

Avian influenza viruses in mammals

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Summary

Highly pathogenic avian influenza viruses of subtype H5N1 are remarkable because of their expanding non-avian host range and wide tissue tropism. They have caused severe or fatal respiratory and extra-respiratory disease in seven naturally infected species of carnivore. However, they are not unique in their ability to cross the species barrier, to cause clinical disease and mortality, or to replicate in extra-respiratory organs. Low pathogenic avian influenza viruses have crossed from birds to swine, horses, harbour seals, whales and mink; have resulted in severe respiratory disease and mortality; and may have spread beyond the respiratory tract in some of these species. They are also transmitted from mammal to mammal in most species, and have become endemic in swine and horse populations, demonstrating their ability to adapt to and become sustained in mammals. Until now, highly pathogenic avian influenza viruses H5N1 have not acquired this ability, but there are concerns that they may adapt to mammalian species and, thus, could spark an influenza pandemic in humans.

Keywords

Avian influenza – Cross-species transmission – H5N1 – Highly pathogenic avian influenza – Host range – Influenza – Mammal – Pathogenesis – Pathology – Species barrier.

Introduction

Phylogenetic evidence strongly suggests that all mammalian influenza A virus lineages originally derive from avian influenza A viruses, after initial cross-species transmission of the viruses from birds to mammals (137, 141). Until the emergence of highly pathogenic avian influenza virus (AIV) H5N1, cross-species transmission of low pathogenic AIV (LPAIV) from birds to mammals was infrequently reported, and for only a limited number of animal species. Such transmission typically results in outbreaks of severe respiratory disease. Since 2003, highly pathogenic AIV (HPAIV) of subtype H5N1 have been transmitted to a wide range of non-human mammalian species. They cause fatal respiratory and extra-respiratory infection, yet with no or only limited mammal-to-mammal transmission. After reviewing known events involving

cross-species transmission of AIV to mammals (excluding humans), these events will be analysed in the light of pathogenesis. The pathogenesis of infection by HPAIV H5N1 and LPAIV will be compared, and important gaps in the understanding of cross-species transmission of AIV to mammals will be identified.

Cross-species transmission of avian influenza viruses to mammals

Known events involving cross-species transmission of LPAIV to mammals and the events involving cross-species transmission of HPAIV H5N1 will be described separately.

Avian influenza viruses in swine

Swine populations are infected with several subtypes of influenza A viruses. Based on phylogenetic analysis of the gene segments, these viruses are classified as classical swine, avian-like, human-like, or reassortants between them (Table I) (8, 38). Multiple lineages have become established in swine populations, and are present endemically in various regions of the world; other influenza viruses have caused one or few epidemics or have been isolated from pigs only occasionally. The clinical signs caused by influenza viruses in swine typically include fever, weight loss, dry cough, laboured breathing and nasal discharge, associated with lesions of tracheo-bronchitis and broncho-interstitial pneumonia. Morbidity is high, but mortality is typically low, unless bacterial infection complicates the course of infection (3).

The isolation of influenza viruses from swine, of the H1N1, H9N2, H4N6 and H3N3 subtypes, with all gene segments of avian origin, strongly suggests cross-species transmission of whole AIV to swine. All of the gene segments of avian-like H1N1 influenza viruses isolated in swine in Europe since 1979 and in Asia in 1993 are phylogenetically related to the gene segments of influenza viruses isolated from Eurasian ducks (38, 99, 111, 112). It remains uncertain whether avian-like H1N1 viruses that emerged in pigs in the south of the People's Democratic

Republic of China have become endemic. These viruses were isolated from tracheal swabs of randomly sampled slaughtered pigs which appeared healthy (38). In contrast, avian-like H1N1 viruses have replaced classical swine H1N1 viruses in Europe and continue to circulate and cause disease (3, 17). This indicates that cross-species transmission of whole AIV can result in the establishment of these viruses in swine populations.

Other AIV subtypes isolated from tracheal swabs or lung tissue samples from swine include H9N2 in China between 1998 and 2000 (98), which appears to continue to circulate to date (21, 142), H4N6 in Canada in 1999 (52) and H3N3 in Canada in 2001 (56). These viruses are whole AIV, belonging to the Eurasian clade (the phylogenetic group comprising AIV isolated mostly from birds sampled in Eurasia) in the case of the H9N2 virus (closely related to contemporary viruses from terrestrial poultry in China) (98), and to the North American clade (phylogenetic group) in the case of the H4N6 and H3N3 viruses, based on phylogenetic analysis (52, 56). Clinical signs similar to those observed in herds affected by endemic influenza viruses were reported following infection with AIV H4N6 and H3N3 (52, 56). Necrotising bronchiolitis and broncho-interstitial pneumonia were observed on post-mortem examination of the fatal cases, but these conditions were likely of mixed viral and bacterial aetiology. Replicating viral and opportunistic

Table I
Influenza A virus subtypes isolated from swine, in chronological order of first isolation

Subtype	Lineage	First isolation	Location	Epidemiology	Ref.
H1N1	Classical swine influenza viruses	1930	Worldwide	Endemic	3
	Avian-like viruses	1979	Europe	Endemic	8, 73
	Avian-like viruses	1993	Asia	Endemic (?)	38
H3N2	Human-like viruses	1970	Worldwide	Endemic	3
	Avian-like viruses (avian H3)	1978	Asia	Repeated isolations	60
	Reassortant human-like/classical swine viruses	1982	Asia	Single epidemic	81, 119
	Reassortant human-like/avian-like viruses	1985	Europe (Italy)	Endemic	18
	Reassortant human-like/classical swine viruses	1998	North America	Single epidemic	92
	Triple reassortant human-like/classical swine/avian-like viruses	1998	North America	Endemic	55, 92, 145
H1N2	Reassortant classical swine/human-like viruses	1978	Asia (Japan)	Endemic	50, 82
	Reassortant avian-like/human-like viruses	1987	Europe	Two epidemics	35
	Triple reassortant human-like/human-like/avian-like viruses	1994	Europe (United Kingdom, Belgium)	Endemic	11, 134
	Reassortant classical swine/human-like viruses	1999	Asia (Taiwan)	Endemic	131
	Triple reassortant classical swine/human-like/avian-like viruses (second generation reassortant)	1999	North America	Endemic	54, 53
H1N7	Reassortant human-like/equine-like viruses	1992	Europe	Single epidemic	9, 12
H9N2	Avian-like viruses	1998	Asia	Endemic	21, 98, 142
H4N6	Avian-like viruses	1999	North America	Single epidemic	52
H3N3	Avian-like viruses	2001	North America	Two epidemics	56
H3N1	Reassortant human-like/classical swine viruses	2001	Asia (Taiwan)	Endemic	131
	Multiple reassortant avian-like/human-like/swine viruses (second generation reassortant)	2004	North America	Repeated epidemics	69, 76

bacterial pathogens (i.e. *Streptococcus suis*, *Pasteurella multocida* and *Arcanobacterium pyogenes*) were isolated from lung tissue samples of dead swine (52, 56). In contrast, the infection of swine with AIV H9N2 did not always result in clinically apparent disease. The virus was repeatedly isolated between 1998 and 2000 from tracheal swabs of randomly sampled slaughtered pigs that appeared healthy (98). The virus was also isolated from diseased pigs in China in 2004 (21), and reassortant H9N2-H5N1 viruses were isolated from diseased pigs in China in 2003 (142). Interestingly, experimental intranasal inoculation of pigs with AIV of different subtypes resulted in productive infections that remained subclinical (59). Additional factors may therefore be involved in the manifestation of clinical disease of influenza in swine, such as the occurrence of secondary bacterial infections (3, 52, 56).

Pigs infected with AIV may not always produce a detectable antibody response, either because of undetectable antibody titres (45) or weak inhibition of haemagglutination (59). Serosurveillance based on the haemagglutination inhibition (HI) test thus may not be suitable for the detection of AIV in swine populations (8), and cross-species transmission of influenza viruses from birds to swine may be under-reported. A suitable alternative is the virus neutralisation test. Through this method, serological evidence of repeated exposure of swine to avian H4, H5 and H9 influenza viruses has been reported in south-east China between 1977 and 1982 and in 1998 (88).

Avian influenza viruses in horses

Two stable lineages of influenza A viruses (i.e. equine-1 or H7N7 subtype, and equine-2 or H3N8 subtype) have become established in horse populations (Table II) (94). Based on phylogenetic analysis (141), both subtypes were probably transmitted from birds to horses. They cause a respiratory disease, which is characterised by a dry cough with nasal discharge and fever. Associated lesions affect both the upper and lower respiratory tract, and include laryngitis, tracheitis, bronchitis, bronchiolitis and interstitial pneumonia. Myocarditis has also been reported in some cases. The disease is highly contagious and morbidity is high, but mortality is typically low, unless

bacterial infections complicate the course of infection (42). The equine influenza H7N7 virus was last isolated in Egypt in 1989 (47), but may circulate at low levels in certain regions of the world (94). In contrast, the equine influenza H3N8 virus is endemic in the global horse population (42, 94).

In March 1989, an outbreak of respiratory disease with 20% mortality occurred among horses in north-east China. This outbreak was caused by infection with an H3N8 influenza virus which was distinguishable from viruses of the equine-2 lineage both antigenically and by molecular make-up (40). Based on phylogenetic analysis, this virus, isolated from the nasal swabs of sick horses, was closely related to avian influenza H3N8 viruses of the Eurasian clade. The polymerase gene was partially sequenced and was the only gene more closely related to genes of the American clade. Clinical signs included fever, dry cough and conjunctival and nasal discharges, associated with conjunctivitis, bronchitis and pneumonia. Death was associated with pneumonia and enteritis. A second outbreak of the same virus occurred in north-east China in spring 1990, but resulted in lower morbidity and no mortality, possibly due to the previous exposure of the horse population of this region to the virus. Serological evidence suggests that the virus continued to circulate in horses in China in 1993 and 1994 (41), but it does not appear to have become established.

In addition to crossing to and becoming established in horses, H3N8 viruses of avian origin appear to have the propensity to adapt to other mammalian species. For example, the H3N8 virus isolated from horses failed to replicate in ducks but caused severe disease in experimentally infected mice and ferrets (40). Moreover, an H3N8 subtype of the equine-2 lineage has recently crossed from horses to domestic dogs in the United Kingdom (UK) and North America, and may have become established in this novel host species (23, 25).

Avian influenza viruses in pinnipeds

Several subtypes of AIV (H7N7, H4N5, H4N6 and H3N3) have caused epidemics in harbour seals (*Phoca vitulina*) but do not appear to have become established in this species

Table II
Influenza A virus subtypes isolated from horses, in chronological order of first isolation

Subtype	Lineage	First isolation	Location	Epidemiology	Ref.
H7N7	Equine-1 influenza viruses	1956	Worldwide	Low-level circulation or extinct	94
H3N8	Equine-2 influenza viruses Two lineages (American and Eurasian)	1963	Worldwide	Endemic	94
H3N8	Avian-like viruses	1989	Asia	Two epidemics and evidence of continued exposure	40

Table III
Influenza A virus subtypes isolated from harbour seals, in chronological order of first isolation

Subtype	Lineage	First isolation	Location	Epidemiology	Ref.
H7N7	Avian-like viruses	1979	New England coast of the United States of America (USA)	Single outbreak	33, 68, 140
H4N5	Avian-like viruses	1982	New England coast of the USA	Two outbreaks	44
H4N6	Avian-like viruses	1991	New England coast of the USA	Single outbreak	15
H3N3	Avian-like viruses	1992	New England coast of the USA	Single outbreak	15

(Table III). The first confirmed outbreak of influenza A virus infection in harbour seals occurred between December 1979 and October 1980 along the New England coast of the United States of America (USA) (33). Influenza H7N7 virus was isolated from the lungs and brain of dead seals, and found to be closely related to avian influenza H7N7 viruses of the American clade, based on HI and neuraminidase inhibition (NI) tests, and competitive ribonucleic acid-ribonucleic acid (RNA-RNA) hybridisation (68, 140). Approximately 600 seals, representing about 25% of the local population, died of the infection (33). Clinical signs included prostration, respiratory distress associated with nasal discharge, and subcutaneous emphysema. Necrotising bronchitis and bronchiolitis and haemorrhagic alveolitis were reported upon post-mortem examination of the fatal cases. Experimental infections of harbour seals with this virus resulted in similar but milder clinical signs (140). The presence of *Mycoplasma* infection in naturally infected seals may have contributed to more severe disease during the outbreak. Interestingly, this H7N7 virus behaved more like mammalian influenza viruses, as it replicated better in experimentally inoculated pigs, cats and ferrets than in ducks, chickens, turkeys or parakeets. Furthermore, five humans who handled infected seals developed conjunctivitis, associated with high titres in their conjunctival swabs, demonstrating the zoonotic potential of the virus (139).

An influenza H4N5 virus closely related to AIV, as determined by HI and NI tests and competitive RNA-RNA hybridisation, was isolated from the lungs and brain of dead harbour seals during an outbreak of respiratory disease from January to March 1983, along the New England coast (44). The virus isolated in 1983 was virtually identical to an AIV H4N5 isolated from the lungs of an emaciated harbour seal found dead in June 1982, suggesting that this virus may have been sustained for several months in the seal population. The outbreak was associated with a three- to four-fold increase in mortality. Animals that died of the infection had a necrotising bronchopneumonia. However, experimental inoculation of a harbour seal, ringed seals (*P. hispida*) and harp seals (*Pagophilus groenlandicus*) with the 1982 H4N5 virus isolate resulted in asymptomatic infections. As in the H7N7 virus outbreak of 1979 to 1980, additional factors, such as co-

infection, probably increased the severity of the disease in naturally infected seals. Experimental infection of ducks with this virus resulted in productive infection (44).

