Rev. sci. tech. Off. int. Epiz., 2009, **28** (1), 69-92

Outbreaks of highly pathogenic avian influenza in Europe: the risks associated with wild birds

M. Artois [(1)*], D. Bicout [(1)], D. Doctrinal [(1)], R. Fouchier [(2)], D. Gavier-Widen [(3)], A. Globig [(4)], W. Hagemeijer [(5)], T. Mundkur [(5)], V. Munster [(2)] & B. Olsen [(6)]

(1) Université J. Fourier, Laboratoire TIMC-IMAG, Unité Environnement et Prévision de la Santé des Populations, F-38000 Grenoble, Ecole nationale vétérinaire de Lyon, 1 avenue Bourgelat, 69280 Marcy l'Etoile, France
E-mail: m.artois@vet-lyon.fr
(2) Institute of Virology, Erasmus MC, PO Box 2040, 3000 CA, Rotterdam, the Netherlands
E-mail: v.munster@erasmusmc.nl
(3) Department of Pathology and Wildlife Diseases, National Veterinary Institute (NVI) & Department of Biomedical Sciences and Veterinary Public Health, University of Agricultural Sciences (SLU), Uppsala, Sweden
E-mail: dolores.gavier-widen@sva.se
(4) Friedrich-Loeffler-Institut, Federal Institute for Animal Health, Insel Riems, Germany
E-mail: anja.globig@fli.bund.de
(5) Wetlands International, PO Box 471, 6700 AL Wageningen, the Netherlands
E-mail: ward.hagemeijer@wetlands.org, taej.mundkur@wetlands.org
(6) Department of Infectious Diseases, Uppsala University, SE-751 85 Uppsala, Sweden
E-mail: bjorn.olsen@medsci.uu.se
*Corresponding author

Summary

The infection of wild birds by highly pathogenic strains of avian influenza (AI) virus was virtually unknown – apart from one instance of the disease appearing in common terns in South Africa in 1961 – before the Asian strain of highly pathogenic AI virus (AIV), H5N1, began to expand across the world.

Outbreaks of clinical disease in Eurasia have resulted in visible mortality among populations of free-ranging wild birds in a multitude of species. The circulation pattern of influenza viruses in natural ecosystems results from a selection pressure towards strains which are indirectly transmitted by droppings from water birds and contaminated fomites, and which exhibit low pathogenicity. Some of these viruses, of the subtypes H5 or H7, can mutate into highly pathogenic strains after being introduced into domestic poultry farms. The maintenance of highly pathogenic AIV (HPAIV) H5N1 in several parts of the world exposes wild birds to infected poultry, resulting in long-distance virus transmission. There is great concern that these wild birds may, in turn, propagate these HPAIV or introduce them into domestic birds. Rigorous disease control and biosecurity measures to protect poultry farms are the only solution presently available to mitigate such a risk.

Keywords

Avian influenza – Control – Ecology – H5N1 – Highly pathogenic avian influenza – Transmission – Wild birds.

Introduction

The recent introduction of highly pathogenic avian influenza (AI) into Asia and Europe in wild waterfowl and other birds is an illustration of the factors that promote the establishment of disease in and disease spread through natural ecosystems. For years this topic has fascinated scientists (4, 146).

Influenza, as an infection or disease, is a more complex model of wildlife-pathogen interactions than others frequently described in the scientific literature, such as rabies (6). Many virus subtypes are involved, which can

evolve very fast (139), and many vertebrate species can be affected (3). Influenza viruses are very successful, express a vast diversity of genes and are permanently evolving, as illustrated elsewhere in this volume.

Recent publications suggest that the generally accepted view that 'co-evolution leads to attenuation' has been challenged by a new concept, since – on the evolutionary scale – pathogens might be viewed as 'negotiating' with their host (123). The 'trade-off' or balance between efficient transmission by virus shedding and the capacity to damage infected tissues is unpredictable in a wide range of virus subtypes and host species.

Within the medical disciplines, the word 'disease' refers to a condition which affects the life expectancy or wellbeing of an individual. However, even so-called 'pathogenic agents' frequently infect a host without obvious clinical effect. Consequently, it is confusing to use the word 'disease' for the mostly inapparent infection of domestic and wild birds by influenza viruses (1, 2). The differentiation of influenza virus strains into low pathogenicity AI (LPAI) and highly pathogenic AI (HPAI) refers almost exclusively to the susceptibility of domestic chickens and turkeys. This differentiation is not so clearly observed in birds involved in the dispersal or maintenance of AI virus (AIV) among populations of free-living (wild) birds. The frequent occurrence of healthy carriers suggests that the induction of clinical disease is detrimental for virus transmission. In other words, in wild birds, clinical 'influenza' is the exception rather than the rule.

Host-pathogen interactions can also be considered at the population level: are these AIV-bird interactions 'sustainable' in the long term? If the pathogen regularly kills its host, it 'commits suicide'. In this case, a minimum number of surviving susceptible hosts are necessary to sustain infection, so that one infected bird can transmit the infection to another (36). As a consequence, a critical community size is required to allow the pathogens to propagate in the long term (121).

Owing to a lack of recorded historical data, the natural history of AIV is, for the most part, unknown. Some historic mass mortality events have been described with clinical signs that suggest AI (15).

A single incident of mass mortality occurred in South Africa in 1961 among common terns (*Sterna hirundo*; Charadriiformes), due to infection by an AIV. This was the only such known occurrence before the 2005 to 2006 outbreak of H5N1 in Eurasia and Africa (12). Thus, this recent outbreak of HPAIV H5N1 appears to be a rare and exceptional epidemiological event in wild birds, in obvious contrast to the previous understanding of AIV-wild bird interactions.

In this paper, the authors update the epidemiological situation of AI among populations of wild birds and clarify their epidemiological role as a reservoir of AIV. Finally, the authors will review the options available to prevent the incursion of AI into domestic poultry.

Important traits of behavioural ecology of birds

The behavioural ecology of birds drives the epidemiology of influenza infection by restraining or expanding the ability of the virus to spread. The risk of a bird species maintaining, introducing or spreading an AIV into a particular area is first correlated with the number of individuals in the susceptible species. This risk should always be considered in relation to a particular geographical area, taking the local (seasonal) abundance of that species into account. Gregariousness during the breeding, migration and non-breeding seasons is another important factor. Water birds play an essential role in AI epidemiology. They have evolved foraging and breeding strategies to exploit natural wetlands and can be found in virtually all types of wetland.

The species affected by the HPAIV H5N1 outbreak in 2006 belonged to the following orders:

– Anseriformes: water birds, such as ducks, geese and swans

– Charadriiformes: shore birds, such as gulls and waders

– Ciconiiformes: large wading birds, such as herons, storks and egrets

– Falconiformes: diurnal raptors, such as buzzards, hawks and kites

– Gruiformes: large waders, such as cranes

– Passeriformes: most of the small songbirds, such as finches, sparrows and crows

– Pelecaniformes: large, fish-eating water birds, such as cormorants and pelicans

– Strigiformes: nocturnal birds of prey, such as owls.

Anseriformes, Charadriiformes, Pelecaniformes and, to a lesser extent, Ciconiiformes are species that depend on wetlands for at least part of their life cycle.

Among the Anseriformes is the family Anatidae, which encompasses all duck species, subdivided into two main groups, according to the way they feed:

– 'dabbling ducks', such as the mallard (*Anas platyrhynchos*), forage in shallow water

– 'diving ducks', such as pochards (*Aythya ferina*) and tufted ducks (*A. fuligula*), swim under the surface.

Duck populations have a high turnover. In mallards, about one-third of the population is replaced each year, implying that this proportion is immunologically naive (13).

Differences in the behaviour of various host species could account for the difference in exposure to viruses and other pathogens among bird families and species. Whereas dabbling ducks feed on the surface of the water, geese and certain swan species graze in pastures and agricultural fields. Human-created and/or altered wetlands are important wildlife habitats as natural wetlands are increasingly being changed and converted to other types of habitat for human use. Wild birds are commonly seen at water reservoirs, salt ponds, dry or flooded agricultural fields, irrigation ditches and farm or aquaculture ponds.

In addition to wetlands, several water bird species can also be found in non-wetland habitats, principally agricultural habitats, where food is often abundant. The substantial loss of natural wetlands and the fact that many wetlands have been converted into intensive rice farms are factors that may result in the concentration of water birds in smaller areas, thereby increasing their density and the risk of virus transmission, primarily between the waterfowl and shore birds that populate these habitats. Through this proximity to agriculture and the sharing of these habitats with domestic birds, the risk of transmission from and to domestic birds is increased (142).

