathbill in Antarctica (*Chionis alba*) (D.S. Jansson, unpublished observations). In addition to mallards, brachyspires colonize the intestinal tract of a wide variety of other anseriform birds including mute swan (*Cygnus olor*), whooper swan (*Cygnus cygnus*), greylag goose (*Anser anser*), barnacle goose (*Branta leucopsis*), Eurasian widgeon (*Anas penelope*), common eider (*Somateria mollissima*), long-tailed duck (*Clangula hyemalis*) and red-breasted merganser (*Mergus serrator*) (D.S. Jansson, unpublished observations). Parameters such as habitat, diet and physiology (e.g. body temperature, age, stress levels) may possibly explain differences in *Brachyspira* spp. prevalence between avian species and populations.

Because available epidemiological data are limited they do not allow any general conclusions on the possible involvement of free-living wild birds as sources of brachyspires for domestic animals and humans. Rodents, flies and cockroaches, free-living wild birds and water are considered as possible sources of brachyspires on pig and chickens farms. Swine dysentery and colonic spirochaetosis, which are diseases of global importance to the pig industry, have not been reported from European wild boar. Molecular data suggest that zoonotic transfer of *B. pilosicoli* may occur[59]. Brachyspires may remain viable in the environment for days to months if protected by faeces, soil or water. Transmission occurs primarily by the faecal-oral route, but cloacal transmission from water or faecally contaminated surfaces cannot be excluded in gallinaceous and anseriform birds.

PATHOGENESIS AND PATHOLOGY

Virulence attributes of brachyspires and mechanisms of pathogenesis are poorly understood. Proposed virulence factors of *B. hyodysenteriae* include motility, chemotaxis, oxygen tolerance, lipooligosaccarides, proteases and haemolysins. *Brachyspira pilosicoli*, and *B. aalborgi* attach in large numbers by one end to the intestinal epithelium, and this dense bacterial layer speculatively interferes with fluid and nutrient absorption. In chickens, colonization by brachyspires may be grossly and microscopically inapparent, or is associated with abnormal caecal contents (yellow and frothy) and non-specific typhlitis. Histologically, brachyspires are randomly dispersed in crypts and on epithelial surfaces (*B. alvinipulli*, *B. intermedia*, *B. pilosicoli*), or the bacteria may adhere to the epithelium as described above (*B. pilosicoli*). Invasive mucosal growth by brachyspires may occur. In common rheas, severe fibrinonecrotic typhlocolitis has been described (*B. hyodysenteriae*). Information on gross and microscopic appearance of brachyspira colonization in free-living wild birds is scarce. *Brachyspira hyodysenteriae* and '*B. suanatina*' induced

epithelial cell changes in mallard caecae by a challenge model, but the birds remained healthy during the trial[60].

CLINICAL SIGNS

Colonization by *B. intermedia*, *B. pilosicoli* and *B. alvinipulli* in laying hens produces diarrhoea, reduced egg production and faecally contaminated egg shells[61], and infection of juvenile common rheas by *B. hyodysenteriae* causes fibrinonecrotic typhlocolitis and high mortality[62]. Clinical disease in *Brachyspira*-colonized free-living wild birds is yet to be reported. Interestingly, the recently proposed species '*B. suanatina*', which naturally colonizes pigs and free-living wild mallards, was shown experimentally to induce diarrhoea in pigs, independent of species of origin of the inoculum[57].

DIAGNOSIS

Because of their small size and failure to stain with the Gram stain and routinely applied cytological and histological stains, brachyspires are barely visible by light microscopy. Hence, clear visualization is dependent on dark-field or phase-contrast microscopy, and Warthin–Starry silver staining should be used for histology.

Highly nutritious selective, solid agar media containing antimicrobials, (e.g. spectinomycin, colistin and vancomycin) and 5–10% blood, and anaerobic incubation for 3–9 days at 37–43°C are applied for isolation. Isolates grow as swarming colonies of varying size and appearance. Species identification may be difficult. Application of phenotypic tests allows presumptive identification, however, more species may colonize avian hosts compared with pigs, and the presence of more than one genotype simultaneously in the same bird is frequently observed. Also, most available diagnostic PCRs tend to lack in sensitivity and/or specificity when applied to avian isolates. Thus, a combination of phenotyping, molecular tests and gene sequencing is often required for definitive identification, and even so, isolates that cannot be assigned to any presently recognized species are occasionally found.

SIGNIFICANCE AND IMPLICATIONS FOR ANIMAL HEALTH

Molecular data are accumulating in support of free-living birds, especially anseriforms, as important reservoirs of *Brachyspira* spp., including those that are pathogenic to domestic animals, and *B. pilosicoli*, which is potentially pathogenic to humans. The clinical significance in free-living wild bird populations remains to be determined.

ACTINOMYCES INFECTIONS

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The actinomycetes comprise a heterologous group sharing the ability to form Gram-positive, branching filaments of less than 1 μm in diameter. *Actinomyces bovis* (previously named *Nocardia bovis*, *Streptothrix bovis*) is an anaerobic, fermentative organism associated with characteristic focal or systemic pyogranulomatous infections of veterinary importance. *Actinomyces marimammalium* has been isolated from marine mammals.

Actinomycosis, or 'lumpy jaw', is caused by infection with *A. bovis*, which results in a destructive osteomyelitis and soft-tissue reaction of the mandible. Actinomycosis is a non-contagious, endogenous, chronic disease of worldwide occurrence.

Actinomyces species are normal residents of the skin, oral mucosa and nasopharynx of their host animal. Infections tend to be endogenous and are favoured by cuts, abrasions of the skin or trauma to the oral mucosa, for example by rough feed. The lower and upper jaws are the most likely sites of infection. Actinomycosis associated with *A. bovis* infection has been described in a herd of captive llama (*Lama glama*) in Switzerland[63]. Chronic granulomatous inflammation in retropharyngeal lymph nodes and osteomyelitis has been described in various deer species (*Odocoileus* spp., *Cervus elaphus*), moose (*Alces alces*), pronghorn (*Antilocapra americana*), caribou (*Rangifer tarandus*) and mountain sheep (*Ovis* spp.), but in many of these descriptions the presence of *Actinomyces* sp. was not confirmed[64]. Disease caused by the organism occurring in wild European ruminants would not be unexpected, and 'lumpy jaw' lesions should be investigated for the presence of *A. bovis*. *Actinomyces marimammalium* has been isolated from multiple organs of a dead male hooded seal (*Cystophara cristata*) that had suffered from pneumonia, from the small intestine of a shot grey seal (*Halichoerus grypus*), and from the lung of a dead harbour porpoise (*Phocoena phocoena*). The

habitat and pathogenic significance (if there is any) of *A. marimammalium* is not known[65].

Actinomyces bovis often gains access through the dental alveoli or lesions in the oral mucosa. It causes chronic rarefying ostemomyelitis of the mandible and suppurative necrosis with progressive destruction of trabeculae and proliferation of periosteal new bone. The reaction may result in loss of teeth and large mandibular deformities. The lesions and tracts filled with pus may contain miliary pale granules (sulphur granules), which are grossly visibly colonies (of up to 3 mm in diameter) of *A. bovis*. These typical 'club colonies' are formed by filamentous and branching bacteria with mineralization of calcium phosphate. Histologically, a pyogranulomatous response develops with surrounding granulation tissue, mononuclear infiltration and fibrosis. The inflammation is usually localized and may be restricted to soft tissues of the oral cavity, but in rare cases other tissues are involved.

Infection with *A. bovis* in ungulates results in noticeable swelling of the jaw, resulting in difficulty with mastication. Severe mandibular swelling and purulent exudate secreted from fistulae was described in affected captive llama[63]. Treatment of actinomycosis in captive individuals includes systemic antibiotic and local disinfectant treatment, and surgical intervention as well as supplementation of vitamins and minerals.

Actinomycosis should be suspected when typical swellings and granulomatous lesions involving the mandibles of ungulates are seen. Specimens for laboratory diagnosis of *Actinomyces* spp. include pus, exudate, aspirates, tissue and scrapings from walls of abscesses. Thin sections of granulomas are useful for histopathology. Direct microscopy can be performed on unstained sulphur granules placed on a microscope slide in a drop of 10% KOH and gently crushed by applying pressure on the cover slip. Gram-stained smears made from granules will show Gram-positive, branching filaments.

Actinomyces bovis infection in free-ranging ungulates occurs only sporadically therefore control is not necessary.

ARCANOBACTERIUM INFECTIONS

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TABLE 37.4 Diseases associated with *Arcanobacterium* (modified after Jost & Billington, 2004)[66].

Organism	Host	Disease
A. pyogenes	Domestic cattle (*Bos taurus*)	Summer mastitis, liver abscesses, endometritis, abortion, endocarditis
	Domestic goat (*Capra hircus*)	Mastitis, miscellaneous abscesses
	Domestic pig (*Sus scrofa domestica*)	Pneumonia, septic arthritis, endocarditis
	Domestic sheep (*Ovis aries*)	Pneumonia, abortion, endocarditis
	Poultry (not specified)	Osteomyelitis, nephritis
A. phocae	Common seal (*Phoca vitulina*), grey seal (*Halichoerus grypus*)	Unknown; isolated from lung, oral and nasal cavities, peritoneal fluid, spleen, lymph nodes, intestine
A. pluranimalium	Common seal; fallow deer (*Dama dama*)	Spleen; lung abscess
A. hippocoleae	Domestic horse	Vaginitis
A. abortisuis	Domestic pig	Abortion

Within the genus *Arcanobacterium*, seven species have been associated with a variety of suppurative infections in animals: *Arcanobacterium pyogenes*, *A. phocae*, *A. pluranimalium*, *A. abortisuis*, *A. bialowiezense*, *A. bonasi* and *A. hippocoleae* (Table 37.4). *Arcanobacterium pyogenes* was formerly known as *Actinomyces pyogenes* and *Corynebacterium pyogenes*. Bacteria of these genera are pleomorphic Gram-positive, non-motile, facultatively anaerobic, oxidase-negative short rods.

With the exception of *A. pyogenes*, little is known about the *Arcanobacterium* spp. that infect animals. *Arcanobacterium pyogenes* is a widely distributed inhabitant of the mucous membranes of the upper respiratory, gastrointestinal and genital tracts. Infection often occurs following injury or a preceding infection. *Arcanobacterium pyogenes* infections have been described in a wide range of mammals, and the agent was isolated from some birds and reptiles. This bacterium is common in mixed infections and has a synergistic relationship with *Fusobacterium necrophorum* and possibly other anaerobic bacteria.

In domestic cattle, *A. pyogenes* can cause severe clinical mastitis. The agent is spread via contaminated objects or is transmitted by *Hydrotea irritans* and other species of fly. Uterine infections, resulting in severe endometritis and infertility, umbilical infections and seminal vesiculitis have

also been described in ruminants and pigs. In liver abscesses in feedlot cattle, *A. pyogenes* is the second most commonly isolated agent.

Foot infection associated with *A. pyogenes* has been described in Spanish fallow deer (*Dama dama*) in the Sueve Regional Hunting Reserve. Foot infections occur in approximately 1% of all fallow deer in the reserve. Mild temperatures, high humidity and exposed bedrock have been suggested as factors contributing to softening of the hoof or minor hoof lesions leading to bacterial infection. A high density of wildlife and domestic livestock sharing subalpine pasture may account for an increased presence of microorganisms in the environment, which may facilitate hoof infection[67].

Intracranial abscessation is a cause of mortality in white-tailed deer (*Odocoileus virginianus*). *Arcanobacterium pyogenes*, as well as other bacteria, are thought to enter via skin wounds or abrasions around the head, or injured/fractured antler pedicles. Penetration of infection into the cranium may then occur through the suture between the parietal and frontal bones. Subcutaneous abscesses on the head or ears due to ticks, bacterial otitis provoked by ear mites, and intraspecific trauma were considered contributory to intracranial abscess formation[68].

Arcanobacterium bialowiezense and *A. bonasi* have both been isolated from European bison (*Bison bonasus bonasus*) bulls at Bialowieza National Park, Poland. Both bacteria were obtained in mixed cultures from the prepuce and penis of individuals suffering from balanoposthitis. Neither healthy bulls nor female bison harboured *A. bialowiezense* and *A. bonasi*; hence, a possible role of both species in the pathogenesis of balanoposthitis in European bison is suggested[69].

Arcanobacterium spp. with so far unknown pathological significance have also been recovered from marine mammals (Table 37.4).

Arcanobacterium pyogenes is one of the most common opportunistic pathogens in domestic ruminants and pigs, but may also act as primary pathogen. Following injury to the host, *A. pyogenes* can disseminate to cause a variety of infections. Haematogenous spread to several body sites results in abscess formation and suppurative conditions. Several known and putative virulence factors are expressed by *A. pyogenes* that are required for adherence and colonization of the host's tissue. Pyolysin (PLO), a cholesterol-dependent exotoxin produced by all *A. pyogenes* strains, is lytic for red blood cells of a variety of animal species, as well as being dermonecrotic and lethal to laboratory animals[66]. PLO is also cytolytic for macrophages and neutrophils. Other virulence factors include collagen-binding protein (CbpA), fibrinogen- and fibronectin-binding protein, DNAse, neuraminidases (NanH, NanP), which facilitate adhesion, and proteases.

Arcanobacterium pyogenes infections in domestic and captive wild ungulate species are frequently found; however, cases in free-ranging wildlife are scarce and probably under-recorded. Lesions may form in any organ system with clinical signs corresponding to the lesion and the host's immune response. Often no specific clinical signs are associated with *A. pyogenes* infection. In conjunction with foot infection in fallow deer, unilateral swelling of extremities and suppurative tissue infection at the coronary band was observed. Radiography revealed slight to significant soft tissue swelling, loss of bone, severe periostal reaction and degenerative joint disease[67]. Animals with intracranial abscesses may show signs of incoordination, blindness, profound depression, torticollis and circling[68].

Local abscess formation is often seen at necropsy. Examination of affected white-tailed deer revealed necrosis, erosion and pitting of cranial bones. Lesions occurred on the external surfaces of the parietal and frontal bones, usually on or near the antler pedicles. Abscesses (1–3 mm in diameter) contained viscous pus, and were surrounded by an intense inflammatory reaction.

Gram-stained smears of pus or mastitic milk reveal small, highly pleomorphic, Gram-positive rods that occur singly, in pairs (V, T and palisade formations) or in clusters.

Arcanobacteria grow aerobically at 37°C on Columbia agar supplemented with 5% sheep blood.

In wildlife, *Arcanobacterium* infection occurs sporadically in individuals. A high density of male deer could be a factor that increases stress and territorial fights, especially during the breeding season, thus facilitating the occurrence of abscess formation associated with injured body sites.

ERYSIPELOTHRIX INFECTIONS

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The genus *Erysipelothrix* contains the species *E. tonsillarum* (9 serotypes), considered to be non-pathogenic, and *E. rhusiopathiae* (19 serotypes), a primary or facultative bacillus pathogen of a wide taxonomic range of hosts. Bacteria of this genus are non-sporulating Gram-positive bacilli of the family Erysipelotrichaceae. Important serovars for humans and swine include 1a, 1b and 2[70].

The geographical distribution of *E. rhusiopathiae* infections in European wildlife is unknown, but the bacterium has a common and worldwide distribution in domestic pigs. It is also an important cause of disease outbreaks in fowl, in particular turkeys, and of polyarthritis in sheep. *Erysipelothrix rhusiopathiae* infection has been reported in a wide range of mammals, birds, reptiles, amphibians, fish and invertebrate species. The agent is often isolated from the tonsils of apparently healthy Eurasian wild boar. Clinical disease and sporadic mortality have been recorded in Eurasian wild boar, Iberian ibex (*Capra pyrenaica*), moose in Canada (*Alces alces*), and many free-living and captive wild bird species[71]. In wild birds, *E. rhusiopathiae* infection occurs mostly sporadically, but it may occasionally cause outbreaks of mortality[71].

Erysipelothrix rhusiopathiae is ubiquitous and able to persist for a long period of time in the environment, including marine environments. Transmission occurs by direct and indirect contact with living infected hosts, or with dead animals or contaminated soil. The infection may be acquired orally or through skin lesions.

Infection by *E. rhusiopathiae* in pigs may cause acute septicaemic infections, mostly fatal, particularly in epidemics. Acute disease is accompanied by disseminated intravascular coagulation, microthrombi, small haemorrhages (petechiae and ecchymoses) and enlarged spleen. With a longer disease course, multifocal purulent tonsillitis, necrosis of the skin (cutaneous infarcts), fibrinous polyarthritis and endocarditis develop. Lesions may develop in other organs, such as lungs, kidneys and brain. Chronic forms may progress into vegetative valvular endocarditis or chronic arthritis. Septic thrombi can detach from the heart valves, and cause septic embolization in various organs.

Erysipelothrix rhusiopathiae infection in birds results in septicaemia with haemorrhages and petechiae in serosae and muscles.

Clinical signs in wild boar include facial oedema, fever and reduced activity. Affected wild boar are often found in or close to waterholes. No specific clinical signs are observed in wild birds.

Erysipelothrix rhusiopathiae isolation on standard culture media is straightforward, this being the most appropriate diagnostic tool. Serological screening tests include enzyme-linked immunosorbent assay (ELISA). PCR techniques are available[72].

Control in domestic animals requires good standards of sanitary hygiene and immunization procedures. Common disinfectants are effective in killing *E. rhusiopathiae*, and penicillin is the drug of choice for antibiotic therapy. Most commercial vaccines for pigs or turkeys are bacterins or attenuated live strains of *E. rhusiopathiae*. Antibiotic treatments and vaccination have been used in wildlife translocations[73].

The infection is readily transmitted to humans by direct contact with infected hosts such as carrier animals, as occurs through occupational exposure in the animal husbandry and meat-processing industries. In humans, this infection is often subclinical, but it can occasionally cause arthritis and severe endocarditis.

Infection by *E. rhusiopathiae* causes severe economic losses to the pig and poultry industry.

ACTINOBACILLUS INFECTIONS

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Members of the genus *Actinobacillus*, family Pasteurellaceae, are small (0.3–0.5 × 0.6–1.4 μm), Gram-negative, pleomorphic rods or coccobacilli that are facultatively anaerobic, non-motile and non-spore-forming. All *Actinobacillus* isolates have complex nutritional requirements. There are two biotypes of *Actinobacillus pleuropneumoniae* (APP), differentiated on the basis of their nicotinamide adenine dinucleotide (NAD) requirement. Thirteen serotypes of biotype 1 and 6 serotypes or biotype 2 have been described. In Europe, serotypes 2 and 9 are most often found.

Species within the genus *Actinobacillus* are always associated with mucous membranes. *Actinobacillus pleuropneumoniae* is considered an obligate parasite of the porcine respiratory tract and is the aetiological agent of porcine pleuropneumonia (synonyms: infectious pleuropneumonia of swine, *Haemophilus* contagious pleuropneumonia), a highly contagious and economically significant respiratory disease in domestic swine worldwide. Transmission

occurs via aerosols or direct contact with infected pigs, and the organism does not survive for long in the environment. *Actinobacillus lignieresii* is a commensal of the ruminant oropharynx and rumen and the oral cavity of healthy horses. In ruminants it causes 'wooden tongue', which consists of chronic inflammatory lesions, forming fibropurulent masses in the tongue and other oropharyngeal soft tissues, but it has also been associated with pyemic processes of soft tissues and stomatitis in horses.

APP seroprevalence in wild boar (*Sus scrofa*) in Slovenia has been reported[74], and *A. minor* and *A. indolicus* were isolated from nasal swabs of wild boar in northeastern Spain[75]. Data regarding prevalence and distribution of this agent among free-ranging wild boar are scarce. In the study in Slovenia, a serological survey was performed on wild boars shot during the hunting season. Of 178 samples tested, 52% produced positive results for antibodies against APP using the Chekit APP-ApxIV enzyme immunoassay kit (Bommeli Diagnostics, Bern, Switzerland)[74].

Clinical disease or lesions related to *Actinobacillus* spp. infection have not been described in wildlife in Europe. Some APP serotypes are more virulent than others but all are capable of causing pleuropneumonia; hence studies on wild boar isolates are needed to clarify their pathogenicity and significance in free-ranging European wild-boar populations.

HAEMOPHILUS INFECTIONS

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Species in the genus *Haemophilus*, family Pasteurellaceae are Gram-negative, small pleomorphic rods or coccobacilli. Many *Haemophilus* are commensals or pathogens of the mucous membranes of animals and humans, most commonly of the upper respiratory and lower genital tracts. Host specificity is a characteristic of *Haemophilus* species. *Haemophilus paragallinarum* causes infectious coryza, with inflammation of the paranasal sinus and nasal cavity, in chickens worldwide. It has also been reported in quail, guinea fowl and pheasants, but not in free-living wild birds.

Haemophilus parasuis inhabits the nasopharynx of healthy pigs. It is the primary agent of Glässer's disease (fibrinous inflammation of serous surfaces), a worldwide disease in young pigs, characterized by polyserositis and occasionally meningitis. In older pigs infectious arthritis and pneumonia are seen. Stress (transport, weather, weaning) predisposes animals to Glässer's disease.

There are two reports regarding *H. parasuis* in European wild boar (*Sus scrofa*). In northeastern Spain, *H. parasuis* was isolated from 2/42 nasal swabs of shot wild boar[75]. Two different strains of *H. parasuis* serotype 2 were obtained. 16S rRNA and multilocus sequence typing (MLST) analyses revealed that these isolates were grouped with non-virulent pig reference strains and were not related to strains isolated from systemic lesions. Owing to the lack of reports regarding Glässer's disease in wild boar, the authors suggested that the boar strains might be less virulent and constitute part of the respiratory microbiota in this species. The other report refers to *H. parasuis* antibody detection in wild boar shot in Slovenia[74]. Eighteen per cent (33/178) of the animals were seropositive. Another serological study conducted in south-central Spain did not find any positive cases[76]. In conclusion, little information is available on *H. parasuis* in wild boar, and further investigations are needed regarding virulence of *Haemophilus* strains as well as susceptibility of the wild boar host.

MORAXELLA INFECTIONS

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There are 10 species within the genus *Moraxella. Moraxella bovis* (synonym: *Haemophilus bovis*) is the agent of infectious bovine keratoconjunctivitis (IBK), and *M. ovis* (synonyms: *Branhamella ovis* and *Neisseria ovis*) has been implicated in infectious keratoconjunctivitis (IKC) in domestic and wild ruminants. Other *Moraxella* spp. (*M. lacunata*, *M. phenylpyruvica*) are only sporadically associated with disease.

Moraxella organisms are non-motile Gram-negative rods (1.0–1.5 × 1.5–2.5 μm), which appear characteristically in pairs but may also be seen in short chains. *Moraxella* are strictly aerobic, asaccharolytic, oxidase-positive and usually catalase-positive. They are susceptible to desiccation and do not survive long outside the animal host.

IBK, also named 'pink eye' or 'New Forest disease', is a highly contagious ocular disease of cattle with worldwide distribution. It causes blepharospasm, epiphora, corneal

opacity, corneal ulceration and conjunctivitis in domestic and wild ruminants.