Influenza H4N6 and H3N3 viruses phylogenetically related to AIV of the North American clade were isolated from the lungs of dead harbour seals along the New England coast in January 1991, and between January and February 1992, respectively (15). Infected seals showed nasal discharge and subcutaneous emphysema, associated with acute interstitial or haemorrhagic pneumonia. An increase in the number of strandings was observed during these periods, compared with previous years, but no severe respiratory outbreaks were reported, in contrast to the 1980s.

In addition to the cases of AIV cited above, harbour seals from the North Sea showing signs of a respiratory disease in spring 1999 were found to be infected with an influenza B virus closely related to strains that had circulated in humans several years earlier (93). Interestingly, antibodies against this influenza B virus were evidenced in archived sera of stranded harbour seals and grey seals (*Halichoerus grypus*), collected between 1995 and 1999. This suggests that the virus had circulated in seal populations for several years. The receptiveness of harbour seals to influenza viruses of both avian and human origin calls for continued surveillance of this species for influenza virus infections (15).

Pinniped species other than harbour seals have also shown serological evidence of influenza virus infection, although infectious virus has not been isolated. Harp seals (*P. groenlandicus*) and hooded seals (*Cystophora cristata*) from the Barents Sea, ringed seals (*P. hispida*) from Alaska and from the Kara Sea, Caspian seals (*P. caspica*), Baikal seals (*P. sibirica*) and sea lions (species not specified) from the Bering Sea were all reported to have antibodies against influenza A viruses (26, 28, 86, 89, 90, 126). A competitive enzyme-linked immunosorbent assay (ELISA) (28, 86, 89, 90, 126) or a double agar immunodiffusion assay (26) were used, which detect antibodies against the nucleoprotein (NP) of influenza A virus. The subtype was further determined in a number of studies, using HI and NI tests. Antibodies against influenza A viruses of the H1,

H3, H4, H7 and H12 subtypes were detected in seals (species not specified) from the North and Bering Seas sampled between 1978 and 1988 (28); antibodies against the H3 and H7 subtypes and the N1, N4, N6 and N8 subtypes were detected in one ringed seal from Alaska sampled in 1984 (26); and antibodies against the H7N7 subtype were detected in one ringed seal from the Kara Sea sampled in 2002 (89). Serum samples of sea lions from the Bering Sea that tested positive by nucleoprotein- (NP) ELISA were all negative by HI test (28). Antibodies against human influenza A and B viruses were also found in Caspian seals sampled between 1993 and 2000, in Baikal seals sampled in 1998, and in ringed seals from the Kara Sea sampled in 2002 (89, 90).

Avian influenza viruses in cetaceans

There is scattered evidence of AIV infection in some species of cetaceans, which comprise whales, dolphins and porpoises. However, the level of exposure to such infection and its impact upon these populations are poorly understood. Influenza H1N3 viruses were isolated from lung and liver samples of Balaenopterid whales (species not specified) collected in 1975 and 1976 in the South Pacific (75). These viruses were antigenically close to AIV. No further details were provided on possible clinical signs or lesions in infected animals.

Influenza H13N2 and H13N9 viruses were isolated from the lungs and hilar node of a pilot whale (*Globicephala melas*) in autumn of 1984 near Maine, USA, when major strandings were reported along the New England coast (43). The haemagglutinins (HA) were antigenically related to H13 of an AIV isolated from gulls, while both neuraminidases (NA) were antigenically related to the prototype N2 and N9 isolates from other avian species. Furthermore, the NP gene of both viruses was closely related to the NP gene of gull viruses of the H13 subtype, as determined by competitive RNA-RNA hybridisation. Clinical signs included skin sloughing and extreme emaciation. The animal had difficulties swimming, diving and surfacing. Post-mortem examination revealed an enlarged hilar node with no histological evidence of a germinal centre, haemorrhagic lungs and a small and friable liver. Although more strandings than usual were reported in the region, no virus could be isolated from the carcasses of other stranded pilot whales, possibly because of advanced autolysis. Thus, it remains unknown whether AIV can cause outbreaks in whales, as it can in harbour seals.

There is only serological evidence for influenza A virus infection in other cetacean species. Using a competitive NP-ELISA, antibodies against influenza A viruses were detected in belugas (*Delphinapterus leucas*) from Arctic

Canada, sampled between 1984 and 1998, and common minke whales (*Balaenoptera acutorostrata*) and Dall's porpoises (*Phocoenoides dalli*) from western North Pacific and Antarctic Oceans, sampled between 2000 and 2003 (80, 85). Further determination of the subtypes was either not possible or unsuccessful. To the knowledge of the authors, there are no reports of influenza virus infection in dolphin species.

Avian influenza viruses in mink

Natural cross-species transmission of AIV from birds to mink, associated with clinical disease, was reported in autumn 1984 in 33 mink farms along the east coast of Sweden (62). Influenza H10N4 virus was isolated from the lungs of sick mink. Based on oligonucleotide fingerprinting, this virus was closely related to the prototype avian H10N7 strain and the recently circulating AIV H10N4 strain isolated from wild waterfowl in the UK (5). In mink, the infection caused an outbreak of severe respiratory disease with almost 100% morbidity and 3% mortality (62). The most pronounced clinical signs were anorexia, sneezing, coughing and nasal and ocular discharges. The main lesion was acute interstitial pneumonia. Experimental infections of mink with the H10N4 virus isolated from sick mink, or with a recently circulating avian H10N4 virus isolated from wild waterfowl, reproduced the disease observed in farmed mink (30, 62). The virus was isolated from lungs, brain and liver samples, and one mink also presented neurological signs, i.e. ataxia (62). In contrast, experimental infection of mink with prototype AIV H10N7 did not result in productive infection (30). Earlier experimental infection of mink with human H3N2 and H1N1, swine H1N1, equine H3N8 and avian H3N8 and H4N6 subtypes of influenza A viruses resulted in productive infection but no clinical signs (80). In addition, AIV of the H3N8, H5N3, H7N7, H8N4 and H11N4 subtypes could be experimentally transmitted from mink to mink (91).

Avian influenza viruses in other mammals

Serological surveys of domestic dogs and cats detected only scarce and limited exposure to human influenza viruses, although both species were found to permit experimental infection without developing clinical disease (87, 95, 96, 130). Similarly, experimental inoculation of domestic cats with AIV resulted in productive infection but no clinical disease (45). However, natural cross-species transmission of LPAIV to domestic carnivores has not yet been reported. An historical report of a diseased cat kept in a laboratory to control a wild rat population may indicate infection with HPAIV (101).

Serological studies suggest that AIV may occasionally cross to ruminants, namely:

- cattle
- sheep
- goats
- yak (*Bos grunniens*)
- water buffalo (*Bubalus bubalis*)
- reindeer (*Rangifer tarandus*)
- fallow deer (*Dama dama*).

The serological test used during most surveys was the HI test, performed for a restricted number of influenza viruses, mostly of human, swine or equine lineages (H1, H3 and H7 subtypes). However, two reports of exposure to influenza viruses with unrecognised HA protein may suggest the exposure of cattle to AIV (74), since these HA proteins differ from those found in the influenza viruses circulating in mammals. Human influenza viruses have occasionally been isolated in some of these species. Experimental infection of cattle with human influenza viruses resulted in asymptomatic infection, but an H3N2 strain isolated from cattle (which possibly originated from early human strains) caused distinctively influenza-like illness in calves (16). In addition to outbreaks of respiratory disease, influenza virus infection may also be associated with outbreaks of milk drop syndrome in cattle (10, 24, 37, 39).

Avian influenza viruses in reptiles and amphibians

There is limited evidence for AIV infection in non-avian animal species besides mammals. Antibodies against influenza viruses of the H1, H3 and H7 subtypes of human and equine lineages were detected in captive and free-ranging snakes (genera *Bothrops* and *Crotalus*), toads (genus *Bufo*) and frogs (genus *Rana*) from Brazil, using the HI test (79). Antibodies against viruses of unknown subtype were also found in captive crocodilians in Florida using an agar gel immunodiffusion assay (27), i.e.:

- a Chinese alligator (*Alligator sinensis*)
- a Schneider's dwarf caiman (*Paleosuchus trigonatus*)
- a Nile crocodile (*Crocodylus niloticus*).

Influenza A virus was detected by reverse transcription–polymerase chain reaction (PCR) in these three reptiles, as well as in a broad-snouted caiman (*Caiman latirostris*). Analysis of the sequence of the non-structural protein (NS) NS1 gene revealed > 99% identity with the NS1 gene of duck isolates of the American clade (27).

Highly pathogenic avian influenza H5N1 viruses in mammals

The HPAIV H5N1 that recently emerged in Asia is remarkable because of its expanding mammalian host range and wide tissue tropism. Natural cross-species transmission of HPAIV H5N1 to non-human mammals was first reported in two captive tigers (*Panthera tigris*) and two captive leopards (*P. pardus*) in a zoo in Thailand in December 2003 (58). A second outbreak occurred in captive tigers in another zoo in Thailand in October 2004 (129). The highly pathogenic AIV H5N1 strains isolated during these outbreaks were phylogenetically similar to each other and virtually identical to the contemporary H5N1 strain circulating in poultry in Thailand (1). Clinical signs included high fever, serosanguineous nasal discharge, respiratory distress and neurological signs. At necropsy, broncho-interstitial or haemorrhagic pneumonia, encephalitis and, in some cases, moderate multifocal necrotising hepatitis were observed. The presence of the virus was determined in the lungs of the leopards, and the lungs, brain and liver of the tigers, by immunohistochemistry and viral isolation (58, 129). A total of 147 tigers died or were euthanased in this zoo during the second outbreak, and probable tiger-to-tiger transmission of the virus was demonstrated (129).

The HPAIV H5N1 strain was isolated from a dead domestic cat in Thailand in February 2004 (120). The virus was phylogenetically related to viruses isolated in Thailand in 2004 from poultry, tigers and humans (2). The cat demonstrated fever and respiratory distress, and lesions of interstitial pneumonia, encephalitis, multifocal necrosis in the liver, tubulonephritis and lymphoid depletion in the spleen. The presence of the virus was found in the lungs, brain, liver, heart, kidneys and spleen by immunohistochemistry and virus isolation, and in the duodenum by virus isolation.

A domestic dog was reported to have died of HPAIV H5N1 infection in Thailand a few months later, in October 2004 (121). Likewise, the virus was phylogenetically related to those viruses isolated in Thailand in 2004 from poultry, tigers and humans. The dog developed high fever and respiratory distress, and, on necropsy, lesions of severe interstitial pneumonia, focal necrosis in the liver and mild nephritis with tubular degeneration were found. The presence of the virus was determined in the lungs, liver and kidneys by immunohistochemistry and viral isolation.

As yet unpublished serological surveys have detected antibodies against H5N1 virus in 8 out of 111 domestic cats and 160 out of 629 domestic dogs in Thailand (14), and in 100 out of 500 domestic cats in Indonesia (103). Domestic cats were also found to be infected with HPAIV H5N1 in Iraq, Germany and Austria in February 2006 (63,

70, 144). The viruses isolated from cats in Iraq and Germany were phylogenetically related to viruses isolated from birds found sick or dead in the vicinity of the cases, i.e. a sick goose from an adjacent household (144), and a dead whooper swan (*Cygnus cygnus*) from the Isle of Rügen (63), respectively. These strains were closely related to a Qinghai-like strain of HPAIV H5N1 circulating at that time. The infected cats from Iraq died with lesions similar to those described for experimentally infected cats (106), as well as severe haemorrhagic pancreatitis (144). The presence of the virus was detected in the lungs, liver and large intestine by reverse transcription- (RT) PCR (144). The infected cats from Germany died with broncho-interstitial pneumonia, multifocal necrosis in the liver and adrenal cortex, lymphoid necrosis in the spleen and Peyer's patches of the small intestine, as well as mild myocardial haemorrhage or fibrinous epicarditis. The presence of the virus was detected in their lungs, liver, adrenal cortex, spleen and brain by immunohistochemistry (64). In contrast, the cats infected with HPAIV H5N1 in Austria remained subclinically infected and presented no lesions at necropsy (70).

Captive Owston's palm civets (*Chrotogale owstoni*) from a breeding programme in a sanctuary in Vietnam were found to be infected with HPAIV H5N1 in June 2005 (107). The virus was phylogenetically related to HPAIV H5N1 of genotype G, which was previously isolated from poultry in mainland China and Vietnam. The animals presented neurological signs, including hind limb paralysis, and lesions of interstitial pneumonia, encephalitis and multifocal necrosis in the liver. The presence of the virus was determined in the lungs and brain by immunohistochemistry and RT-PCR or viral isolation, and in the kidneys and intestine by RT-PCR or viral isolation. A second outbreak of HPAIV H5N1 infection was reported in the same sanctuary in March 2008, killing four Owston's palm civets (104).

Lastly, in March 2006, cross-species transmission of HPAIV H5N1 was reported in free-living wild carnivores, i.e. a stone marten (*Mustela foina*) in Germany (64) and an American mink (*M. vison*) in Sweden (102). The virus isolated from the stone marten was phylogenetically related to a virus isolated from a dead whooper swan from the Isle of Rügen (64), and belonged to the Qinghai-like lineage. The stone marten presented neurological signs, including ataxia and circling, and was euthanased. At necropsy, moderate-to-severe encephalitis was observed in the cerebrum, cerebellum and brain stem. Inflammation was reported in the pancreas, lungs, liver and kidneys. The presence of the virus was determined in the brain and pancreas by immunohistochemistry (64).