Birds can fly fast over long distances. Different types of movement across country borders are called 'migration' (14). A large number of birds undertake annual migrations. Many of these migrations follow one or more 'flyways', which are best defined as: a geographical region within which a species or constellation of species, or some populations of a species or constellation of species, complete their annual cycle (17). This region includes the following areas:

– where the birds breed

– the areas of the main non-breeding or contra-nuptial range

– migration stopovers or staging areas

– areas where birds that have not yet reached breeding maturity may spend the breeding season

– moulting areas

– post-breeding expansion areas.

Traditionally, migration is believed to happen from north to south (and vice versa), within or between continents. However, such generalisations underestimate the importance of east to west movements (or vice versa), which happen within each continent and flyway.

During their annual migrations, wild birds regularly interact and mix on their breeding grounds, stopover and non-breeding sites, which offers the opportunity for viruses to be exchanged. This includes the exposure of juvenile, immunologically 'naïve' individuals to the virus pool of adult individuals in the breeding grounds. Moreover, many populations of species that follow different flyways may have overlapping breeding ranges and stopover areas, providing an opportunity for the mixing and movement of viruses between flyways.

In addition to annual long-distance migration patterns, wild bird species may undertake short-to-medium-distance movements to escape unusual weather or seasonal conditions, including severe storms, freezing conditions, heavy snows or even drought.

Human activities have wide impacts on the use of habitats by wild birds and on their local movement patterns. Agricultural practices have direct and indirect influences on the use of these habitats and the movement patterns of birds. Such practices include:

– the types of crops planted

– cropping patterns

– the use of wild bird deterrents

– the types and intensities of fishing activities

– hunting birds.

The practice of feeding wild birds in parks, nature reserves or other areas that may be close to human habitation leads to an increase in concentrations of wild birds, thereby also increasing their interactions with people as well as with native, sedentary or migratory bird species and domestic poultry. This and other cultural and recreational activities may increase the risk of spreading viruses among domestic and wild animals.

Descriptive epidemiology

Influenza A viruses have been isolated from many mammals and bird species (3, 138). They are classified on the basis of the haemagglutinin (HA) and neuraminidase (NA) glycoproteins. In wild birds, all currently known influenza A viruses representing the 16 HA and 9 NA antigenic subtypes have been detected in numerous combinations (1, 51, 91, 94, 135, 149).

Species preference

Influenza A viruses have been isolated from at least 105 wild bird species from 26 different families (94), on all continents except Antarctica, from where there is only serological evidence (10, 90, 134). Although almost all possible subtype combinations have been found in free-living wild birds, some subtypes of HA have only been isolated from certain species (51, 110). Birds from wetlands and aquatic environments, such as the Anseriformes (particularly ducks, geese and swans) and Charadriiformes (particularly gulls, shore birds, terns and waders), constitute the most frequently reported hosts of AIV. Isolations of virus strains from 'terrestrial' birds are, in contrast, relatively rare (75, 76, 80, 91, 96, 111, 135, 139).

Geographic variability

Different genetic lineages of influenza A viruses have evolved in bird populations which are separated by oceans, resulting from limited interaction between the populations of Eurasia and the Americas. Avian influenza virus strains from North, Central and South America can thus be separated from those of the rest of the world (40, 106, 130). However, limited transmission of genes between the North American and Eurasian populations has been reported (84, 88, 131, 133), indicating that the interaction that does take place is sufficient for exchange to occur.

There are also inter-regional differences in the prevalence of subtypes in different duck populations ranging over different parts of the continent (65); probably as the result of limited interaction with distant populations. Nevertheless, a phylogeographic analysis of AIV genome sequences in North America revealed no strong species effect, suggesting that AIV can infect several bird species that share the same area (34).

Seasonal variation

Surveys for AIV yield variable results, depending on the season during which samples were collected. Data on this aspect have been obtained almost exclusively from mallard ducks. In general, the prevalence of AIV in ducks in the Northern Hemisphere peaks in late summer and early autumn (134). This pattern, which is clearly seen in mallards in North America, is associated with pre-migration and post-breeding staging (64, 65, 115). The results of wild bird surveillance in Europe (57, 120) and North America (76) have shown that the prevalence of AIV in wild ducks is less than one percent during most of the year, but rises to levels of around 30% during August and September until December.

The higher virus prevalence in ducks observed in Northern Europe in spring (132, 135), as compared with ducks in North America, could be explained by differences in virus/host ecology between these two continents but could also be due to data bias. A high virus prevalence early in the autumn migration probably declines gradually as the migration proceeds, thus forming a north-south gradient of virus prevalence, even within a single species (Fig. 1) (91, 134).

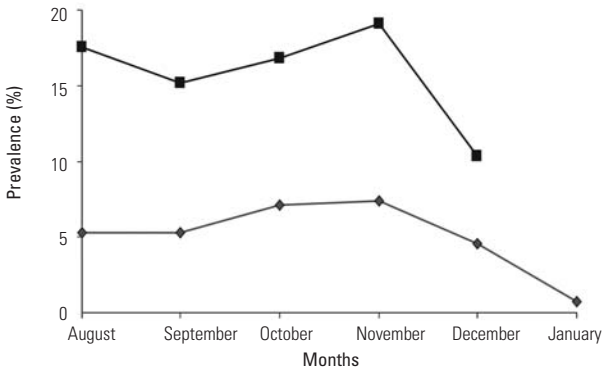

Fig. 1
Trend lines for the prevalence of influenza A virus in mallards sampled in Sweden and the Netherlands during the autumn migration
The squares represent the proportion (%) of mallards caught and sampled at Ottenby Bird Observatory in Sweden, between 2002 and 2005, which tested positive for the presence of antibodies against influenza A virus. The diamonds represent mallards caught at various locations in the Netherlands, between 1998 and 2005. Redrawn with permission from Munster *et al.* (91)

Yearly variation

In North America, at least, isolation rates and the occurrence of subtypes can vary considerably from year to year (65, 116). Sharp *et al.* (110) and Krauss *et al.* (76) have shown a cyclic pattern in wild ducks on this continent, since a peak in virus isolation can be followed, one or two years later, by a reduced frequency of isolation. Interestingly, the peak in virus prevalence in ducks parallels a period of low prevalence in shore birds.

Subtype distribution

A great diversity of subtype combinations has been detected in the few long-term studies performed in North America and Europe (76, 91, 94, 120). In mallards (*A. platyrhynchos*), NA and HA were found in 40 different combinations (Table I). The most prevalent were H4N6, H7N7 and H6N2. A summary is found in Table II.

It should be kept in mind that these differences may be biased by limited access to wild birds for sampling and a small number of available sampling sites. Thus, these differences may disappear as more studies are performed.

Table I
Haemagglutinin and neuraminidase subtypes and subtype combinations, found in mallards sampled at Ottenby Bird Observatory, Sweden, from 2002 to 2004

Haemagglutinin subtype	Neuraminidase subtype									Total
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	
1	5	2				1				**8**
2	2		7		1					**10**
3		1				1		5		**7**
4		3				19				**22**
5		5	3			1			3	**12**
6	2	12			1	1		3		**19**
7							16		1	**17**
8				4						**4**
9										**0**
10		2		1	1	2	2	2	3	**13**
11	1	1	2		1	1		1	7	**14**
12					2				1	**3**
13										**0**
14										**0**
15										**0**
16										**0**
Total	**10**	**26**	**12**	**5**	**6**	**26**	**18**	**11**	**15**	**129**

Table II
Comparison of findings between longitudinal studies of ducks in North America, Germany and Sweden

Study	Sweden	Germany	North America
Prevalence in autumn	13.50%	8.7%	22.2%
Prevalence in spring	3.30%	No data	0.03%
Most prevalent HA	H4, H6, H7	H4, H2, H1, H6, H7	H6, H3, H4
HA not found	H9, H13, H14, H15, H16	H5, H12, H13, H14, H15, H16	H13, H14, H15, H16
Most prevalent NA	N2, N6, N7	N1, N3, N6	N8, N2, N6
NA found	N1-9	N1-9	N1-9
Most prevalent subtype combinations	H4N6, H7N7, H6N2	H2N3, H4N6, H1N1, H6N2, H7N7	H3N8, H6N2, H4N6

HA: haemagglutinin
NA: neuraminidase
Source: Wallensten *et al.* (135)

Of the subtypes known to cause HPAI outbreaks in poultry, the subtype H7 was commonly found, but the relative frequency of H5 appears more variable. In comparison with North America, H5 and H7 strains were isolated more often in Europe. It is intriguing that some subtype combinations seem to be not only prevalent throughout the years, but also in different geographic areas of the world.