In a study on Spanish ibex (*Capra pyrenaica hispanica*), *M. bovis* was isolated from conjunctiva (1.5%) and nasal swab (0.6%) samples from apparently healthy animals. In the same study, *M.* (*Branhamella*) *ovis* was detected in 5.3% of conjunctival swabs and in 1.5% of the nasal samples investigated[26]. Both agents have been associated with IKC in roe deer (*Capreolus capreolus*)[26] and were isolated from clinically healthy bighorn sheep (*Ovis canadensis*) in the USA. *Moraxella bovis* was also isolated from a moose with keratitis in Canada[77], and *M. ovis* has been implicated in epizootics of IKC in mule deer (*Odocoileus hemionus*) and moose (*Alces alces*) in the USA[78].

Moraxella bovis is maintained in the conjunctiva, nasopharynx or possibly vagina of clinically normal cattle older than 2 years. *Moraxella ovis* is considered a commensal of the conjunctiva and upper respiratory tract in sheep, cattle and goats. Virulent *M. bovis* strains possess pili, and produce a haemolysin and a cytotoxin that damages bovine neutrophils. Lipopolysaccharides, a collagenase and a hyaluronidase may also contribute to virulence. Predisposing environmental factors such as irritation of the eye by ultraviolet light, dust, long vegetation and flies are implicated in IBK[79]. Pathogenicity of *M. ovis* is considered low, and the pathogenesis of *M. ovis* infection remains poorly understood, but haemolytic, non-piliated isolates have been involved in epizootic IKC in free-ranging cervids[78]. Transmission is by direct contact or via insects. The following eye lesions have been described in *M. bovis* or *M. ovis* infections: inflammation of the conjunctiva and cornea, corneal ulceration, oedema surrounding the ulcer, vascularization of the cornea, characteristic red cone of granulation tissue (in cattle), corneal rupture and anterior synechia, loss of the lens, anterior uveitis and hypopyon[77–79].

Clinical signs of *Moraxella* infection include excessive lacrimation, blepharospasm, corneal scars, corneal opacity, keratitis and mucopurulent conjunctivitis.

For diagnosis, swab samples for bacterial culture should be taken deeply from the inner corner of the eye. Inoculation on agar plates must be done as soon as possible after collection of samples. If this is not possible, a transport medium to prevent desiccation is recommended. Gram-stained smears are of little practical use, but the fluorescent antibody technique has been used to demonstrate and identify *M. bovis*. Serological assays are not available[79].

There are no suitable measures for the control and treatment of IBK and IKC in wild animals.

LAWSONIA INTRACELLULARIS INFECTIONS

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Lawsonia intracellularis is an obligate intracellular bacterium that does not grow in cell-free media and can only be propagated in tissue cultures. It occurs worldwide and is the causative agent of porcine proliferative enteropathy. It is able to infect a large variety of animals. Free-ranging wild animal species only exceptionally develop disease, but PCR data indicate the carrier status of the house mouse (*Mus musculus*), striped field mouse (*Apodemus agrarius*), yellow-necked mouse (*A. flavivcollis*), common vole (*Microtus arvalis*), fox (*Vulpes vulpes*), wolf (*Canis lupus*), red deer (*Cervus elaphus*) and fallow deer (*Dama dama*). Wild animals kept in game enclosures, wildlife parks or fur farms have more frequently been reported to show clinical disease (blue fox (*Alopex lagopus*), rabbit (*Oryctolagus cuniculus*), wild boar (*Sus scrofa*), ostrich (*Struthio camelus*), emu (*Dromaius novaehollandiae*) and Rhesus macaque (*Macaca mulatta*)). The disease is also known in hamsters (*Mesocricetus* spp.) and is increasingly recognized in young horses, but has also been sporadically reported in dogs, rats (*Rattus norvegicus*) and ferrets (*Mustela putorius furo*).

The bacterium colonizes enterocytes and replicates within their cytoplasm. As a reaction the affected portions of the intestine show proliferation of undifferentiated, hypertrophic enterocytes with formation of branching crypts. The changes are generally transient and may completely recede after elimination of the agent.

Clinical signs are usually associated with poor growth, diarrhoea and hypoproteinaemic oedema. Lesions include irregular thickening of the intestine, sometimes with focal necrosis. Histologically, there is a proliferation of undifferentiated enterocytes with lack of goblet cells. Abundant intracellular bacteria are present adjacent to the luminal cell surface and can be visualized by silver impregnation or specific detection assays, such as IHC or *in situ* hybridization.

Post mortem, the agent is best demonstrated by IHC. For diagnosis in the live animal, a number of PCR assays on faeces are available. Serology is not recommended for

diagnosis in individual animals, because it does not indicate the actual presence of the bacteria.

Wild animals do not play a role as reservoirs of the agent for domestic pigs kept in facilities with high biosecurity levels. For free-ranging pigs, infected wild animals may be a potential source of infection. The agent is non-pathogenic for humans.

AEROMONAS SPECIES INFECTIONS

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Aeromonas spp. are Gram-negative, rod-shaped bacteria. Some species are saprophytic and free-living in fresh water. *Aeromonas hydrophila* is an opportunistic pathogen that on rare occasions causes disease in mammals, including wild mammals.

BORDETELLA SPECIES INFECTIONS

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Bordetella spp. are small, Gram-negative, rod-shaped bacteria. They are strict aerobes. The species with known pathogenicity, *B. bronchiseptica* and *B. avium* are both motile. Some *Bordetella* spp. were previously called *Alcaligenes*.

The organisms are inhabitants of the upper respiratory tract of humans, animals (*B. bronchiseptica*) and birds (*B. avium* – principally turkeys). *Bordetella bronchiseptica* attaches to the respiratory epithelium and has antiphagocytic and toxigenic activities and can depress the respiratory clearance mechanisms – for example, by causing immobility of the respiratory cilia. These actions facilitate invasion of the respiratory tract by other organisms. Diagnosis is based on culture from lesions, on sheep blood and MacConkey agars, incubated aerobically at 37°C for 24–48 hours. As mixed culture is always possible from respiratory tract lesions, the use of selective media such as Smith–Baskerville media is advised. Colonies are identified by morphology and biochemical properties[80].

Bordetella bronchiseptica causes a respiratory tract infection in commercial rabbits and rodents known as 'snuffles'. *Bordetella avium* causes respiratory disease in turkeys and occasionally other avian species. *Bordetella* spp. are not associated with specific diseases in European wildlife; however, the organisms may be isolated from respiratory tract lesions and occasionally other lesions, often in association with other pathogens from wild mammals including aquatic mammals. Whether *Bordetella* spp. are primary or secondary pathogens in these cases is not readily discernible. The organism is occasionally isolated from lesions in tissues other than the respiratory tract.

SUTTONELLA ORNITHICOLA SP. NOV. INFECTIONS OF SPECIES OF TIT AND LONG-TAILED TITS

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Gram-negative rod-shaped organisms were isolated from the lungs of blue tit (*Cyanistes caeruleus*), coal tit (*Periparus ater*), great tit (*Parus major*) and long-tailed tit (*Aegithalos caudatus*), the latter species being unrelated to the other members of the *Paridae* tit family. This organism was found to be a new bacterium, *Suttonella ornithocola*, belonging to the family Cardiobacteriaceae. *Suttonella ornithicola* has been isolated from birds from mortality incidents with a wide geographical distribution within the UK[81]. Incidents have occurred sporadically and at low incidence, suggesting that the infection is endemic in native bird populations with a seasonal spring peak. Pulmonary congestion was seen in the birds at necropsy, and histopathology showed multiple foci of acute pulmonary necrosis associated with the bacteria, indicating that *S. ornithocola* is a primary pathogen of these passerine species in the UK.

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SECTION

3

Fungal and Yeast Infections

CHAPTER

38

ASPERGILLOSIS

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Aspergillus is a large ascomycetous genus of saprobic filamentous fungi that is of great importance in human and veterinary medicine, agriculture, biotechnology, the food industry and genetic research. Invasive and non-invasive infections of vertebrates by *Aspergillus* spp. are collectively named aspergillosis. Aspergillosis is the most important fungal respiratory infection in birds and a major cause of mortality in free-living, captive and domestic birds worldwide. Sporadic cases also occur in wild and domestic mammals. Aspergillosis is also named fungal, or mycotic, pneumonia, pneumonomycosis, bronchomycosis and brooder pneumonia; and it is caused by infection with *A. fumigatus*, or more rarely *A. flavus*, *A. niger*, *A. nidulans*, *A. terreus*, and others.

AETIOLOGY

Aspergillus spp. are fungi that recycle carbon and nitrogen from organic sources, particularly decaying vegetation in soil, compost heaps, litter and crops. They are cosmopolitan multicellular organisms consisting of elongated, septate, 3–6 μm thick, dichotomously branching cells (hyphae) that grow by apical extension to form a network (mycelium) on and within substrates. When exposed to air some hyphae differentiate into T- or L-shaped foot cells, which produce specialized structures called conidiophores (spore-bearing branches) in a perpendicular orientation to the axis of the foot cell. At the apex of the conidiophore, a vesicle is formed on which chains of mitotic (asexual) single-celled smooth- or rough-walled spores called conidia or conidiospores are formed. Conidia are released into air, and when they settle on a suitable substrate and water is available, they germinate and grow into a mycelium. Sexual reproduction by ascospores is known in a subset of *Aspergillus* spp., and was only very recently identified in *A. fumigatus*[1].

The genus *Aspergillus* contains approximately 250 species. Fungal nomenclature is governed by the International Code on Botanical Nomenclature, based on phenotypic features, most importantly the morphological appearance of sexual and asexual reproductive structures. According to nomenclatural rules, the teleomorph (sexual) state takes precedence over the anamorph state (asexual form). Hence, aspergilli that lack a known teleomorph state are classified as fungi imperfecti, i.e. within the artificial form-phylum *Deuteromycota*, genus *Aspergillus*. However, when sexual reproduction is recognized the species are classified within the phylum *Ascomycota*. A single species may thus be known under two different names that are used interchangeably, e.g. *Neosartorya fumigata* and *A. fumigatus*, but by strict interpretation of the

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code, *N. fumigata* is the correct name. A polyphasic approach, which considers data on morphology, physiology, metabolism and multilocus sequence information, is gaining in importance. By this system, all aspergilli are considered to belong to phylum *Ascomycota*, genus *Aspergillus*, and the genus is subdivided into eight subgenera, numerous sections and species.

Complete genome sequences are available for several *Aspergillus* spp., including *A. fumigatus.* The genome size is between 29 and 37 Mb, and comparative genomics has shown extensive interspecies variation, but low levels of genetic diversity between strains of *A. fumigatus*, speculatively because of its largely clonal population structure[2]. The *A. fumigatus* genome is divided into eight chromosomes, each between 1.8–4.9 Mb in size, and there are 9,632 protein-coding genes, most of which are of unknown function[3].

EPIDEMIOLOGY

Aspergillus fumigatus is the predominant agent in both mammalian and avian aspergillosis, but approximately 40 species are known opportunistic pathogens of vertebrates. Based on molecular characterization of a large number of environmental and clinical isolates of human origin, it has been suggested that, given a susceptible host, all *A. fumigatus* strains should be considered capable opportunistic agents[4]. However, interstrain variation in virulence has been observed. Moreover, polyclonal infection by *A. fumigatus* has been reported from both humans and birds in recent years, which may have potentially important repercussions on diagnostics and therapy[5]. *Aspergillus* spp. are aetiologic agents of a range of diseases in humans and in domestic animals, such as bovine mycotic abortion, sinonasal infections in dogs, guttural pouch mycosis in horses and respiratory infections in poultry and other captive birds. Aspergillosis in poultry is a particularly important and costly disease, which is associated with reduced welfare and financial losses from mortality and carcass condemnation, especially in the turkey industry.

Aspergillosis is a rarely reported condition in free-living and captive wild mammals including cervids such as red deer (*Cervus elaphus*) and roe deer (*Capreolus capreolus*)[6,7] cetaceans (dolphins and whales)[8–10], and hare (*Lepus* spp.)[11]. Malnutrition and immunocompromisation from anthropogenic toxins, heavy metals or viral infections have been suggested as possible predisposing factors to pulmonary aspergillosis or mycotic encephalitis caused by *A. fumigatus* in cetaceans. Aspergillosis is a potentially underdiagnosed infection in marine mammals and much remains to be learned about its epidemiology.

Information on prevalence of aspergillosis among wild birds is fragmentary and shows broad variations between studies and avian species. All avian species should probably be considered susceptible, but there seems to be a species predilection. Most reported cases among free-living birds involve waterfowl (particularly Anatidae), gulls (Laridae) and birds of prey (Accipitridae and Falconidae), which may reflect true species differences, a higher risk of exposure or a higher likelihood of detection. Among captive wild birds disease has been reported in a much wider range of families and species. Aspergillosis is a non-contagious disease. The main route of entry of conidia in both mammals and birds is the airways. However, in birds aspergilli may penetrate egg shells during incubation, and growth *in ovo* is a well-known cause of embryonic and hatchling mortality, i.e. brooder pneumonia. Age dependency has been reported, and most cases of aspergillosis in poultry involve young birds, following massive exposure to conidia during incubation or post-hatch from aerosolized conidia from mouldy litter, straw or feed. More rarely, conidia or hyphal fragments may be introduced by puncture wounds or during surgery. Aerosolized conidia may induce keratitis. Data on the infective dose of *A. fumigatus* in poultry and wild birds are contradictory and may reflect differences between avian species, age, immunity, strain virulence and experimental conditions. After transcutaneous air sac inoculation in turkey poults, inflammatory lesions are apparent within 24 hours and progress rapidly[12].

There are two main forms of aspergillosis occurring among free-living birds: an acute epizootic form, and sporadic disease in individual birds. Acute aspergillosis is sometimes manifested as sudden mass-mortality events in a restricted area. Such outbreaks usually involve anseriform birds, and are infrequently reported mainly in the North American literature, but could most probably occur elsewhere. The timing and location of these outbreaks are highly suggestive of a common source of conidia and exposure within a limited time frame. Outbreaks are often, but not invariably, associated with adverse weather conditions and availability of mouldy agricultural waste crops. Otherwise healthy birds appear to be overwhelmed by massive conidial exposure and develop acute epizootic aspergillosis, but it is unclear if immunocompromisation is a necessary

prerequisite. Outbreaks of acute aspergillosis are also seen in poultry and on game farms. The other form of aspergillosis in free-living birds is much more common and involves incidental and usually subacute to chronic cases. Affected birds often show signs of trauma, inanition or concomitant disease such as parasite infections[13], mycobacteriosis[14], lead toxicity[15], carbofuran toxicity[16] or oiling[17]. There is circumstantial evidence that aspergillosis may be a significant cause of mortality in certain free-living avian populations, such as in immature white storks (*Ciconia ciconia*) in Germany[18]. Occasional reports of aspergillosis involve near-threatened, conservation-dependent or endangered species. It cannot be ruled out that this disease may have a negative impact on such free-living populations, especially if combined with other infectious diseases or toxins. Also, because aspergillosis is a particularly common sequel to captivity in zoological exhibits, during research projects, in relocation programmes or at rehabilitation centres, it is a potentially important cause of failure of conservation and translocation programmes of avian species.

PATHOGENESIS, PATHOLOGY AND IMMUNITY

The past decade has seen remarkable progress in the understanding of mammalian host–pathogen interactions and virulence determinants of *Aspergillus* spp. The current view is that aspergilli make use of their saprobic lifestyle, thermotolerance and competitiveness, and that these help ensure survival both in their complex environmental niche and during infection. Conidia of *A. fumigatus* are widely dispersed in air because of prolific asexual reproduction, conidial longevity and small size, which allows them to remain airborne. Conidial numbers may reach particularly high levels in poultry barns and stables, at composting plants and in damp buildings. Because of their small size, conidia will readily penetrate airways, including alveoli in mammals and air sacs in birds. It is generally assumed that conidia are continuously inhaled and rendered innocuous by innate immunity mechanisms. Many conidia get expelled by mucociliary action, whereas others are internalized by respiratory epithelial cells or adhere to exposed extracellular matrix components. Alveolar macrophages recognize and kill conidia, and they trigger a polymorphonuclear immune response. Based on studies of the pathogenesis in mammalian animal models it is known that neutrophils extravasate and migrate towards the site of infection in response to chemokines that are generated during complement activation, and they use a range of pathogen recognition receptors to recognize and eliminate hyphae by reactive oxygen and nitrogen species and enzymatic granule components, and by phagocytosis. Further, dendritic cells seem to trigger an adaptive immune response[19]. In mammals, neutropenia, defective phagocyte function, and corticosteroids that impair macrophage function and epithelial integrity, are recognized risk factors for aspergillosis.

Aspergilli make use of both active and passive mechanisms to circumvent innate immunity, e.g. cilio-inhibitory, cytotoxic and immunomodulatory mechanisms, and the majority of hitherto identified putative virulence determinants in aspergilli do not display classical virulence attributes. Candidate pathogenicity determinants include secondary metabolites such as catalase, proteases, elastase and the immunomodulatory substance gliotoxin, genes responsible for thermotolerant growth, a variety of cell wall components, enzymes involved in the pyrimidine biosynthesis, histidine kinases, a Ras-related protein, cAMP signalling pathways and calcineurin, nutrient sensing system components, which ensure versatility of nutrient availability, and substances that are involved in resistance to phagocytosis and scavenging of reactive oxygen species. Recent reviews expand upon virulence determinants and mammalian host–pathogen interactions[20–22].

Immunosuppression or transgenic models of immune deficiency combined with high infective doses are necessary prerequisites in mammalian experimental models of aspergillosis. In contrast to mammalian experimental models, immune modulation is not a necessary prerequisite in avian challenge models. Aspergillosis is readily induced by inhalation of aerosolized conidia, by intravenous and intratracheal routes, and by transcutaneous inoculation into air sacs in otherwise healthy birds. Knowledge on predisposing factors in avian aspergillosis relies mostly on anecdotal evidence. It is tempting to speculate that the apparent higher susceptibility of birds when compared with mammals may be attributed to anatomic and physiologic characteristics of the avian respiratory system, immune mechanisms and/or a higher body temperature that allows quicker fungal growth. Birds have small, non-expanding lungs, and air sacs that act like bellows and hold most of the respiratory volume. The avian air sacs usually constitute a primary nidus of infection because the air (and conidia) reaches the caudal air sacs before it passes through

those parts of the lungs in which the gas exchange takes place. The mucociliary system is unevenly distributed in the air sacs, and macrophage numbers in distal airways are low, although there is presently no scientific proof that this explains the apparent higher susceptibility of birds. In addition, avian heterophils primarily depend on non-oxidative mechanisms[23]. There is generally little information available on immunity mechanisms in avian aspergillosis.

Acutely affected birds are often in normal body condition, but as the disease progresses, birds lose condition and may become emaciated. Lesions may be confined to air sacs and lungs, but trachea, syrinx and bronchi may also be primary targets. Virtually any organ and tissue can become secondarily involved from direct extension of contiguous respiratory lesions, and/or by haematogenous dissemination of fungal elements contained within macrophages. Birds with acute aspergillosis present with pulmonary congestion, oedema, haemorrhage, consolidation and miliary 1–10 mm diameter, coalescing, round to irregular, caseous, parenchymatous and pleural nodules (Figure 38.1), sometimes accompanied by white luminal dome-shaped nodules or plaques in air sacs. The more common subacute to chronic presentation is characterized by diffusely thickened, opaque, and prominently vascularized air sac membranes with scattered flat, white to tan, adhering caseonecrotic plaques of varying size, sometimes with umbilicated centres, in single or multiple air sacs (Figure 38.2). In advanced cases, there is extensive, confluent mycotic growth with severely thickened air sacs, accumulation of fibrinopurulent exudates within air sacs, and extension of lesions into surrounding tissues. Fungal reproduction within air sacs is a common feature, upon which plaques become velvety and change colour to shades of green, olive, brown or black depending on the fungal species involved, which leads to further dispersal of conidia within the airways. Gross lesions of varying age and severity may present simultaneously in the same bird. Asphyxiation from focal invasive growth in proximal airways may occur.

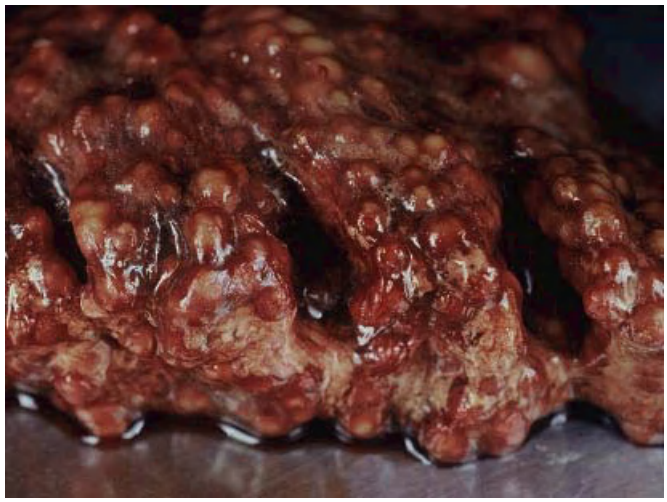

FIGURE 38.1 Acute aspergillosis caused by *Aspergillus fumigatus* in an immature bean goose (*Anser fabalis*). The lung contains large numbers of coalescing pale nodules. Photo: Bengt Ekberg, SVA.

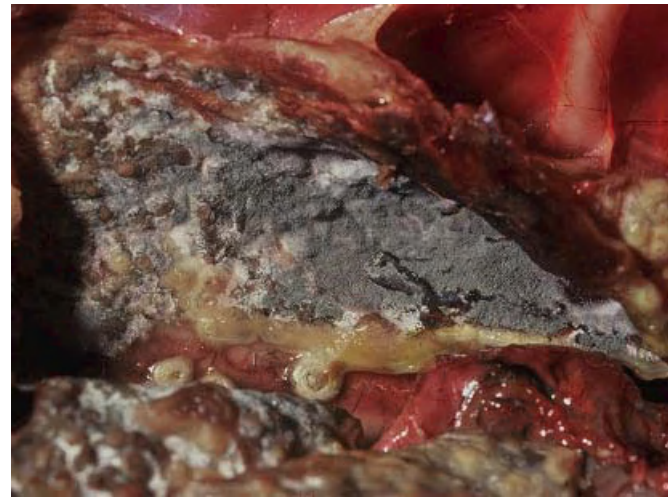

FIGURE 38.2 Severe chronic aspergillosis (*Aspergillus fumigatus*) in a young white-tailed eagle (*Haliaeetus albicilla*). The air sac is lined by confluent, grey-green sporulating fungal growth. Photo: Bengt Ekberg, SVA.