No outbreaks of HPAIV H5N1 infection have been reported in swine or horses, despite their general

susceptibility to infection with AIV. No experimental infections or serological surveys aimed at determining the level of exposure of horses to HPAIV H5N1 have yet been carried out. Swine experimentally infected with HPAIV H5N1 typically develop mild disease (20, 72, 118). Reports of isolating H5N1 viruses from swine remain scarce. No H5N1 viruses were isolated during a surveillance of swine in Hong Kong from 1997 to 2000 (118), and swine raised in contact with infected poultry did not become infected (116). Two HPAIV H5N1 strains were isolated from swine during routine surveillance in the Fujian province of China in 2001 and 2003, and were closely related to the HPAIV H5N1 strain isolated from a duck (146). In addition, a multiple reassortant influenza H5N1 virus was isolated from swine in the Shandong province of China, in 2003 (114). However, a very low exposure rate (0.25%) to HPAIV H5N1 was detected during a serological survey of swine in Vietnam in 2004 (20). These studies suggest that swine may not become easily infected with HPAIV H5N1. Little is known on the susceptibility of ruminants to infection with HPAIV H5N1. Calves infected with HPAIV H5N1 did not present clinical signs and shed low amounts of virus for up to two days post inoculation (51).

Species likely to prey on or scavenge infected poultry and wild birds, e.g. carnivores and rodents, may also be exposed to HPAIV H5N1. Little is known on the susceptibility of such species to infection with HPAIV H5N1, and few serological studies aimed at determining their level of exposure in the wild have been reported. During the initial outbreak of HPAIV H5N1 in Hong Kong in 1997, various mammals living around poultry markets, including rats (*Rattus* sp.) and mice (*Mus* sp.), were screened for infection. Although no virus was isolated, haemagglutination-inhibiting activity was detected in some of the rat sera (116). Rats and mice were shown to be susceptible to experimental infection with the 1997 HPAIV H5N1 isolate (118), but the rats and mice used in experimental studies are typically laboratory breeds, which may differ in their susceptibility to infection and disease from their free-ranging counterparts (e.g. 133). Free-ranging carnivore species with opportunistic feeding habits, such as the red fox (*Vulpes vulpes*), are also likely to be at risk of infection with HPAIV H5N1. There is no confirmed report of natural HPAIV H5N1 infection in red foxes. However, this species has been shown to be susceptible to experimental infection (105). They can develop severe lesions of broncho-interstitial pneumonia, encephalitis and myocarditis, associated with the presence of the virus in lungs, brain and heart. Interestingly, red foxes fed on infected bird carcasses developed only mild lesions, while excreting the virus for several days, and may potentially play a limited role in local dispersal of HPAI H5N1 viruses in the wild (105).

Pathogenesis of avian influenza viruses in mammals

Cross-species transmission of AIV to mammals requires the pathogens to overcome several barriers (66), including:

- effective exposure of mammals to AIV
- infection at the portal of entry
- production of progeny viruses
- transmission to new hosts.

These barriers limit the extent of the host range of AIV in mammals. The pathogenesis of infection by AIV in mammals determines the character, distribution and severity of lesions, and influences all steps involved in cross-species transmission of the viruses.

Effective exposure of mammals to avian influenza viruses

The exact route of exposure of mammals to AIV is not precisely known. Avian influenza viruses replicate mostly in the intestinal tract of birds and are thought to be transmitted by a faecal-oral route in these species (138). By contrast, mammalian influenza viruses replicate in the respiratory tract of mammals and are transmitted via the respiratory route (3, 7, 42). Transmission of influenza viruses in humans is thought to occur primarily through respiratory droplets generated by coughing and sneezing, although direct physical contact, indirect contact with a contaminated environment or airborne transmission of exhaled virus particles have also been suggested (7). Cross-species transmission of AIV to mammals may thus occur through:

- direct physical contact between mammals and bird reservoirs of AIV, including predation and ingestion of infected bird carcasses
- indirect contact with bird faeces or a contaminated environment, including ingestion of contaminated food or water
- inhalation of virus particles excreted by birds as aerosols.

Proximity to and contact with bird reservoirs are the factors that have probably driven the cross-species transmission of LPAIV to swine, horses, harbour seals, whales and mink, although the precise route of exposure remains unknown (Fig. 1). Contact may occur between farmed mammals and poultry or wild birds. Integrated farming is a common practice in Southeast Asia, where

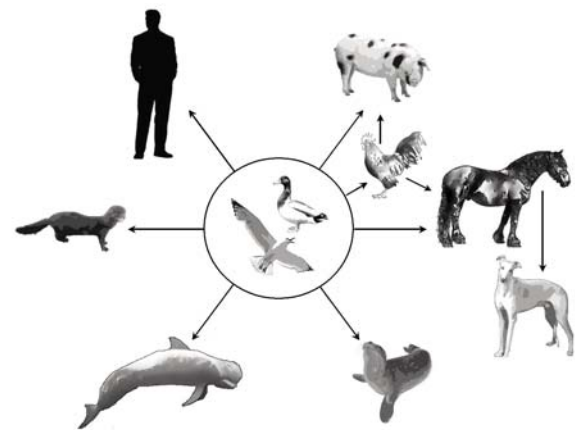


Fig. 1
Schematic representation of known events involving cross-species transmission of low pathogenicity avian influenza viruses to mammals besides humans

Cross-species transmission of low pathogenicity avian influenza viruses (LPAIV) to swine, horses, harbour seals, whales and mink. The source of infection is not precisely known but is thought to be wild bird reservoirs (Anseriformes, such as ducks, and Charadriiformes, such as gulls). Poultry can become infected with LPAIV and may transmit the viruses to swine and horses, when reared together. Horses have transmitted equine influenza H3N8 virus to domestic dogs

pigs and poultry are reared together, and this method is increasingly integrated with fish farming, fertilising fish ponds with their manure (110). Such husbandry practices have likely favoured the emergence of influenza virus in swine and the repeated cross-species transmission of various subtypes in this species (117). An example is the identification in swine from Asia of avian-like H9N2 viruses, which are endemic in terrestrial poultry in China (98) (Table I). In Western countries, the most used swine husbandry practices are intensive indoor pig rearing and free-range outdoor pig rearing. Intensive indoor rearing practices may allow cross-species transmission of AIV, if optimal biosecurity is not maintained. Outbreaks of AIV H4N6 and H3N3 infections in pigs occurred in a barn where the water supply for pigs was pumped out of an adjacent lake used by wild waterfowl (52, 56). Low pathogenic AIV can survive in water for long periods, up to several months (122, 123). Water contaminated with virus particles may thus have been the source of swine infection (52, 53). Free-range outdoor pig rearing may also provide multiple opportunities for virus transmission between wild birds or poultry and pigs, e.g. on pastures where birds and pigs forage together. Likewise, other animals, such as horses and ruminants, grazing on pastures used by foraging poultry or wild birds, may also be exposed to AIV. Finally, the avian H10N4 virus outbreak in farmed mink in Sweden occurred among animals kept in outside enclosures, where they had direct contact with gulls. The gulls were seen eating mink food placed on top of the enclosures and are believed to have transmitted the AIV H10N4 to the mink (62).

Contact may also occur between wild mammals and wild birds. Pinnipeds and cetaceans may have close contact with seabirds ashore or at sea. Pinnipeds spend time ashore at haul-out sites, which are typically used by seabirds for roosting. Such interactions may have contributed to outbreaks of AIV infections in harbour seals (33). Similarly, cetaceans and seabirds may feed on the same fish species at sea, and so facilitate cross-species transmission of AIV between these species. Examples are viruses of the H13 subtype (H13N2 and H13N9), isolated from a single pilot whale, which were found to be closely related to gull viruses (43). Close contact also occurs during predation or scavenging of birds by wild mammals. It remains unclear why species that prey or scavenge on poultry or wild waterfowl have yet to be reported as being infected with LPAIV.

The situation differs markedly for cross-species transmission of HPAIV H5N1. Ingestion of infected bird carcasses appears to be the primary route of exposure in affected animal species so far. Thus, all mammals (except humans) found to be infected with HPAIV H5N1 to date are carnivore species (except for the rare reports of the isolation of HPAIV H5N1 in swine) (Fig. 2). Poultry carcasses were the likely source of infection for captive tigers and leopards, which were fed on chickens (58, 129), and for domestic cats and dogs, which probably scavenged on dead poultry remains (121, 144). Wild bird carcasses were reported to be the source of infection for free-ranging domestic or wild carnivores. The domestic cat that died of

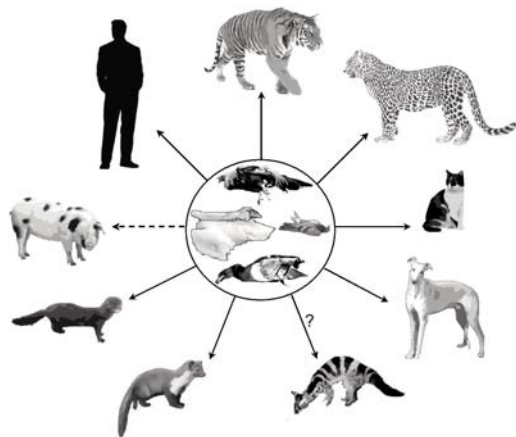


Fig 2.
Schematic representation of known events involving cross-species transmission of highly pathogenic avian influenza viruses to mammals besides humans

Cross-species transmission of highly pathogenic avian influenza virus (HPAIV) H5N1 to tigers, leopards, domestic cats, a domestic dog, Owston's palm civets, a stone marten, a mink and swine. The source of infection is typically traced to wild bird and domestic poultry carcasses that the animals fed on. The route of infection for Owston's palm civets is unknown. Isolation of HPAIV H5N1 in swine has only rarely been reported

HPAIV H5N1 infection in Thailand was reported to have eaten a dead pigeon (*Columba livia*), and infected pigeons were found in the area where the cat lived (120). Similarly, domestic cats, a stone marten and a mink, infected with HPAIV H5N1 in Europe in early 2006, were found in the vicinity of outbreaks occurring in wild bird populations, and also probably scavenged on bird carcasses (63, 64). Owston's palm civets, however, were not fed on bird carcasses at the sanctuary in Vietnam. The source of their infection remains unknown, although small mammals and birds could have entered the civet enclosures (107).

Infection at the portal of entry

The respiratory portal of entry

The respiratory tract of mammals is considered the portal of entry and primary target for replication of AIV in these species, based on the distribution and preferred tissue tropism of AIV in mammals. Avian influenza virus infection in mammals typically results in respiratory disease, and both LPAIV and HPAIV H5N1 were recovered from the respiratory tracts of most infected animals. Since necropsy is usually performed several days after infection, it is difficult, if not impossible, to determine which cells are initially infected upon natural cross-species transmission of AIV to mammals. Detailed studies on the tissue distribution of AIV at early time-points following the experimental infection of mammals remain scarce but may provide such information. The experimental inoculation of mink with AIV H10N4 demonstrated extensive infection of both the nasal mucosa and lungs on the first day after aerosol exposure, as determined by immunohistochemistry (30). Tracheal cells were found to be infected two days after exposure, thus the viruses may have spread upwards from the lungs, or downwards from the upper respiratory tract, to infect the trachea. Aerosol exposure may therefore have seeded viruses directly into the lungs of experimentally infected mink, and both the cells of the nasal mucosa and the cells of the pulmonary parenchyma may have been initially infected upon inoculation. Similarly, on the first day post inoculation, HPAIV H5N1 was present at high titres in the nasal turbinates and lungs of ferrets inoculated intranasally (147). However, local virus replication in these tissues was not confirmed by immunohistochemistry or *in situ* hybridisation.

The preferred tissue tropism of AIV for the respiratory tract of mammals may be explained by:

- its accessibility as a portal of entry
- the presence of trypsin-like proteases, which are necessary for full infectivity of LPAIV
- the presence of cellular receptors, which are recognised by AIV.

The HA protein of influenza viruses mediates the attachment and fusion of virus particles with host cells, and needs to be cleaved by host proteases for full infectivity (124). Only cleaved HA can undergo an irreversible conformational change resulting in the fusion of the virus envelope with the infected cell membrane. The HA of LPAIV is cleaved by extracellular trypsin-like proteases present in a limited number of tissues (124). In consequence, trypsin-like proteases must be present at the portal of entry for infection with LPAIV to take place. Such trypsin-like proteases have been shown to occur in the respiratory tract of some mammals (46, 61). In contrast, the HA of HPAIV is cleaved by ubiquitous intracellular subtilisin-like proteases (124), and HPAIV are typically released from infected cells with cleaved HA proteins (6). This may explain the wider tissue tropism of HPAIV H5N1 in mammals (see below).

The presence of cellular receptors for AIV at the portal of entry is essential for successful infection, and undoubtedly partly determines the host range of AIV in mammals (83, 125). These viruses bind preferentially to sialic acid residues with alpha 2,3 linkage to galactose (SA α 2,3Gal) (108, 109). The presence of such sialic acids has been demonstrated in:

- the trachea of swine (48) and horses (127)
- the lungs of a seal and whale (species unspecified) (49)
- the bronchial epithelium of domestic dogs (77).

Studies of the binding of AIV on tissues of the respiratory tract of mammals further demonstrated the presence of target cells for attachment in the lower respiratory tract (mainly bronchioles and alveoli) of swine, ferrets and domestic cats (136). Interestingly, a rare binding of AIV was detected in the trachea of pigs (136), which contrasts with the reported presence of SA α 2,3Gal, as determined by lectin histochemistry (48).