As well as the classification based on the envelope glycoproteins HA and NA, AIV have also been classified by their pathogenicity in chickens. Avian influenza virus of the subtypes H5 and H7 (but no other subtypes) may become highly pathogenic after being introduced into poultry and can cause outbreaks of fowl plague. The transition from an LPAI virus (LPAIV) into the HPAI phenotype results from the introduction of basic amino acids into the H5 or H7 cleavage site. This introduction facilitates systemic virus replication, resulting in an acute generalised disease in domestic chickens and turkeys, with mortality reaching 100% (1, 138, 139). All other AIV strains, including the H5 and H7 subtypes without a basic cleavage site, are LPAIV. Highly pathogenic virus strains are rarely isolated from wild birds (95).

Recent outbreaks of the highly pathogenic avian influenza virus H5N1 in wild birds

An HPAI H5N1 virus began circulating in Hong Kong in the late 1990s and, since 2005, has subsequently spread beyond Asia into Europe and Africa (Fig. 2). This strain was first isolated from a flock of diseased domestic geese in the Guangdong province of the People's Republic of China in 1996 (150). The H5N1 virus continued to evolve and spread to humans in Hong Kong in February 2002. This genotype then expanded across Southeast Asia (141). At the end of 2002, it occurred in wild waterfowl, captive wild birds and then in terrestrial birds in Hong Kong (44).

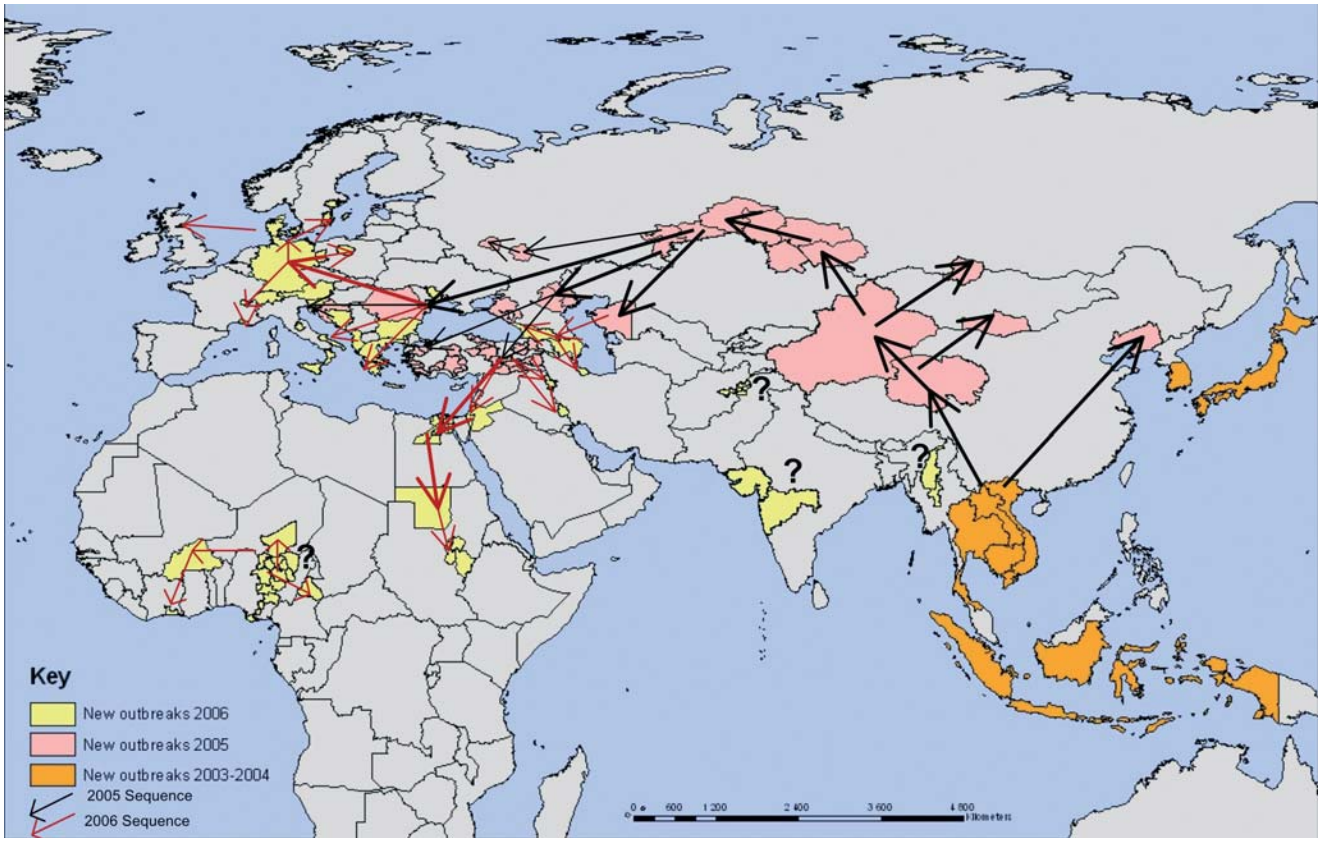

Fig. 2

The spatial and temporal sequence of spread of the H5N1 highly pathogenic avian influenza virus during 2005 and 2006

Note: the arrows indicate the apparent sequence of spatial (geographic) spread during the years 2005 and 2006. Redrawn and adapted from Sabirovic *et al.* (105), with permission

A large outbreak in May 2005 on Lake Qinghai, China, affected high numbers of wild birds, such as bar-headed geese (*Anser indicus*), brown-headed gulls (*Larus brunnicephalus*), great black-headed gulls (*L. ichthyaetus*) and great cormorants (*Phalacrocorax carbo*) (31, 32, 83). The virus then rapidly reached north-western Asia, Europe, the Middle East and Africa (Fig. 1).

Affected wild birds have been reported from several countries, predominantly:

– mute swans (*Cygnus olor*)
– whooper swans (*C. cygnus*)
– tufted ducks (*A. fuligula*).

However, other infected species have also been reported, such as:

– mergansers (*Mergus merganster*)
– raptors (Accipitridae)
– gulls (Laridae)
– white storks (*Ciconia ciconia*)
– herons (Ardeidae) (41, 58, 124, 148).

While swan deaths were the first indicator for the presence of H5N1 in several European countries, they may only represent 'sentinel' birds infected by other species (22, 62, 124). In proportion, relatively few ducks of the genus *Anas* have been found killed by this virus (53).

Data from the OIE, gathered from official country notifications of disease, demonstrate that, in spring and summer 2007, new outbreaks were recorded in Central and Western Europe, in which another wild bird species, black-necked grebes (*Podiceps nigricollis*), not previously reported to be susceptible, were lethally affected. Outbreaks were recorded in southern England in mute swans, late in 2007 and early in 2008. Finally, it is worth mentioning that, in February 2008, an apparently healthy pochard duck (*A. ferina*) was found to be infected with HPAIV H5N1 during routine surveillance on a lake close to Lucerne (Switzerland). The latest recorded outbreak, which was still continuing when this paper was completed, has affected several swans near Kosaka, Japan, since April of 2008. In January of 2009, 35 mallards were shot by hunters in Bayern (Germany) and submitted for testing, as part of routine active HPAI surveillance. One was found to be infected with HPAIV H5N1.

Signs of disease in wild birds

Few reports are available on AI as a clinical disease in free-living wild birds, except for the description of lesions and clinical signs recorded in birds affected by the recent HPAIV H5N1 outbreak, or in the 1961 outbreak of tern/S. Africa/61 virus. In wild species, HPAIV have been restricted to subtype H5, although most AIV of these subtypes cause mild, inapparent or no disease.

The information below, unless otherwise stated, comes from published descriptions and from observations by the authors on naturally infected wild birds which died or were euthanased *in agonum*.

Variability

A wide range of wild avian species died during outbreaks of natural infection by HPAIV H5N1 in Asia and Europe, demonstrating the broad host range of the virus. Experimental studies indicate that there is a high variation in the innate susceptibility of avian species, even within the same order (72, 99). The disease can vary greatly in the:

– severity of lesions
– extent of organ involvement
– duration
– clinical features
– ultimate mortality or recovery.

Nevertheless, the associated disease syndromes are remarkably similar across species (29).