Histopathologically, lung lesions in the acute forms of aspergillosis consist of pleuritis, interstitial pneumonia with infiltrates consisting of predominantly heterophils, admixed with lymphocytes, macrophages and fibrin, and coalescing necrotic to pyogranulomatous lesions, which efface the normal pulmonary architecture and compress surrounding tissue. Pyogranulomas have an eosinophilic centre consisting of degenerating heterophils and/or central necrosis, surrounded by heterophils admixed with lymphocytes, and palisading epithelioid and multinuclear giant cells at the periphery. The amount of fibrous tissue at the periphery of pyogranulomas increases as lesions age. Severe suppurative and/or necrotizing pneumonia sometimes occurs in birds. Air sac membranes display infiltration with heterophils, macrophages and to a lesser degree lymphocytes and plasma cells, prominent vasculature,

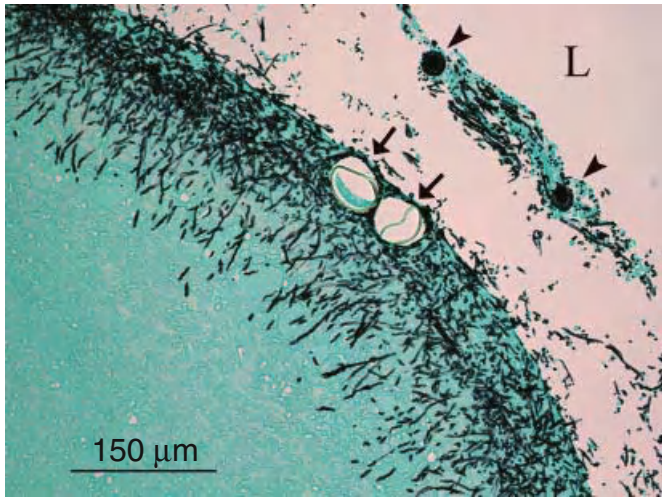

FIGURE 38.3 Hyphae (*Aspergillus fumigatus*) growing in a severely thickened air-sac membrane of a white-tailed eagle (the same bird as in Figures 38.2 and 38.4). Note the presence of nematode eggs (arrows) and conidiophores (arrowheads). L: air sac lumen. Grocott's methenamine silver stain. Photo: Désirée Jansson.

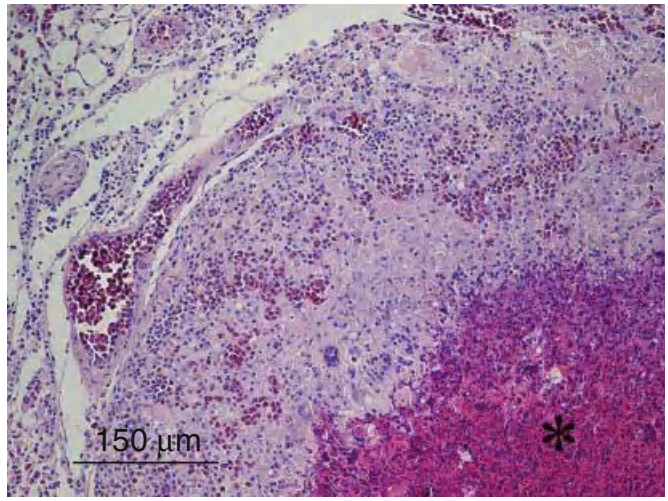

FIGURE 38.4 Microscopic appearance of lung tissue from a white-tailed eagle (the same bird as in Figures 38.2 and 38.3). A necrotic centre (*) of a pyogranuloma is seen in the lower right corner, and the lesion is surrounded by multinucleated giant cells and epithelioid macrophages. Note hyperaemia and the mixed interstitial inflammation. Haematoxylin and eosin. Photo: Désirée Jansson.

perivasculitis and oedema. As the disease progresses, air sac membranes become thickened with fibroplasia, massive leucocyte infiltration, formation of pyogranulomas and the presence of caseous exudates. Visualization of conidia, germinating conidia and hyphae is improved by various special stains (Figure 38.3). Fungal elements are predominantly located in pyogranulomas, in necrotic areas and within epithelioid and multinucleate giant cells. Hyphae appear as dichotomously branching, septate structures, having parallel cell walls. Signs of angioinvasion, i.e. hyphal invasion of blood vessels, subendothelial and mural mononuclear to polymorphonuclear invasion (Figure 38.4), thrombosis and coagulative necrosis may be observed in some birds. Upon sporulation in aerated tissues, conidiophores are present microscopically in exudates. Brain lesions are rarely reported from free-living birds, but when they occur they consist of solitary abscesses with necrotic centres and heterophil and macrophage infiltration at the periphery.

CLINICAL SIGNS AND TREATMENT

Affected free-living wild birds are usually found moribund or dead. When clinical signs are observed they are non-specific and vary with the severity of the disease, but are often subtle. Severely affected birds may be lethargic, dehydrated, are unable to fly and withdraw socially. Respiratory signs include nasal discharge, bill opening, gasping, dyspnoea, changes in vocalization, accelerated breathing and cyanosis. Neurological signs such as ataxia, torticollis and opisthotonos are occasionally reported from wild birds.

Treatment of aspergillosis in birds generally meets with a low success rate because of the often advanced disease stage when the diagnosis is confirmed, the lack of pharmacokinetic data of antifungal drugs in birds, the failure of drugs to reach target tissues (especially encapsulated granulomatous lesions), the fact that birds possess elaborate airways and pneumatized bones to which the infection may spread, and the presence of concurrent diseases and immunosuppression. Moreover, drug resistance to antifungal drugs has been reported in *A. fumigatus* isolates of avian origin[24]. Antifungal agents that may be used in birds include amphotericin B, terbinafine HCL, 5-fluorocytosine, itraconazole, fluconazole, ketoconazole, miconazole, clotrimazole, enilconazole and voriconazole. Drugs can be administered topically by nebulization, flushing of proximal airways and/or air sacs at surgery, or systemically by intravenous or oral routes, either alone or in different combinations. Surgical debridement of caseous

material and granulomas may be attempted if the bird can tolerate anaesthesia.

DIAGNOSIS

Ante mortem diagnosis of aspergillosis is difficult and relies on a combination of clinical signs, culture and cytology on tracheal, bronchoalveolar or air sac lavage, haematology, serology, protein electrophoresis, endoscopic findings and radiology. A positive culture must be interpreted with caution because aspergilli are frequent airway contaminants. Heterophilic leucocytosis and increased β and γ globulins are indicative of aspergillosis. Enzyme-linked immunosorbent assays and galactomannan analysis (fungus-specific polysaccharide) have been applied to avian samples.

The *post mortem* diagnosis relies on gross findings and microscopic features of the characteristic hyphal growth pattern in wet mounts in 20% KOH and ink dye, or by histology. Confirmation is achieved by isolation on mycologic media such as Sabouraud dextrose agar, Czapek Dox agar or potato dextrose agar. Diagnostic key characters should be evaluated using standardized culture conditions, and include characteristics such as the colour of the aerial mycelium and conidia, appearance of the colony margin and surface texture, growth rate *in vitro*, thermotolerance and, in particular, the size and morphology of conidiophores. Most aspergilli are fast-growing and display a powdery to cotton-like texture. The colour of colonies is highly variable between species. Hyphae and conidiophores may be collected from culture plates by cellophane tape, which is placed in a drop of lactophenol cotton blue and examined microscopically. Immunohistochemistry on formalin-fixed tissues can be used retrospectively. Molecular methods, especially polymerase chain reaction on DNA extracted from cultures, are useful tools for species identification.

MANAGEMENT, CONTROL AND REGULATIONS

Prevention is by far the best way to control high-risk situations such as captivity of wild birds in zoological exhibits, in relocation programmes or at rehabilitation centres. Animal facilities, transport crates, incubators and hatchers should be cleaned and disinfected with antifungal agents before use, and potential sources of conidia such as mouldy litter materials and feed should never be introduced. Care should be taken to minimize any stress. Incubators and areas around feeders and drinkers should be kept clean and dry to prevent fungal growth. To prevent outbreaks in wildlife, mouldy agricultural waste should be covered, ploughed under or cut and removed. Bird feeders should be kept clean and dry.

PUBLIC HEALTH CONCERN AND SIGNIFICANCE AND IMPLICATIONS FOR ANIMAL HEALTH

Aspergilli are rarely pathogenic to healthy people and domestic animals, with the exception of poultry in heavily contaminated environments. However, care should be taken to avoid heavy environmental exposure of humans and domestic animals to conidia. Protective face masks should preferably be used when removing mouldy feed and litter from poultry barns and stables, when visiting densely populated seabird colonies, and when performing necropsies on affected animals. Sporulating cultures should be handled with care at the laboratory.

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CHAPTER

39

YEAST INFECTIONS

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INTRODUCTION

Yeasts are eukaryotic, unicellular, round or oval microorganisms that develop blastoconidia during asexual reproduction. They form either pseudohyphae – when blastoconidia are produced without separation – or true hyphae in animal tissues. Yeasts are classified into the Ascomycota or Basidiomycota phyla in their perfect stage, and form colonies in Sabouraud dextrose agar resembling those of bacteria, although under microscopic examination their cells are much larger, ranging from 2 to 10 µm.

Whereas some yeasts can be retrieved from the environment, others are considered to be normal biota of the gastrointestinal tract, skin and urinary tract in animals and humans.

As opportunistic pathogens, yeasts require impairment of host defence mechanisms to establish clinical infections. Infections are categorized as exogenous when derived from the environment, or endogenous when resulting from overgrowth of commensal organisms. Since the 1960s, several new yeast species have been reported as human and animal pathogens, owing to the increase in immunodeficiency syndromes and immunosuppressant chemotherapy in cancer treatment. Domestic and wild animals are known to act as carriers of human pathogenic fungi, with bird droppings considered the main source worldwide for such pathogenic yeasts as *Candida* spp. and *Cryptococcus neoformans*. Notable yeasts in animal diseases include *Candida* spp., *Cryptococcus* spp. and *Malassezia* spp. Others, such as *Trichosporon* spp., *Rhodotorula* spp., *Sporobolomyces* spp., *Saccharomyces* spp. and *Geotrichum* spp. rarely cause infections.

CANDIDA INFECTIONS

Currently over 200 ascomycetous yeasts make up the genus *Candida*, but only a few are opportunistic pathogens of humans and animals. *Candida albicans* is thought to be the major fungal pathogen of animals, but *C. krusei*, *C. famata*, *C. parapsilosis* and *C. guilliermondii* are also isolated from clinical samples.

Candida yeasts are commensal organisms, inhabiting the skin, upper respiratory, alimentary and genital tracts of humans and warm-blooded animals. *Candida* counts of up to 10^3 cfu/g in the mammalian intestinal tract are regarded as normal flora[1]. *Candida* spp. are also present in the environment, e.g. in soil or water, or on plants and fruit. Contaminated environments, including litter from poultry- and bird-rearing facilities, refuse-disposal areas, discharge sites for poultry operations and areas contaminated with human waste have all been suggested as sources for *Candida* exposure in birds.

Infectious Diseases of Wild Mammals and Birds in Europe, First Edition. Edited by Dolores Gavier-Widén, J. Paul Duff, and Anna Meredith.

There are few reports of candidiasis causing disease in free-ranging wild animals and few investigations of its prevalence. Superficial infections have most frequently been described in birds, dogs and cats[1], while systemic infections are described in captive wild mammals such as alpacas (*Lama pacos*)[2]. Additionally, infected animals could be transmission vectors or reservoirs of strains causing human disease and may affect immunocompromised patients[3]. Occurrence is generally not seasonal, and young animals are more susceptible to infection.

Candida spp. require impairment of host defence mechanisms to establish clinical infections. Depending on the host's immune status, infection is either restricted to the skin and mucous membranes as a superficial mycosis or it may spread haematogenously, resulting in systemic mycosis.

Disturbances of the cutaneous and mucosal microenvironment are required for tissue invasion by the fungus, a process usually accompanied by phenotypic switching from yeast form to filamentous form in *C. albicans* infections. Systemic immunosuppression, long-term glucocorticoid therapy, and parenteral drug administration facilitate the invasion of deeper tissue layers and capillaries and subsequent haematogenous dissemination of *Candida* spp.

There are no unique signs of disease. In superficial candidiasis, lesions are generally confined to the digestive or urogenital tracts in mammals. In birds, the mouth, oesophagus, and, primarily, the crop, may have greyish-white, loosely attached, plaque-like areas on their internal surfaces. Mycotic stomatitis has been reported in pups, kittens and foals, gastro-oesophageal ulceration has been reported in pigs and foals, and abortion and mastitis have been reported in cows[1]. No data on superficial candidiasis has been published in wild mammals, whereas systemic fungal infections have been reported in wild mammals and birds, especially Psittacidae[2]. Multifocal erosive dermatitis associated with thoracic and abdominal serofibrinous effusions (following haematogenous spread), and multiple suppurative foci in lung, myocardium, kidney, pancreas and brain have been described in wild mammals[2].

Diagnosis requires histopathological examination, confirmed with fungal culture. Tissue sections, stained with periodic acid-Schiff (PAS) or methylamine silver methods may reveal budding yeast cells or hyphae. *Candida* spp. show oval, budding blastospores 3–8 µm in diameter. Suppurative to necrotizing inflammation with necrotizing vasculitis might be present. Culture is carried out aerobically at 37°C for 2–5 days on Sabouraud dextrose agar with chloramphenicol (0.5 g/l). Colonies are white, round and convex with a diameter up to 4–5 mm. Identification requires biochemical profiles and chlamydospore and germ-tube formation. Germ tubes are visible after 2–4 hours incubation at 37°C in sheep, beef, rabbit or human serum and should help identify *C. albicans.*

Antifungal agents are employed in the treatment of human or pet candidiasis, but should be administered only after the results of antifungal susceptibility tests. Oral/topical nystatin, oral or injectable fluconazole or daily application of clotrimazole are the treatments most frequently employed in veterinary medicine. No information is available on treatment in free-ranging wild animals.

CRYPTOCOCCAL INFECTIONS

Cryptococcus is a fungal genus, of which two species, *Cryptococcus neoformans* and *Cryptococcus gattii*, cause nearly all human and animal cryptococcal infections. These species are characterized by a mucopolysaccharide capsule, whose antiphagocytic properties make it an important virulence factor. On the basis of capsular antigen, five different serotypes (i.e. *C. neoformans* var. *neoformans* (serotypes D and AD), *C. neoformans* var. *grubii* (serotype A) and *C. gattii* (serotypes B and C) are accepted.

Cryptococcus gattii is geographically restricted to tropical and subtropical areas, but has also recently been isolated in Southern Italy[3]. *Cryptococcus gattii* is associated with trees of the genus *Eucalyptus*, usually causing infection in immunocompetent animals. By contrast, *C. neoformans* vars. *neoformans* and *grubii* are commonly found worldwide in association with soil and the droppings of birds such as psittacidae, passeriformes, columbiformes and falconiformes[3] and generally cause cryptococcosis in immunocompromised individuals. The association with bird excreta is because of the presence of creatine and uric acid, which are selectively metabolized by *C. neoformans*. Cryptococcosis occurs worldwide and is reported in various non-domestic animal species. Infection generally occurs exogenously by inhalation of aerosolized cells.

The primary reproduction site is the respiratory tract, where granulomas can form. *Cryptococcus* then reaches brain, eyes and other organs haematogenously, lymphogenously or via direct extension through the nasal mucosa across the cribiform plate. Purulent haemorrhagic rhinosinusitis with nasal discharge and dyspnoea have frequently

been described in cheetah (*Acinonyx jubatus*)[4]. Granulomatous pneumonia associated with meningoencephalomyelitis is most frequently observed in cheetah, non-human primates and free-living toads[5]. Localized granulomatous pneumonia is described in wild foxes (*Vulpes vulpes*)[6]. Even though birds are considered the most important healthy reservoir of *C. neoformans*, 26 clinical cases have so far been described, in parrots, pigeons and kiwis outside European countries[7]. Infection in immunocompetent birds is restricted to the upper respiratory tract, resulting in signs indicative of mycotic rhinitis or involvement of structures contiguous with the nasal cavity, such as the beak, sinuses, choana, retrobulbar space and palate[7]. In immunocompromised birds, infections involve the lower respiratory tract (e.g. trachea, lungs, air sacs) and give rise to disease, which may then disseminate to other sites. Pigeons develop localized subcutaneous infections, probably owing to inoculation of skin wounds with large doses of yeast.

Diagnosis of infections mainly requires histopathological examination, which should be confirmed by fungal culture. Myelography, in mammals, indicating the presence of an intramedullary compressive mass at various spinal cord levels, may help to diagnose meningoencephalomyelitis. Suitable specimens for laboratory examination include exudate, cerebrospinal fluid and biopsy/*post mortem* tissues. Budding yeasts with characteristically thick capsules can be demonstrated in fluid samples using India ink preparations. In tissue sections, yeast capsules are detected by Mayer's mucicarmine stain. Fontana-Masson staining can be employed to detect melanin in *Cryptococcus* spp. cell walls. In all cases, *Cryptococcus* cells are round or oval with a diameter of 3.5–8 μm. Culture is performed aerobically at 37°C for 1–2 weeks on Sabouraud dextrose agar with chloramphenicol (0.5 g/l). Niger seed agar as a selective and differential medium is useful. Identification is based on microscopic morphology, urea hydrolysis and sugar assimilation. *Cryptococcus gattii* can be identified using canavanine glycine bromothymol blue agar medium because of its selective growth. A latex agglutination test that detects capsular material from *Cryptococcus* spp. can be used on cerebrospinal fluid, serum and urine samples.

In domestic animals, itraconazole and fluconazole are probably the drugs of choice for systemic treatment of most cases of invasive cryptococcosis, both being suitable for oral administration. Fluconazole is less likely to produce significant side effects than itraconazole, and has the additional advantage of reaching higher concentrations in the eye and central nervous system[8]. Treatment should extend several months beyond resolution of clinical disease. Topical or intralesional therapy with amphotericin B may also be helpful, e.g. using a high concentration as topical ointment or as intralesional infusion in cases of avian cryptococcosis involving the beak and nearby tissues[7].

MALASSEZIA INFECTIONS

Malassezia spp. are lipophilic, non-mycelial, unipolar, budding yeasts belonging to the normal cutaneous microflora of most warm-blooded animals. These yeasts sometimes act as opportunistic pathogens under the influence of predisposing factors, mainly related to changes in the cutaneous microenvironment and/or alterations in host defence mechanisms[9]. Currently, 13 species (*M. furfur*, *M. obtusa*, *M. globosa*, *M. slooffiae*, *M. sympodialis*, *M. restricta*, *M. dermatis*, *M. japonica*, *M. nana*, *M. yamatoensis*, *M. equina*, *M. caprae* and *M. cuniculi*), are recognized as lipid-dependent, with only *Malassezia pachydermatis* not requiring lipid supplementation for *in vitro* growth[9]. *Malassezia* yeasts are currently regarded as new emerging pathogens, as the incidence of their infections has increased during the last decade.

The lipid-dependent species are frequently associated with human skin disorders, whereas the non-lipid-dependent *M. pachydermatis* is considered an opportunistic pathogen growing on animal skin surfaces and ear canals, even in wild animals[9]. In particular, *M. sympodialis* is the most frequent species isolated from the ear canal of large felids (56.9%) and of adult pigs (63.6%), which may also carry *M. furfur* (22.7%). By contrast, *M. pachydermatis* was the only species isolated from wild boar (12.9%), Cinta Senese swine (20.7%), juvenile pigs (13.6%) and small felids (43.1%)[10,11].

The pathogenic role of *Malassezia* yeasts is unknown and seems to be related to changes in the skin's normal physicochemical or immunological mechanisms, which may enhance or downregulate the molecular production of yeast virulence factors or antigens. In addition, *Malassezia* yeast proliferation is likely to be a preliminary step towards dermatitis and/or otitis. Superficial hyperplastic dermatitis with a predominance of lymphocytes and macrophages are described as histological features of *Malassezia* dermatitis. Epidermis and hair follicles show spongiosis, exocytosis and parakeratotic hyperkeratosis. Budding yeast cells ranging from 3 to 8 μm in diameter located within the

keratin can be differentiated by PAS from other pyknotic nuclei of the *stratum corneum*.

Malassezia dermatitis may present with pruritus, inflammation and epidermal hyperplasia, whereas otitis is characterized by the presence of brown and malodorous cerumen[12].

Diagnosis of infection usually requires cytological and cultural techniques and/or histopathological procedures. For cytology, samples can be collected using sterile cotton swabs moistened with sterile saline solution (NaCl 0.9%), rolled on a clean glass slide and stained using May-Grünwald Giemsa. Results can be considered positive if large populations of *Malassezia* cells are counted at 40× magnification (i.e. >5 *Malassezia* yeasts for skin sites and >10 for the ear canal, in dogs). Because of the lipophilic characteristic of these yeasts, many fungal media supplemented with a range of lipid sources have been used for culture. Dixon's medium is now the commonest used. A large *Malassezia* yeast population in the medium could be indicative of *Malassezia* spp. infection. *Malassezia* yeast can be identified at species level by its morphology and physicochemical characteristics as well as using molecular-biology tools.

Malassezia dermatitis or otitis is treated with antifungal drugs. Systemic therapy is often necessary, in particular when clinical signs are severe and widespread. Ketoconazole and itraconazole are the most commonly used drugs. Topical therapy is an alternative in case of localized lesions and otitis. The commonest topical drugs are imidazole antifungals, chlorhexidine and lime sulphur.

Wild animals are known to act as carriers of human pathogenic fungi, with bird droppings considered the main source worldwide for such pathogenic yeasts as *Candida* spp. and *Cryptococcus neoformans*.

Among the yeasts species, herein presented, *Cryptococcus* spp. is the cause of systemic fungal infections in humans or in animals that have contact with avian roosts or nests.

By contrast, *Candida* spp. and *Malassezia* spp., even if potentially transmitted by infected animals via direct contact, do not pose a human health risk. However, animals known to have infections from these yeasts should be properly handled, and cages, equipment and other material in contact with infected animals should be disinfected.

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CHAPTER

40

OTHER FUNGAL INFECTIONS

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INTRODUCTION

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Mycoses are infections caused by fungal organisms that usually grow as saprophytes in the environment. Most of these fungi are opportunistic and rarely cause disease in healthy animals, but they can affect immunocompromised individuals. In this chapter, the most common emerging fungal pathogens of animals are considered. A limited number of antifungal agents are licensed for use in animals; however, many of those available for the treatment of mycoses in humans are used by veterinary practitioners. The practical applications and the therapeutic regimens that have proved successful in the treatment of fungal infections in animals are summarized in Table 40.1[1].

ADIASPIROMYCOSIS

Adiaspiromycosis is a pulmonary disease of small mammals and occasionally humans, and is caused by dimorphic fungi belonging to the genus *Emmonsia*. *E. crescens* (renamed *Chrysosporium parvum* var. *crescens*) is widespread in Europe, and *Emmonsia parva* (renamed *Chrysosporium parvum* var. *parva*) is found in the Americas, Central Asia and Africa. These fungi are ubiquitous in the environment, grow on soil and produce 2 to 4 µm conidia in the saprophytic form and thick-walled spherules, designated as adiaconidia, in the infective form. *Emmonsia crescens* produces multinucleate adiaconidia that reach diameters in excess of 500 µm, whereas *E. parva* produces mononucleate adiaconidia of 20–40 µm in diameter.