Studies on the distribution of cellular receptors for AIV, or the attachment patterns of AIV, in the respiratory tracts of mink, leopards, tigers, Owston's palm civets and stone martens, are lacking. Furthermore, little is known on the distribution of SA α 2,3Gal residues in the upper respiratory tract of mammals besides the trachea (84). Biopsies from the upper respiratory tract of humans (nasopharyngeal, adenoid and tonsillar tissues) have been successfully infected with HPAIV H5N1 following inoculation at relatively high titres (85). Although the cells of both the upper and lower respiratory tract may become infected initially, the lower respiratory tract appears to be one of the main target sites for viral attachment (136). Mucins secreted by mucous cells along the respiratory tract, which can bind to and trap AIV particles (136), together with the activity of the ciliated respiratory

epithelium, which may propel virus particles upwards away from the lower respiratory tract, may prevent AIV from reaching and infecting their target cells (128). This may explain the relative rarity of cross-species transmission of AIV in mammals.

Other potential portals of entry

There is evidence that the intestinal tract may also represent a portal of entry for AIV in mammals. The HPAIV H5N1 was occasionally isolated from the intestine of ferrets, on the first day after intranasal inoculation (147). When inoculated intranasally, HPAIV H5N1 may be swallowed and infect cells of the intestinal tract directly from the lumen of the intestine. Neuronal cells from the wall of the intestinal tract were found to be infected with HPAIV H5N1 in cats fed on infected bird carcasses but not in cats infected intra-tracheally, as determined by immunohistochemistry. This further suggests a potential intestinal portal of entry (106). A number of LPAIV have also been isolated from the ileum and colon of intranasally infected ferrets (57), from the liver of Balaenopterid whales naturally infected with AIV H1N3 (75), and from the liver of mink experimentally infected with AIV H10N4 (62). The liver was found to be infected with HPAIV H5N1 in tigers, domestic cats and a domestic dog that had ingested infected bird carcasses (63, 106, 120, 121, 129, 144).

Unlike mammalian influenza viruses, AIV are resistant to high temperature and low pH (31). Thus, the acid environment of the stomach lumen may not form such a strong barrier. Swallowed viruses may enter mammals via the intestinal tract and infect the liver after entering the portal system, which transports blood directly from intestine to liver (129). Although the above results are suggestive, the intestine as a portal of entry for the influenza virus in mammals is yet to be formally proven. This is because it cannot be excluded that the virus detected in the intestine and liver of the above cases originated from the respiratory tract, which became infected during feeding.

Avian influenza viruses have also been recovered from the brains of infected mammals, that is:

- from the brain of harbour seals naturally infected with LPAIV H7N7 and H4N5 (44, 68, 140)
- from the brain of a pilot whale naturally infected with LPAIV H13N2 and H13N9 (43)
- from the brain of a mink experimentally infected with LPAIV H10N4 (30)
- from the brains of tigers (129), domestic cats (63, 106, 120, 144), Owston's palm civets (107) and a stone marten (64), all either naturally or experimentally infected with HPAIV H5N1.

These viruses may have spread to the brain following viraemia and crossing of the blood-brain barrier. However, in addition to viraemia, neuronal transmission of HPAIV H5N1 from the nasal cavity to the olfactory bulb, along the olfactory nerves, has been experimentally demonstrated in mice (97). Interestingly, the cells of the intestinal tract infected with HPAIV H5N1 in cats fed on infected carcasses were neuronal cells of the submucosal and myenteric plexi (106). These findings suggest that HPAIV H5N1 is neurotropic and that, both in the nose and intestine, which are putative portals of entry, the first infected cells may be neuronal rather than epithelial.

Within-host replication and associated lesions

Within the respiratory tract

The respiratory tract is considered the primary site for replication of influenza viruses in mammals. Avian influenza viruses were isolated from the lungs of all naturally infected mammals, with the exception of a stone marten infected with HPAIV H5N1 (Table IV). Binding of AIV was demonstrated predominantly on type II pneumocytes and alveolar macrophages, and occasionally on non-ciliated cuboidal bronchiolar cells in swine, cats and ferrets (136). Attachment to bronchial and tracheal

Table IV

Tissue tropism of low pathogenic avian influenza viruses and highly pathogenic avian influenza H5N1 viruses, and associated lesions in naturally and experimentally infected mammals

Mammal species	Virus subtype	Distribution of main microscopic lesions	Distribution of LPAIV and HPAI H5N1 virus detected by RT-PCR (R), viral isolation (V), or immunohistochemistry (I)								Ref.
			Brain	Lungs	Liver	Pancreas	Intestine	Kidney	Heart	Spleen	
Swine	LPAIV H4N6	Lungs	... ^(a)	V-I ^(b)	52, 56
	LPAIV H3N3										
Horse	LPAIV H3N8	Lungs, intestine	...	V	40
Harbour seal	LPAIV H7N7	Lungs	V ^(c)	V	15, 33, 44,
	LPAIV H5N4										68, 140
	LPAIV H4N6										
	LPAIV H3N3										
Balaenopterid whale	LPAIV H1N3	Not conducted	...	V	V	75
Pilot whale	LPAIV H13N2	Brain, lungs, liver	V	V	43
	LPAIV H13N9										
Mink	LPAIV H10N4	Upper respiratory tract, lungs	V	V-I	V	5, 30, 62
Leopard	HPAIV H5N1	Brain, lungs	...	V-I	58
Tiger	HPAIV H5N1	Brain, lungs, liver	I	V-I	I	129
Domestic cat	HPAIV H5N1	Brain, lungs, liver, pancreas, intestine, kidney, heart, spleen, adrenal gland	V-I	R-V-I	R-V-I	R	R-V-I	V-I	V-I	V-I	63, 106, 120, 144
Owston's palm civet	HPAIV H5N1	Brain, lungs, liver	R-V-I	R-V-I	R-V	R-V	107
Domestic dog ^(d)	HPAIV H5N1	Lungs, liver, kidney	–	V-I	V-I	–	–	V-I	–	–	121
Stone marten	HPAIV H5N1	Brain, lungs, liver, pancreas, kidney	R-I	–	–	I	–	–	–	–	64
Red fox	HPAIV H5N1	Brain, lungs, heart	I	V-I	–	–	–	–	I	–	105

LPAIV: low pathogenic avian influenza viruses

HPAIV: highly pathogenic avian influenza viruses

RT-PCR: reverse transcription–polymerase chain reaction

a) In various reports, the list of organs tested was not explicitly stated, thus negative results could not be inferred

b) Viral replication of only avian influenza H3N3 viruses has been demonstrated by immunohistochemistry, in naturally infected swine

c) Only avian influenza H7N7 and H5N4 viruses were isolated from the brains of naturally infected harbour seals which died of the infection

d) Negative results apply to viral isolation in this case – no immunohistochemistry was performed on organs found negative by viral isolation. All organs found positive by viral isolation also tested positive by immunohistochemistry

cells was rare. The attachment patterns of AIV were consistent with the distribution of respiratory tract lesions most often observed in naturally and experimentally infected mammals (136).

The cell types infected by AIV in the respiratory tracts of most naturally and experimentally infected mammals have rarely been precisely identified, but include bronchial, bronchiolar and alveolar epithelial cells (30, 58, 107, 120, 129). More specifically, ciliated and non-ciliated bronchiolar epithelial cells, type I pneumocytes and type II pneumocytes, as well as occasional alveolar macrophages, were found to test positive for infection by immunohistochemistry in cats that were naturally and experimentally infected with HPAIV H5N1 (63, 106). There are very few reports of target cells for the replication of AIV in the upper respiratory tract, including the trachea. The trachea appears rarely to be infected with AIV in mammals, in accordance with the paucity of attachment of AIV to this organ (136). However, epithelial cells of both the nasal cavity and trachea were reported infected with LPAIV H10N4 in experimentally infected mink, one and two days post aerosol exposure, respectively (30).

The primary pathological findings were: bronchitis, bronchiolitis, interstitial pneumonia or a combination of these in swine, horses, harbour seals, a pilot whale and mink infected with LPAIV, and in most carnivore species infected with HPAIV H5N1 (15, 33, 40, 43, 44, 52, 56, 58, 62, 63, 107, 120, 121, 129). Both the cytolytic effect of viral replication and the indirect effects of the induced host immune response may be responsible for the damage observed in the lungs of infected animals (13). Lesions of epithelial necrosis in the bronchi, bronchioli and alveoli and the presence of pulmonary oedema or haemorrhage may be associated with the cytolytic effect of viral replication in these epithelia. In the lungs, type I pneumocytes prevent the leakage of fluid across the alveolar–capillary barrier and reabsorb fluid from the alveolar lumen, and type II pneumocytes reabsorb fluid from the alveolar lumen and produce lung surfactant that is important for reducing alveolar surface tension. Therefore, damage to these cells allows fluid from the alveolar capillaries, as well as fibrin and, in severe cases, erythrocytes, to flood into the alveolar lumina. This causes severe, and in some cases fatal, respiratory dysfunction (19). Infiltration by inflammatory cells in response to infection further hampers the respiratory function of the affected respiratory tract.

Accordingly, in most mammals infected with AIV, respiratory distress was observed, and the lungs were frequently reported haemorrhagic or oedematous, i.e.:

- in harbour seals infected with LPAIV H7N7 (33)
 - in the pilot whale infected with LPAIV H13N2 and H13N9 (43)
 - in all carnivore species naturally infected with HPAIV H5N1 (58, 63, 64, 105, 106, 107, 120, 121, 129).
- In addition, inflammatory cells were frequently present in massive quantities around the bronchi and bronchioli, and within the pulmonary parenchyma of infected mammals. Interestingly, the severity of the pulmonary lesions induced by LPAIV and HPAIV H5N1 viruses in mammals appears comparable, although pathological examination was typically more thorough in mammals infected with HPAIV H5N1 (58, 63, 64, 105, 106, 107, 120, 121, 129).
- Secondary bacterial infections are thought to contribute to more severe disease in mammals infected with influenza A viruses (3, 42, 65). This appears to be the case for swine infected with LPAIV H4N6 and H3N3 (52, 56). Both the influenza virus and opportunistic bacterial pathogens were present in the lungs of dead swine, and mortality was reduced with antibiotic treatment. Similarly, co-infection with *Mycoplasma* may have contributed to the severity of the disease caused by infection with LPAIV H7N7 and H4N5 in harbour seals (44, 140). The absence of secondary bacterial infections was not systematically reported, but they appear to be rare in mammals infected with HPAIV H5N1 so far (67).
- ### Outside the respiratory tract
- Low pathogenic AIV and HPAIV have been isolated from extra-respiratory organs in mammals, suggesting that other sites for replication occur. The extra-respiratory organs from which AIV are most frequently isolated are the brain and liver (Table IV). However, only *in situ* detection of the virus (by direct immunofluorescence, immunohistochemistry or *in situ* hybridisation) can prove viral replication (65). Thus, the replication of AIV in the extra-respiratory organs of mammals has so far been proven only for HPAIV H5N1, namely, in the:
- brain (mostly neuronal and glial cells but occasionally choroid epithelial cells, ependymal cells and leptomeningeal epithelial cells)
 - liver (hepatocytes)
 - heart (cardiomyocytes)
 - kidney (tubular epithelial and glomerular cells)
 - spleen (mononuclear and reticular cells)
 - intestine (ganglion cells and Schwann cells of the submucosal and myenteric plexi, mononuclear cells in the Peyer's patches)
 - pancreas (pancreatic acinar cells)
 - adrenal gland (adrenocortical cells) (58, 63, 64, 106, 107, 120, 121) (Fig. 3).

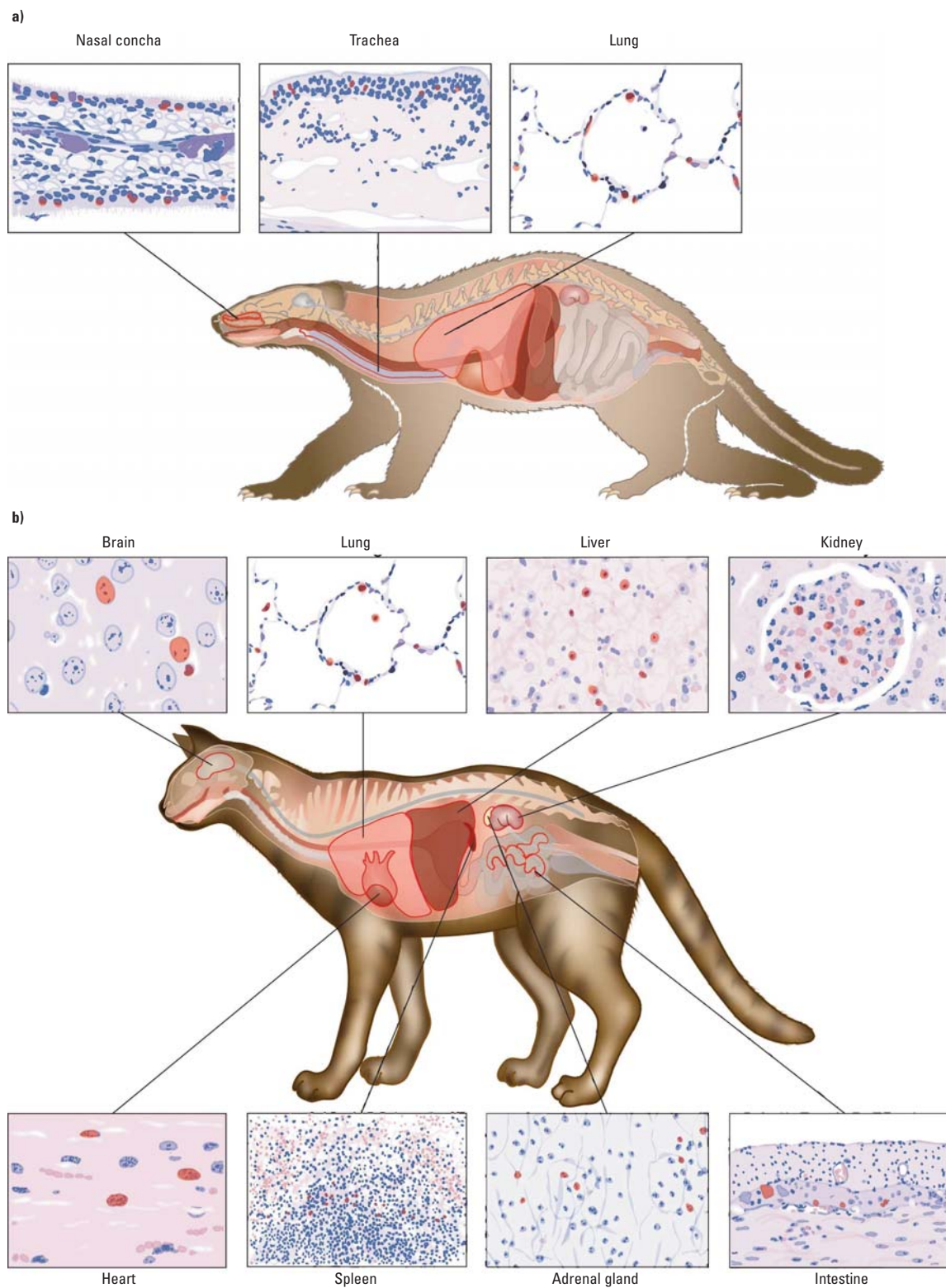


Fig. 3
Schematic distribution of avian influenza virus replication sites

a) Replication sites of low pathogenicity avian influenza viruses in mammals, based on data from experimental avian influenza H10N4 virus infection in mink

b) Replication sites of highly pathogenic avian influenza virus (HPAIV) H5N1 in mammals, based on data from experimental HPAIV H5N1 infection in domestic cats.