Clinical signs

After experimental infection of *Aythya* species, clinical signs developed at three to four days post infection (72). Wild birds affected by HPAI may die rapidly without showing any clinical signs (44). In general, the birds show neurological disturbances, including paresis, paralysis, tremors, opisthotonus, head tilt and circling (44).

Pathology

Natural infection by HPAIV causes multifocal, disseminated acute-to-sub-acute lesions, suggestive of a viraemic phase resulting in haematogenous spread of the virus. Gross lesions, consisting of focal to large areas of necrosis, or necrosis and haemorrhage in the pancreas, are not always present. Haemorrhages and lung congestion are often observed. The most predominant and consistent histological lesions are:

a) multifocal, non-suppurative encephalitis, with:
– neuronal degeneration and necrosis
– perivascular cuffing
– glial and inflammatory reaction

b) focal areas of necrosis and inflammation in the pancreas

c) lung congestion and oedema.

Through immunohistochemistry, virus antigen can be demonstrated in the nucleus and cytoplasm of neurons and glial cells in the brain (Fig. 3) and in the neurons of peripheral ganglia and sub-mucosal and myenteric plexi. The neurotropism appears to be the most salient feature of natural HPAIV H5N1 infection in wild birds. The pancreas (Fig. 4) is also a preferential site of viral infection and viral antigen can be demonstrated in association with the lesions (122, 124). Other sites of viral replication resulting in focal necrosis and inflammation are the adrenal glands, kidneys, liver and heart.

In wild birds naturally infected with HPAIV H5N1, the involvement of the lungs appears to be less marked than in naturally infected poultry and experimentally infected wild birds. In naturally infected wild birds, pulmonary circulatory changes, congestion and oedema are frequent, but inflammatory reactions are in general mild or absent.

Isolated reports describe the presence of viral antigen in the mucosal epithelial cells of the small intestine in crows

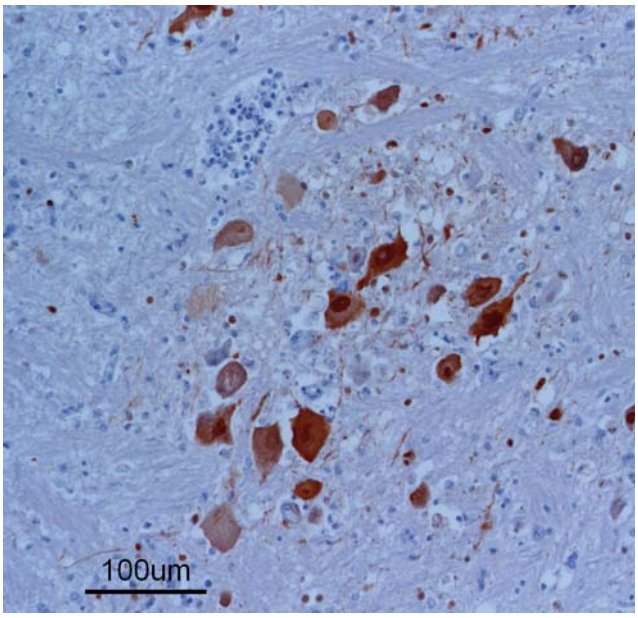

Fig. 3
The brain of a swan (*Cygnus* sp.) that died of infection from highly pathogenic avian influenza H5N1 virus
The large amount of virus (shown by red-brown pigment) is demonstrated in the nucleus and cytoplasm of neurons and glial cells in an area of encephalitis. Test conducted by immunohistochemistry

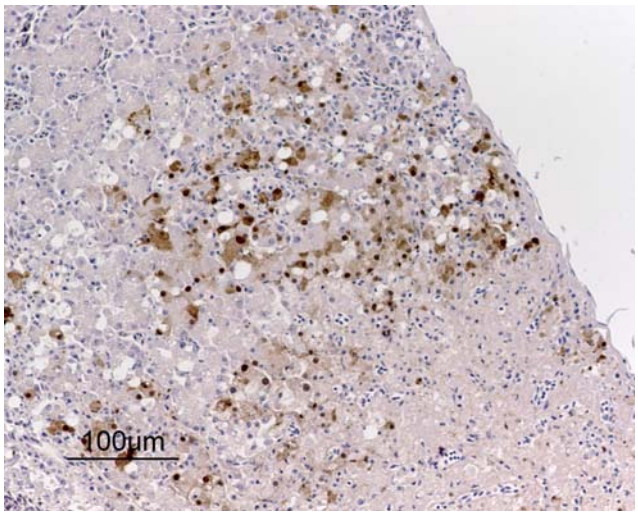

Fig. 4
The pancreas of a mute swan (*Cygnus olor*) that died of infection from highly pathogenic avian influenza H5N1 virus
Viral antigen is demonstrated (brown pigment) in the nucleus and/or cytoplasm of acinar cells in a focus of necrotising pancreatitis. Test conducted by immunohistochemistry

(*Corvus macrorhynchos*) (122) and magpies (*Pica pica sericea*) (77).

Viral shedding

Cloacal shedding is demonstrated in most cases of HPAIV infection in wild birds. The largest amount of virus probably derives from the pancreas and, in some cases, from the kidneys and liver. Until now, there has been little evidence of viral replication in the intestinal epithelia. The highly pathogenic AIV H5N1 has also been demonstrated in tracheal swabs from most birds that die of the infection. However, in a significant proportion of these birds, the virus can be demonstrated in the brain but not in the lungs, or else can be detected in the lungs only with a highly sensitive method (44). Terminally ill birds and their carcasses can be considered a source of the virus for predators and scavengers and are also likely to contaminate the environment.

Outcome of infection and mortality

Little information is available on non-fatal infection by HPAIV and on morbidity and mortality rates in wild birds. Experimental studies indicate that susceptibility and mortality are related to species (50). This is probably true also in natural outbreaks which affected multiple avian species (20, 74, 124, 153). Observations of birds in parks (44) indicate that a variable proportion of the wild birds exposed to and most probably infected by HPAIV H5N1 recover. After experimental infection, ducks of the genus *Anas* developed subclinical infection and recovered by seven to eight days post infection, while all the tufted ducks and most Eurasian pochards (*A. ferina*) died or were euthanased by the fourth day after infection (72).

Assessment of the potential risks of avian influenza associated with wild birds

The control of AIV rests principally on the ability to stop transmission from infected wild birds to poultry. However, an understanding of the role of wild birds in the maintenance and spread of HPAIV strains remains incomplete, as does the epidemiology of most multihost pathogens (63). This deficiency of understanding is a particular problem with emerging diseases, such as 'bird flu', since most of the responsible pathogens can infect multiple hosts. Several different and often conflicting definitions of the word 'reservoir' exist. Haydon *et al.* (63) proposed that a reservoir be defined as: 'one or more epidemiologically connected populations or environments in which the pathogen can be permanently maintained and from which infection is transmitted to the defined target population'. The authors now attempt to follow this definition to clarify the role played by wild birds in the evolution, maintenance and propagation of AIV.

The target population

The transmission of AIV from birds to non-avian species, including sea mammals, mink and pigs, occurs fairly frequently but these are usually temporary events and do not establish lasting lineages (140). In these host species, the condition resulting from infection can be serious and can affect the health of individuals.

Humans

Before the first recorded outbreak of HPAIV H5N1 in 1997, the proposal that birds could directly pass a virulent influenza virus to humans met with little acceptance (37, 137). Even now, the direct transmission of HPAIV from wild birds to humans is an extremely rare event, with only one case reported from Azerbaijan in 2006. Thus, the potential of wild birds to transmit HPAIV to humans will not be discussed further.

Domestic poultry and wild birds

The transmission of AIV from wild birds, in particular, waterfowl, to domestic poultry can result in considerable economic losses.