Infection is widespread, especially among the families Cricetidae, Muridae and Mustelidae[2]. The infection occurs when dust-borne adiaconidia are inhaled by the host and reach the alveoli. Adiaconidia thereafter increase their size and form thick-walled, non-replicating adiaconidia that elicit extensive granulomatous reactions, which may bear a resemblance to those of mycobacterial granulomas. Clinical signs of adiaspiromycosis include coughing, progressive dyspnoea and lung failure.

The diagnosis of adiaspiromycosis is based on the observation of adiaconidia on histopathological examination using periodic acid-Schiff (PAS), Gomori's methenamine silver (GMS), and mucicarmine stains. The large (up to 300 µm) fungal spherules are composed of fine granular eosinophilic material and are surrounded by a thick trilamellar wall capsule, PAS- and GMS-positive but mucicarmine-negative.

Infectious Diseases of Wild Mammals and Birds in Europe, First Edition. Edited by Dolores Gavier-Widén, J. Paul Duff, and Anna Meredith.

TABLE 40.1 Antifungal agents of use in animal health.

Chemical class	Drugs[a]	Target	Indication	Dosage[b] and note
Polyenes	Grisefulvin (o)	Inhibits microtubule sliding	*Microsporum, Trichophyton*	50 mg/kg once daily or 25 mg/kg twice daily; should be administered with a fatty meal
	Amphotericin B (iv, t, o)	Membrane barrier function	*Aspergillus, Candida, Histoplasma, Coccidioides, Sporothrix, Mucorales, E. crescens, Blastomyces, Coccidioides*	Sometimes amphotericin B is used in conjunction with an azole antifungal. The drug requires intensive patient monitoring because of the risk for adverse effects (nephrotoxicity).
	Natamycin (t)		*Trichophyton, Microsporum*	Topical 100 ppm suspension; two times, with interval of pimaricin 4 days or spray suspension on the horse; repeat after 4–5 days, and 14 days later
	Nystatin (t, o)		*Candida, Microsporum, Trichophyton*	100,000–300,000 IU/kg, every 12 hours, 7–10 days with antibiotic therapy in *Candida* infections in birds
Azole Imidazoles	Clotrimazole (t)	Ergosterol biosynthesis	*Microsporum, Trichophyton, Pneumocystis Aspergillus*	Intranasal infusion of clotrimazole is an effective treatment for canine nasal aspergillosis; association of trimethoprim and sulfamethoxazole in *Pneumocystis pneumonia*
	Econazole (t)		*Microsporum, Trichophyton*	
	Enilconazole (t)		*Microsporum, Trichophyton, Malassezia, Aspergillus*	2% solution, whole-body applications with 3- to 4-day intervals; intranasal infusion of enilconazole is an effective treatment for canine nasal aspergillosis
	Ketoconazole (t, o)		*Microsporum, Trichophyton, Malassezia, Coccidioides*	10 mg/kg per day for 3–4 weeks; tablets should be given with a meal
	Miconazole (t)		*Microsporum Trichophyton Malassezia*	Shampoo containing 2% miconazole + 2% chlorhexidine or other topical human formulations
Triazoles	Fluconazole (o, iv)		*Aspergillus, Cryptococcus Blastomyces*	2.5–5 mg/kg daily
	Itraconazole (o, iv)		*Microsporum, Trichophyton, Malassezia, Sporothrix, E. crescens, Blastomyces, Coccidioides, Histoplasma capsulatum, Mucorales*	5–20 mg/kg daily or with 48-hour intervals
Allylamines	Terbinafine (t, o)	Squalene epoxidase	*Microsporum, Trichophyton, Mucorales*	8–40 mg/kg once daily
Benzimidazoles	Thiabendazole (o, t)	Microtubule assembly	*Trichophyton, Microsporum*	4% solution
Pyrimidine	Flucytosine (o, iv)	DNA and RNA synthesis	*Aspergillus, Candida*	Oral 75–120 mg/kg, every 6 hours; 50 mg/kg, every 12 hours for 2–4 weeks (mainly in birds)
Iodine compounds	Saturated solution of potassium iodide (SSKI)	Enhances the halide-perioxidase killing system of phagocytic cells	*Sporothrix, Entomophtorales*	20–40 mg/kg once daily intravenously or parenterally

[a] o – oral; t – topical; iv – intravenous
[b] Dosage varies, depending on the animal species

BLASTOMYCOSES

Blastomycosis is one of the principal systemic mycoses, caused by a dimorphic fungus, *Blastomyces dermatitidis*, which grows as a 'mycelial' form at room temperature and as a 'yeast' form at 37°C. The mycelial form grows in sandy, acidic soils and along waterways. Blastomycosis is endemic in North America but has been described in circumscribed African regions and sporadically reported in European human patients. The disease has been described in dogs, and occasionally in wild animals (i.e. wolves and lions)[3,4]. Mammals become infected by inhaling conidia. At 37°C in the host, conidial spores transform into yeast-phase cells, which multiply within the lung and may disseminate via the bloodstream and lymphatic system to visceral organs.

Canine blastomycosis is characterized by chronic cough, dyspnoea, weakness, lethargy, lameness, anorexia, weight loss, nasal and ocular discharge and cutaneous lesions. The infection is diagnosed by fungal culture, which should be restricted to biosafety level 3 laboratories. Enzyme-linked immunosorbent assay (ELISA) serology techniques are also available. At necropsy, in wild animals it is possible to observe many firm nodules up to 2.0 cm in diameter in the lungs, which histologically correspond to focal concentrations of macrophages and yeast-like round cells with a thin wall.

COCCIDIOIDOMYCOSIS

Coccidioidomycosis is a fungal disease caused by *Coccidioides* spp., dimorphic pathogenic fungi that cause infections characterized by respiratory, dermatological, musculoskeletal, neurological and ophthalmological signs.

Two genetically distinct species have been recently identified: namely *Coccidioides immitis* and *Coccidioides posadasii*. These two species are ubiquitous in the endemic regions, where they grow as moulds on soil, producing arthroconidia.

They have different geographic distributions: *C. immitis* is found in California, whereas *C. posadasii* is found in Central and South America.

Coccidioides spp. is considered infectious for all mammals and at least some reptiles. However, infections by *Coccidioides* spp. have been reported in no avian or animal species in Europe. Primates are particularly susceptible, and disseminated coccidioidomycosis has been reported to cause death in many species. Coccidioidal infections have also been described in free-ranging wildlife, such as as western cougars (*Puma concolor*), coyotes (*Canis latrans*), bottlenose dolphins (*Tursiops* spp.) and California sea lions (*Zalophus californianus*)[5].

The most important risk factors for acquiring the infection are dust exposure, pregnancy, age, sex and immunosuppression. The infection occurs when dust-borne arthroconidia are inhaled. Upon inhalation, the fungi convert to spherules, which contain endospores that are released by rupture and lead to new endospore-producing spherules. Approximately 60% of incidents of exposure to the fungus result in subclinical infection. The clinical form of coccidioidomycosis is characterized by several signs that range from a primary (usually benign) pulmonary infection, to a progressive pulmonary or extrapulmonary disease. Clinical signs include fever, lethargy, weight loss associated with anorexia, dullness, coughing and lameness. Diagnosis is based on the detection of endosporulating spherules in areas of lesion. Culture of specimens is recommended along with histopathological studies, although cultural examination should be restricted to biosafety level 3 laboratories.

HISTOPLASMOSIS

Histoplasmosis is a fungal infection caused by the dimorphic fungus *Histoplasma capsulatum* and can affect both humans and animals. The disease occurs in North, Central and South America, Africa, India and Southeast Asia. Although clinical cases in humans have been reported in Italy and in France, the presence of this fungus in Europe is yet to be ascertained.

Histoplasma capsulatum occurs in soil contaminated with bird and bat droppings. The host range of *H. capsulatum* includes many animal species, but the clinical cases reported in European animals are few and mainly related to dogs, chinchillas[6] and Dorcas gazelle (*Gazella dorcas neglecta*) kept at a Spanish zoo[7].

Infection most commonly occurs via the inhalation or ingestion of microconidia. The organism is transformed into the yeast phase at body temperature and phagocytized by alveolar macrophages, which can disseminate the infection to other organs.

There are three main clinical manifestations: subclinical, respiratory and disseminated disease. The clinical signs observed in affected animals are: anorexia followed by weight loss, constipation and increased respiratory rate. Gastrointestinal signs characterize the disseminated disease. Generalized lymphadenopathy and pleural and peritoneal

effusions have also been documented in disseminated forms. Anaemia and hypoalbuminaemia are the most common haematological abnormalities of disseminated histoplasmosis. At necropsy, numerous 3–6 mm white foci can be seen throughout the pulmonary parenchyma, liver, spleen and skeletal muscles.

Histoplasmosis is diagnosed by performing a cytological evaluation of tissues and effusions using haematoxylin and eosin (H&E) and PAS stains. A definitive diagnosis can be achieved by fungal culture.

PNEUMOCYSTIS INFECTIONS

The genus *Pneumocystis* includes non-cultivable fungi that are able to induce severe pneumonitis, especially in severely immunocompromised hosts. The following species are described: *P. jirovecii* Frenkel in humans, *P. carinii* Frenkel and *P. wakefieldiae* in rats, *P. murina* in mice and *P. oryctolagi* sp. nov. in rabbits from the Old World[8]. *Pneumocystis* spp. exist in three forms: trophic, sporocytes and mature cysts. Trophic forms are the most abundant and localize in the lungs of hosts. Infection by *Pneumocystis* spp. appears to be a relatively common finding in many species of the orders Rodentia and Soricomorpha[9]. In Europe, acute *Pneumocystis* pneumonia is reported in different mammalian species of the orders Rodentia (house mouse*, Mus musculus)*, Carnivora (weasel*, Mustela nivalis*), and in captive Pilosa (brown-throated sloth, *Bradypus variegatus*). Also, it has been shown that rabbits can develop spontaneous *Pneumocystis* pneumonia at weaning (about 1 month after birth) that develops for 7–10 days followed by recovery within 3–4 weeks.

The diagnosis of infection is performed by sampling pulmonary material by bronchoalveolar lavage (BAL) or by *post mortem* homogenization of lungs. *Pneumocystis* organisms are detected using toluidine blue O (TBO), GMS or Giemsa-like stains. Additionally, *Pneumocystis*-specific fluorescein-labelled antibodies are used to identify *Pneumocystis* organisms in impression smears or lung-homogenate, air-dried smears.

SPOROTRICHOSIS

Sporotrichosis is a chronic, granulomatous and usually lymphocutaneous infection affecting humans and animals. The aetiologic agent, *Sporothrix schenckii,* is a dimorphic fungus usually found as a mould in plant debris, soil and water, and as a yeast in infected tissues of the hosts. Sporotrichosis is distributed worldwide, mainly in areas with high humidity and mild temperatures. In Europe, sporotrichosis is endemic in Spain and Italy. The disease has been described in horses, dogs, cats, cattle, camels, fowls, swine, rats, mice, hamsters, chimpanzees and humans. No data are available on sporotrichoses in wild animals in Europe. Data collected from wild mammals in Latin America show seropositive responses to the sporotrichin test in 6% of Cebidae, 64% of Procyonidae and 30% of Felidae, suggesting that these animals could represent reservoirs of sporotrichosis in these areas. Severe necrotizing granulomatous lymphadenitis caused by *S. schenckii* has been diagnosed in a Pacific white-sided dolphin (*Lagenorhynchus obliquidens*), and systemic sporotrichosis has been reported in nine-banded armadillos (*Dasypus novemcinctus*)[10,11].

The main route of sporotrichosis infection is through skin wounds and, more sporadically, through the inhalation of conidia. The most common presentation of sporotrichosis is a chronic granulomatous lymphocutaneous infection that, in immunocompromised individuals, can spread systemically. The lesions consist of small, firm, non-painful, non-pruritic, cutaneous or subcutaneous nodules that develop at the site of inoculation after an incubation period of 1–3 months. Typically a single nodule is present that then ulcerates. Additionally lymphangitic nodules may develop and a regional lymphadenopathy may occur. Definitive diagnosis of infection is based on cytological examination of exudates, histological examination of a biopsy specimen, or isolation of *S. schenckii* by fungal culture. Although cases of spontaneous resolution of sporotrichosis have been reported, most patients require long-term treatment.

ZYGOMYCOSES

Zygomycosis is caused by fungi belonging to the class of Zygomycetes comprising the orders Mucorales and Entomophthorales. Genera within Mucorales cause an angio-invasive infection called mucormycosis, whereas genera within Entomophthorales produce a chronic subcutaneous infection called entomophthoramycosis.

MUCORMYCOSIS

Mucormycosis is an infection caused by saprophytic fungi belonging to the genera *Mucor*, *Rhizopus*, *Rhizomucor*, *Absidia*, *Apophysomyces*, *Cunninghamella*, *Saksenaea*,

Mortierella and *Syncephalastrum*. Infections by Mucorales are usually aggressive and frequently fatal angio-invasive diseases in immunocompromised humans and animals, with an acute onset and rapid progression.

Mucorales infections occur in many animal species, but reports in European wild animals are rare and those available are related to infections in marine mammals and birds[12].

Known risk factors for Mucorales infections are associated with exposure to a large amount of fungal spores that colonize the host through inhalation, percutaneous inoculation or ingestion. The clinical signs vary according to the immunological status of the host.

Four main clinical manifestations of Mucorales infections are known: cutaneous or subcutaneous, gastrointestinal, pulmonary and rhinocerebral mucormycoses.

Cutaneous or subcutaneus infections have been described in birds and amphibians. In birds, the clinical signs are feather loss on legs, dorsum, neck and head, and hyperkeratosis of the feet.

Pulmonary infections have been described in horses and cattle, whereas air-sac infections are known to occur in grey parrots (*Psittacus erithacus*). Clinical signs include apathy, fever, lacrimation and dyspnoea.

Gastrointestinal mucormycosis with high mortality rates has been described in sika deer (*Cervus nippon*). Clinical signs of rhinocerebral mucormycosis include lethargy, hypermetria, ataxia and blindness.

Mucorales infections can be diagnosed by microscopic examination and fungal culture of the lesions. Specimens should be aseptically collected, and a large amount of tissue is needed for culture.

ENTOMOPHTHORAMYCOSIS

Entomophthoramycoses are subcutaneous zygomycoses caused by fungi belonging to the genera *Basidiobolus* and *Conidiobolus*, which cause localized subcutaneous granulomas in immunocompetent humans and animals.

These organisms reside in decaying plant material, soil and leaves from deciduous trees. In addition, *Basidiobolus* spp. are frequently isolated from the intestines of fish, frogs, toads, insects, reptiles and insectivorous bats. Although these genera are distributed worldwide, the diseases in wild animals are reported in Africa, India, Saudi Arabia, South and North America and Australia.

In animals, entomophthoramycoses are reported mainly as cutaneous or subcutaneous infections in horses, llamas (*Lama glama*) and toads (*Bufo hemiophrys*), and as rhinofacial, nasopharyngeal or oral infections in horses, dogs and sheep[14,15].

Basidiobolus infections cause cutaneous or subcutaneous lesions localized on the head, neck, chest or trunk, characterized by a granulomatous mass with an erythematous and haemorrhagic surface.

Conidiobolus infections occur as nasopharyngeal infections with or without local dissemination into tissues of the face, retropharyngeal region, retrobulbar spaces or cerebrum. They are characterized by nasal obstruction, nasal discharge or chronic sinusitis. The infections can be diagnosed by microscopic histopathological examination and by fungal culture with tissue samples.

TRICHOPHYTON MENTAGROPHYTES INFECTION IN EUROPEAN HEDGEHOGS

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Ringworm caused by *Trichophyton mentagrophytes* var. *erinacei* is the only significant dermatophyte infection of European hedgehogs (*Erinaceus europeaus*). It is endemic in European hedgehogs and is carried by up to 50% of individuals, often as a subclinical infection, and is found throughout the range of this species in Europe[16]. Transmission can be direct and infection is more evident in urban hedgehogs, which typically have smaller home ranges, and in males with larger home ranges and greater social interactions[17]. Infected spines may be driven into another hedgehog when fighting[18]. Older hedgehogs appear to have a much higher incidence compared with those under 1 year of age[18]. *Caparinia tripilis* mites have been implicated in transmission of *T. mentagrophytes* var. *erinacei*, as fungi have been recovered from their faeces[19]. The fungal spores persist in nests and transmission may occur indirectly via shared nesting sites and home ranges[20]. Transmission from mother to offspring is also possible[18].

Trichophyton mentagrophytes var. *erninacei* infection can lead to a subclinical infection with no skin lesions evident, or spine loss, scabs and scurf, particularly in the skirt area

or spine fur margin. Affected spines can easily be epilated.

In severe cases cracked crusty lesions can occur, typically on the snout, ears and head, which bleed when the scabs are lifted. Chronic disease can lead to thickening and hyperpigmentation. Pruritis is seen infrequently. Concurrent disease, with *Caparinia tripilis* mites or bacterial infections, are common findings[21]. The incubation could be over a 3 month period and the disease can take several weeks to months to progress[18].

Confirmation of infection is by isolation and culture. Treatment typically involves the use of systemic antifungals such as griseofulvin (30–50 mg/kg once daily), itraconazole (10 mg/kg once daily) or terbinafine (10–30 mg/kg once daily) given orally for 2–6 weeks. Topical antifungal therapy is also commonly employed using enilconazole 0.2% dilution, F10® disinfectant (Health and Hygiene (Pty) Ltd) or miconazole. Repeat culture is required to confirm clearance of the infection.

This has been a well-recognized zoonotic disease since the late 19th century, with a number of reports of infected human handlers – resulting from handling without gloves. Lesions may not be typical of human ringworm, however, but are intensely pruritic.

OTHER DERMATOPHYTE INFECTIONS

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Dermatophytoses (also referred to as ringworm) are superficial, cutaneous mycoses caused by filamentous fungi – the dermatophytes – which invade keratinized tissues (i.e. skin, hair and nails) of humans and animals, causing mild to severe, localized and/or diffuse infections. Dermatophytes are grouped into anthropophilic, zoophilic and geophilic species according to their habitat. Zoophilic dermatophytes infect both animals and humans, whereas anthropophilic dermatophytes mainly infect humans. Geophilic dermatophytes can induce both animal and human infections. Animal dermatophytoses are caused by fungi of the genera *Microsporum* and *Trichophyton*. Among these, *Microsporum canis* and species belonging to *Trichophyton mentagrophytes* complex are zoophilic species pathogenic to humans. In wild animals, dermatophyte fungi have been recovered mainly from healthy animals, including rodents, insectivores, lagomorphs, foxes (*Vulpes vulpes*), wild boar (*Sus scrofa*), wolves (*Canis lupus*), polecats (*Mustela putorius*) and badgers (*Meles meles*), and have only occasionally been reported in diseased animals (i.e. Eastern cottontail rabbit (*Sylvilagus floridanus*) and chamois (*Rupicapra rupicapra*))[22,23]. *Microsporum canis* and *T. mentagrophytes* complex species are the most frequently retrieved species, followed by geophilic dermatophytes such as *Microsporum cookie*, *M. gypseum, Trichophyton ajelloi* and *T. terrestre*. Dermatophyte infection is acquired by contact between healthy and diseased animals but also from the environment. Generally the clinical signs include mild to severe alopecia associated with erythema. Hairs, if present, are easily epilated. Laboratory diagnosis consists of direct microscopic examination of the clinical specimen followed by *in vitro* culture techniques. On direct microscopic examination, chains of spherical, translucent spores (arthroconidia), with a diameter ranging from 2 to 18 µm, can be observed using 10% potassium hydroxide. Colonies grown onto Sabouraud dextrose agar supplemented with chloramphenicol 0.05 g/l and cycloheximide 0.5 g/l are identified to species based on their morphology and the microscopic characteristics of the hyphae, macroconidia and microconidia.

Mammalian dermatophytosis usually resolves spontaneously (self-cure) within 1 to 4 months, but the contagious and zoonotic nature of this disease makes treatment mandatory for companion animals. Miconazole, chlorhexidine, lime sulphur dips and enilconazole are useful topical treatments. Griseofulvin, ketoconazole and itraconazole are the most frequently used therapeutic agents for the systemic treatment of dermatophytosis. The treatment should be continued for 2 to 4 weeks after clinical resolution, and until two negative cultures have been obtained. For environmental decontamination, hypochlorite bleach and enilconazole environmental spray are frequently employed.

MACRORHABDUS ORNITHOGASTER INFECTION IN BIRDS

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Macrorhabdus ornithogaster (formerly known as 'megabacterium') is a yeast that colonizes the gastric tissues of some birds. The disease is called megabacteriosis, 'going light', 'chronic wasting disease' or 'thin bird disease'.

Macrorhabdus ornithogaster is an ascomycetous yeast and the sole known member of the genus *Macrorhabdus*[24]. The organism is filamentous and 2–3 × 20–80 µm in size. Cells possess a single nucleus, a deeply invaginated plasma membrane, a thick chitinous cell wall, and septae, but they seem to lack mitochondriae[24–26]. Sequence data of partial 18S rRNA genes are available from two budgerigars and show 97% sequence similarity[24,27]. It is assumed that morphologically similar organisms from other species belong to the same or a closely related taxon.

Organisms indistinguishable from *M. ornithogaster* have been reported from passerine and psittacine companion birds, domestic birds (chickens, turkeys, ostriches, partridges), free-living finches (order Passeriformes, family Fringillidae) and sulphur-crested cockatoos (*Cacatua galerita*). Transmission occurs orally. Presence of this yeast in faeces indicates a faecal source, but bird-to-bird transmission from mutual feeding during courtship or feeding of hatchlings cannot be excluded. An environmental source has not been identified. The mechanisms of pathogenesis are unknown.

Colonization in domestic and companion birds is associated clinically with weight loss, ill thrift, dehydration, fluffed-up plumage, anorexia or apparent polyphagia, regurgitation and diarrhoea or dry faeces of varying degrees of severity. Sudden death from proventricular haemorrhage or rupture occurs sporadically. The clinical significance in wild finches remains to be determined. Colonization of the gastrointestinal tract by this yeast in wild finches is often observed concurrently with colibacillosis or salmonellosis[28] (Jansson et al., unpublished observations).

Gross lesions consist of an enlarged and hyperaemic proventriculus with a viscous mucoid luminal cover (Figure 40.1). Microscopically, the organism is visualized by H&E stains (Figure 40.2), which improves with silver and PAS stains, but it stains weakly or variably by Gram stain. Microscopically, lymphoplasmocytic and/or heterophilic inflammation, epithelial hyperplasia, disrupted koilin layer, goblet cell hyperplasia, micro-abscesses and focal necroses may be observed.

Ante mortem diagnosis depends on identification of the organism in faeces or crop/gastric washings. *Post mortem* diagnosis is achieved by demonstration of the organism in the proventriculus by microscopy. Isolation requires liquid or semi-solid Basal Medium Eagle at pH 3–4 with 20% fetal calf serum, glucose or sucrose as growth substrate, and microaerophilic incubation at 42°C for 3 days[27]. Treatment is possible with oral amphotericin B, and can resolve clinical signs but will not always eliminate the organism.