Infected organs are outlined in red. Inserts represent histological details of infected organs. Infected cells are coloured in red
 Artwork by Samantha J. Elmhurst (www.livingart.org.uk)

Such wide tissue tropism may be explained by the ability of the HA protein of HPAIV H5N1 to be cleaved by ubiquitous intracellular proteases. However, it remains unknown whether extra-respiratory spread is particular to HPAIV H5N1 or – given the rare reports of the isolation of LPAIV in the brain or liver (or both) of harbour seals, whales and mink – whether other AIV also have the ability to spread and infect organs beyond the respiratory tract. Little is known about the distribution of cellular receptors (SA α 2,3Gal) recognised by AIV in extra-respiratory tissues of mammals, although these have been identified on cells of the liver, kidney, spleen, brain and intestine in humans (143).

Avian influenza virus may reach extra-respiratory organs via the blood, lymph or nerves. In the case of HPAIV H5N1, both viraemia and neuronal transmission have been suggested as routes of spread beyond the respiratory tract. Experimental infection of mice suggests that HPAIV H5N1 may infect the brain from the nasal cavity through neuronal transmission along the olfactory nerves, which penetrate the cribriform plate and extend to the olfactory bulb (97). Avian influenza virus also may enter the brains of infected mammals after viraemia and crossing of the blood-brain barrier. Viraemia may occur after viruses cross the alveolar-capillary barrier when it is damaged by influenza viral pneumonia (65). Viraemia has rarely been measured or reported in natural or experimental infection of non-human mammals with influenza viruses (71).

In contrast, the isolation of the virus from the blood of two patients, and the detection of H5N1 viral RNA in the blood of 9 out of 16 patients, indicates that viraemia can occur at reasonably high levels and for prolonged periods in humans with symptomatic HPAIV H5N1 infection (71). Furthermore, the pattern of virus infection in the kidneys, liver and adrenal gland in experimentally infected cats strongly suggested that the spread of HPAIV H5N1 was blood borne (106).

Elevated levels of cytokines, including interferons (IFN) and tumour necrosis factor α (TNF- α), have been associated with high viral loads (29) in fatal cases of HPAIV H5N1 infection in humans. The non-structural (NS) NS1 protein of HPAIV H5N1 has been shown to confer resistance to the antiviral effects of IFN and TNF- α , and contributes to prolonged viraemia in swine (113). It is possible that elevated levels of cytokines are caused or promoted by a stronger host cytokine response to HPAIV H5N1, which escapes the antiviral effects of cytokines and continues to replicate (113). Viral replication resulting in high HPAIV H5N1 titres and high levels of pro-inflammatory cytokines in the lower respiratory tract may induce sufficient damage to the alveoli for viral entry into the bloodstream. This may then result in systemic viral spread and the infection of extra-respiratory organs.

Surprisingly, endothelial cells of humans, and possibly other mammals, harbour SA α 2,3Gal residues (143). Infection of the endothelium is a significant pathological finding in chickens infected with HPAIV H5N1 (100), but has rarely been documented in mammals, e.g. the replication of HPAIV H5N1 in occasional endothelial cells within the heart and pulmonary vein of experimentally infected cats (106).

Severe lesions of necrosis and inflammation are observed in most infected extra-respiratory tissues, and may result from both the cytolytic effect of viral replication and the indirect effects of the induced host immune response. The presence of replicating virus in the cells of affected organs is typically detected at the edge of focal lesions of necrosis, suggesting a radial spread of the virus within the organs (Fig. 4). Furthermore, massive infiltration by inflammatory cells may not always be observed. This may further support the role of the cytolytic effects of influenza virus replication in the formation of reported lesions.

Extra-respiratory clinical signs associated with such lesions are most often subtle, but may be observed in more advanced stages of organ failure. Thus, one cat experimentally infected with HPAIV H5N1 presented generalised icterus, possibly resulting from liver failure associated with viral infection (106). Neurological signs are the exception as these are often conspicuous, associated with small lesions of necrosis and inflammation in the brain and, notably, in the cerebellum. Thus, neurological

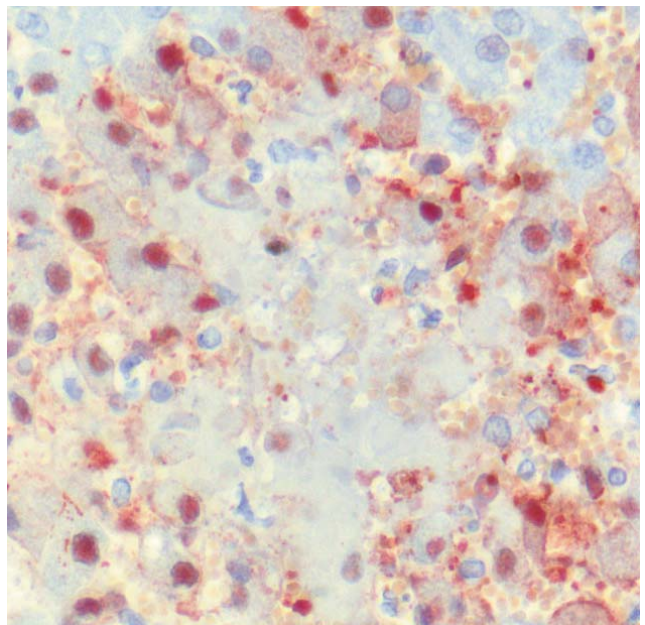


Fig. 4
The focal lesion of necrosis in the liver of a cat experimentally infected with highly pathogenic avian influenza virus H5N1, and the presence of infected hepatocytes at the transition between necrotic and normal liver parenchyma, as detected by immunohistochemistry

signs, such as ataxia and circling, have often been reported in mammals infected with HPAIV H5N1, i.e. in naturally infected leopards and tigers (58, 129), Owston's palm civets (107) and a stone marten (64), but also in a mink that was experimentally infected with LPAIV H10N4 (30).

Mammal-to-mammal transmission of avian influenza viruses

Infection with LPAIV has resulted in epidemics with mammal-to-mammal transmission in:

- swine (52, 56)
- horses (40)
- harbour seals (15, 33, 44)
- mink (62).

Mammal-to-mammal transmission of HPAIV H5N1 is rare, but has been suggested in captive tigers (129), and demonstrated experimentally in cats (106). Interspecific transmission from experimentally infected cats to naïve domestic dogs did not occur (34). Multiple factors may determine mammal-to-mammal transmission of AIV (66). These are likely to include the presence of progeny AIV at the portal of exit and their release into the environment, which are both related to the pathogenesis of AIV infection in the mammalian host.

Respiratory portal of exit

The location of virus replication in the respiratory tract is considered important for the ability of influenza viruses to be transmitted to new hosts. The highly pathogenic H5N1 strains, which replicate mostly in the lower respiratory tract, may not be easily excreted, and may not easily reach their target cells in new hosts (115, 135, 136). This may explain, at least partly, the poor ability of these viruses to be transmitted. In contrast, mammalian influenza viruses (swine, equine and human), which mostly replicate in a higher location in the respiratory tract, are efficiently transmitted between individuals. Clinical signs associated with mammalian influenza virus infection, such as coughing and sneezing, create respiratory droplets, which contain virus particles that can be transmitted to secondary hosts (7). Human influenza viruses and classical and human-like swine influenza viruses attach to different cellular receptors from AIV, namely, sialic acids with alpha 2,6 linkage to galactose (SA α 2,6Gal) (108). These sialic acids are present mostly in the upper respiratory tract, including the nasal, pharyngeal and tracheal epithelia of pigs and humans (22, 48, 84). Binding studies further demonstrate that human influenza viruses attach to different cell types from AIV in swine, ferrets and domestic cats, i.e. mostly to ciliated epithelial cells in the trachea and bronchi (136). Accordingly, when ferrets were

experimentally infected with influenza viruses with a receptor specificity for SA α 2,6Gal residues, they transmitted viruses to naïve animals, while ferrets infected with influenza viruses with a receptor specificity for SA α 2,3Gal residues did not (132).

However, mammal-to-mammal transmission of several LPAIV has been observed in swine, horses, harbour seals and mink. Low pathogenic AIV H10N4 was shown to replicate in the upper respiratory tract, including the trachea and nasal epithelium, of mink which developed rhinitis and presented with coughing and sneezing (30). In addition, other AIV were experimentally shown to be transmitted from mink to mink (91). Other unknown factors may therefore favour or limit the ability of AIV to be transmitted from mammal to mammal. Importantly, AIV that were naturally transmitted from mammal to mammal in swine, horses, harbour seals and mink may have already adapted to these species, at least partially. Thus, the AIV H7N7 and H3N8, which caused respiratory outbreaks in harbour seals and horses, respectively, appeared to be more mammalian-like than avian-like viruses, as they replicated experimentally in various mammals, and only poorly in birds (40, 140). These viruses may have acquired mutations that facilitated their transmission from mammal to mammal. Interestingly, the AIV H4N6 virus that infected swine in Canada in 1999 presented two amino acid substitutions in the HA protein (52), one of which was shown to confer receptor specificity for SA α 2,6Gal residues (4).

At present, there is a lack of consensus about the target cells for AIV in the mammalian respiratory tract. There are several possible reasons for this. First, the specificity of influenza virus for the glycan receptor on the host cell is determined not only by the type of glycan-sialic acid linkage, but also by glycan modifications, such as fucosylation, sulphation and additional sialylation (32, 125), and so cannot be determined by techniques that only measure glycan-sialic acid linkages. Secondly, the affinity of AIV for respiratory tissues has been determined by different techniques (lectin histochemistry, virus histochemistry, infection), each with its own advantages and limitations. Thirdly, the respiratory cells or tissues tested have different histories (*in vitro* cell culture, *ex vivo* tissues, *in vivo* tissues). This history may have an important effect on the receptor expression on the cell surfaces. Therefore, further research is required to determine the affinity of AIV and other influenza viruses for different parts of the mammalian respiratory tract, including standardisation of the methodology used to determine target cells.

Other portals of exit

Avian influenza viruses may also be excreted through other portals of exit. The HPAIV H5N1 strains have been isolated

from the intestinal tract and faeces, as well as the urinary tract and urine, of a number of infected mammals, i.e. both experimentally and naturally infected cats (106, 120, 144), experimentally infected ferrets (36, 78, 147), and naturally infected Owston's palm civets (107). Low pathogenic AIV of various subtypes have also been isolated from the ileum and colon of experimentally infected ferrets (57). Whether digestive and urinary tracts are also portals of exit remains speculative, and little is known on the risk of mammal-to-mammal transmission via these routes.

Perspective

Although HPAIV H5N1 is remarkable because of its expanding host range and wide tissue tropism, it is not unique in its ability to cross the species barrier, to result in clinical disease and mortality in mammals, or to replicate in extra-respiratory organs of mammals. Other AIV have crossed to swine, horses, harbour seals, whales and mink. These viruses have resulted in outbreaks of severe respiratory disease and mortality in most of these species, and have been isolated from extra-respiratory organs – namely, the brain and liver – of harbour seals, whales and mink. Avian influenza viruses have also become endemic in swine and horse populations, demonstrating their ability to adapt to and become sustained in mammalian host species. As of today, HPAIV H5N1 has not acquired this ability.

Important gaps remain in the understanding of cross-species transmission of AIV to mammals. In particular, the distribution of cellular receptors and the attachment patterns of AIV in the upper and lower respiratory tract and other tissues are yet to be described in detail for most

mammals. The target cells for replication of AIV, as determined by the *in situ* detection of replicating virus, are poorly known and need further attention. The intestinal tract has been suggested as a portal of entry for HPAIV H5N1, and this needs to be confirmed. Present understanding of the pathogenesis of AIV infection in mammals is very limited, in particular: the relative proportion of damage attributable to the direct cytolytic effect of virus replication versus the indirect effect of host immune response. Finally, the factors influencing the ability of AIV to be transmitted from mammal to mammal, and so become sustained in mammal populations, are poorly understood. Knowledge of these factors is crucial in the effort to mitigate the effects of cross-species transmission of AIV to mammals, including humans.