However, the H5N1 epizootic on Qinghai Lake in May 2005 caused an estimated 10% decrease in the entire

Table III
Number of outbreaks of highly pathogenic avian influenza virus H5N1, recorded per year and per country, from 2005 to November 2008
Over all, the deaths of more than 6,000 birds have been notified to the World Organisation for Animal Health (OIE), which is an underestimation of the real number of deaths due to H5N1. No case has been recorded from the Americas or Oceania, and very few from wild birds in Africa

Country name	Number of outbreaks per year				
	2005	2006	2007	2008	Total
Afghanistan		1			**1**
Austria		1			**1**
Azerbaijan		4			**4**
Bangladesh			5		**5**
Bulgaria		1			**1**
China		7			**7**
Côte d'Ivoire		1			**1**
Croatia	3	6			**9**
Czech Republic		11	2		**13**
Denmark		26			**26**
Egypt		19	6		**25**
France		38	4		**42**
Georgia		1			**1**
Germany		127	301		**428**
Greece		15			**15**
Hong Kong	2	11	5		**18**
Hungary		3			**3**
Indonesia		1			**1**
Iran		1			**1**
Israel		2			**2**
Italy		13			**13**
Japan			4	6	**10**
Kazakhstan	1	1			**2**
Kuwait	1		2		**3**
Mongolia	2	2			**4**
Nigeria		2			**2**
Pakistan			2		**2**
Poland		14	1		**15**
Romania	5	7			**12**
Russia	3	2	1		**6**
Saudi Arabia			1		**1**
Serbia and Montenegro		2	NA	NA	**2**
Slovakia		2			**2**
Slovenia		5			**5**
Spain		1			**1**
Sweden		5			**5**
Switzerland		10		1	**11**
Turkey	3	7			**10**
Ukraine	14	1		1	**16**
United Kingdom		1	3		**4**
Vietnam		3	2	1	**6**
Total	**34**	**354**	**339**	**9**	**736**

NA: not applicable
Source: statistics courtesy of Francesco Berlingieri, OIE Animal Health Information Department (148)

species population of bar-headed geese (*A. indicus*). Since then, the deaths of more than six thousand other wild birds, mostly in China (Table III), have been attributed to HPAIV, an unusual figure for an influenza mortality among wild birds and, most probably, largely underestimated, thus highlighting the potential detrimental effects of this virus on susceptible wildlife (94).

The source

The AIV reservoir in wild birds constitutes a constant source for AI strains. However, since an acute influenza infection in a given individual is short in duration, the sustainability of virus circulation is crucial.

Environment

Avian influenza virus can remain infectious for long periods of time in surface water (24, 117), where the survival of the virus is influenced by physical conditions, e.g. a shorter duration of persistence in acidic conditions, warmer temperatures and high salinity (21). For these reasons, shore bird species generally present lower LPAI prevalence than freshwater birds (52). Avian influenza viruses remain infectious in water for up to four days at 22°C, and for more than 30 days at 0°C. Some strains retained infectivity in water for up to 207 days at 17°C. Based on predictions from linear regression models, virus persistence in ice is even longer. Indirect evidence indicates that AIV could resist repeated freezing and thawing. The detection rate of AIV in pond sediments can be as high as or even exceed 50%. Characterisation of these viruses showed that there is a vast range of subtypes found in such sediments (78). Avian influenza virus left by waterfowl in Arctic and other lakes can remain infectious until after winter and infect susceptible migrating birds the following summer (152). Consequently, the high AIV prevalence in water birds may be associated with transmission via the faecal/oral route from surface waters (50) contaminated by wild bird droppings (143).

Wild water birds

All AIV can be found in wild water birds (139) but strains are extremely variable. Avian influenza virus of all subtypes causes disease only rarely, and the virus is predominantly shed by the intestinal tract, in the faeces. The apparent prevalence of influenza viruses in ducks and shore birds falls to levels that are difficult to detect in spring (in the Northern Hemisphere). It is assumed that AIV are maintained from autumn to spring in the breeding areas of Alaska and Siberia. Nevertheless, a second hypothesis is that AIV are maintained at low levels in flocks and brought back each year (76). There is evidence from the field to support both explanations, which are not mutually exclusive.

Terrestrial birds

Reports indicate that passerine and psittacine birds can carry both LPAIV and HPAIV. Both infections have been identified in free-flying birds on several occasions (73). Experimental studies seem to show that, although terrestrial birds (18), such as sparrows, can act as an intermediate host and potentially transmit AIV to both poultry and mammals, they cannot serve as a source for extended shedding of HPAIV H5N1. However, experimental infections in starlings, which resulted in a non-fatal course of infection with extended virus shedding, suggest that this species could play a role in the maintenance and circulation of AIV. Pigeons may have a minor role in maintenance and transmission of the virus (82). However, these conclusions are based on only a limited number of surveys or experimental studies.

In conclusion, water birds should be considered as the main hosts of AIV, with notable differences observed in the taxonomy and ecology of involved species in Eurasia and the Americas (115).

However, due to the low prevalence of AIV infection in terrestrial birds, more effort should be dedicated to studying the epidemiology of LPAIV in these bird populations to clarify their potential role in the maintenance and propagation of influenza.

Maintenance: preservation and persistence

Avian influenza virus prevalence differs between continents, as follows:

- 15% in Asia
- 27% in Australia
- 19% in Europe
- 37% in North America (52, 91, 94).

Differences in the pathogenicity of AI strains might induce different bird mortality which, in turn, would cause a lower rate of detection of virulent strains in live birds in acutely infected regions (102).

Avian influenza virus has been described as being in evolutionary stasis in wild birds (11, 111, 139), which limits the induction of clinical signs. Virus genes identified from wild ducks that had been preserved in museums since the early 20th Century showed almost no antigenic drift when compared to modern AI strains (103).

Natural history traits influence the balance between AIV and their bird hosts (52): dabbling ducks are more likely to inhabit fresh waters so are more frequently found infected (since virus survival is far more likely in fresh water). If the smallest populations could support the perpetuation and maintenance of a limited number of AIV strains (91), a larger population (for example, tens of millions of mallards) (67) could therefore be hypothesised to support the perpetuation of a broad range of short-lived influenza infections, as there would be enough susceptible individuals at any given time, since several bird populations in Europe are linked (9).

Furthermore, as ducks infected with LPAIV do not appear to be severely affected clinically, the infection does not seem to limit any interaction between infected birds and other birds or the environment (129). In addition, infection with LPAI strains does not appear to limit the capability for long-distance flights that could spread the virus, although experimental inoculation of wild Bewick's swans (*Cygnus columbianus bewickii*) with LPAIV has led to reduced performance in these birds (129). However, depending on the AIV strain and the bird species, this phenomenon may be variable.

Avian influenza virus strains from Eurasia and the Americas are quite distinct and can be divided into two super families (140). The most important difference between them is the absence of a significant flow of AIV in European shore birds (gulls and waders). The predominant isolation of the H13 and H16 strains from different gull species (Laridae) (51, 71) suggests that these viruses exhibit distinct genetic, ecological and epidemiological properties which must be studied in more detail.

Maintenance of virulent strains

Although HPAIV H5N1 has been isolated from wild birds (44), it is now recognised that HPAIV emerge in domestic poultry from LPAIV that have occurred in wild bird populations (19, 28, 92, 113). Low pathogenicity H5 or H7 strains are precursors of HPAIV. So far, there has been no indication that these viruses are perpetuated in aquatic birds (18). Given the lack of evidence that HPAIV can be maintained within wild bird populations, these animals may be 'dead-end' hosts for HPAIV acquired from poultry (33).

Highly pathogenic avian influenza spread and migration

Nevertheless, it is important to consider whether wild birds, notably migrating birds, might have the potential to transport virulent AIV over long distances.

A relationship between migration behaviour and LPAIV prevalence does appear in retrospective studies (52). Apparently, frequent stopovers associated with interactions with other species en route increase the probability for migrating species to be exposed to AIV. This probability appears to be more accurately predicted by bird interactions rather than by long-distance movement *per se*.

It has been much debated whether wild birds play an active role in the geographic spread of HPAI. It has been argued that infected birds would be too severely affected to continue migration and would thus be unlikely to spread HPAIV H5N1 over considerable distances (136). Although this may be true for some wild birds, it has also been shown that, in experimental infections, several bird species survive infection and shed HPAIV H5N1 without apparent disease (23, 31, 72, 119), or with clinical signs that are limited in duration and intensity (79). In addition, many wild birds may be partially immune, due to previous exposure to LPAIV, as has been shown with chickens (108).

The present situation in Europe, where infected wild birds have been found in several countries that have not reported outbreaks among poultry, suggests that wild birds can indeed carry the virus to previously unaffected areas (19). Based on analysis of the spread of HPAIV H5N1, nine of 21 introductions of the virus into Asia were most probably caused by poultry and three by wild birds, whereas, in Europe, 20 of 23 introductions were most probably caused by migrating wild birds (54).

In addition to 'natural' movements of birds, trade in domestic poultry and poultry products, as well as exotic birds and/or game release, presents a great risk of spreading HPAIV H5N1 from continent to continent (73). This mode of transmission may have played a more significant role in the spread of H5N1 within and beyond Asia (53, 113).