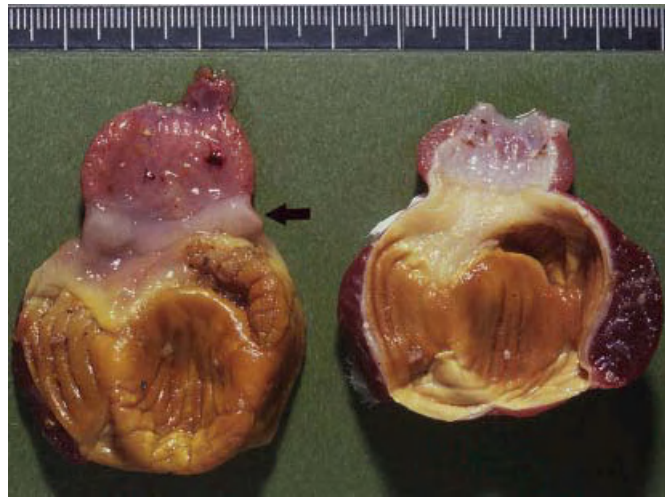

FIGURE 40.1 Hyperaemic proventriculus and mucinous covering of the isthmus region (arrow) in a partridge (*Perdix perdix*) colonized by presumed *Macrorhabdus ornithogaster* (left), and a normal organ (right). Photo: Bengt Ekberg, SVA.

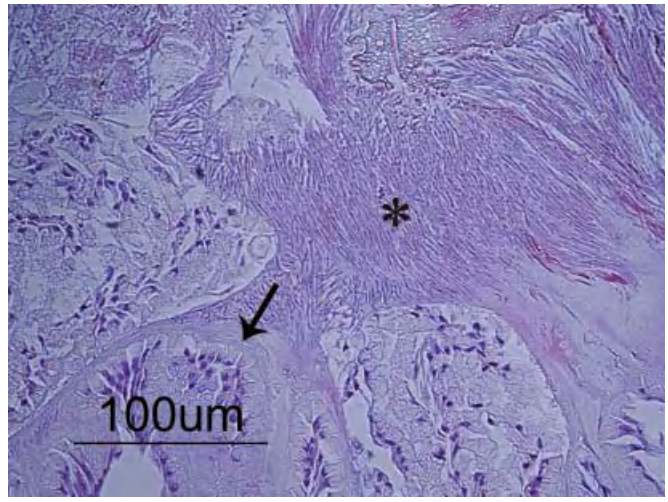

FIGURE 40.2 *Macrorhabdus ornithogaster*-like organisms in the isthmus region of a free-living wild common redpoll (*Carduelis flammea*) diagnosed with salmonellosis. The organisms display disorganized superficial growth and profuse oriented growth (*) in the mucinous layer that covers the epithelium (arrow). (H&E) Photo: Désirée Jansson.

Colonization of mammals has not been confirmed. *Macrorhabdus ornithogaster* was successfully transferred from budgerigars to chickens[27,29]. Hence, natural transmission from companion birds and wildlife to domestic birds should be considered as a potential disease risk.

GEOMYCES DESTRUCTANS – WHITE-NOSE SYNDROME IN HIBERNATING BATS

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White-nose syndrome (WNS) is named for the visible growth of fungus on the muzzle of hibernating bats. Within 2 years of emergence in 2007, this disease had spread 900 km along the eastern USA and affected seven bat species[30]. In 2010 WNS was identified along 1500 km of cave habitat from Ontario, Canada to Tennessee in the USA, and bat mortality estimates exceeded 1 million.

The visible fungal growth on bats with WNS is *Geomyces destructans*, a recently identified obligate psychrophile in the phylum Ascomycota. *Geomyces destructans* colonizes the skin of bats, and in North America, causes ulcerations on wing membrane, muzzle and ears.

In Europe white fungus on the muzzles of single live bats has been noted at least since the 1980s. *Geomyces destructans* has been confirmed on six species of European bats (*Myotis myotis*, *M. dasycneme, M. daubentonii, M. brandtii, M. blythii, M. mystacinus)* in Belgium, Czech Republic, Estonia, France, Germany, Hungary, Poland, Switzerland, Slovakia, the Netherlands and Ukraine, with historic photographic evidence of white fungus on hibernating bats in several other European countries[31,32]. The genetic sequence of European and US isolates of *G. destructans* at the ITS and SSU genes are identical. However, disease or mortality associated with *G. destructans* has not been confirmed in European bats (disease reported at time of book publication).

The microclimate of affected cave hibernacula, as well as the body temperature of the bats hibernating in these caves, provides an optimal host for infection of glabrous skin by *G. destructans*.

Currently both bat-to-bat transmission and mechanical dissemination of the fungus by humans are thought to contribute to the spread of *G. destructans*. There is also evidence of environmental persistence of this organism in cave hibernacula in the absence of bats.

Bats infected with *G. destructans* may not have white fungal growth on the muzzle, and the white muzzle is not diagnostic for infection with *G. destructans*. Wing membrane affected with the *G. destructans* can adhere to other wing folds and may lead to tearing of the wing membrane as the wings are extended. Microscopically, the characteristic pattern of 'cupping erosion' is formed by dense, focal aggregates of fungal hyphae at the interface of the skin[33]. As these colonies of fungal hyphae proliferate and expand, they erode the epidermis and eventually invade all layers of skin with ulceration and invasion of deep dermal structures. PAS stain is essential to identify these clusters of fungal hyphae and the distinct pattern of erosion and invasion that is currently the diagnostic feature of WNS (Figure 40.3). The invasion of living tissue distinguishes *G. destructans* from typical dermatophytes of mammals. The lack of cellular response to *G. destructans* as it erodes and invades the skin is a likely consequence of physiological immunosupression in normal hibernating mammals. Recovery from WNS as bats become euthermic has been documented in rehabilitation studies[34]. Wing membrane is critical to physiological homeostasis in hibernating bats.

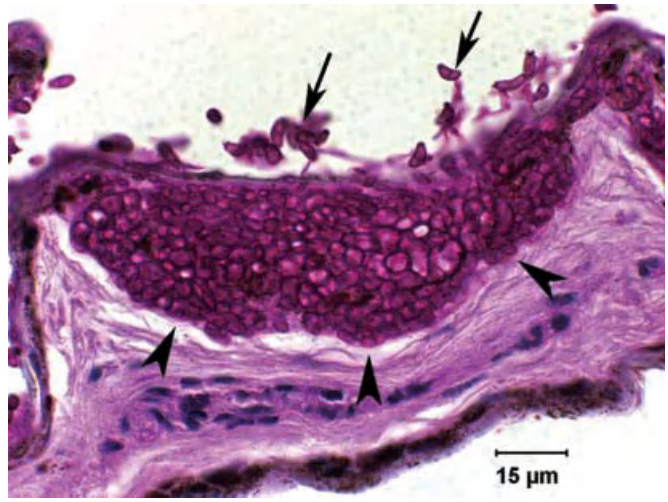

FIGURE 40.3 Wing membrane from *M. lucifugus* with WNS collected in the USA (PAS stain). Typical cupping erosion of skin at the advancing margin of fungal hyphae (arrowheads). Conidia characteristic of *G. destructans* are present (arrows). Note the lack of cellular inflammatory response to the fungus. Photo: Carol U. Meteyer, USGS, NWHC.

Damage to the integrity of wing membrane with subsequent disruption of hydration, circulation, cutaneous respiration and thermoregulation is the proposed mechanism by which infection with *G. destructans* kills hibernating bats[35].

Behavioural signs of bats with WNS include increased frequency and duration of arousal cycles, shifts from normal roost sites deep in the caves towards cave entrances, and early emergence from hibernation with day flights during winter. These abnormal behaviours lead to premature consumption of energy reserves, and can result in emaciation, which is another finding often associated with WNS.

Because *G. destructans* spores can be present on bats without causing infection, the current confirmatory test for WNS is microscopic identification of the typical histologic skin lesions described above. If the muzzle of a dead bat is not visibly affected, wings are the best tissue for histology, fungal isolation and polymerase chain reaction (PCR). Growth on Sabouraud dextrose or cornmeal agar is optimal, although slow, at 5–10°C. Microscopic identification of *G. destructans* conidia on fungal tape collections from a clinically affected muzzle can provide a non-lethal screening tool for *G. destructans* with subsequent confirmation using histopathology, culture or PCR.

Current management strategies are directed at reducing the potential for human spread of *G. destructans* through decontamination of footwear and equipment, and closing caves and mines to human access. There is no currently accepted treatment for bats that is efficacious, reasonable to administer without disrupting hibernation, a viable option for treating large numbers of bats, and known to be safe. Treatment of hibernacula is currently untenable because of the risk to other cave biota and delicate cave ecosystems. Because of the unique environmental and host physiology requirements for successful pathogenicity of *G. destructans*, there appears to be little risk of this disease spreading to non-hibernating warm-blooded animals, including humans. Potential for spread to other hibernating mammals, insects and exotherms is unknown.

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CHAPTER

41

HARMFUL ALGAL BLOOMS INCLUDING CYANOBACTERIAL TOXICOSIS

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INTRODUCTION

A number of aquatic microalgal species are known to cause harmful toxic blooms and animal intoxications (phycotoxicosis). These include a variety of cyanobacteria (blue-green algae) as well as dinoflagellate and diatom species. Although dinoflagellates have been associated with seabird mortalities, e.g. along the English coast[(1)], cyanobacteria are by far the most common group associated with animal toxicosis. Despite their aquatic origin, cyanotoxins appear to be more hazardous to terrestrial animals than to aquatic biota[(2)].

Toxic cyanobacterial blooms have long been associated with intoxication events in domestic animals all over the world, and they are also posing a human health hazard (e.g.[(3)]). Some reports mention that wild mammals and birds were also affected. Specific reports of suspected cyanotoxic die-offs in wildlife are, however, mostly restricted to mass mortality events in birds[(4,5)]. Owing to the accidental nature of exposure and limitations of diagnostic methodology, the general frequency of cyanotoxicosis in wildlife is likely to have been greatly underestimated in the past.

AETIOLOGY

Cyanobacteria (Class Cyanophyceae) are prokaryotic (no nucleus) photosynthetic microorganisms containing chlorophyll-a[(6)]. They also contain accessory photosynthetic pigments such as phycocyanin, allophycocyanin and phycoerythrin. Cyanobacteria are important primary producers that may flourish in fresh, brackish and marine water ecosystems. Growth is dependent on carbon dioxide, inorganic compounds and light, and may take place both in the pelagic (water column) and benthic (sediment) water domains. Their basic morphology is very diverse, including unicellular, colonial and multicellular filamentous forms. Cell colour varies from blue-green to violet-red, depending on the composition of photosynthetic pigments. Around 2,000 species of cyanobacteria, belonging to 150 genera, have been described, but only 40 species are associated with animal toxicoses.

Cyanobacterial toxins constitute a diverse group of compounds from both a chemical and toxicological point of view[(2)]. They can be classified according to their mode of toxicity into three different groups:

1. hepatotoxins, including microcystins and nodularins
2. neurotoxins, including anatoxins and saxitoxins
3. dermatotoxins.

Microcystins include 80 structural variants of cyclic peptides, among which microcystin-RR and -YR are recognized as the most toxic. Microcystins are produced by a relatively wide range of fresh and brackish water cyanobacteria, the most important belonging to the genera *Micro-*

Infectious Diseases of Wild Mammals and Birds in Europe, First Edition. Edited by Dolores Gavier-Widén, J. Paul Duff, and Anna Meredith.

cystis, Anabaena, Planktothrix and *Oscillatoria*. Production of nodularins is restricted to the species *Nodularia spumigena*, which occurs in brackish waters and is an important species in the Baltic Sea.

The anatoxins involved in animal cyanotoxicoses are anatoxin-a, homoanatoxin-a and anatoxin-a(s). The anatoxins are mainly produced by cyanobacteria within the genus *Anabaena*, but also by other genera, including *Microcystis, Oscillatoria* and *Planktothrix*.

The dinoflagellate *Karenia brevis* produces brevitoxin, a potent marine neurotoxin.

EPIDEMIOLOGY

GEOGRAPHICAL DISTRIBUTION AND HOSTS

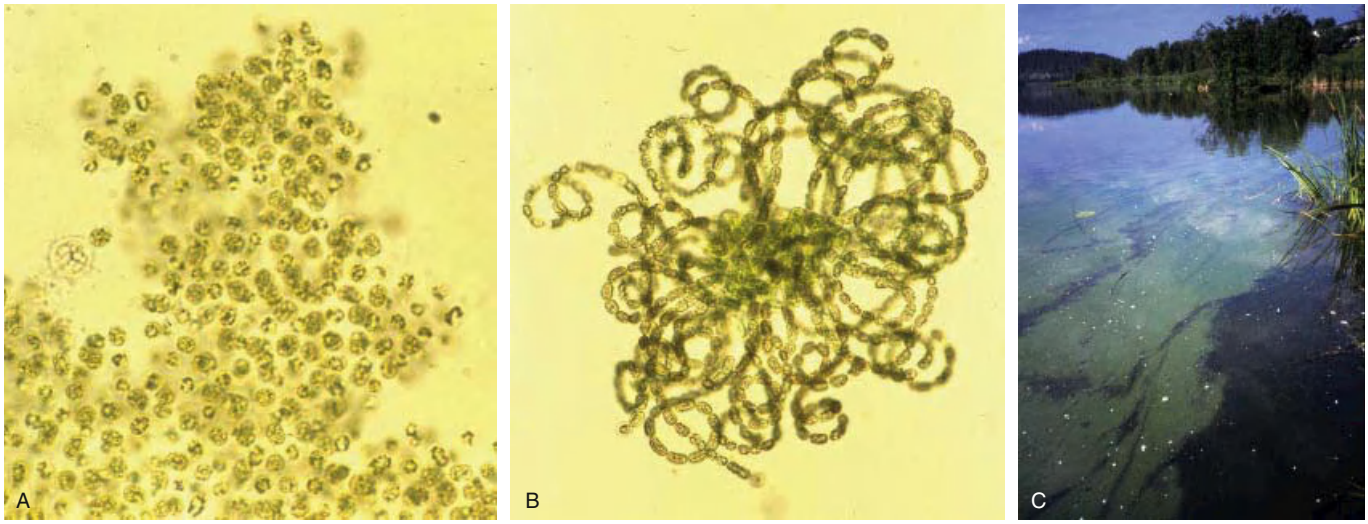
Most cases of cyano-intoxication associated with cyanobacterial blooms have been reported from livestock and dogs. In Europe, domestic animal intoxication has been reported in Germany, Denmark, Sweden, Norway, England, Scotland, Ireland and Switzerland (e.g.[3]). Although associated with deaths in various wild mammal species on other continents[7], cyano-intoxication in wild European mammals seems to be restricted to a case report in roe deer (*Capreolus capreolus*) in Norway[8]. This diagnosis was based on demonstration of typical liver lesions and the presence of high concentrations of microcystin-YR in the liver tissue. The suspected source of intoxication was algal growth in a drainage ditch.

Mortalities in wild waterfowl associated with cyanobacterial bloom have been registered in several European countries including Denmark[9,10]; Belgium[11], Spain[12,13], Sweden and Finland[5]. The Danish outbreaks were associated with anatoxin-a(s) producing *Anabaena* blooms in freshwater lakes. The Spanish outbreaks occurred in the Doñana National Park, which is a major waterfowl refuge in Southern Europe. Here, many mass mortality events have occurred since 1973, and at least two were associated with heavy cyanobacterial blooms. In the first recorded outbreak, a large number of waterfowl, especially greater flamingos (*Phoenicopterus ruber*), died[5]. This outbreak was associated with a mixed bloom, consisting of *Microcystis aeruginosa* and *Anabaena flos-aquae.* Laboratory examination revealed hepatotoxicosis and high levels of microcystins in the liver of dead birds. The second outbreak was associated with a bloom of *Microcystis aeruginosa*, and at least 6,000 waterfowl belonging to 47 species were killed[13]. This outbreak initially affected herbivorous waterfowl and fish, followed by a die-off in piscivorous birds. Laboratory findings included typical liver lesions in dead birds, and the presence of high concentrations of microcystins in the livers of both dead birds and fish. The authors suggested this cascade of waterfowl die-offs could be explained by accumulation of cyanotoxins in the food web: firstly affecting herbivorous avian species consuming cyanobacterial bloom water (scum), and subsequent poisoning of piscivorous birds feeding on fish that had bioaccumulated the toxins through predation on contaminated zooplankton and aquatic invertebrates. Bioaccumulation of cyanotoxins in the food web (mussels, fish, waterfowl) has also been demonstrated for nodularins produced by *Nodularia spumigena* in the Baltic Sea[14].

Episodes of mortality associated with brevetoxicosis affected endangered Florida manatees (*Trichechus manatus latirostris*) and bottlenose dolphins (*Tursiops truncatus*) that had consumed toxic seagrass and fish, respectively[15]. Southern sea otters (*Enhydra lutris nereis*), a threatened species, were reported to have died of cyanotoxicosis in the Pacific coast of California[16]. Mortality of marine mammals caused by phycotoxicosis has not been reported in Europe.

ENVIRONMENTAL FACTORS

Planktonic (pelagic) cyanobacterial blooms are characterized by water discoloration and formation of a blue or green surface scum. Blooms occur in eutrophic waters rich in mineral nutrients (phosphorous, nitrate) during summer or autumn, when water temperature is high and light conditions favourable for photosynthesis. Cyanobacteria are slow-growing compared with other competing planktonic microalgae, and require stagnant water to proliferate in large numbers. They have, however, the advantage of needing less light (accessory photosynthetic pigments) than other microalgae, allowing them to outcompete other species under turbid water conditions. Under stagnant and turbid water conditions one or a few species of cyanobacteria may completely dominate, and if the dominating species include one or several toxin-producing cyanobacteria, intoxication events may occur (Figure 41.1). Toxic cyanobacterial blooms occur mostly in freshwater ecosystems (lakes, ponds, rivers) and have been reported from a large number of European countries[17]. In Europe, some 53% of fresh water lakes are considered eutrophic and thus constitute potential sites of toxic blooms[18]. Brackish (marine) ecosystems may also be eutrophic and develop



FIGURES 41.1 Mixed cyanobacterial bloom of *Microcystis aeruginosa* (**A**) and *Anabaena flos-aquae* (**B**) occurring in lake Østensjøvannet (**C**), Oslo, Norway in July 1989. The bloom was associated with mass mortalities in ducks, geese and gulls. Some birds showed microscopic liver lesions consistent with hepatotoxicosis, whereas others showed less distinct liver lesions. Thus, the bird die-off may have been associated with both hepatotoxic and neurotoxic cyanotoxins. Photos: Olav M. Skulberg, Norwegian Institute of Water Research.

algal blooms, e.g. the Baltic Sea[6]. Although some water sources are naturally eutrophic, in many others the excess nutrient levels are caused by human activity, resulting from wastewater discharges or run-off from agricultural areas.

Animals are most often exposed to toxic amounts of algae as a result of drinking water or scum from a lake or pond in which prevailing winds have concentrated the bloom. Cyano-intoxication may also occur following water consumption from smaller water sources such as waterholes, small dams and dugouts containing stagnant water, in which thick coats of algae may form[19]. There are indications that animals do not avoid cyanobacterium-contaminated drinking water[20].

Intoxication may also be associated with benthic aggregations (coherent mats) of cyanobacteria. During periods of high photosynthesis, production of oxygen bubbles may increase the buoyancy of the mats, causing them to tear loose and rise to the surface, where if ingested, they may cause animal intoxication. This type of cyanotoxicosis has been reported in domestic animals in Scotland[21] and Switzerland[22]. Another route of intoxication is through consumption of organisms (fish, molluscs, zooplankton) that have themselves fed on cyanobacteria or otherwise bio-accumulated cyanotoxins[2]. In this way, mortality can occur weeks after the disappearance of the bloom.

PATHOGENESIS AND PATHOLOGY

Cyanobacterial toxins are released following algal cell destruction in the animal's stomach, but may also exist free in the water body as a result of cyanobacterial cell autolysis. Microcystins (and nodularins) are rapidly absorbed in the intestinal tract and transported to the liver, which constitutes the target organ[23]. It has also been demonstrated that microcystins may be efficiently absorbed via the respiratory route. In the liver, microcystins and nodularins function as potent inhibitors of protein phosphatases 1 and 2A, leading to actin filament alterations and disruption of the hepatocyte cytoskeleton, as well as damage to the sinusoidal endothelium[24].

The toxicokinetics of the neurotoxic anatotoxins have not been described. However, based on the peracute clinical course of this intoxication, intestinal absorption must be rapid. Anatoxin-a and homoanatoxin-a act as potent cholinergic agonists at nicotinic acetylcholine receptors in neurons and neuromuscular junctions, leading to respiratory paralysis. Anatoxin-a(s) is an irreversible acetylcholinesterase inhibitor, which leads to increased concentrations of acetylcholine in the synapse, resulting in neuromuscular blockage and respiratory arrest. The anatoxins do not cause cell-morphological changes.

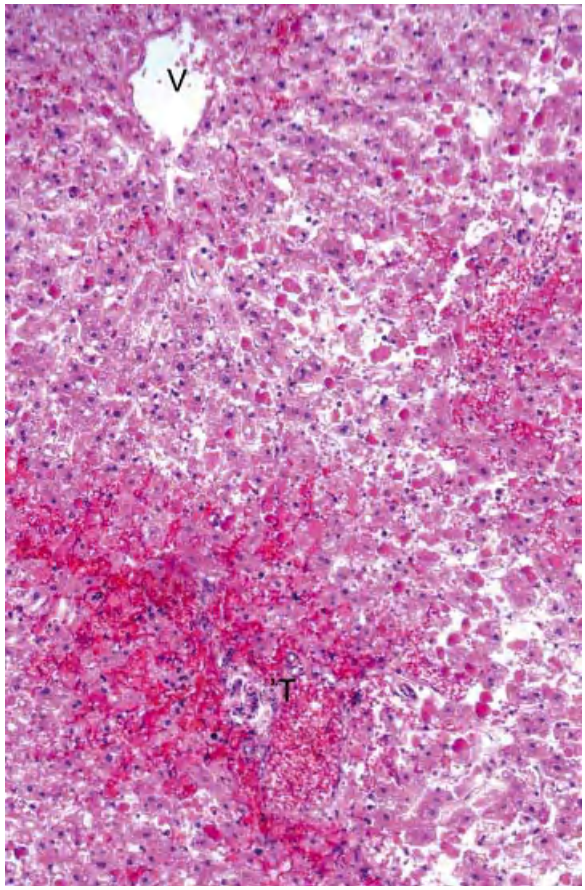


FIGURE 41.2 Liver from a microcystin-intoxicated roe deer, showing diffuse hepatocyte dissociation, degeneration and necrosis and perisinusoidal haemorrhage. T = portal tract; V = central vein. Haematoxylin-eosin stain. From Handeland & Østensvik, 2010[8].