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Les virus de l'influenza aviaire et les mammifères

L.A. Reperant, G.F. Rimmelzwaan & T. Kuiken

Résumé

Les virus de sous-type H5N1 de l'influenza aviaire hautement pathogène sont remarquables car ils possèdent un spectre d'hôte s'étendant aux espèces non aviaires et un tropisme tissulaire diversifié. Ces virus sont responsables d'affections létales respiratoires et extra-respiratoires chez sept espèces de carnivores contractant l'infection naturellement. En revanche, ils ne sont pas les seuls à franchir la barrière d'espèce, à causer des infections cliniques et létales, ni à se répliquer dans les organes extra-respiratoires. Les virus de l'influenza aviaire faiblement pathogène se transmettent des oiseaux aux porcs, aux chevaux, aux phoques communs, aux baleines et aux visons ; ils sont alors à l'origine d'affections respiratoires graves et souvent létales ; dans certaines de

ces espèces, ils peuvent se propager en dehors du système respiratoire. La transmission entre mammifères est également constatée chez la plupart des espèces ; ces virus circulent désormais à l'état endémique dans les populations de porcs et de chevaux, ce qui confirme leur capacité à s'adapter et à persister chez les mammifères. Jusqu'à présent, les virus de l'influenza aviaire hautement pathogène de type H5N1 n'ont pas acquis ces propriétés, mais le risque qu'ils puissent un jour s'adapter aux espèces de mammifères et déclencher par la suite une pandémie de grippe humaine ne peut être totalement exclu.

Mots-clés

Barrière d'espèce – Influenza – Influenza aviaire – Mammifère – Pathogénie – Pathologie – Spectre d'hôte – Transmission entre espèces.



Virus de la influenza aviar en mamíferos

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Resumen

El incremento de los hospedadores no aviares del subtipo H5N1 del virus de la influenza aviar altamente patógena, así como su elevado tropismo tisular, son sorprendentes. Ese subtipo provocó enfermedades graves o mortales de las vías respiratorias y otros órganos en siete especies de carnívoros infectados naturalmente. Pero no es el único que se transmite entre distintas especies, provoca enfermedades clínicas o muertes, y se replica fuera de los órganos respiratorios. Los virus de la influenza aviar de baja patogenicidad se transmiten de aves a cerdos, caballos, focas comunes, ballenas y visones; provocan enfermedades respiratorias graves y muertes, y se sospecha que en algunos casos podrían haberse diseminado más allá del tracto respiratorio. También se propagan entre mamíferos de la mayoría de las especies. Además, se han vuelto endémicos en poblaciones de cerdos y caballos, lo que demuestra su capacidad para adaptarse y sobrevivir en mamíferos. Hasta la fecha, el subtipo H5N1 del virus de la influenza aviar altamente patógena no ha adquirido esa capacidad, pero se teme que se adapte a los mamíferos y desencadene una pandemia de influenza humana.

Palabras clave

Barrera interespecífica – Hospedador – Influenza – Influenza aviar – Mamífero – Patología – Patogénesis – Transmisión entre especies.



References

1. Amonsin A., Payungporn S., Theamboonlers A., Thanawongnuwech R., Suradhat S., Pariyothorn N., Tantilertcharoen R., Damrongwantanapokin S. *et al.* (2006). – Genetic characterization of H5N1 influenza A viruses isolated from zoo tigers in Thailand. *Virology*, **344** (2), 480-491.
2. Amonsin A., Songserm T., Chutinimitkul S., Jam-On R., Sae-Heng N., Pariyothorn N., Payungporn S., Theamboonlers A. & Poovorawan Y. (2007). – Genetic analysis of influenza A virus (H5N1) derived from domestic cat and dog in Thailand. *Arch. Virol.*, **152** (10), 1925-1933. E-pub.: 18 June 2007.
3. Bachmann P.A. (1989). – Swine influenza virus. *In* Virus infection of pigs. Elsevier Science, London, 193-207.
4. Bateman A.C., Busch M.G., Karasin A.I., Bovin N. & Olsen C.W. (2008). – Amino acid 226 in the hemagglutinin of H4N6 influenza virus determines binding affinity for alpha2,6-linked sialic acid and infectivity levels in primary swine and human respiratory epithelial cells. *J. Virol.*, **82** (16), 8204-8209. E-pub.: 11 June 2008.
5. Berg M., Englund L., Abusugra I.A., Klingeborn B. & Linné T. (1990). – Close relationship between mink influenza (H10N4) and concomitantly circulating avian influenza viruses. *Arch. Virol.*, **113** (1-2), 61-71.
6. Bosch FX., Orlich M., Klenk H.D. & Rott R. (1979). – The structure of the hemagglutinin, a determinant for the pathogenicity of influenza viruses. *Virology*, **95** (1), 197-207.
7. Brankston G., Gitterman L., Hirji Z., Lemieux C. & Gardam M. (2007). – Transmission of influenza A in human beings. *Lancet infect. Dis.*, **7** (4), 257-265.
8. Brown I.H. (2000). – The epidemiology and evolution of influenza viruses in pigs. *Vet. Microbiol.*, **74** (1-2), 29-46.
9. Brown I.H., Alexander D.J., Chakraverty P., Harris P.A. & Manvell R.J. (1994). – Isolation of an influenza A virus of unusual subtype (H1N7) from pigs in England, and the subsequent experimental transmission from pig to pig. *Vet. Microbiol.*, **39** (1-2), 125-134.
10. Brown I.H., Crawshaw T.R., Harris P.A. & Alexander D.J. (1998). – Detection of antibodies to influenza A virus in cattle in association with respiratory disease and reduced milk yield. *Vet. Rec.*, **143** (23), 637-638.
11. Brown I.H., Harris P.A., McCauley J.W. & Alexander D.J. (1998). – Multiple genetic reassortment of avian and human influenza A viruses in European pigs, resulting in the emergence of an H1N2 virus of novel genotype. *J. gen. Virol.*, **79** (12), 2947-2955.
12. Brown I.H., Hill M.L., Harris P.A., Alexander D.J. & McCauley J.W. (1997). – Genetic characterisation of an influenza A virus of unusual subtype (H1N7) isolated from pigs in England. *Arch. Virol.*, **142** (5), 1045-1050. Erratum: *Arch. Virol.*, **142** (7), 1519.
13. Bruder D., Srikiatkachorn A. & Enelow R.I. (2006). – Cellular immunity and lung injury in respiratory virus infection. *Viral Immunol.*, **19** (2), 147-155.
14. Butler D. (2006). – Thai dogs carry bird-flu virus, but will they spread it? *Nature*, **439** (7078), 773.
15. Callan R.J., Early G., Kida H. & Hinshaw V.S. (1995). – The appearance of H3 influenza viruses in seals. *J. gen. Virol.*, **76** (Pt 1), 199-203.
16. Campbell C.H., Easterday B.C. & Webster R.G. (1977). – Strains of Hong Kong influenza virus in calves. *J. infect. Dis.*, **135** (4), 678-680.
17. Campitelli L., Donatelli I., Foni E., Castrucci M.R., Fabiani C., Kawaoka Y., Krauss S. & Webster R.G. (1997). – Continued evolution of H1N1 and H3N2 influenza viruses in pigs in Italy. *Virology*, **232** (2), 310-318.
18. Castrucci M.R., Donatelli I., Sidoli L., Barigazzi G., Kawaoka Y. & Webster R.G. (1993). – Genetic reassortment between avian and human influenza A viruses in Italian pigs. *Virology*, **193** (1), 503-506.
19. Cheng I.W. & Matthay M.A. (2003). – Acute lung injury and the acute respiratory distress syndrome. *Crit. Care Clin.*, **19** (4), 693-712.
20. Choi Y.K., Nguyen T.D., Ozaki H., Webby R.J., Puthavathana P., Buranathal C., Chaisingh A., Auewarakul P. *et al.* (2005). – Studies of H5N1 influenza virus infection of pigs by using viruses isolated in Vietnam and Thailand in 2004. *J. Virol.*, **79** (16), 10821-10825.
21. Cong Y.L., Pu J., Liu Q.F., Wang S., Zhang G.Z., Zhang X.L., Fan W.X., Brown E.G. & Liu J.H. (2007). – Antigenic and genetic characterization of H9N2 swine influenza viruses in China. *J. gen. Virol.*, **88** (7), 2035-2041.
22. Couceiro J.N., Paulson J.C. & Baum L.G. (1993). – Influenza virus strains selectively recognize sialyloligosaccharides on human respiratory epithelium; the role of the host cell in selection of hemagglutinin receptor specificity. *Virus Res.*, **29** (2), 155-165.
23. Crawford P.C., Dubovi E.J., Castleman W.L., Stephenson I., Gibbs E.P., Chen L., Smith C., Hill R.C. *et al.* (2005). – Transmission of equine influenza virus to dogs. *Science*, **310** (5747), 482-485. E-pub.: 26 September 2005.
24. Crawshaw T.R., Brown I.H., Essen S.C. & Young S.C. (2007). – Significant rising antibody titres to influenza A are associated with an acute reduction in milk yield in cattle. *Vet. J.*, **178** (1), 98-102.
25. Daly J.M., Blunden A.S., Macrae S., Miller J., Bowman S.J., Kolodziejek J., Nowotny N. & Smith K.C. (2008). – Transmission of equine influenza virus to English foxhounds. *Emerg. infect. Dis.*, **14** (3), 461-464.

26. Danner G.R., McGregor M.W., Zarnke R.L. & Olsen C.W. (1998). – Serologic evidence of influenza virus infection in a ringed seal (*Phoca hispida*). *Mar. Mammal Sci.*, **14** (2), 380-384.
27. Davis L.M. & Spackman E. (2008). – Do crocodilians get the flu? Looking for influenza A in captive crocodilians. *J. experim. Zool., Pt A, Ecol. Genet. Physiol.*, **309** (10), 571-580.
28. De Boer G.F., Back W. & Osterhaus A.D. (1990). – An ELISA for detection of antibodies against influenza A nucleoprotein in humans and various animal species. *Arch. Virol.*, **115** (1-2), 47-61.
29. De Jong M.D., Simmons C.P., Thanh T.T., Hien V.M., Smith G.J., Chau T.N., Hoang D.M., Van Vinh Chau N. *et al.* (2006). – Fatal outcome of human influenza A (H5N1) is associated with high viral load and hypercytokinemia. *Nature Med.*, **12** (10), 1203-1207. E-pub.: 10 September 2006.
30. Englund L. & Hård af Segerstad C. (1998). – Two avian H10 influenza A virus strains with different pathogenicity for mink (*Mustela vison*). *Arch. Virol.*, **143** (4), 653-666.
31. Fiszon B., Hannoun C., Garcia-Sastre A., Villar E. & Cabezas J.A. (1989). – Comparison of biological and physical properties of human and animal A (H1N1) influenza viruses. *Res. Virol.*, **140** (5), 395-404.
32. Gambaryan A., Yamnikova S., Lvov D., Tuzikov A., Chinarev A., Pazykina G., Webster R., Matrosovich M. & Bovin N. (2005). – Receptor specificity of influenza viruses from birds and mammals: new data on involvement of the inner fragments of the carbohydrate chain. *Virology*, **334** (2), 276-283.
33. Geraci J.R., St Aubin D.J., Barker I.K., Webster R.G., Hinshaw V.S., Bean W.J., Ruhnke H.L., Prescott J.H. *et al.* (1982). – Mass mortality of harbor seals: pneumonia associated with influenza A virus. *Science*, **215** (4536), 1129-1131.
34. Giese M., Harder T., Teifke J., Klopffleisch R., Breithaupt A., Mettenleiter T. & Vahlenkamp T.W. (2008). – Experimental infection and natural contact exposure of dogs with avian influenza virus (H5N1). *Emerg. infect. Dis.*, **14** (2), 308-310.
35. Gourreau J.M., Kaiser C., Valette M., Douglas A.R., Labie J. & Aymard M. (1994). – Isolation of two H1N2 influenza viruses from swine in France. *Arch. Virol.*, **135** (3-4), 365-382.
36. Govorkova E.A., Rehg J.E., Krauss S., Yen H.L., Guan Y., Peiris M., Nguyen T.D., Hanh T.H. *et al.* (2005). – Lethality to ferrets of H5N1 influenza viruses isolated from humans and poultry in 2004. *J. Virol.*, **79** (4), 2191-2198. Erratum: *J. Virol.*, **80** (12), 6195.
37. Graham D.A., Calvert V. & McLaren E. (2002). – Retrospective analysis of serum and nasal mucus from cattle in Northern Ireland for evidence of infection with influenza A virus. *Vet. Rec.*, **150** (7), 201-204.
38. Guan Y., Shortridge K.F., Krauss S., Li P.H., Kawaoka Y. & Webster R.G. (1996). – Emergence of avian H1N1 influenza viruses in pigs in China. *J. Virol.*, **70** (11), 8041-8046.
39. Gunning R.F., Brown I.H. & Crawshaw T.R. (1999). – Evidence of influenza A virus infection in dairy cows with sporadic milk drop syndrome. *Vet. Rec.*, **145** (19), 556-557.
40. Guo Y., Wang M., Kawaoka Y., Gorman O., Ito T., Saito T. & Webster R.G. (1992). – Characterization of a new avian-like influenza A virus from horses in China. *Virology*, **188** (1), 245-255.
41. Guo Y., Wang M., Zheng G.S., Li W.K., Kawaoka Y. & Webster R.G. (1995). – Seroepidemiological and molecular evidence for the presence of two H3N8 equine influenza viruses in China in 1993-94. *J. gen. Virol.*, **76** (8), 2009-2014.
42. Hannant D. & Mumford J.A. (1996). – Equine influenza. In *Virus infections of equines* (M.J. Studdert & M.C. Horzinek, eds). Elsevier Health Sciences, Amsterdam, 285-293.
43. Hinshaw V.S., Bean W.J., Geraci J., Fiorelli P., Early G. & Webster R.G. (1986). – Characterization of two influenza A viruses from a pilot whale. *J. Virol.*, **58** (2), 655-656.
44. Hinshaw V.S., Bean W.J., Webster R.G., Rehg J.E., Fiorelli P., Early G., Geraci J.R. & St Aubin D.J. (1984). – Are seals frequently infected with avian influenza viruses? *J. Virol.*, **51** (3), 863-865.
45. Hinshaw V.S., Webster R.G., Easterday B.C. & Bean W.J. Jr (1981). – Replication of avian influenza A viruses in mammals. *Infect. Immun.*, **34** (2), 354-361.
46. Horimoto T. & Kawaoka Y. (2001). – Pandemic threat posed by avian influenza A viruses. *Clin. Microbiol. Rev.*, **14** (1), 129-149.
47. Ismail T.M., Sami A.M., Youssef H.M. & Abou Zaid A.A. (1990). – An outbreak of equine influenza type I in Egypt in 1989. *Vet. med. J. Giza*, **38** (2), 195-206.
48. Ito T., Couceiro J.N., Kelm S., Baum L.G., Krauss S., Castrucci M.R., Donatelli I., Kida H. *et al.* (1998). – Molecular basis for the generation in pigs of influenza A viruses with pandemic potential. *J. Virol.*, **72** (9), 7367-7373.
49. Ito T., Kawaoka Y., Nomura A. & Otsuki K. (1999). – Receptor specificity of influenza A viruses from sea mammals correlates with lung sialyloligosaccharides in these animals. *J. vet. med. Sci.*, **61** (8), 955-958.
50. Ito T., Kawaoka Y., Vines A., Ishikawa H., Asai T. & Kida H. (1998). – Continued circulation of reassortant H1N2 influenza viruses in pigs in Japan. *Arch. Virol.*, **143** (9), 1773-1782.
51. Kalthoff D., Hoffmann B., Harder T., Durban M. & Beer M. (2008). – Experimental infection of cattle with highly pathogenic avian influenza virus (H5N1). *Emerg. infect. Dis.*, **14** (7), 1132-1134.