Transmission to target species: domestic and wild bird interactions

The use of common habitats by domestic poultry and wild birds increases the potential for the exchange of viruses and other infectious agents. Contact between these two groups may be direct or indirect. Free-range terrestrial (e.g. chickens, turkeys and guinea fowl) or aquatic (e.g. ducks and geese) poultry may forage in a variety of habitats that are also used by a wide range of wild bird species, which may include flying species or species that are largely terrestrial with limited flight capacity (including guinea fowl and ostrich). They may be sedentary (resident in the area for most or all of the year), locally migratory or long-distance international migrants. Species which can provide a bridge for AIV between wild and domestic birds must fulfil two conditions:

– they must have a relatively high chance of getting infected and of shedding an HPAIV

– they must have a relatively high chance of coming into contact with poultry (or humans).

Direct contacts with poultry

Wild bird species that feed/forage, roost or nest in or around domestic poultry holdings or on open grazing sites will have a higher likelihood of interacting with domestic poultry. Some of the common species include:

– crows (*Corvus* spp.)

– ducks (*Anas* spp.)

– egrets (*Egretta* spp.)

– kites (*Milvus* spp.)

– pigeons (*Columba* spp.)

– sparrows (*Passer* spp.)

– starlings and mynahs (*Acridotheres* or *Sturnus* spp.).

If wild birds gain access to food or water provided for poultry, this enables direct contact. Domestic and wild birds may also feed in the same crop fields or pasturelands, either together or at different times, with domestic birds using the sites during the day and wild birds feeding there at night.

Research in Southeast Asia has demonstrated that duck rearing in rice fields, as the ducks are moved from field to field to graze, increases the incidence of outbreaks in chickens. This type of open grazing system is believed to increase the opportunity for interactions between domestic ducks and wild birds, and to sustain the virus in the farming system, although synchronised testing of wild birds has been scarce to date, and has thus far failed to confirm this hypothesis (32, 55).

Indirect contacts with poultry

Where biosecurity of poultry holdings or behavioural barriers preclude direct contact between domestic and wild birds, there are a wide range of mechanisms through which an AIV could be transferred between these two groups. Sourcing water for poultry from water bodies used by wild birds, especially without any pre-treatment to reduce contamination, provides an indirect method of contact. Wild birds gaining access to stored poultry food sources on the farm or along the supply chain can provide a source of viral contamination. At the other end of the production system, release of untreated contaminated materials into the environment can also provide a source of infection for wild birds.

In conclusion, regarding the 'reservoir' role played by wild birds, since LPAIV are primarily transmitted by the faecal/oral route, effective transmission is indeed possible between susceptible domestic and wild birds (117, 139). By contrast, since recent Asian strains of HPAIV H5N1 are frequently transmitted by the aerial route (50), the exposure of poultry farms is dependent on the type of production. Most poultry species are exposed to indirect transmission through contaminated matter (3), but poultry

kept outdoors are, in addition, exposed to direct contact, mostly with infected ducks, provided that their premises and behaviour allow them to gain sufficiently close contact and that the ducks are attracted by the resources available on the farm (38, 115, 119). Duck farms are thus more at risk than chicken and turkey farms.

Identification of those wild bird species that may be considered a higher risk to poultry should therefore be based on criteria that include the characteristics mentioned above. Such criteria are based on the assumption that the chances of infection and further spread of HPAIV H5N1 are relatively high in:

– species that frequent freshwater wetland habitats and agricultural areas

– species that occur in groups that are large and/or dense

– species that show a high degree of mixing with other species.

In addition, the following 'specific risk factors' may be considered:

– the likelihood of breeding in colonies

– the likelihood of exhibiting predator behaviour

– the likelihood of exhibiting scavenging behaviour.

Nevertheless, on balance, the various arguments canvassed by the authors above would seem to demonstrate that, while wild birds are a reservoir of HPAI, they are not a *permanent* reservoir for these virus strains, as previously thought.

Epidemiological modelling as a tool for risk assessment

Modelling uses accumulated knowledge to predict the evolution of an infection within a population (i.e. a given species) or community (e.g. 'waterfowl') to:

– understand the processes of transmission and pathogen circulation

– design research studies or conduct 'risk assessment'

– assess the efficiency of control strategies.

One of the characteristics of AI is the interplay of parameters and variables that enter the modelling process:

– the unlimited possibilities of virus variants under unstable selective pressure

– a broad range of clinical pictures (including routes of virus shedding)

– uncertainty about the protection conferred by previous exposure to related subtypes

– the diversity of host species, with an imperfect capacity to categorise individuals by age, sex and social habits (49).

Nevertheless, modelling offers a way to deal with this complexity by clarifying priorities, notably when anticipating the advent of vaccines to protect the human population (43). In comparison with public health modelling, much less effort has been dedicated to predicting AI in poultry or wild bird populations. In this field, most of the recent efforts have been focused on analysing the conditions for efficient control of HPAI outbreaks in domestic poultry (47, 48, 85, 86, 109).

To risk oversimplification, two approaches can be used in epidemiological modelling. One relies on cellular automata or using individual-based models for simulations of stochastic process (114). The second is known as a 'box or compartment model' (4), in which the population of interest is subdivided into compartments, according to the individual infection stage. This is known as the 'SIR' paradigm, in which 'susceptible', 'infectious' and 'recovered' are the commonly used compartments.

Simulation models are typically employed to portray the dynamics of HPAI transmission. They are particularly useful for assessing the impact of control measures after the introduction of an HPAI strain into domestic poultry. These models enable researchers to improve the current understanding of AI epidemiology, allowing risk assessment to be comprehensive and rigorous (100). In the context of HPAIV H5N1, simulation has not been used explicitly to model the epidemiology of infection in wild birds. Most current epidemiological models consider the risk of an HPAI outbreak in wildlife to be unpredictable.

Thus, there is a need to anticipate the evolution of strains in a natural environment. For example, in 2002, Gog and Grenfell (59) simulated the dynamics of four subtypes interacting with the cross-immunity of hosts, which can (to simplify somewhat) become immune or susceptible after exposure. This model predicts that the relative lifetime of the host influences the persistence of the strains, since lifelong immunity tends to reduce the variety of strains in circulation. The model also reproduces the observed tendency to cluster influenza haemagglutinin into aggregates. This occurs even without introducing variations from seasons or geographic space into the model, meaning that it is principally the balance between virus infection and host immunity that directs the variations in strain occurrence.

Avian population turnover clearly plays a central role in the balance between AIV and water birds. An SIR model has been developed (61) which combines a dabbling duck population and LPAI infection dynamics to simulate LPAIV

circulation throughout the entire year. According to the simulation, the critical community size necessary to maintain a virus over winter is close to 1,200 ducks. When a population size falls under this threshold, the probability of a strain becoming extinct before the following spring and hatching of a new generation of susceptible birds is high.

The window favourable for maintenance and circulation of the AIV strains in clustered natural populations of wild birds seems, accordingly, very narrow. Doctrinal *et al.* and Simon *et al.* (39, 112) suggest that the favourable period for introducing HPAI strains into these types of populations is strongly dependent on the phenology of migration and the duration of AI infectivity. Provided that the lessons learned from the epidemiology of HPAI in domestic flocks can be transposed, the carrier state is a fundamentally important factor in the maintenance of AIV in populations (109). If so, isolation from the silent spread of AI in wild waterfowl remains the most efficient defence against the incursion of these strains into poultry farms. However, biosecurity efficiency is demanding and not always sustainable in all poultry production, notably outdoor farming, backyard and subsistence operations and even public and private zoos, wildlife parks and aviaries. As global AI surveillance programmes provide modellers with more detailed and long-standing results, such information can be integrated into predictive modelling to allow researchers to anticipate the emergence of strains that may become highly pathogenic in wild populations.

Management and control

Surveillance and monitoring: lessons learned from highly pathogenic avian influenza H5N1

'Surveillance' is usually accepted as meaning: a close, continuous examination of a suspected danger among exposed populations (7). In veterinary epidemiology, 'surveillance' is aimed at demonstrating the absence or estimating the prevalence of disease or infection but has a range of nuances, depending on the importance assigned to the task. Thrusfield (125) counted 12 different definitions for surveillance. For the purposes of the *Terrestrial Animal Health Code* of the World Organisation of Animal Health (OIE) (147), surveillance is defined as: 'The systematic ongoing collection, collation, and analysis of data, and the timely dissemination of information to those who need to know so that action can be taken' (147). For the purpose of HPAI surveillance, it is essential to understand how the AI viruses can circulate in wild birds.