Animals found dead after drinking toxic bloom may show evidence of green- or blue-coloured cyanobacteria on the hair or feathers. Gross pathological findings reported following acute microcystin and nodularin intoxication include a pale, swollen and sometimes haemorrhagic liver, as well as ascites and widespread small haemorrhages. Microscopic liver lesions include hepatocellular dissociation, degeneration and necrosis, as well as areas of perisinusoidal haemorrhage or massive bleedings (Figure 41.2). No specific gross or microscopic lesions are found in animals dying from anatoxin intoxications.

CLINICAL SIGNS

Clinical signs of cyanotoxicosis may be linked to either hepatotoxic or neurotoxic effects. However, it is important to keep in mind that mixed cyanobacterial blooms, with multiple associated toxins, are common, and may complicate the clinical picture. Moreover, the effect of interactions between the different toxins involved is not clearly understood. Thus, the clinical signs resulting from naturally occurring intoxication events may be complex.

The clinical course of fatal microcystin and nodularin hepatotoxicoses is acute, with death normally occurring within a few hours post-exposure. Clinical signs include general weakness, vomiting, diarrhoea, pallor and shock. Animals living beyond a few hours post-exposure may develop hyperkalaemia, hypoglycaemia, nervousness, recumbancy and convulsions.

The course of fatal neurotoxicoses caused by anatoxin-a, homoanatoxin-a and anatoxin-a(s) is peracute, with death due to respiratory paralysis. Death normally occurs within a few minutes to a few hours post-exposure. Clinical signs following anatoxin-a and homoanatoxin-a ingestion include rigidity, muscle tremor, cyanosis and paralysis and, in birds, opisthotonos. Clinical signs following anatoxin-a(s) intoxication include tremor, inco-ordination, and convulsion, as well as excess salivation ('s' stands for salivation), lacrimation and urinary incontinence.

DIAGNOSIS

Specific diagnosis of cyanobacterial toxicosis has been difficult, as a result of the lack of appropriate diagnostic procedures, and remains challenging. In the past, diagnosis was based upon algal bloom identification and disease history, possibly in combination with toxin analyses of algae and bioassays in experimental animals. Toxin production is, however, not constant during a bloom, and many algal blooms, even when dominated by species known to be capable of producing toxicosis, may not be hazardous.

A definitive diagnosis of cyanotoxicosis should be based upon a thorough *post mortem* examination, followed by demonstration of high tissue cyanotoxin levels. The *post mortem* examination should aim to demonstrate the presence of typical liver lesions by cyanobacterial hepatotoxicosis or absence of morphological lesions by cyanobacterial neurotoxicosis, as well as consideration of other possible aetiologies. One important differential diagnosis in avian events is botulism, a common cause of waterfowl death in eutrophic waters. Methodologies for the determination of cyanotoxins in *post mortem* tissues include liquid

chromatography and mass spectrometric systems, as well as enzymatic assays and immunoassays[3]. Liquid chromatography and mass spectrometric methods have been employed on tissue samples for quantitative analyses of specific cyanotoxin accumulation or intoxication in wild birds and mammals in Europe[8,14,25]. Commercial kits are also available for determination of cyanotoxins (microcystin) in the tissues and have been used to confirm cyanotoxicosis in birds[12,13].

MANAGEMENT, CONTROL AND REGULATIONS

The temporary management of phycotoxicosis is based on mitigation and/or removal of the bloom, by applying algicides, by physical removal of surface scums and by mixing of the water column. However, possible adverse effects on the ecosystem have to be considered. Exposure to toxic water, by domestic animals for example, should be avoided whenever possible.

Process-based and statistical models have been developed to predict cyanobacterial bloom occurrence, and their concentration and distribution in water bodies can be monitored.

Anthropogenic input of nutrients into sensitive waters largely affects the rate of blooming of harmful algae. Effective long-term control and management of blooms requires reducing eutrophication, including constraints of human-made supply of nutrients, mostly nitrogen and phosphorus. The European Commission, Institute for Environment and Sustainability (IES) Global Environment Monitoring Unit has developed two eutrophication indices, which allow the assessment of the coastal and marine ecosystem's status and physical sensitivity to eutrophication, as part of the Research Action 2121 (Monitoring and Assessment of Marine Ecosystems)[26].

PUBLIC HEALTH CONCERN

Mass populations of toxic cyanobacteria present important environmental and human health hazards. Phycotoxicoses in humans are mainly associated with consumption of contaminated shellfish and include, among others, paralytic shellfish poisoning (caused by a saxitoxin), neurotoxic shellfish poisoning (caused by brevetoxin), and amnesic shellfish poisoning (caused by domic acid). Algal dermatotoxins and gastrointestinal toxins also affect humans. Hepatotoxins, such as microcystin, cause major liver damage in humans. Nodularin is a carcinogen. Cases may occur simultaneously in humans and animals when both are exposed to the same source.

SIGNIFICANCE AND IMPLICATIONS FOR ANIMAL HEALTH

Phycotoxicosis may cause important mortality events in wild animal populations, in particular in wild waterfowl.

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CHAPTER

42

MYCOTOXICOSIS

CLAUDIA CAFARCHIA, KJELL HANDELAND AND TURID VIKØREN

INTRODUCTION

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Mycotoxicoses are acute or chronic intoxications caused by mycotoxins produced by moulds (fungi) that contaminate plant materials.

More than 100 fungal species are known to produce mycotoxins. Many of these fungi belong to the genera *Aspergillus*, *Penicillium* and *Fusarium*. The growth of the fungi and the production of toxins occur in the presence of a suitable substrate (mainly starch), moisture (water activity = 0.90) and temperature (from 4 to 31°C, depending on the type of mycotoxin).

Examples of mycotoxins of great significance for both public health and agro-economic perspectives include aflatoxins, ochratoxins, trichothecenes, zearalenone, fumonisins, patulin and ergot alkaloids. Feedstuffs most frequently contaminated with mycotoxins include nuts, oilseeds and grains.

Some species of fungi can produce several mycotoxins, which may differ in their biological activities, resulting in complex clinical effects. The severity of the clinical signs depends upon the length of exposure to the contaminated feed and the quantity of mycotoxins ingested.

The diagnosis may prove challenging because the clinical signs are often similar to those caused by other agents or pathogens. Presently, the European Community has imposed limits or guidance levels for several mycotoxins in human food and feed for domesticated animals[1]. Conversely, no limits are indicated in the case of feed for wild animals, thus allowing contaminated grain to be fed to wildlife.

However wildlife should not be fed, or should be prevented having access to, grain with levels of mycotoxins exceeding those recommended for use in domestic animal food. When non-specific signs of ill health are seen in wildlife (both mammals and birds) epidemiologically associated with arable fields or consuming grain, the possibility of mycotoxicosis should be considered, even though reported cases of toxicosis in European wild species are rare. Wherever possible, grain derived from fields in which wildlife is present should be carefully checked for mycotoxins by specialized laboratories. In cases of heavily contaminated fields, deep ploughing can effectively remove the grain from access by most wildlife species.

Handling mycotoxin-poisoned ill or dead animals does not present a risk for human health. However, meat or

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TABLE 42.1 Mycotoxins and toxicity in animals.

Mycotoxins	Fungal spp.	Animal spp. affected	Toxicity	Limits for total mycotoxins in food and feed
Ergot alkaloids	*Claviceps purpurea*	Cattle, sheep, deer, horses, pigs, poultry	Neurotoxicity, vasoconstriction	0.1% (feed with cereals – European Directive 2002/32/EC)
Ochratoxins A, B, C	*Aspergillus ochraceus*, *Penicillium viridicatum*	Pigs and poultry	Degenerative renal changes, fall in egg production	From 3 to 50 µg/kg (cereals and cereal products)
Patulin	*Penicillium expansum*	Laboratory animals	Teratogenic effects in chicken embryos, exophthalmia	From 10 to 50 µg/kg (baby food and fruit products)
Satratoxin, roridin, verrucarin	*Stachybotrys atra*	Horses, cattle, sheep, pigs	Cytotoxicity, coagulopathy, immunosoppression	NR
Sterigmatocystin	*Aspergillus nidulans*, *Aspergillus versicolor*	Rodents, cattle, monkeys, fish	Carcinogenicity, mutagenicity, teratogenicity	NR

NR – not reported

products from animals known to have died of mycotoxicosis should not be consumed.

Until now, intoxications by only two types of mycotoxicosis (aflatoxicosis and fusariotoxicosis) have been reported in free-ranging animals (wild birds).

Although there are reports of mycotoxin detection in food and feed in many European countries (Germany, France, Italy, Greece, Belgium) the occurrence of cases of poisoning in European wild animals appears to be rare or unreported.

In this chapter aflatoxicosis and fusariotoxicosis are described; see Table 42.1 for the toxicity associated with other relevant mycotoxins.

AFLATOXICOSIS

Aflatoxicosis occurs following the ingestion of aflatoxins, a group of difuranocoumarins produced by toxigenic strains of *Aspergillus flavus* or *Aspergillus parasiticus*. The major aflatoxins are B1, B2, G1 and G2 and aflatoxin M1, an hydroxylated metabolite found primarily in animal tissues and fluids (milk and urine) as a metabolic product of aflatoxin B1.

Aspergillus spp. are saprophytic organisms, which are disseminated via their conidia and carried by wind or insects to the growing crop. Initially fungi can grow in grains and can form sufficient moisture from metabolism to allow further growth and the production of mycotoxins. Grain stored under high moisture/humidity (>14%) at warm temperatures (>20°C) and/or inadequately dried can potentially become contaminated.

Aflatoxicosis has been documented worldwide and in many animal species, with a considerable variation in susceptibility between species and within age groups. Among domestic animals, calves, pigs and dogs are highly sensitive to toxic effects; among avian species, ducklings and turkey poults are the most sensitive, whereas chickens are the most resistant[2]. In general, birds are more susceptible than mammals, and young birds are more susceptible than adult birds. Mortality caused by the exposure to aflatoxins has been reported in free-ranging birds, mainly from North America, including a variety of duck species (mallard (*Anas platyrhynchos*), lesser scaup (*Aythya affinis*), gadwall (*Anas strepera*) and blue- and green-winged teal (*Anas carolinensis*)), snow geese (*Chen caerulescens*) and sandhill cranes (*Grus canadensis*). Aflatoxin concentrations of 800 mg/kg have been found to produce subclinical hepatic injury in young white-tailed deer (*Odocoileus virginianus*)[3].

The aflatoxins are primarily hepatotoxic, immunosuppressive, carcinogenic, teratogenic and mutagenic. Aflatoxicosis can occur in two forms (acute and subacute) depending upon the concentration of toxins in food and length of exposure. The subacute form is usually associated with prolonged exposure to low concentration of toxin and is generally characterized by a slowly developing illness and reduced growth rate. Chronic exposure can also result in a shrunken, fibrous liver with regenerative nodules, or it may result in tumours in birds. Immunosuppression can

also occur in affected groups of animals. Acute aflatoxicosis is associated with a high concentration of toxin, and clinical signs are characterized by ataxia, opisthotonos and death. Hepatopathy and cutaneous haemorrhages have been documented in young birds older than 3 weeks of age. Liver haemorrhages can occur as multiple focal areas, or as diffuse areas. Haemorrhages in many organs and excessive fluid in the thorax and abdomen may be observed.

The diagnosis is difficult because clinical signs are not specific. Tissues can be examined for gross lesions and typical microscopic lesions (e.g. liver necrosis and/or proliferation of lesions in the bile ducts). Measurement of aflatoxin levels in ingesta and tissues collected from affected animals and from grain are important for confirming the diagnosis. Aflatoxin intoxications can be prevented by appropriate measures, particularly the careful handling of grains and feed, especially following harvesting and during storage. Treatment with ammonia gas at high temperature and pressure has been used to detoxify contaminated batches of feed, whereas addition of hydrate sodium calcium aluminosilicate to feed has been reported to reduce aflatoxin toxicity.

FUSARIOTOXICOSIS

The disease is caused by toxins produced by fungi belonging to the genus *Fusarium*, which infect cereals in the summer and autumn while crops are in the field. These toxins can be produced at relatively cool temperatures and can reach high levels during storage, where conditions for fungal growth and mycotoxin formation are favourable. Usually moisture concentrations >14% and temperatures ranging from 6 to 24°C are ideal for the production of fusariotoxins. Corn and small grains such as wheat, oats, barley and rye are frequently contaminated.

Fusariotoxins of great socio-economic importance include zearalenone, trichothecenes and the fumonisins.

Zearalenone is produced by *Fusarium graminearum* or *Fusarium culmorum*. Effects of intoxication by zearalenone include precocious development of mammae and other oestrogenic effects in young gilts and prepucial enlargement in young boars. Zearalenone is thought to bind to oestrogen receptors, thus leading to hormonal changes. Swine are significantly affected, together with cattle and poultry and laboratory rodents. Effects of zearalenone fed to sows during gestation can include weak piglets and reduced litter size. Levels of 0.5 to 1.0 μg/g of dietary zearalenone have been associated with the latter effects, whereas hyperoestrogenism has been associated with dietary levels of 1.5 to 5 μg/g[4].

Trichothecene comprises more than 50 different toxins, with deoxynivalenol (DON) being the commonest one followed by T-2 toxin. DON, also known as vomitoxin, is produced principally by *Fusarium graminearum*[4]; this toxin is immunosuppressive and may cause inflammation and tubular damage in the animal kidney. Swine are the species most frequently affected. Levels above 1 μg/g are considered potentially harmful to these animals. Conversely, birds seem to be less sensitive to intoxication by DON. In particular ducks ingesting grain containing moderate levels of DON (5.8 ppm) for ~14 days do not show any adverse effects, whereas broiler chickens fed a diet containing 82.8 ppm for ~27 days show small erosions of the gizzard mucosa[5].

T-2 toxin is produced by *Fusarium sporotrichioides*. The major effect of T-2 toxin is the inhibition of protein synthesis, followed by a secondary destruction of DNA and RNA synthesis. This results in an impaired function of the actively dividing cells such as those lining the gastrointestinal tract, skin, lymphoid and erythroid cells and can lead to decreased levels of antibodies and cytokines[4]. The clinical signs of disease include weight loss, bloody diarrhoea, dermal necrosis or beak and mouth lesions, haemorrhage and decreased production of milk and eggs. Yellow caseous plaques, occurring at the margin of the beak, mucosa of the hard palate, and angle of the mouth and tongue, represent typical oral lesions in poultry. These lesions can occur at dietary levels of 4 mg/kg after 1 week, 0.4 mg/kg after 7 weeks.

Fumonisins are a group of mycotoxins produced primarily by *Fusarium verticillioides* and *Fusarium proliferatum.* Horses are most frequently affected and develop liquefactive necrosis of the white matter in the cerebrum (leucoencephalomalacia). Lung oedema in swine can also occur. Other manifestations such as liver disease, and tumours of the liver and kidney, have been reported experimentally using rodents. Experimentally, poultry and cattle have shown a much lower sensitivity to the toxic action of fumonisins when compared with horses and pigs[6]. Regardless of the effects on animals, the fumonisins are often responsible for liver toxicity, and their major mode of action is by interference with the metabolism of sphingolipids.

Diagnosis of fusoriotoxicosis is challenging because clinical signs are not specific. The observation of appropri-

ate gross and microscopic lesions, and the detection of toxins in grains, forages or the ingesta of affected animals can help in formulating a diagnosis. The samples of choice for diagnosis should be frozen, as toxins are produced under cold temperatures. However, the diagnostic tests required to detect these toxins are complex and available only in a few specialized diagnostic laboratories.

GANGRENOUS ERGOTISM

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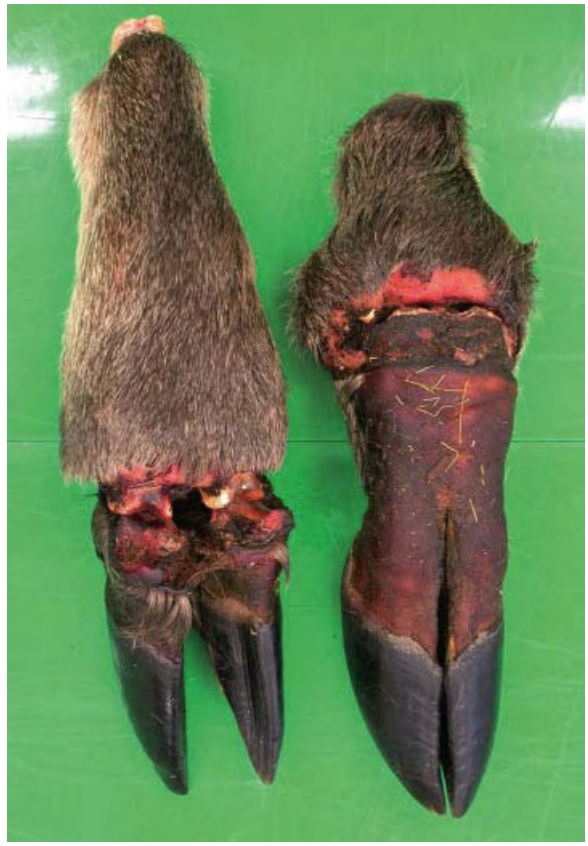
Ergotism refers to disease conditions associated with ingestion of ergots produced by the fungus *Claviceps purpurea*. Ergots occur in the seed heads of grasses and cereals during late summer, and contain pharmacologically active alkaloids that stimulate vasoconstriction, which in turn may lead to thrombosis and ischaemic necrosis (gangrene). Although gangrenous ergotism is a well-known disease in domestic mammals and poultry, reports in wildlife are restricted to single cases in roe deer (*Capreolus capreolus*) from England and Norway, and multiple moose (*Alces alces*) cases from Norway[7,8].

Although a browsing ruminant, Norwegian moose are known to eat the upper part of mature, tall grasses during the late summer and autumn. This grazing behaviour and high cutting level of the moose, compared with that of other wild cervids in Norway, suggests that moose are at greater risk of ingesting ergot-containing seed heads. Wild grasses are commonly infested with ergots in Norway, and their alkaloid content has been demonstrated through chemical analyses[9].

Affected moose display multiple distal limb (Figure 42.1) and ear-tip lesions. Whereas lesions observed in moose found during the autumn present as dry gangrene with a prominent demarcation line between viable and dead tissue, those found during the winter show advanced open lesions or loss of the distal part of limbs and ears.

A definite diagnosis of ergotism based on toxin identification is not possible because of the time lag between ergot ingestion and the manifestation of gangrenous lesions. Thus, the diagnosis is presumptive, relying on gross pathology – specifically, the presence of typical peripheral dry gangrene and exclusion of differential diagnoses such as trauma and bacterial infections. Affected animals found alive should be destroyed for animal welfare reasons.



FIGURES 42.1 Limbs of a moose calf, showing gangrenous phalanges with partial sloughing through the physis of the metacarpus (right) and at the proximal interphalangeal joint (left). Photo: Norwegian Veterinary Institute.

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SECTION

4

Prion Infections

CHAPTER

43

TRANSMISSIBLE SPONGIFORM ENCEPHALOPATHIES

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Transmissible spongiform encephalopathies (TSE), or prion diseases, are a group of infectious neurodegenerative diseases caused by a transformed host protein. These transformed proteins are called prions (proteinaceous infectious particles), a novel type of infectious agent that is devoid of nucleic acid.

In animals TSE include bovine spongiform encephalopathy (BSE), atypical BSE, scrapie, atypical scrapie or Nor98, chronic wasting disease (CWD), transmissible mink encephalopathy (TME), feline spongiform encephalopathy (FSE); in humans TSE include, among others, Creutzfeldt–Jakob disease, with the sporadic (sCJD), variant (vCJD), iatrogenic and familial subtypes.

AETIOLOGY

The cellular prion protein (PrP^{C}) is a glycosylphosphatidylinositol-linked copper-binding membrane protein of approximately 220 amino acid residues typically in α-helical conformation. The cellular prion protein is a normal cell surface protein of neurons and other cells encoded by a unique *Prnp* gene; it is highly conserved across diverse species. The disease-causing isoform of PrP^{C} is named PrP scrapie (PrP^{Sc}), and the terms PrP^{CWD} and PrP^{BSE} are used to denote the source. This abnormal relatively protease-resistant isoform of the PrP protein is rich in β-pleated sheets. PrP^{C} is converted into PrP^{Sc} during a process of conformational change, which results in replacement of some of the α-helical structure by β-sheets. This form can adopt a fibrillar aggregated structure, which is a feature of many of the deposits observed in the brain of animals with prion diseases. This post-translational alteration of PrP^{C} into the pathogenic infectious isoform, PrP^{Sc}, is considered to be the principal molecular basis of TSE.

The 'protein only' hypothesis proposed that PrP^{Sc} was the cause of prion diseases, that it was infectious on its own and that it replicated by interacting with the cellular prion protein of the host so that PrP^{C} would undergo a conformational conversion into $PrP^{Sc(1)}$. This proteinase K (PK)-resistant PrP^{Sc} accumulated in the tissues, as it could not be degraded as the normal cellular prion protein[1] and became the most effective marker of prion infection. The fraction resulting from the treatment of PrP^{Sc} with PK is termed PrP^{res} ('res' meaning resistant) and most diagnostic methods are based on its detection. PrP^{Sc} cannot be inactivated with formalin, is highly resistant to traditional sterilization methods such as autoclaving and disinfecting and it has an extraordinary environmental persistence. The BSE agent, like all the human TSE agents, is categorized as a Hazard Group 3 pathogen[2], but as there is no

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evidence of airborne transmission, full containment Level 3 is not required.

Biochemically, the properties of PrP^{Sc} appear to be determined by the three-dimensional shape of the molecule, changes to which could result in different forms, or 'strains'.

EPIDEMIOLOGY

GEOGRAPHICAL DISTRIBUTION AND HOSTS

The first naturally occurring TSE to be recognized was scrapie, with descriptions dating back to the 18th century. The susceptibility of sheep to scrapie has been shown to be heavily dependent on the *Prnp* genotype of the host. Programmes for selective breeding for scrapie resistance have been implemented in many countries as part of a control strategy and current EU control policies advocate culling on a selective genotype basis. Nor98, or atypical scrapie, is a novel TSE type first recognized and described in Norway in 1998[3] and later identified as sporadic cases in most European countries as well as in the USA, Canada and New Zealand.[4] It has been postulated to be a spontaneous form of TSE in animals. Nor98 affects old sheep and goats of more scrapie-resistant genotypes but not of the most susceptible genotypes. No cases of scrapie or atypical scrapie have been reported in wild Caprinae or Ovidae in any continent.

BSE was first identified in the UK in 1986[5] and was propagated within cattle by recycling meat and bone meal from infected cattle. This resulted in a large-scale epidemic in the mid 1990s, which has to date affected approximately 184,600 cattle in the UK and more than 7,900 cases outside the UK, including 13 European member countries, Japan, Canada and the USA. In 1996, vCJD in humans was shown to be caused by BSE, probably by consumption of contaminated beef products. In the EU, the BSE concern led to the introduction of a series of measures aimed at its eradication, including large-scale testing in EU member states.