52. Karasin A.I., Brown I.H., Carman S. & Olsen C.W. (2000). – Isolation and characterization of H4N6 avian influenza viruses from pigs with pneumonia in Canada. *J. Virol.*, **74** (19), 9322-9327.
53. Karasin A.I., Carman S. & Olsen C.W. (2006). – Identification of human H1N2 and human-swine reassortant H1N2 and H1N1 influenza A viruses among pigs in Ontario, Canada (2003 to 2005). *J. clin. Microbiol.*, **44** (3), 1123-1126.
54. Karasin A.I., Landgraf J., Swenson S., Erickson G., Goyal S., Woodruff M., Scherba G., Anderson G. & Olsen C.W. (2002). – Genetic characterization of H1N2 influenza A viruses isolated from pigs throughout the United States. *J. clin. Microbiol.*, **40** (3), 1073-1079.
55. Karasin A.I., Schutten M.M., Cooper L.A., Smith C.B., Subbarao K., Anderson G.A., Carman S. & Olsen C.W. (2000). – Genetic characterization of H3N2 influenza viruses isolated from pigs in North America, 1977-1999: evidence for wholly human and reassortant virus genotypes. *Virus Res.*, **68** (1), 71-85.
56. Karasin A.I., West K., Carman S. & Olsen C.W. (2004). – Characterization of avian H3N3 and H1N1 influenza A viruses isolated from pigs in Canada. *J. clin. Microbiol.*, **42** (9), 4349-4354.
57. Kawaoka Y., Bordwell E. & Webster R.G. (1987). – Intestinal replication of influenza A viruses in two mammalian species. Brief report. *Arch. Virol.*, **93** (3-4), 303-308.
58. Keawcharoen J., Oraveerakul K., Kuiken T., Fouchier R.A., Amonsin A., Payungporn S., Noppornpanth S., Wattanodorn S. *et al.* (2004). – Avian influenza H5N1 in tigers and leopards. *Emerg. infect. Dis.*, **10** (12), 2189-2191.
59. Kida H., Ito T., Yasuda J., Shimizu Y., Itakura C., Shortridge K.F., Kawaoka Y. & Webster R.G. (1994). – Potential for transmission of avian influenza viruses to pigs. *J. gen. Virol.*, **75** (9), 2183-2188.
60. Kida H., Shortridge K.F. & Webster R.G. (1988). – Origin of the hemagglutinin gene of H3N2 influenza viruses from pigs in China. *Virology*, **162** (1), 160-166.
61. Kido H., Yokogoshi Y., Sakai K., Tashiro M., Kishino Y., Fukutomi A. & Katunuma N. (1992). – Isolation and characterization of a novel trypsin-like protease found in rat bronchiolar epithelial Clara cells. A possible activator of the viral fusion glycoprotein. *J. biol. Chem.*, **267** (19), 13573-13579.
62. Klingeborn B., Englund L., Rott R., Juntti N. & Rockborn G. (1985). – An avian influenza A virus killing a mammalian species – the mink. Brief report. *Arch. Virol.*, **86**, 347-351.
63. Klopffleisch R., Wolf P.U., Uhl W., Gerst S., Harder T., Starick E., Vahlenkamp T.W., Mettenleiter T.C. & Teifke J.P. (2007). – Distribution of lesions and antigen of highly pathogenic avian influenza virus A/swan/Germany/R65/06 (H5N1) in domestic cats after presumptive infection by wild birds. *Vet. Pathol.*, **44** (3), 261-268.
64. Klopffleisch R., Wolf P.U., Wolf C., Harder T., Starick E., Niebuhr M., Mettenleiter T.C. & Teifke J.P. (2007). – Encephalitis in a stone marten (*Martes foina*) after natural infection with highly pathogenic avian influenza virus subtype H5N1. *J. comp. Pathol.*, **137** (2-3), 155-159. E-pub.: 8 August 2007.
65. Kuiken T. & Taubenberger J.K. (2008). – Pathology of human influenza revisited. *Vaccine*, **26** (Suppl. 4), D59-D66.
66. Kuiken T., Holmes E.C., McCauley J., Rimmelzwaan G.F., Williams C.S. & Grenfell B.T. (2006). – Host species barriers to influenza virus infections. *Science*, **312** (5772), 394-397.
67. Kuiken T., Rimmelzwaan G., van Riel D., van Amerongen G., Baars M., Fouchier R. & Osterhaus A. (2004). – Avian H5N1 influenza in cats. *Science*, **306** (5694), 241. E-pub.: 2 September 2004.
68. Lang G., Gagnon A. & Geraci J.R. (1981). – Isolation of an influenza A virus from seals. *Arch. Virol.*, **68** (3-4), 189-195.
69. Lekcharoensuk P., Lager K.M., Vemulapalli R., Woodruff M., Vincent A.L. & Richt J.A. (2006). – Novel swine influenza virus subtype H3N1, United States. *Emerg. infect. Dis.*, **12** (5), 787-794.
70. Leschnik M., Weikel J., Mostl K., Revilla-Fernandez S., Wodak E., Bago Z., Vanek E., Benetka V. *et al.* (2007). – Subclinical infection with avian influenza A (H5N1) virus in cats. *Emerg. infect. Dis.*, **13** (2), 243-247.
71. Likos A.M., Kelvin D.J., Cameron C.M., Rowe T., Kuehnert M.J. & Norris P.J. (2007). – Influenza viremia and the potential for blood-borne transmission. *Transfusion*, **47** (6), 1080-1088.
72. Lipatov A.S., Kwon Y.K., Sarmiento L.V., Lager K.M., Spackman E., Suarez D.L. & Swayne D.E. (2008). – Domestic pigs have low susceptibility to H5N1 highly pathogenic avian influenza viruses. *PLoS Pathog.*, **4** (7), e1000102.
73. Loeffen W.L., Kamp E.M., Stockhofe-Zurwieden N., van Nieuwstadt A.P., Bongers J.H., Hunneman W.A., Elbers A.R., Baars J. *et al.* (1999). – Survey of infectious agents involved in acute respiratory disease in finishing pigs. *Vet. Rec.*, **145** (5), 123-129.
74. Lopez J.W. & Woods G.T. (1984). – Influenza virus in ruminants: a review. *Res. Commun. chem. Pathol. Pharmacol.*, **45** (3), 445-462.
75. Lvov D.K., Zhdanov V.M., Sazonov A.A., Braude N.A., Vladimirtseva E.A., Agafonova L.V., Skljanskaja E.I., Kaverin N.V. *et al.* (1978). – Comparison of influenza viruses isolated from man and from whales. *Bull. WHO*, **56**, 923-930.
76. Ma W., Gramer M., Rossow K. & Yoon K.J. (2006). – Isolation and genetic characterization of new reassortant H3N1 swine influenza virus from pigs in the midwestern United States. *J. Virol.*, **80** (10), 5092-5096.

77. Maas R., Tacken M., Ruuls L., Koch G., van Rooij E. & Stockhofe-Zurwieden N. (2007). – Avian influenza (H5N1) susceptibility and receptors in dogs. *Emerg. infect. Dis.*, **13** (8), 1219-1221.
78. Maines T.R., Lu X.H., Erb S.M., Edwards L., Guarner J., Greer P.W., Nguyen D.C., Szretter K.J. *et al.* (2005). – Avian influenza (H5N1) viruses isolated from humans in Asia in 2004 exhibit increased virulence in mammals. *J. Virol.*, **79** (18), 11788-11800.
79. Mancini D., Mendonça R., Cianciarullo A., Kobashi L., Trindade H., Fernandes W. & Pinto J. (2004). – Influenza in heterothermic animals [in Portuguese]. *Rev. Soc. bras. Med. trop.*, **37** (3), 204-209.
80. Matsuura Y., Yanagawa R. & Noda H. (1979). – Experimental infection of mink with influenza A viruses. *Arch. Virol.*, **62** (1), 71-76.
81. Nerome K., Kanegae Y., Shortridge K.F., Sugita S. & Ishida M. (1995). – Genetic analysis of porcine H3N2 viruses originating in southern China. *J. gen. Virol.*, **76** (3), 613-624.
82. Nerome K., Kanegae Y., Yoshioka Y., Itamura S., Ishida M., Gojobori T. & Oya A. (1991). – Evolutionary pathways of N2 neuraminidases of swine and human influenza A viruses: origin of the neuraminidase genes of two reassortants (H1N2) isolated from pigs. *J. gen. Virol.*, **72** (3), 693-698.
83. Neumann G. & Kawaoka Y. (2006). – Host range restriction and pathogenicity in the context of influenza pandemic. *Emerg. infect. Dis.*, **12** (6), 881-886.
84. Nicholls J.M., Bourne A.J., Chen H., Guan Y. & Peiris J.S. (2007). – Sialic acid receptor detection in the human respiratory tract: evidence for widespread distribution of potential binding sites for human and avian influenza viruses. *Respir. Res.*, **8** (1), 73.
85. Nicholls J.M., Chan M.C., Chan W.Y., Wong H.K., Cheung C.Y., Kwong D.L., Wong M.P., Chui W.H. *et al.* (2007). – Tropism of avian influenza A (H5N1) in the upper and lower respiratory tract. *Nature Med.*, **13** (2), 147-149. E-pub.: 7 January 2007.
86. Nielsen O., Clavijo A. & Boughen J.A. (2001). – Serologic evidence of influenza A infection in marine mammals of arctic Canada. *J. Wildl. Dis.*, **37** (4), 820-825.
87. Nikitin T., Cohen D., Todd J.D. & Lief F.S. (1972). – Epidemiological studies of A/Hong Kong/68 virus infection in dogs. *Bull. WHO*, **47**, 471-479.
88. Ninomiya A., Takada A., Okazaki K., Shortridge K.F. & Kida H. (2002). – Seroepidemiological evidence of avian H4, H5, and H9 influenza A virus transmission to pigs in southeastern China. *Vet. Microbiol.*, **88** (2), 107-114.
89. Ohishi K., Kishida N., Ninomiya A., Kida H., Takada Y., Miyazaki N., Boltunov A.N. & Maruyama T. (2004). – Antibodies to human-related H3 influenza A virus in Baikal seals (*Phoca sibirica*) and ringed seals (*Phoca hispida*) in Russia. *Microbiol. Immunol.*, **48** (11), 905-909.
90. Ohishi K., Ninomiya A., Kida H., Park C.H., Maruyama T., Arai T., Katsumata E., Tobayama T. *et al.* (2002). – Serological evidence of transmission of human influenza A and B viruses to Caspian seals (*Phoca caspica*). *Microbiol. Immunol.*, **46** (9), 639-644.
91. Okazaki K., Yanagawa R. & Kida H. (1983). – Contact infection of mink with 5 subtypes of avian influenza virus. Brief report. *Arch. Virol.*, **77** (2-4), 265-269.
92. Olsen C.W. (2002). – The emergence of novel swine influenza viruses in North America. *Virus Res.*, **85** (2), 199-210.
93. Osterhaus A.D.M.E., Rimmelzwaan G.F., Martina B.E.E., Bestebroer T.M. & Fouchier R.A.M. (2000). – Influenza B virus in seals. *Science*, **288** (5468), 1051-1053.
94. Paillot R., Hannant D., Kydd J.H. & Daly J.M. (2006). – Vaccination against equine influenza: quid novi? *Vaccine*, **24** (19), 4047-4061.
95. Paniker C.K. & Nair C.M. (1972). – Experimental infection of animals with influenzavirus types A and B. *Bull. WHO*, **47** (4), 461-463.
96. Paniker C.K.J. & Nair C.M.G. (1970). – Infection with A2 Hong Kong influenza virus in domestic cats. *Bull. WHO*, **43** (6), 859-862.
97. Park C.H., Ishinaka M., Takada A., Kida H., Kimura T., Ochiai K. & Umemura T. (2002). – The invasion routes of neurovirulent A/Hong Kong/483/97 (H5N1) influenza virus into the central nervous system after respiratory infection in mice. *Arch. Virol.*, **147** (7), 1425-1436.
98. Peiris J.S., Guan Y., Markwell D., Ghose P., Webster R.G. & Shortridge K.F. (2001). – Cocirculation of avian H9N2 and contemporary 'human' H3N2 influenza A viruses in pigs in southeastern China: potential for genetic reassortment? *J. Virol.*, **75** (20), 9679-9686.
99. Pensaert M., Ottis K., Vandeputte J., Kaplan M.M. & Bachmann P.A. (1981). – Evidence for the natural transmission of influenza A virus from wild ducks to swine and its potential importance for man. *Bull. WHO*, **59** (1), 75-78.
100. Perkins L.E. & Swayne D.E. (2001). – Pathobiology of A/chicken/Hong Kong/220/97 (H5N1) avian influenza virus in seven gallinaceous species. *Vet. Pathol.*, **38** (2), 149-164.
101. ProMED-mail (2004). – Avian influenza – eastern Asia (32). Archive no. 20040224.0581. Available at: www.promedmail.org (accessed on 10 September 2008).
102. ProMED-mail (2006). – Avian influenza – worldwide (70): Asia, Europe. Archive no. 20060328.0943. Available at: www.promedmail.org (accessed on 10 September 2008).
103. ProMED-mail (2007). – Avian influenza (17): Indonesia (feline), Japan, Hungary. Archive no. 20070126.0347. Available at: www.promedmail.org (accessed on 10 September 2008).