Experience from HPAIV H5N1 outbreaks in wild birds in 2006 and 2007 in Europe revealed that the cases remained restricted in:

– the size of the outbreaks
– the species found to be affected
– the duration of the outbreaks
– location.

This restricted prevalence was observed despite the presence of hundreds of thousands of susceptible wild birds, highlighting the limited exposure of these birds to the virus.

One of the limiting factors for the emergence of a massive epidemic among water birds might be the mode of transmission. Experimental infections in Anseriformes species (24, 68, 72, 83, 119) demonstrate that HPAIV H5N1 is predominantly shed by the respiratory tract whereas shedding of LPAIV occurs via the enteric tract (139). Consequently, transmission of this virus may be more dependent on close contacts between birds. This might explain why, in Europe, the HPAIV H5N1 infections remained restricted and occurred mainly between birds of the same species, such as swans or grebes.

Although many factors are likely to influence the scale of observed mortality events, such as bird density and whether conditions are favourable for virus survival, pre-infection with LPAIV could have another important impact. Experiments in swans and geese showed that even heterosubtypic immunity raised by previous LPAIV infections has a protective effect on exposure to HPAIV H5N1 (68, 97).

While some bird species, including swans, grebes, diving ducks and scavenging birds, were frequently found dead after HPAIV H5N1 infection, there are indications that dabbling ducks have some resistance against H5N1 disease, which may possibly even be independent of any pre-existing H5-specific immunity (23, 72). Thus, dabbling ducks could serve as the ideal vehicle for infection or even as a local and transient reservoir host.

The discovery of HPAIV H5N1 in dead wild birds seemed to occur as limited events in time, taking place most often in conjunction with other abiotic or biotic stressing factors, such as:

– frost
– food shortage (93)
– summer moult
– bacterial and parasitic co-infections
– toxic factors
– long-distance migration.

Species displaying mortality are not necessarily those species that brought the virus to a specific location and it is not possible to differentiate between birds that introduced the virus to a location (vehicle) and birds that subsequently succumbed to the infection (indicator/sentinel) (62).

Despite intensive wild bird surveillance, HPAIV H5N1 was found in apparently healthy wild birds only on very rare occasions. The main deficiency of active surveillance is that, at a given instant, the virus prevalence (whatever the subtype) can be very low (see above). At such a point, the probability of detecting any strain is low if not enough bird samples are investigated (refer to Table IV for more details). Moreover, bird capture and swabbing require expertise. Staff who perform sampling must have the skills to handle the birds properly and identify them correctly. In many countries, birds are protected by law and cannot be captured without permission and this limits the range of samples that can be collected. A recent guideline provides technical advice on the proper collection of samples from healthy, sick and dead birds for the investigation of AIV (104).

As a consequence of all this, the current objective of AI surveillance in wild birds is to ensure early detection of HPAIV H5N1 by investigating increased mortality (meaning that normal mortality rates must have been previously recorded). However, the larger purpose is to gain information on the circulation of LPAIV, particularly subtypes H5 and H7, which both have the potential to become highly pathogenic. For this reason, both active/scanning surveillance and passive/diagnostic surveillance are needed to better prepare the farming industry for any incursion of HPAIV. Since the word 'passive' surveillance is inappropriately pejorative, the term 'diagnostic' surveillance could be substituted, to refer to the examination of birds which have been found with clinical signs, injured or dead, while 'scanning' surveillance could be used for examining captured, apparently healthy birds which present no clinical signs of disease.

To increase the probability of detecting HPAIV, diagnostic surveillance of dead or diseased birds should include a range of species, but focus on dead water birds (Anseriformes) and scavenging/predator birds (Laridae, Accipitriformes, Falconiformes, Corvidae), especially when mortality at a particular location is clustered around or above a previously determined threshold (indicator monitoring) (60).

Since some of the migratory species that introduce HPAIV may not display clinical signs, and thus may not be detected through diagnostic surveillance, monitoring live birds of the orders Anseriformes and Charadriiformes is the best tool for early detection of birds that are more likely to introduce the virus, and to identify infection before it spills over to other species (vehicle monitoring).

However, the results of past European Union (EU) surveillance programmes indicate that active surveillance is not an efficient use of resources for the early detection of HPAIV H5N1, but does provide valuable data on the occurrence and circulation of LPAIV subtypes H5 and H7.

The limitations on active wild bird monitoring – for instance, the inaccessibility of many water bird species – can be overcome by more targeted approaches and should focus on high-risk species. (See the results of a large ornithological survey by the EU Directorate General for Health and Consumer Affairs and Wetland International, detailed in 46.) The use of sentinel birds in areas which are highly frequented by wild water birds might help to economise on surveillance efforts.

A current EU research project, New FluBird (www.new-flubird.eu), is a multi-disciplinary approach, involving ornithologists, virologists, epidemiologists and data managers. New FluBird is implementing more targeted active surveillance for the early detection of incursions of HPAIV H5N1 into Europe through wild birds (45).

From a global perspective, several systems have enhanced HPAI surveillance in wild birds and domestic poultry (151). This information is currently collected by several organisations and distributed worldwide. Significant sources for official information on the occurrence of HPAI include OFFLU (www.offlu.net/), a joint venture of the OIE and Food and Agriculture Organization of the United Nations intended as a network of expertise on avian influenza (89).

For Veterinary Services, surveillance is ultimately intended: '... to meet the objectives of mitigating, controlling or even eradicating a disease in a population' (147). Consequently,

Table IV
The theoretical number of ducks that would need to be sampled to have a 95% and 99% chance of detecting an avian influenza strain, using a perfect test

This table assumes a random dispersal of the virus within the population, and is calculated upon the number of birds in the sample being below 10% of the entire studied population. Refer to the text to find the range of avian influenza prevalence found in previous surveys

Strain prevalence	Number of ducks to be sampled	
	95%	99%
0.1%	~ 3,000	(> 4,600)
1%	~ 300	(~ 460)
2%	~ 150	(~ 230)
5%	~ 60	(~ 90)
10%	~ 30	(~ 45)

After Toma *et al.* (126)

the detection of an HPAIV, even in a migrating bird, brings the risk of an immediate ban on the trade of birds or bird products on the local or international market. The underlying difficulty lies in the regulatory implications of detecting a potentially pathogenic strain in wild birds. This might dissuade a country from conducting sufficiently large official surveillance programmes, in case a listed disease case is found and exports of that country are banned. This issue must be resolved, since an efficient global early warning system is crucial when potentially virulent strains are circulating in wild birds.

However, AIV are also natural components of the ecosystem and, for this reason too, it remains important to monitor them on a regular basis. Since most strains do not induce visible clinical signs in infected wild birds, even when they shed virus, monitoring must also rely on 'scanning' surveillance.

Disease control through targeting the host

Highly pathogenic AI is a danger for domestic poultry, and can be a zoonotic risk. Control primarily aims to protect farms and humans by the most efficient means. From a wildfowl perspective, the aim of control might be to mitigate the risk at the level of the natural reservoir or to limit or hamper the transmission from this reservoir to domestic birds or even humans. In the recent past, rabies in foxes, classical swine fever in wild boar and tuberculosis in badgers (to mention examples from Europe) have allowed wildlife management services, including Veterinary Services, to gather practical experience in applying disease control measures to wildlife (8). In many circumstances, severe restrictions on the deployment of control measures may be wise (56). The complexities of disease transmission in animals (notably when they are gregarious or social) and the importance of host ecology limit the usefulness of most field technologies for controlling wildlife diseases (87). The authors explore the disease control and medical tools available to mitigate the impact of HPAI, below.

Biosecurity

Natural bird movements cannot be controlled. As a consequence, isolation measures must target poultry facilities to block direct contact or indirect transmission. Industrial farming and poultry bred indoors can be efficiently protected from contact with wild birds. However, there is little, if any, official regulation of the biosecurity of poultry raised outdoors. It is usually recommended that access to the outside is restricted by ensuring that outdoor enclosures are covered with solid roofs and have wire mesh or netted sides. It is wise to provide feed and drinking water in an indoor area, to avoid faecal contamination by wild birds. Finally, poultry should not be provided with circulating surface water for drinking, bathing or swimming in their enclosures (127).

Culling

Population reduction has been attempted to control several diseases in wild mammals (5, 144). Cutting the host population size should, in theory, reduce the density of both infected and susceptible individuals until a threshold density is reached, at which point the infection will disappear. Most of the previous attempts to reduce the population of a wild species that acted as a pathogen reservoir by culling were often compensated for by the effects of social attraction and immigration, or compensatory reproduction (30, 35, 145). It is out of the scope of this paper to discuss in detail the reasons why the depopulation of wild birds cannot be sustainable over the long term, has deleterious effects on natural ecosystems and, finally, will not be tolerated by the public (81, 94, 140). As a result, the only acceptable solution for local or global AI outbreaks is to stop the virus spread at its domestic source (55, 140).