BSE is unique among the TSE in that it has extensively crossed the species barrier. Besides the human form, vCJD, natural BSE infection has occurred in seven species from the family Bovidae, four Felidae and four non-human primates in zoological collections[6–8]. FSE also has been shown to be a BSE infection of domestic cats[9]. Sheep and goats orally inoculated with BSE develop a TSE pathologically and clinically similar to scrapie[10]. It is also well established that small ruminants in Europe received feed supplements containing meat and bone meal over the time period that cattle were exposed. This finding raised the concern that possibly cases diagnosed as scrapie could actually be BSE infection in sheep, and that endemic scrapie may mask BSE infections within the sheep population, and it questioned the safety of consuming sheep meat. This resulted in obligatory large-scale active surveillance of small ruminants in the EU, implemented in 2002[11]. Since 2005, all the TSE cases in small ruminants in the EU are required to undergo discriminatory testing to determine if the TSE is scrapie or BSE [11]. Up until October 2011 only one TSE-positive case in a small ruminant, a domestic goat in France, has been confirmed to be BSE[12].

Atypical BSE is a novel presentation of TSE in old cattle, with unusual molecular and pathological features. Two main types have been identified: BSE-L and BSE-H. 'L' stands for low and 'H' for high, making reference to the differences in the molecular size of the PrP^{Sc} unglycosylated protein band seen on western blot (WB) assays compared with that of BSE. More than 60 cases of atypical BSE have been detected globally. It has been hypothesized that atypical BSE is a spontaneous form of TSE. No cases of BSE or atypical BSE have been reported in free-ranging wildlife.

TME was at first recognized as a food-borne disease of ranch-raised mink (*Mustela vison*) in the USA and was thereafter detected from time to time in several other countries, but it has not been detected in wild or farmed mink in Europe.

CHRONIC WASTING DISEASE

CWD was first recognized as a clinical entity of unknown cause in cervids in 1967 in captive mule deer (*Odocoileus hemionus*), derived from free-living populations in Colorado, USA[13]. A similar syndrome was identified in Wyoming, USA, in 1978 and in the same year spongiform encephalopathy was found in affected animals from Colorado[13]. Since its first recognition as a TSE, the geographical distribution and number of cases of CWD in free-ranging and captive deer herds within North America has increased significantly. As far as it is known, CWD is still restricted to North America, with the exception of a report in South Korea in captive elk imported from Canada. In North

America, CWD (*Cervus elaphus nelsoni*) occurs in 17 states in the USA and in the provinces of Saskatchewan and Alberta in Canada. However, it is likely that if CWD occurs in countries or areas not known to be affected it will not be identified unless specific surveillance and testing for CWD is done.

Hosts

Chronic wasting disease has been identified in the following cervid species in North America: mule deer, white-tailed deer (*Odocoileus virginianus*), Rocky Mountain elk (*Cervus elaphus nelsoni*) and Shira's moose (*Alces alces shirasi*). CWD is not known to affect other species naturally, including carnivores and scavenger species. CWD has been transmitted to several other species, including cattle, by intracerebral inoculation, but oral experimental inoculation with CWD did not result in infection[14,15].

In Europe, three species from the family Cervidae have been considered as possibly susceptible to CWD owing to their taxonomic relation to the known target species. These are the European red deer (*Cervus elaphus elaphus*), the European moose (*Alces alces alces*), and the white-tailed deer, which were introduced into Finland from North America in the 1930s.

Few experimental studies have been conducted to assess the susceptibility of European cervids to TSE. In a study in Canada, CWD was transmitted to red deer (*Cervus elaphus elaphus)* of the same subspecies as the European red deer by oral inoculation, indicating likely susceptibility of red deer in Europe[16]. In the UK, red deer (*Cervus elaphus elaphus)* were inoculated with BSE orally or intracerebrally. This resulted in a TSE with different immunohistochemical and biochemical properties from CWD[17,18].

As in other TSEs, susceptibility to CWD also appears to be related to polymorphisms in the *Prnp* gene. A study on *Prnp* variability was conducted on 715 wild animals in Europe, including European red deer (*Cervus elaphus*), roe deer (*Capreolus capreolus*) and chamois (*Rupicapra rupicapra*). In the red deer, the study identified a number of polymorphisms giving rise to 12 haplotypes. One of the haplotypes was identical to the *Prnp* of Rocky Mountain elk. In chamois one silent mutation at codon 119 was found. In roe deer, no single nucleotide polymorphisms were detected[19]. The study concluded that the species investigated had a *Prnp* genetic background compatible with susceptibility to TSE.

THE EUROPEAN CWD SURVEY

A survey for CWD and other TSE in farmed and wild cervids in the EU was set up by the European Commission[20]. This was the first time a European-wide survey for TSE was conducted in wild animals. Until then, little information was available on TSE diagnosis in cervids in Europe and the occurrence of CWD could not be known without specific testing. Also, it was known that farmed cervids in Europe had been fed compound feed (containing ruminant protein); therefore, the risk for BSE exposure could not be ruled out[21]. The design of the survey was based on European Food Safety Authority (EFSA) opinion in 2004[21] which at that time indicated that in Europe red deer and white-tailed deer were the only two species considered most likely to be susceptible. North American moose were found to be susceptible to natural CWD first in 2005[22], and therefore the susceptibility of European moose was postulated only after the survey had been designed[20].

All EU member states were required to test as many farmed and free-living cervids as possible from all species and older than 18 months, of the following risk categories: cervids with signs of disease (such as neurological signs or poor body condition); cervids fallen (found dead) or culled and road-injured or road-killed cervids. Additionally, member states with large populations of farmed or free-living red deer, and Finland, the only European country with known white-tailed deer populations, needed to test a minimum number of healthy animals harvested by hunting.

All rapid tests approved at that time for BSE monitoring could be used for screening for CWD/TSE in European cervids and immunohistochemistry (IHC) or WB would be used for confirmation. Even though EFSA recommended testing of both brainstem and lymph node[21], the European Commission decided to conduct the survey testing brainstem samples only, probably as a result of practical and logistic reasons, as most member states had well-developed routines for brainstem sampling based on the experience of BSE and scrapie surveillance.

The survey was conducted largely during the years 2007, 2008 and 2009, with some samples collected during 2006 and 2010. On the whole, 14 member states and Norway tested 3,274 farmed cervids, including the following numbers and species: 2,781 red deer, 348 fallow deer (*Dama dama)*, 44 reindeer (*Rangifer tarandus tarandus)*, 3 sika deer

(*Cervus nippon)*, 4 moose and 94 cervids of unknown species; and 21 member states and Norway tested 10,049 wild cervids, including 7,329 red deer, 1,089 roe deer, 611 white-tailed deer, 262 moose, 165 fallow deer, 30 reindeer, 2 wild forest reindeer (*Rangifer tarandus fennicus)*, 1 sika deer and 560 deer of unknown species. No TSE-positive cases were detected[20].

It was concluded that the results indicated that there was not a TSE epidemic in cervids in the EU. However, the survey had several limitations. These included, among others, difficulties in the collection of samples (not all member states achieved the required sample size), the limited number of potentially susceptible cervids tested, the fact that the sampling could not be assumed to be random and that the testing had been done in brainstem only (not including lymph node), which may have affected the sensitivity of detection. Owing to the limitations explained above, and also taking into account that CWD spreads from and within clusters and that CWD susceptible cervids are present in Europe, it was concluded that occurrence of cases of TSE, particularly in remote areas and areas not sampled in the survey, may not be excluded[20].

Few other large-scale studies on the occurrence of CWD/TSEs in cervids have been conducted in Europe. A large survey was done in Germany from 2002 to 2005. In total, 7,056 wild cervids (red deer, fallow deer and roe deer) and 248 farmed cervids were tested with a rapid test on samples of brainstem (obex) and medial retropharyngeal lymph nodes. Eighty-seven per cent of the administrative districts in Germany were sampled, including different hunting areas and including as many deer populations as possible, attempting to identify potential clusters of CWD. No positive results were found, and it was concluded that TSE was unlikely to occur in free-ranging cervids in Germany, and if it occurred it was not extensively distributed[23].

TRANSMISSION

BSE is transmitted by the oral route. Horizontal or vertical transmission has not been detected; however, the offspring of clinical cases of BSE have shown an increased risk for BSE.

Scrapie is maintained and disseminated by lateral transmission. PrP^{Sc} may be demonstrated in the placenta of infected ewes, which is a source of infection for the offspring and other sheep in the flock.

CWD is contagious and is transmitted horizontally and through the environment. Pastures and enclosures contaminated by saliva and excreta and by carcasses of animals that died from CWD are the primary source of infection[24]. PrP^{CWD} binds with high affinity to clay constituents of soil and contamination persists for years[25]. PrP^{CWD} has also been demonstrated in water and can even be transmitted through bedding material and water from pens used by infected animals[26]. CWD is also transmitted by direct contact and transmission can occur among deer of the same species and of different species. CWD is easily transmitted among captive deer and elk concentrated in enclosures, and it is believed that artificial feeding and baiting facilitates transmission by concentrating animals.

In atypical scrapie and atypical BSE there are no indications of transmission. Only single sporadic cases with a wide geographical distribution occurred.

PATHOGENESIS AND PATHOLOGY

TSE in animals are mainly acquired by oral infection. During the early phase, PrP^{Sc} crosses the mucous membranes and is deposited in the tonsils, Peyer's patches and lymph nodes draining the gastrointestinal tract. Experimentally, the early involvement of lymphoid tissue in CWD has been observed as early as 6 weeks post-infection[27] and in natural scrapie infection in lambs from 3 months of age. PrP^{Sc}/PrP^{CWD} replicates in the lymphoreticular system, including the spleen and lymph nodes, during a prolonged phase of months to years. In TSE the kinetics of the infection and distribution of prions is affected by the host species and its *Prnp* genotype. In BSE and in some scrapie cases there is little involvement of the lymphoreticular system.

At later stages of TSE infection, PrP^{Sc} extends to the brain, where it progressively accumulates, causing fatal neurodegenerative changes. The mechanisms of transit of the PrP^{Sc} from the gastrointestinal tract or tonsils into the brain are not yet fully understood. Two routes of transit have been implicated: the haematogenous route and the retrograde axonal routes, involving fibres innervating lymphoid tissues or the autonomic nervous fibres of the digestive tract. The parasympathetic dorsal motor nucleus of the vagus nerve (DMNV) is the site of earliest involvement in the brain in scrapie and CWD and occurs before onset of clinical disease. In BSE, the first site of prion accumulation in the brain is the nucleus of the solitary tract. Both the DMNV and the solitary tract nuclei are located in the caudal brainstem, at the level of the obex. From these early

sites the infection extends to other parts of the brain, targeting specific locations, which depend on the type of TSE, host species, genotype and prion strain.

During late phases of infection, PrP^{CWD} is extensively distributed in the body and is present in tonsils, spleen, retina, skeletal muscle, fat, peripheral nervous system, adrenal glands, blood, saliva, lymph nodes, brain and spinal cord and antler velvet. PrP^{CWD} is shed in faeces, urine and saliva, mainly during terminal disease[28,29].

The TSE are characterized by two types of vacuolar lesions in the grey matter, affecting specific target nuclei/areas. One is vacuolation of neuronal processes, called neuropil vacuolation or spongiosis, and the other is intraneuronal vacuolation. The PrP^{Sc} accumulation in the brain is characteristic of TSE and can be detected at earlier stages than vacuolar lesions. No inflammatory reaction and no immune response appear to be developed against the pathogenic PrP, presumably because it is not perceived as foreign by the immune system. Amyloid plaques may occur in some TSE. No histological lesions are observed in lymphoid tissues in any of the TSE, even with advanced PrP^{Sc} accumulation.

CLINICAL SIGNS AND TREATMENT

In general, TSE are clinically manifested by neurological signs and loss of body condition.

Clinical signs of CWD characteristically appear after 1.5 years of age and are most frequent in animals 3 to 5 years old. The signs include behavioural changes, such as depression, aggression, somnolence and separation from other animals; signs of neurological disturbance such as paralysis, dysphagia, hind limb ataxia, head tremors and recumbency. Other signs are increased salivation, progressive weight loss, polyuria and polydypsia, and signs related to aspiration pneumonia[15].

CWD has a long incubation time; following oral experimental infection, the average time from infection to death is 23 months (with a range of 20–25 months). Most animals that test positive to CWD do not exhibit clinical signs[15].

DIAGNOSIS

The selection of diagnostic techniques for TSE depends on the purpose of the testing: screening, confirming TSE or typing a strain. For CWD it was stated by the US Department of Agriculture that the available tests could only be used as a surveillance tool and not for food safety purposes. Similarly, the EU has also approved TSE tests only for surveillance. Current TSE tests are not considered to be suitable for food safety control, because there is not enough knowledge on the infectious dose for natural TSE in animals and humans, on the PrP^{Sc} tissue distribution and infectivity, and on the stage in the incubation period at which the tests are able to detect natural infection.

The *post mortem* diagnosis of well-established TSE poses no major difficulties. However, tests applied to cases with minimal accumulation of PrP^{Sc} or at just about the detection limits of the test, such as during early-stage infection, may give false negative or inconclusive results. Screening tests with high sensitivity may either detect such cases as positive or may classify them as 'suspects', and their confirmation with another test may be more difficult and may result in discrepancies in results. Different test results may also derive from differences in protocols, especially for in-house developed IHC and WB techniques. Commercial tests for TSE have been validated and are officially approved. The EFSA evaluated data provided by companies producing tests for diagnosis of CWD and deduced that all the tests evaluated would be suitable for surveillance in Europe[30]. Direct comparisons of sensitivity and specificity of the tests are often not correct because of the high variation in the design of the validation studies.

DIAGNOSTIC SAMPLES

PrP^{Sc} accumulates in lymphoid tissues in TSE in deer, North American elk, sheep and goats at earlier stages than in the brain; therefore, sampling lymphoid tissue allows detection of early infection. However, in some cases, mostly in elk and in sheep with genotypes with no peripheral PrP^{Sc} accumulation, PrP^{Sc}/PrP^{CWD} is detected by IHC in the brainstem but not in single sections of either tonsils or retropharyngeal lymph nodes. This is probably due to the lower levels of accumulation of PrP^{CWD} in lymphoid tissues in early phases of infection, often focal and restricted to a few follicles. Therefore, the highest sensitivity of detection of CWD is achieved by testing both lymphoid tissue and brainstem at the level of the obex.

Brain

The medulla oblongata at the level of the obex is an optimum sample for diagnosing BSE, CWD and typical

FIGURE 43.1 Sampling of a moose for testing for CWD. A sample of brainstem (a piece of cerebellum is left attached) was obtained by introducing a disposable instrument in the foramen magnum. Photo: Bengt Ekberg, SVA.

scrapie because of its consistent early accumulation of PrP^{Sc} and vacuolar lesions. For BSE an appropriate sample for diagnosis should include the solitary and the trigeminal tract nuclei. For CWD and scrapie it is critical that the DMNV is represented, particularly to detect early or subclinical infections. For atypical scrapie, the trigeminal tract nuclei and particularly the cerebellum are required for confirmation. Brainstem samples are conveniently obtained by introducing a commercially available long spoon-shaped metal or disposable instrument with cutting edges, through the foramen magnum (Figure 43.1).

Lymphoid Tissues

Because of the convenience for sampling and the close association with the gastrointestinal tract, the medial retropharyngeal lymph nodes, the tonsils and rectal biopsies are the lymphoid tissues of choice for testing for CWD and scrapie. As PrP^{Sc} accumulates in lymphoid follicles, it is important that they are well represented in the sample; for example, in lymph nodes the cortex (which contains follicles) has to be included.

Testing tonsillar or rectal biopsies allows the *ante mortem* diagnosis of CWD and scrapie, even in pre-clinical stages of infection. The methods to obtain rectal biopsies are well developed, easily performed and allow sampling of several biopsies over different time intervals, without causing significant damage to the mucosa. Special disposable instruments are commercially available to obtain the rectal biopsies. As the number of lymphoid follicles in the recto-anal lymphoid tissues decreases with age, the diagnosis performs best in elk younger than 8.5 years[31].

Autolysis

The PrP^{Sc} is very resistant to degradation, and its detection is not significantly compromised by autolysis. However, the early accumulation of PrP^{Sc} in the brain is very restricted neuroanatomically, and the biggest threat to sensitivity in the autolysed tissues is the uncertainty as to whether the target areas were actually tested.

Diagnostic Methods

Most of the diagnostic methods for PrP^{Sc} detection in tissues rely on its protease resistance and identification by PrP antibodies.

To date, there are no reliable non-invasive tests to diagnose TSE in live animals. Progress has been made on the development of *in vivo* tests for urine or blood, but sufficient sensitivity and consistency have not yet been obtained. Even though some tests for live animals are already commercially available their performance is not yet well assessed. As immunological response to PrP^{Sc} is not developed, immunological tests cannot be used.

Rapid tests or screening tests are tests of high sensitivity, which can be completed usually in a few hours, and are suitable for high throughput testing, for example, for testing hunter killed populations. Most of them are based on enzyme-linked immunosorbent assay (ELISA) or WB methods, and many are commercially available, validated and approved for screening (see information about their application in the survey section above).

Confirmatory tests are tests of high specificity used to confirm clinical suspicion of disease, or positive or inconclusive rapid test results. In most countries, only the confirmed BSE and scrapie cases are considered to be positive. The methods that are recommended as confirmatory tests consist of histopathology, IHC, detection of scrapie-associated fibril (SAF) and WB.

Immunohistochemistry

IHC recognizes the presence of distinctive PrP^{Sc} deposition in the target areas or cells and is therefore the most specific test for TSE. IHC is usually done on formalin-

fixed samples. It applies a pre-treatment of the sections with formic acid and hydrated autoclaving for epitope demasking and to remove the normal PrP^{C}. Numerous anti-PrP monoclonal and polyclonal antibodies have been developed and many are commercially available. IHC has also been used as a screening test for CWD in automated high output systems. For brainstem samples, the target nuclei have to be well represented and recognized in the section. The early distribution of PrP^{Sc} in the lymphoid tissue is not homogenous; therefore, it is considered that when applying IHC a minimum number (usually 4 to 6) of follicles with germinal centres need to be examined to provide a reliable negative diagnosis.

In the early days histopathology and the detection of SAF by negative contrast electron microscopy were the only available tests for diagnosis of TSE. Today SAF is rarely used because of its low sensitivity compared with the rapid tests, but it may be a method of choice when only very autolysed or only formalin-fixed sample is available.

Strain Discriminatory Methods

In vitro methods to typify prion strains have been developed to differentiate BSE from scrapie in sheep. A recent study, albeit on a small number of samples, showed that it was also possible to discriminate between CWD in North American elk and white-tailed deer, and BSE and scrapie cases[32]. The methods are based on the different sensitivity of the prion strains to PK digestion. The precise basis for discrimination is the location of the N-terminal cleavage site for proteinase K digestion of PrP^{res} between BSE and scrapie. Although *in vitro* methods give a preliminary molecular description of the isolate, they cannot actually identify a strain. The ultimate typing of the prion strain is still determined by bioassay, most commonly in mice, as molecular characterizations are not fully specific.

MANAGEMENT, CONTROL AND REGULATIONS

The BSE concern prompted large-scale testing in Europe and elsewhere. For a prolonged period, European regulations required rapid screening for BSE on all cattle aged over 30 months slaughtered for human consumption. More than 87 million cattle have been tested since 2001. The TSE control measures implemented in the EU resulted in a marked decline in the number of cases and the regulations have been adapted accordingly for example by increasing the age limit of obligatory TSE testing of cattle to 72 months.

With the application of TSE screening rapid tests to large-scale testing of animals, and a battery of confirmatory and strain-typing methods, new atypical forms of TSE were identified in sheep and cattle. It is hypothesized that atypical TSE may represent spontaneous forms of disease related to old age, caused by mutations in the *Prnp* gene equivalent to sCJD in humans, rather than prion infections contracted horizontally or orally. It can be speculated that such spontaneous diseases could occur in other species, including cervids and other wild animals. If any type of prion disease is detected in a wild animal in Europe it will need to be confirmed and fully characterized. The confirmation is done by the EU reference laboratory for TSE[33].

In North America, surveillance of CWD includes targeted surveillance, testing of cervids with clinical suspicion, and hunter harvest-based surveillance – testing of healthy animals done on the heads submitted voluntarily by hunters. Additionally, road-killed cervids and cervids found dead are tested in parallel. CWD is contagious, is also transmitted through environmental contamination and migrates over large distances, so its eradication from free-living cervids is considered unlikely.

PUBLIC HEALTH CONCERN

The finding that vCJD, a new variant of CJD, was associated with PrP^{BSE} was alarming. More than 160 cases of vCJD have been diagnosed in the UK and smaller numbers in other countries. The public fear increased when likely iatrogenic vCJD transmission via blood products was reported[34]. However, as the EU-wide control strategies were successful and the number of BSE cases in Europe has decreased drastically, the risk for humans has also decreased considerably.

Fortunately, BSE in small ruminants was found to be only a rarity and therefore potential infectivity to humans of BSE from species other than cattle is no longer of major concern. The infectivity for humans and other animals of the atypical strains of TSEs in cattle and sheep is not fully established.

The BSE experience and the unknowns about TSE resulted in concerns about a potential food-borne zoonotic role for other prion diseases of animals, and CWD was an example. CWD as a possible cause of CJD has been questioned, but fortunately, a higher incidence of CJD in hunters, their relatives and venison consumers has not been found. It is reassuring that until today, no human TSE infection acquired through consumption of venison or sheep meat has been shown. Still, caution through human avoidance of consumption of TSE-infected products is recommended[35].

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SECTION

5

Appendices

Appendices compiled by J Paul Duff, Anna Meredith, Javier Millán and Marie-Pierre Ryser-Degiorgis with help from text authors.

Notes

The Appendices and Tables summarize some of the information in the text and are intended to be of use to diagnosticians, field investigators and epidemiologists.

The information summarized here is not intended to be comprehensive; it is selective, to give general guidance.

Infectious Diseases of Wild Mammals and Birds in Europe, First Edition. Edited by Dolores Gavier-Widén, J. Paul Duff, and Anna Meredith.

APPENDIX

1

SOME WILDLIFE RELATED EMERGING DISEASES (WIREDS) IN EUROPE

Name of disease	Species of wildlife affected	Notes on possible sources of emergence or if disease probably historically present but not detected due to lack of surveillance (a) anthroprogenic (not domesticated animals); (d) infection from domesticated animals; (ls) lack of surveillance; (unkn) unknown (mutational); (ow) infection from other wild species
VIRAL DISEASES		
Hedgehog herpesvirus infection	hedgehog	ls
Cervid herpesvirus infections	red deer and reindeer	unkn
High path avian influenza	mute swans, other waterbirds	unkn mutational
Pigeon paramyxovirus infection	pigeons, other species	ow
Rabbit haemorrhagic disease	European wild rabbit	unkn mutational and d
European brown hare syndrome	brown and mountain hare	unkn mutational, ls
Calicivirus infection of seals	seal species	unkn
European bat lyssavirus infection	*Myotis* sp. bats	ls
Morbillivirus infections in aquatic mammals	common seal	unkn, ow?
Canine distemper virus	red fox primarily	unkn
Blue tongue disease	roe deer	d and a
West Nile fever	raptors, magpie	unkn
Usutu virus infection	blackbird, owls	unkn
Pestivirus infection of chamois	Pyrenean chamois	d
Aleutian disease	mink,otter,	d?
Porcine parvovirus infection in boar	wild boar	d?
Squirrelpoxvirus disease	red squirrel	ow + a
Myxomatosis	European rabbit	ow + a
Adenovirus infection of squirrels	red squirrel	ls
Feline leukaemia	Iberian lynx	d
Hantavirus	voles	ls

(*Continued*)

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Name of disease	Species of wildlife affected	Notes on possible sources of emergence or if disease probably historically present but not detected due to lack of surveillance (a) anthroprogenic (not domesticated animals); (d) infection from domesticated animals; (ls) lack of surveillance; (unkn) unknown (mutational); (ow) infection from other wild species
Ljungan virus	voles	ls
Hepatitis E	wild boar	d?
African swine fever	wild boar	d
BACTERIAL AND FUNGAL DISEASES		
Bovine tuberculosis	badger, deer, wild boar	d
Brucella in aquatic mammals	several aquatic mammal species	ls
Borreliosis	several species including humans	ls + unkn?
Salmonellosis	passerine birds	d? + a?
Avian botulism	waterbirds	unkn
Necrotic enteritis	swans and geese	a
Suttonella sp. infection	paridae/tits	ls
Adiaspiromycosis	otters, other mammals	ls
Gangrenous ergotism	moose	ls

Notes

1. WiREDS here defined as an emerging disease in Europe or a significant change in a disease (WiREDS = Wildlife Related Emerging DiseaseS).
2. The majority of the diseases selected cause significant mortality in wildlife, domesticated animals or human populations.

APPENDIX

2

SELECTED ZOONOTIC PATHOGENS WITH EUROPEAN WILDLIFE RESERVOIRS/HOSTS

Pathogen	Primary wildlife host	Clinical disease in European wildlife?	Human disease acquired from European wildlife?
VIRAL PATHOGENS			
Avian influenza viruses	waterbirds	yes	?
Lyssavirus, rabies viruses	fox, raccoon dog	yes	yes – rare
European bat lyssa virus	*Myotis* spp. bats	yes	yes – rare
West Nile virus	partridge, magpie, raptors	yes	yes
Louping ill virus	red grouse	yes grouse	yes – rare, via anthropods
Tick-borne encephalitis virus	small mammals	no-reservoir hosts	yes – via arthropod vectors
Hantavirus	bank vole and other rodents	no	yes
Hepatitis E virus	wild boar	no?	not known
Lymphocytic choriomeningitis	rodents	not reported	not known
Ljungan virus	rodents	no?	not confirmed
Borna virus	mammalian and avian species	yes	not confirmed
Sindbis virus	migratory birds	no	yes
BACTERIAL and FUNGAL PATHOGENS			
Mycobacterium bovis	badger, wild boar, deer	yes	not known
Paratuberculosis (*M. a. paratuberculosis*)	deer, rabbits	yes	not proven
Yersinia pestis	rodents	yes	yes – now very rare
Yersinia species (yersiniosis)	rodents, lagomorphs	yes	probably
Francisella tularensis	rodents, lagomorphs	yes	yes
Pasteurella spp.	lagomorphs, many other species	yes	yes
Brucella spp.	boar, hares, ruminants	yes	yes – rare
Marine brucellosis	seals and cetaceans	cetaceans	not known
Anthrax (*B. anthracis*)	no animal reservoir	deer	no
Chlamydial Chlamydophila spp.	pigeons, other avian species	yes	yes
Borrelia sp.	range of wild species	no	yes
Rickettsiales, *Anaplasma*	range of wild species	yes	yes

(*Continued*)

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Pathogen	Primary wildlife host	Clinical disease in European wildlife?	Human disease acquired from European wildlife?
M. phocicerebrale	seals	probable	yes
E. coli	wide range of wild species	yes + reservoir hosts	yes (e.g. 0157 in rabbits)
Salmonella spp.	passerines and others	yes	probably
Campylobacter spp.	passerines and others	yes	yes
Leptospira spp.	wide range of wild species	yes	yes
Coxiella burnettii	ungulates, rabbits, other species	not known	unconfirmed
Listeria spp.	wide range of wild species	yes	contamination of food
Staphylococcus spp.	wide range of wild species	yes	unconfirmed
Streptococcus spp.	wide range of wild species	yes	unconfirmed
Erysipelothrix rhusiopathiae	wild boar	yes	probable
Trychophyton mentagrophytes	hedgehog	yes	yes

Notes

1. The table gives a selection of zoonotic pathogens present in European wildlife that present a health risk for humans.
2. Those selected are the more endemic (present in a predictable pattern) pathogens found in Europe. Many more exotic and sporadically occurring zoonotic pathogens are included in the text.

APPENDIX

3

SELECTED SOCIO-ECONOMICALLY IMPORTANT WILDLIFE RELATED PATHOGENS AND DISEASES IN EUROPE

Diseases selected generally due to wildlife populations serving as sources of pathogens that are a risk to domesticated animal health. The majority listed are notifiable or reportable to the OIE (see Note at end of table).

Disease or pathogen	Wild species affected	Comment
VIRUS		
Aujeszky's disease	wild boar	wild boar as source of disease for pigs and hunting dogs
Duck virus enteritis	wild duck, geese and swans	waterbirds as source of virus for domestic ducks
Avian influenza virus	waterbirds	waterbirds as a source of infection for poultry
Newcastle disease virus	pigeons primarily	wild birds source of virus for domestic birds
Rabbit haemorrhagic disease	rabbit	wild rabbit source of virus for farmed rabbit; hunting losses
Rabies	red fox, racoon dog	disease risks to other mammals
Bluetongue	roe, deer	wild ungulates are potential reservoirs of virus
West Nile virus	several bird species	wild bird reservoirs for other species
Louping-ill virus	grouse	disease threat to grouse shooting, UK
Bovine viral diarrhoea	deer	wild mammals as source of virus for domestic ruminants
Border disease virus	chamois, caprinae, deer	wild mammals as possible source of virus for domestic ruminants
Classical swine fever virus	wild boar	wild mammals as source of virus for domestic pigs
Myxomatosis virus	rabbit	wild mammals as source of virus for domestic rabbits
Hepatitis E	wild boar	wild boar as possible source of virus for pigs
African swine fever	wild boar	wild boar as possible source of virus for pigs

(*Continued*)

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Disease or pathogen	Wild species affected	Comment
BACTERIA AND FUNGI		
Mycobacterium bovis	badger, wild boar, deer species	wild mammals as source of infection for other species
Mycobacterium avium	many wild birds, esp. waterbirds	wild birds as sources of infection for other domestic species
Paratuberculosis	wild rabbits	possible wild reservoirs for domestic animals
Yersinia spp.	several species	wild reservoirs for domesticated species
Pasteurellosis (*Pasteurella multocida*)	several species	wild reservoirs for domestic birds
Brucellosis	boar, hare	wild reservoirs for domestic species
Chlamydiosis	several avian species	wild reservoirs for domestic birds and mammals
Avian mycoplasmosis	several species	wild reservoirs for domestic birds
E. coli	several species	wild reservoirs for domestic birds and mammals
Salmonella spp.	several species	wild reservoirs for domestic birds and mammals
Campylobacter spp.	several species	wild reservoirs for domestic birds and mammals

Notes: The World Organisation for Animal Health (the OIE) requests the notification by Member Countries of OIE-listed diseases in domestic and wild species (approximately 80 diseases for terrestrial animals are listed). The OIE also asks Member Countries for facultative notification, done on a voluntary basis, for non-OIE-listed diseases specific to wildlife (composed of approximately 50 infectious and non-infectious diseases of wild mammals and wild birds). OIE Member Countries are obliged to provide information on the presence or absence of the OIE-listed diseases in domestic and wild animals in six-monthly reports. Additionally the OIE requests countries to notify annually on a voluntary basis on the List of diseases specific to wild species that are deemed important. This is meant to protect biodiversity and could be used as an early warning system to protect domestic species and human health. Further information is available at – information.dept@oie.int and – http://www.oie.int/en/international-standard-setting/specialists-commissions-groups/working-groups-reports/working-group-on-wildlife-diseases/

APPENDIX

4

WILDLIFE PATHOGENS WITH ARTHROPOD VECTORS

Pathogen	Arthropod vector – r: required for transmission ne: not essential; vector and non-vector transmission occur	Vector f: flies m: mosquito mv: multiple vector oa: other anthropods t: tick
VIRAL		
Rabbit haemorrhagic disease virus	ne	f
Bluetongue virus	r	oa – midges
Flaviviruses:		
West Nile virus	r	m – several species
Usutu virus	r	m – assumed, species not known
Louping-ill virus	r	t
Tick-borne encephalitis virus	r	t
Pox viruses	ne	mv?
Myxomatosis virus	ne	oa – fleas
African swine fever virus	ne	t-soft ticks
Alphaviruses:		
Sindbis virus	r	m
BACTERIAL AND FUNGAL PATHOGENS		
Yersinia pestis	ne	oa – fleas
Francisella tularensis	ne	mv
Bacillus anthracis	ne	flies
***Borrelia* spp.**	r	t
***Rickettsia* spp.**	r (usually)	t
***Anaplasma* spp.**	r	t
Mycoplasma conjunctivae	ne	flies?
E. coli, Salmonella	ne	mv
Coxiella burnetii	ne	t
***Bartonella* spp.**	r	mv
Staphylococci	ne	t, mv
Arcanobacterium spp.	ne	f

Notes

Pathogen families in which arthropod transmission is essential or important are marked in bold.

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APPENDIX

5

PATHOGENS SUSPECTED OF CAUSING WILD POPULATION DECLINES, OR OF CONSERVATION IMPORTANCE

Pathogens in Europe that are of conservation importance, causing disease that threatens or reduces some wild populations either pathogens of threatened species, or pathogens causing significant population declines locally

Pathogen	Wild species affected	Reservoir/host species	Comment
Viral pathogens			
Rabbit haemorrhagic disease virus	rabbit	n/a	may cause local population declines
Rabies virus	red fox (not at risk)	n/a	rabies probably reduced fox populations
Phocine distemper virus	common seal	not known	epidemics periodically reduce populations
Porpoise morbillivirus	Mediterranean monk seal	not known	threatened species, disease occurrence suspected in remaining population
Louping-ill virus	red grouse	not known	disease can reduce some grouse populations
Pestivirus of chamois	Pyrenean chamois	sheep?	epidemics cause local population declines
Squirrelpox virus	red squirrel	grey squirrel	threatened extirpation of UK metapopulations
Myxoma virus	rabbit	none	may cause local population declines
Squirrel adenovirus	red squirrel	not known	suspected reductions in local populations in the UK
Feline leukaemia virus	lynx species	domestic cat	FeLV from domestic cats killed 5 Iberian lynx in Spain
Aleutian disease virus	European mink and other riparian carnivores	American mink	suspected but not confirmed cause of decline
Bacterial and fungal pathogens			
Mycobacterium bovis	Iberian lynx	cattle, wild boar, deer	threatened species, disease occurrence in remaining population
Mycoplasma conjunctivae	Chamois, ibex	sheep?	may cause local population declines
Salmonella	passerines	not known	transmission at feeding sites reduction in English greenfinch population

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APPENDIX

6

DISEASES BY CLINICAL PRESENTATIONS, MAMMALS

Clinical disease not as yet recorded in European wild mammals but which may occur are included for awareness

note 1 – presenting clinical signs are given as **bold headers**
note 2 – non-infectious disease and parasitic disease should also be considered in the diagnostic processes
note 3 – 'Found dead' may apply to any wildlife disease, the diseases listed as 'found dead' are acute diseases that are rapidly fatal

Skin disease and skin lesions
Bluetongue, morbillivirus infections (canine distemper), pestivirus infections, classic swine fever, foot-and-mouth disease, swine vesicular disease, myxomatosis, squirrelpox, contagious ecthyma, other pox infections, papillomavirus – particularly cervids, brucella (as subcutaneous abscesses), borreliosis, fusobacterium infections, staphylococcosis, dermatophilosis, dermatophytosis, white-nose syndrome in bats, gangrenous egotism in cervids

Nasal discharge, including blood. Also see Respiratory disease.
Malignant catarrhal fever, cervid herpes, phocid herpesvirus, influenza in marine mammals, morbillivirus infections including marine mammals and canine distemper virus, rabbit haemorrhagic disease, phocid calicivirus, pestivirus infections, bluetongue, classical swine fever, infectious canine hepatitis, Rift valley fever, porcine reproductive and respiratory syndrome (PRRS), anthrax, chlamydial infections, streptococcal infections, aspergillosis

Buccal cavity lesions
Phocid herpesvirus, rabies and lyssavirus infections (salivation), bluetongue, pestivirus infections, contagious ecthyma, squirrel pox, myxomatosis, contagious ecthyma, papillomavirus infection (cervids), pasteurellosis, fusobacterium diseases, actinomycosis, yeast infections.

Eyes – occular disease and discharge
Herpes virus infections in general, cervid herpesvirus (red deer, reindeer), malignant catarrhal fever, influenza virus infections, morbillivirus infections, bluetongue, pestivirus infections, foot-and-mouth disease, squirrelpox virus infection, infectious canine hepatitis, feline infectious peritonitis, pasteurellosis, chlamydial infections, infectious kerato-conjunctivitis and other mycoplasmal infections, staphylococcal infections, haemophilus infections, and moraxella infections

Musculo-skeletal/lameness
Bluetongue, pestivirus infections including classical swine fever, foot-and-mouth disease, swine vesicular disease, brucella arthritis, mycoplasma arthritis, fusobacterium

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diseases, staphylococcal and streptococcal arthritis, gangrenous ergotism, salmonella arthritis

Wasting disease – chronic ill thrift
Post-weaning multisystemic syndrome (suids), pestivirus infections including swine fever, foot-and-mouth disease, retroviral infections, mycobacterial infections, yersiniosis, salmonellosis, aspergillosis

Respiratory disease
Phocid herpes and other herpesvirus infections, influenza in carnivores and pigs, influenza in seals, canine distemper virus, Aleutian disease, infectious canine hepatitis, feline infectious peritonitis, mycobacterial infections, tularaemia, pasteurellosis, chlamydial infections, mycoplasma infections, Q fever, aspergillosis and other fungal infections

Enteric disease – diarrhoea
Malignant catarrhal fever, influenza virus (carnivores), morbillivirus infections in marine mammals and canine distemper, pestivirus infections, canine parvovirus infection feline panleucopenia, infectious canine hepatitis, squirrel adenovirus, feline infectious peritonitis, moose retrovirus, paratuberculosis, yersiniosis, *E. coli* infections, salmonellosis, campylobacteriosis, listeriosis, clostridial infections, Tyzzer's disease

Reproductive – abortion, vaginal discharge
Ruminant alphaherpesvirus (abortion), morbillivirus infections in marine mammals, bluetongue, pestivirus infections, porcine reproductive and respiratory syndrome, brucellosis, chlamydial infections, salmonellosis, campylobacteriosis, leptospirosis, Q fever, listeriosis

Nervous signs, fitting, paralysis, incoordination, lack of fear
Aujesky's disease, malignant catarrhal fever, rabbit haemorrhagic disease, European brown hare syndrome, rabies, bat lyssavirus, morbillivirus infections in marine mammals and canine distemper, pestivirus infections, infectious canine hepatitis, flavivirus infections – West Nile fever, louping-ill, tick-borne encephalitis, infectious canine hepatitis, feline panleucopenia, moose retrovirus, borna disease, tularaemia, listeriosis

Found dead
Rabbit haemorrhagic disease, European brown hare syndrome, African swine fever, yersiniosis, tularaemia, pasteurellosis, anthrax, salmonellosis, clostridial toxaemia, Tyzzer's disease, algal toxicity

General malaise/depression
Herpesvirus infections, morbillivirus infections, bluetongue, influenza virus infections, rabies/lyssavirus infections, retrovirus infections, pestivirus infections, feline panleucopenia, feline infectious peritonitis, Aleution disease, bunyavirus infections, African swine fever, mycobacterial infections, salmonellosis, pasteurellosis, brucellosis, rickettsial infections, tularaemia, yersinial infections, Q-fever, fungal infection

APPENDIX

7

DISEASES BY CLINICAL PRESENTATIONS, BIRDS

Clinical disease not as yet recorded in European wild birds but which may occur are included for clinician awareness

note 1 – presenting clinical signs are given as bold headers
note 2 – non-infectious disease and parasitic disease should also be considered as part of the diagnostic process
note 3 – 'Found dead' may apply to any wildlife disease, the diseases listed as 'found dead' are acute diseases that are rapidly fatal

Skin disease, lesions, feather and beak deformities
Avian circovirus infections, avian poxvirus infections, papillomaviruses, staphylococcal infections

Eyes – occular disease and discharge, discharge from nares. Also see Respiratory disease.
Herpesvirus infections e.g. infectious laryngotracheitis, avian influenza, paramyxovirus infections including Newcastle disease, avian pox, infectious bronchitis, pasteurellosis, chlamydial infections, mycoplasmal infections, staphylococcal infections, *Bordetella avium* infection, *Haemophilus* spp. infections

Buccal cavity lesions
Herpesvirus infections including Smadel's disease, avian influenza, avian pox, salmonellosis (passerines), candidiasis, *M. ornithogaster* infection

Musculoskeletal/lameness
Avian poxvirus infections, finch papillomavirus infections, avian polyomavirus infection, mycoplasmal arthritis, botulism (limb paralysis), staphylococcal infections (bumble foot and arthritis)

Wasting disease – chronic ill thrift, thin body condition
Circovirus infection, avian retroviral infections, avian tuberculosis, yersinial infections, chlamydial infections, mycoplasmal infections, *E. coli* infections, aspergillosis and other fungal infections

Respiratory disease – pneumonia, airsacculitis, tracheitis
Avian influenza, avian pox, avian tuberculosis, pasteurellosis, chlamydial infections, mycoplasmal infections, aspergillosis

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Enteric disease – diarrhoea
Herpesvirus infections including pigeons – owls – raptors, avian influenza, avian paramyxovirus infections – Newcastle disease, pasteurellosis, *E. coli* infections, salmonellosis, yersiniosis, campylobacteriosis, clostridial disease (necrotic enteritis)

Nervous signs, fitting, paralysis, incoordination, lack of fear
Avian influenza, paramyxovirus infection including Newcastle disease, flavivirus infections and encephalitides, including West Nile fever, Usutu, louping-ill, borna disease, borreliosis, listeriosis, pasteurellosis, botulism

Found dead, acute disease no obvious clinical signs
Avian influenza, Usutuvirus infection and other flavivirus infections, avian polyomavirus infection, tularaemia, pasteurellosis, salmonellosis, clostridial disease including necrotic enteritis, algal and cyanobacterial toxicosis

APPENDIX

8

SPECIES (FAMILY)-SPECIFIC WILDLIFE DISEASES IN EUROPE

Some wild species have species-specific disease, particularly in one-species families such as boar

This table is intended to alert the diagnostician to species/family-specific infectious diseases

Note 1 – some families of pathogens are frequently host-species-specific e.g. herpesviruses, poxviruses, papillomaviruses, avian circoviruses, rotaviruses, retroviruses and *Bartonella* spp.

Note 2 – *Herpesvirus* infections – many (possibly most) species of mammal and bird have species-specific herpesviruses, not all of which cause disease (many birds also appear to have species-specific circoviruses).

Disease	Pathogen	Wild host species/family
Herpesvirus infection of hedgehogs	Erinaceid herpesvirus 1 (EsHV1)	hedgehog
Cervidherpesvirus infection in red deer	Cervid herpesvirus 1 (CvHV1)	red deer
Cervidherpesvirus infection in reindeer	Cervid herpesvirus 2 (CvHV2)	reindeer
Pigeon herpesvirus (Smadel's disease)	Columbid herpesvirus 1 (CoHV1)	pigeons and doves
Inclusion body disease of owls	Strigid herpesvirus 1 (StHV1)	owls
Inclusion body disease of falcons	Falconid herpesvirus 1 (FaHV1)	falcons, raptors
Avian herpesvirus of storks	Ciconiid herpesvirus 1 (CiHV1)	white and black stork
Rabbit haemorrhagic disease (RHD)	RHD virus (RHDV)	rabbit
European brown hare syndrome (EBHS)	EBHS virus (EBHSV)	brown hare, mountain hare
Post-weaning multisystemic wasting syndrome (PMWS)	Porcine circovirus 2 (PCV2)	wild boar
European bat lyssavirus	European bat lyssavirus 1 and 2 (EBLV 1 and 2)	bats (*Myotis* sp.)
Pestivirus of chamois	Chamois border disease virus (Chamois BDV)	chamois
Classic swine fever (CSF)	CSFV	wild boar
Canine parvovirus	Canine parvovirus 2 (CPV 2)	red fox, wolf
Parvovirus of wild boar	Porcine parvovirus (PPV)	wild boar
Squirrelpoxvirus disease (SQPD)	SQP virus (SQPV)	red and grey squirrel
Sealpox disease	Seal parapoxvirus (unclassified)	common and grey seals
Myxomatosis	Myxomavirus	rabbit, rarely in brown hare
African swine fever (ASF)	African swine fever virus (ASFV)	wild boar
Contagious dermatitis in mountain hare	Pox virus (not named)	mountain hare

(*Continued*)

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Disease	Pathogen	Wild host species/family
Squirrel adenovirus disease	Squirrel adenovirus (SqAdV)	red squirrel
Feline leukaemia	Feline leukaemia virus (FeLV)	wild cat, Iberian lynx
Moose wasting syndrome	Alces leucotropic oncovirus (ALOV)	moose
Deer fibromatosis	Delta papillomaviruses of cervids	roe and other deer species
Chaffinch papillomatosis	Fringilla papillomavirus (FPV)	chaffinch and brambling
Feline infectious peritonitis (FIP)	Feline coronavirus (FCoV)	wild cat, Eurasian lynx
Brucellosis	*Brucella suis* biovar 2	wild boar, brown hare
Brucellosis	*Brucella suis* biovar 4	reindeer (rarely moose)
Infectious keratoconjunctivitis	*Mycoplasma conjunctivae*	ibex and chamois
Ringworm	*Trichophyton mentagrophytes*	hedgehog
Gangrenous ergotism in moose	*Claviceps purpurea*	moose

SUBJECT INDEX

Note:
Animals and birds are indexed by groups e.g. coot(s), owl(s), deer, sheep or seal(s) etc. For example, red squirrels and grey squirrels are listed as 'squirrel(s)'.
Page numbers in *italics* refer to figures, whilst those in **bold** refer to tables.
vs denotes comparison or differential diagnosis.

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