104. ProMED-mail (2008). – Avian influenza (44): Viet Nam, civet. Archive no. 20080312.0991. Available at: www.promedmail.org (accessed 10 September 2008).
105. Reperant L.A., van Amerongen G., van de Bildt M.W.G., Rimmelzwaan G., Dobson A.P., Osterhaus A.D.M.E. & Kuiken T. (2008). – Highly pathogenic avian influenza virus (H5N1) infection in red foxes fed infected bird carcasses. *Emerg. infect. Dis.*, **14** (12), 1835-1841.
106. Rimmelzwaan G.F., van Riel D., Baars M., Bestebroer T.M., van Amerongen G., Fouchier R.A., Osterhaus A.D. & Kuiken T. (2006). – Influenza A virus (H5N1) infection in cats causes systemic disease with potential novel routes of virus spread within and between hosts. *Am. J. Pathol.*, **168** (1), 176-183.
107. Robertson S.I., Bell D.J., Smith G.J., Nicholls J.M., Chan K.H., Nguyen D.T., Tran P.Q., Streicher U. *et al.* (2006). – Avian influenza H5N1 in viverrids: implications for wildlife health and conservation. *Proc. Biol. Sci.*, **273** (1595), 1729-1732.
108. Rogers G.N. & Paulson J.C. (1983). – Receptor determinants of human and animal influenza virus isolates: differences in receptor specificity of the H3 hemagglutinin based on species of origin. *Virology*, **127** (2), 361-373.
109. Rogers G.N., Pritchett T.J., Lane J.L. & Paulson J.C. (1983). – Differential sensitivity of human, avian, and equine influenza A viruses to a glycoprotein inhibitor of infection: selection of receptor specific variants. *Virology*, **131** (2), 394-408.
110. Scholtissek C. & Naylor E. (1988). – Fish farming and influenza pandemics. *Nature*, **331** (6153), 215.
111. Scholtissek C., Burger H., Bachmann P.A. & Hannoun C. (1983). – Genetic relatedness of hemagglutinins of the H1 subtype of influenza A viruses isolated from swine and birds. *Virology*, **129** (2), 521-523.
112. Schultz U., Fitch W.M., Ludwig S., Mandler J. & Scholtissek C. (1991). – Evolution of pig influenza viruses. *Virology*, **183** (1), 61-73.
113. Seo S.H., Hoffmann E. & Webster R.G. (2002). – Lethal H5N1 influenza viruses escape host anti-viral cytokine responses. *Nature Med.*, **8** (9), 950-954. E-pub.: 26 August 2002.
114. Shi W.F., Gibbs M.J., Zhang Y.Z., Zhang Z., Zhao X.M., Jin X., Zhu C.D., Yang M.F. *et al.* (2008). – Genetic analysis of four porcine avian influenza viruses isolated from Shandong, China. *Arch. Virol.*, **153** (1), 211-217. E-pub.: 15 November 2007.
115. Shinya K., Ebina M., Yamada S., Ono M., Kasai N. & Kawaoka Y. (2006). – Avian flu: influenza virus receptors in the human airway. *Nature*, **440** (7083), 435-436.
116. Shortridge K.F., Gao P., Guan Y., Ito T., Kawaoka Y., Markwell D., Takada A. & Webster R.G. (2000). – Interspecies transmission of influenza viruses: H5N1 virus and a Hong Kong SAR perspective. *Vet. Microbiol.*, **74** (1-2), 141-147.
117. Shortridge K.F. & Stuart-Harris C.H. (1982). – An influenza epicentre? *Lancet*, **320** (8302), 812-813.
118. Shortridge K.F., Zhou N.N., Guan Y., Gao P., Ito T., Kawaoka Y., Kodihalli S., Krauss S. *et al.* (1998). – Characterization of avian H5N1 influenza viruses from poultry in Hong Kong. *Virology*, **252** (2), 331-342.
119. Shu L.L., Lin Y.P., Wright S.M., Shortridge K.F. & Webster R.G. (1994). – Evidence for interspecies transmission and reassortment of influenza A viruses in pigs in southern China. *Virology*, **202** (2), 825-833.
120. Songserm T., Amonsin A., Jam-On R., Sae-Heng N., Meemak N., Pariyothorn N., Payungporn S., Theamboonlers A. & Poovorawan Y. (2006). – Avian influenza H5N1 in naturally infected domestic cat. *Emerg. infect. Dis.*, **12** (4), 681-683.
121. Songserm T., Amonsin A., Jam-On R., Sae-Heng N., Pariyothorn N., Payungporn S., Theamboonlers A., Chutinimitkul S. *et al.* (2006). – Fatal avian influenza A H5N1 in a dog. *Emerg. infect. Dis.*, **12** (11), 1744-1747.
122. Stallknecht D.E., Kearney M.T., Shane S.M. & Zwank P.J. (1990). – Effects of pH, temperature, and salinity on persistence of avian influenza viruses in water. *Avian Dis.*, **34** (2), 412-418.
123. Stallknecht D.E., Shane S.M., Kearney M.T. & Zwank P.J. (1990). – Persistence of avian influenza viruses in water. *Avian Dis.*, **34** (2), 406-411.
124. Steinhauer D.A. (1999). – Role of hemagglutinin cleavage for the pathogenicity of influenza virus. *Virology*, **258** (1), 1-20.
125. Stevens J., Blixt O., Glaser L., Taubenberger J.K., Palese P., Paulson J.C. & Wilson I.A. (2006). – Glycan microarray analysis of the hemagglutinins from modern and pandemic influenza viruses reveals different receptor specificities. *J. molec. Biol.*, **355** (5), 1143-1155. E-pub.: 18 November 2005.
126. Stuen S., Have P., Osterhaus A.D., Arnemo J.M. & Moustgaard A. (1994). – Serological investigation of virus infections in harp seals (*Phoca groenlandica*) and hooded seals (*Cystophora cristata*). *Vet. Rec.*, **134** (19), 502-503.
127. Suzuki Y., Ito T., Suzuki T., Holland R.E. Jr, Chambers T.M., Kiso M., Ishida H. & Kawaoka Y. (2000). – Sialic acid species as a determinant of the host range of influenza A viruses. *J. Virol.*, **74** (24), 11825-11831.
128. Sweet C. & Smith H. (1980). – Pathogenicity of influenza virus. *Microbiol. Rev.*, **44** (2), 303-330.

129. Thanawongnuwech R., Amonsin A., Tantilertcharoen R., Damrongwatanapokin S., Theamboonlers A., Payungporn S., Nanthapornphiphat K., Ratanamungklanon S. *et al.* (2005). – Probable tiger-to-tiger transmission of avian influenza H5N1. *Emerg. infect. Dis.*, **11** (5), 699-701. Erratum: *Emerg. infect. Dis.*, **11** (6), 976.
130. Todd J.D. & Cohen D. (1968). – Studies of influenza in dogs. I. Susceptibility of dogs to natural and experimental infection with human A2 and B strains of influenza virus. *Am. J. Epidemiol.*, **87** (2), 426-439.
131. Tsai C.P. & Pan M.J. (2003). – New H1N2 and H3N1 influenza viruses in Taiwanese pig herds. *Vet. Rec.*, **153** (13), 408.
132. Tumpey T.M., Maines T.R., Van Hoeven N., Glaser L., Solorzano A., Pappas C., Cox N.J., Swayne D.E. *et al.* (2007). – A two-amino acid change in the hemagglutinin of the 1918 influenza virus abolishes transmission. *Science*, **315** (5812), 655-659.
133. Tumpey T.M., Szretter K.J., Van Hoeven N., Katz J.M., Kochs G., Haller O., Garcia-Sastre A. & Staeheli P. (2007). – The Mx1 gene protects mice against the pandemic 1918 and highly lethal human H5N1 influenza viruses. *J. Virol.*, **81** (19), 10818-10821. E-pub.: 25 July 2007.
134. Van Reeth K., Brown I.H. & Pensaert M. (2000). – Isolations of H1N2 influenza A virus from pigs in Belgium. *Vet. Rec.*, **146** (20), 588-589.
135. Van Riel D., Munster V.J., de Wit E., Rimmelzwaan G.F., Fouchier R.A., Osterhaus A.D. & Kuiken T. (2006). – H5N1 virus attachment to lower respiratory tract. *Science*, **312** (5772), 399. E-pub.: 23 March 2006.
136. Van Riel D., Munster V.J., de Wit E., Rimmelzwaan G.F., Fouchier R.A., Osterhaus A.D. & Kuiken T. (2007). – Human and avian influenza viruses target different cells in the lower respiratory tract of humans and other mammals. *Am. J. Pathol.*, **171** (4), 1215-1223. E-pub.: 23 August 2007.
137. Webby R.J. & Webster R.G. (2001). – Emergence of influenza A viruses. *Philos. Trans. roy. Soc. Lond., B, biol. Sci.*, **356** (1416), 1817-1828.
138. Webster R.G., Bean W.J., Gorman O.T., Chambers T.M. & Kawaoka Y. (1992). – Evolution and ecology of influenza A viruses. *Microbiol. Rev.*, **56** (1), 152-179.
139. Webster R.G., Geraci J.R., Petursson G. & Skirnisson K. (1981). – Conjunctivitis in human beings caused by influenza A virus of seals. *N. Engl. J. Med.*, **304**, 911.
140. Webster R.G., Hinshaw V.S., Bean W.J., van Wyke K.L., Geraci J.R., St Aubin D.J. & Petursson G. (1981). – Characterization of an influenza A virus from seals. *Virology*, **113**, 712-724.
141. Wright P.F. & Webster R.G. (2001). – Orthomyxoviruses. In *Fields Virology* (D.M. Knipe & P.M. Howley, eds), 4th Ed. Lippincott Williams & Wilkins, Philadelphia, 1533-1579.
142. Xu C., Fan W., Wei R. & Zhao H. (2004). – Isolation and identification of swine influenza recombinant A/swine/Shandong/1/2003 (H9N2) virus. *Microbes Infect.*, **6** (10), 919-925.
143. Yao L., Korteweg C., Hsueh W. & Gu J. (2008). – Avian influenza receptor expression in H5N1-infected and noninfected human tissues. *FASEB J.*, **22** (3), 733-740.
144. Yingst S.L., Saad M.D. & Felt S.A. (2006). – Qinghai-like H5N1 from domestic cats, Northern Iraq. *Emerg. infect. Dis.*, **12** (8), 1295-1297.
145. Zhou N.N., Senne D.A., Landgraf J.S., Swenson S.L., Erickson G., Rossow K., Liu L., Yoon K., Krauss S. & Webster R.G. (1999). – Genetic reassortment of avian, swine, and human influenza A viruses in American pigs. *J. Virol.*, **73** (10), 8851-8856.
146. Zhu Q., Yang H., Chen W., Cao W., Zhong G., Jiao P., Deng G., Yu K. *et al.* (2008). – A naturally occurring deletion in its NS gene contributes to the attenuation of an H5N1 swine influenza virus in chickens. *J. Virol.*, **82** (1), 220-228. E-pub.: 17 October 2007.
147. Zitzow L.A., Rowe T., Morken T., Shieh W.J., Zaki S. & Katz J.M. (2002). – Pathogenesis of avian influenza A (H5N1) viruses in ferrets. *J. Virol.*, **76** (9), 4420-4429.