Banning movements and translocations

In 2004, the HPAIV H5N1 virus was isolated from two mountain eagles (*Spizaetus nipalensis*), illegally imported to Belgium from Thailand, by a traveller who carried them in two baskets as cabin baggage (as described elsewhere in this issue). Other examples of the illegal importation of wild birds include passerines shipped to the United Kingdom. These incidents could have led to devastating consequences (42). The global movement of exotic/wild animals for the companion animal trade or other purposes is estimated at some 350 million live animals per year. Approximately one-quarter of this trade is thought to be illegal, and thus is not inspected or tested (70). Disease outbreaks from this trade in wildlife have caused both health and economic damage (69). Translocations (the movement of wild birds for release) and captive breeding raise the possibility that captive wild birds might be the source of AI outbreaks in surrounding ecosystems (25). Thus, it would be wiser not to move or breed wild birds without efficient disease control measures, and always to take the AI risk into consideration when attempting to release wild birds. For general procedures on disease control assessment during the translocation of wildlife, see the Canadian Cooperative Wildlife Health Centre/OIE guidelines (26).

Medical control through targeting the virus

Vaccination of wildlife has been successful in controlling rabies in Europe (98, 118) and is now used to control classical swine fever (66) in wild boar. Despite the technical and ethical difficulties (16), wildlife vaccination must be considered when evaluating AIV management.

From a preventive and economic point of view, it may be valid to consider vaccinating key wildfowl reservoirs instead of millions of poultry farms (128). The problems are both practical and theoretical. Efficient vaccines administered by the parenteral route are available, and the results of vaccinating zoo birds in Europe indicate that most vaccinated wild birds produce a significant immune response after an appropriate vaccination (27, 101). However, the efficacy of subtype vaccine strains in inducing solid immunity when the same subtypes are already circulating in wild bird populations remains unclear. More importantly, it is not obvious how the vaccine could be delivered to millions of free-moving birds to achieve sufficient mass immunity, without exerting a counterproductive selective pressure. At present, no practical solution exists to immunise wild and free-moving birds.

Conclusion

The epidemiology of AI in wild birds remains a complex topic and requires further research to be properly understood. Despite considerable progress gained in studies prompted by the spread of HPAIV H5N1, it is still difficult to evaluate the probability that a virulent strain of AI can be sustained in wild birds. It is also difficult to predict accurately the exposure risk presented by poultry farms (and human populations) situated in the neighbourhood of an HPAI outbreak in wild birds. Nevertheless, the European situation during the years 2006 to 2007 suggests that the probability of transmission is extremely low and related to obvious breaks in biosecurity.

Infections of wildlife by HPAIV or H5/H7 LPAIV should not be recognised as a case or outbreak in the sense of the OIE *Terrestrial Animal Health Code*. Avoiding the economic and commercial consequences following the notification of a case or outbreak in wild birds would encourage national Veterinary Services to exchange information about unusual mortality events in the wild, which, in turn, would facilitate early warning surveillance.

The control of HPAI outbreaks on poultry farms, whatever their origin, is costly and difficult, as no technology is available to limit the spread of AI strains between poultry and free-ranging wild birds. The only practical solution for the farming industry is to develop efficient and sustainable prevention methods. The OIE concepts of zoning and compartmentalisation (107) must be translated into practical rules, which means that efforts must be made to implement efficient and economically viable biosecurity measures, in particular, to maintain, where desirable, backyard and outdoor poultry breeding.

Surveillance can be improved as tests to detect AIV become easier to use, particularly in the field. Improvements would include the ability to store the tests and swabs at room temperature, increased specificity of scanning tests and the rapid detection of highly pathogenic strains.

Finally, the H5N1 outbreak has demonstrated that the Veterinary Services of most countries must improve both communication and co-operation with behavioural ecologists, nature conservationists and all those who deal with wildlife in the field.

Acknowledgements

Professor Marc Artois & Dr Delphine Doctrinal were supported by a grant from the National Game Agency (ONCFS), Reference No. 2005/16/6171. The research on avian influenza conducted by Marc Artois and Dominique Bicout is supported by a grant by the Agence Inter établissement de Recherche et de développement (AIRD) and the Institut Technique de l'Aviculture (ITAVI).

The pathology research mentioned in this article was partly supported by the Swedish Research Council for the Environment, Agricultural Sciences and Spatial Planning (FORMAS). The ornithological and epidemiological studies of Professor Björn Olsen were supported by grants from the Swedish Environmental Protection Agency, and the Swedish Research Council FORMAS, as well as from the EU-funded New FluBird project. This is contribution no. 229 from the Ottenby Bird Observatory.

Le risque de survenue en Europe de foyers d'influenza aviaire hautement pathogène liés à l'avifaune

M. Artois, D. Bicout, D. Doctrinal, R. Fouchier, D. Gavier-Widen, A. Globig, W. Hagemeijer, T. Mundkur, V. Munster & B. Olsen

Résumé

Avant que la souche asiatique H5N1 du virus de l'influenza aviaire hautement pathogène (IAHP) ne se propage dans le monde, l'infection par des souches hautement pathogènes du virus de l'influenza aviaire était pratiquement inconnue chez les oiseaux sauvages, à l'exception d'un foyer survenu en 1961 chez des sternes communes d'Afrique du Sud.

Les foyers de maladie clinique survenus en Eurasie sont à l'origine d'une mortalité affectant visiblement les populations d'une multitude d'espèces d'oiseaux sauvages vivant en liberté. Le mécanisme de circulation des virus de l'influenza dans les écosystèmes naturels résulte d'une pression sélective en faveur de souches faiblement pathogènes, qui se transmettent indirectement par les déjections des oiseaux aquatiques et par des matières contaminées. Certains de ces virus, appartenant aux sous-types H5 ou H7, subissent une mutation après s'être établis dans des populations de volailles domestiques, et deviennent alors hautement pathogènes. La persistance du virus H5N1 de l'IAHP dans plusieurs endroits du monde expose l'avifaune aux volailles infectées, avec comme conséquence la transmission du virus sur de longues distances. Le risque que ces oiseaux sauvages puissent à leur tour disséminer les virus de l'IAHP ou les introduire dans d'autres populations d'oiseaux domestiques est extrêmement préoccupant. La seule solution actuellement envisageable pour minimiser ce risque consiste à pratiquer des mesures rigoureuses de prophylaxie et de biosécurité afin de protéger les exploitations avicoles.

Mots-clés

Avifaune – Écologie – H5N1 – Influenza aviaire – Influenza aviaire hautement pathogène – Prophylaxie – Transmission.

Riesgo de brotes de influenza aviar altamente patógena asociados a las aves salvajes en Europa

M. Artois, D. Bicout, D. Doctrinal, R. Fouchier, D. Gavier-Widen, A. Globig, W. Hagemeijer, T. Mundkur, V. Munster & B. Olsen

Resumen

Antes de que la cepa asiática H5N1 de la influenza aviar altamente patógena (IAAP) empezara a propagarse rápidamente por el mundo, la infección de aves salvajes por cepas de tales características era un fenómeno prácticamente desconocido (aparte de un episodio que afectó a la golondrina de mar común en Sudáfrica en 1961).

Los brotes de infección clínica en Eurasia han provocado una notoria mortalidad en poblaciones de aves salvajes en libertad de numerosas especies. El patrón de circulación de los virus de la influenza en ecosistemas naturales es fruto de una presión selectiva en favor de cepas poco patógenas que se transmiten indirectamente por los excrementos de aves acuáticas y fómites contaminados. Algunos de esos virus, de los subtipos H5 o H7, pueden mutar y adquirir gran poder patógeno al ser introducidas en explotaciones avícolas. La persistencia de la cepa H5N1 de la IAAP en varias partes del mundo expone a las aves salvajes al contacto con aves de corral infectadas, lo que trae consigo una transmisión del virus a larga distancia. Lo más preocupante es la posibilidad de que esas aves salvajes, a su vez, propaguen los virus de la IAAP o los introduzcan en poblaciones de aves domésticas. La única solución a la vista para reducir tal riesgo estriba en rigurosas medidas de seguridad biológica y control sanitario para proteger las explotaciones avícolas.

Palabras clave
Aves salvajes – Control – