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

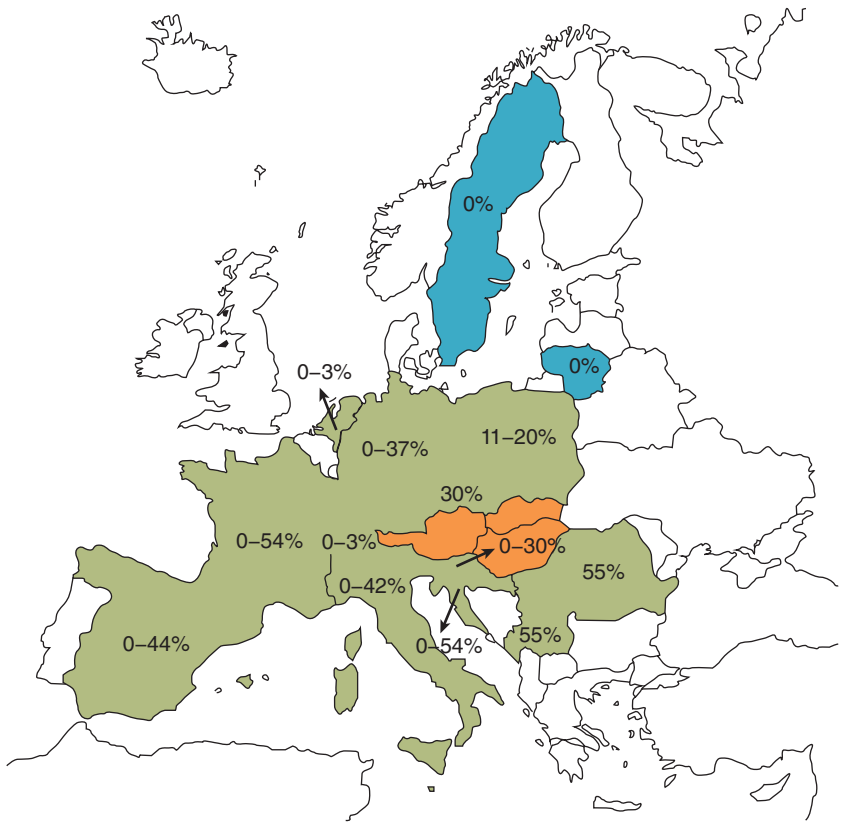

FIGURE 1.1 European countries where reports of ADV surveillance in wild boar populations have been published in the scientific literature[3] or reported in national wildlife surveillance programmes between 1987 and 2011. Countries shaded in green represent ADV-positive wild boar populations, whereas 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

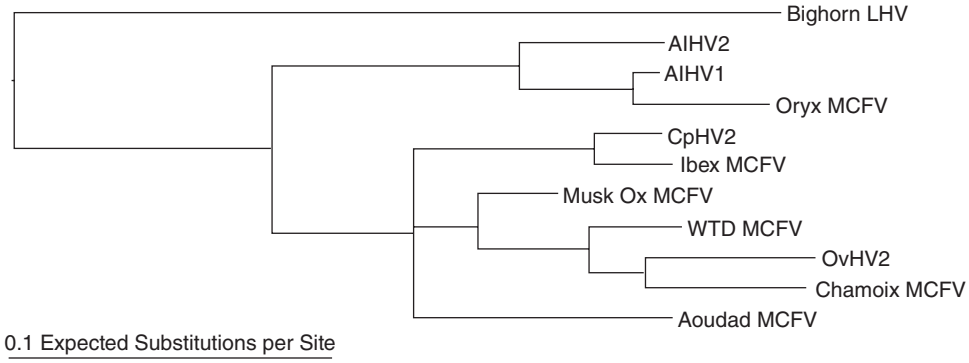

FIGURE 1.2 Phylogenetic analysis of MCF viruses was based on a 177 bp fragment of the DNA polymerase gene, for which the widest range of sequences were available. The DNA sequences were aligned using ClustalV, based on the translated amino acid sequences, and the phylogenetic analysis was done using 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.

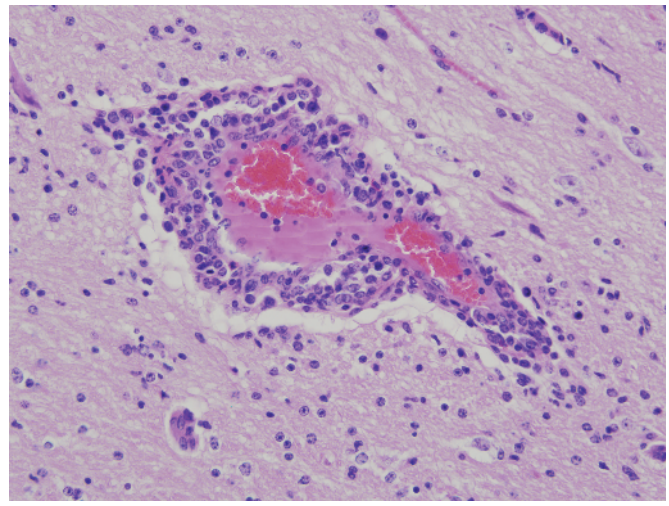

FIGURE 1.3 Histological section of farmed red deer brain with MCF, showing non-suppurative encephalitis. Note the characteristic accumulation of predominantly lymphoid inflammatory cells around the blood vessel (peri-vascular cuff) along with those free in the neuropil (gliosis, to the left of the blood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

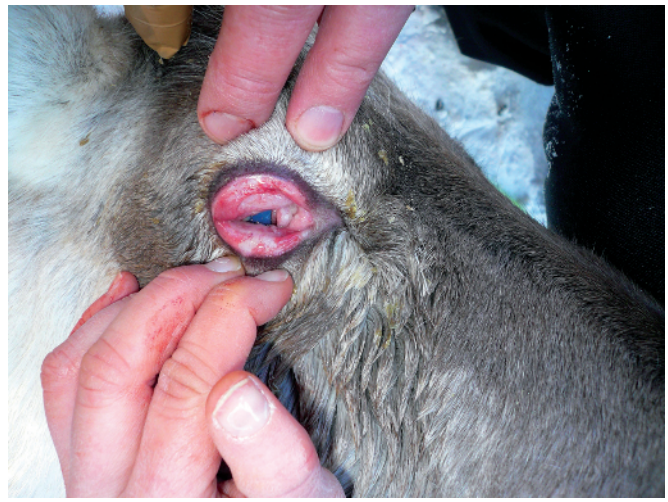

FIGURE 1.4 Infectious keratoconjunctivitis caused by *Cervid herpesvirus 2* infection in semi-domesticated reindeer during an outbreak in Norway in 2009. Severe degree of periorbital oedema. With permission from Das Neves, C.G. et al., 2010[48].

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

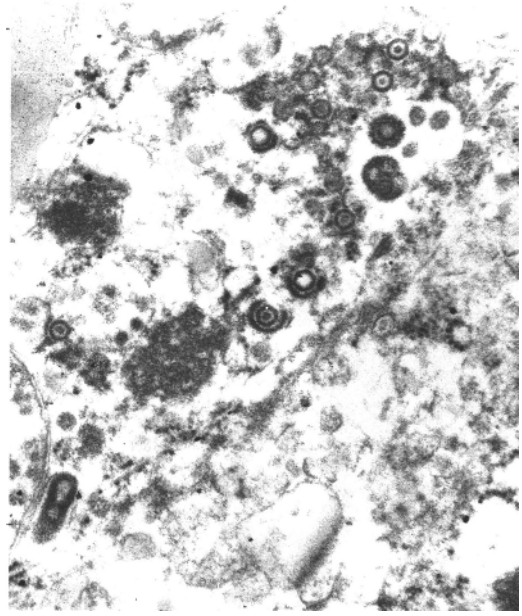

FIGURE 1.5 Electron microscopy of herpesvirus from a hedgehog with hepatitis cultured on primary bovine foetal skin cells.

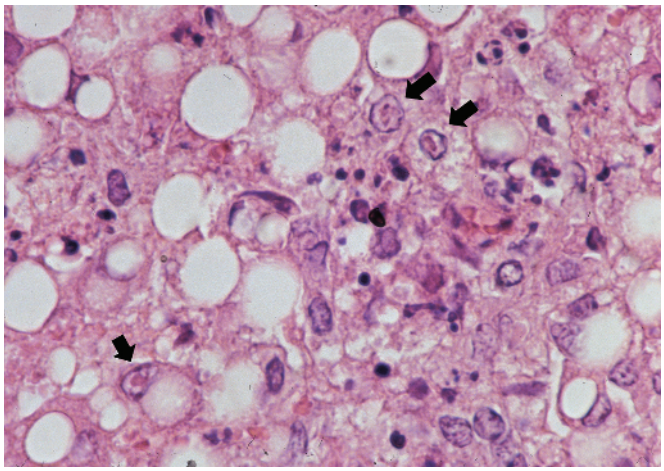

FIGURE 1.6 Histological section of liver of a European hedgehog with fatal herpesviral hepatitis, showing necrosis of hepatocytes and fatty degeneration. Intranuclear acidophilic inclusion bodies are observed in hepatocytes (arrows). Photo: D. Gavier-Widén, SVA.

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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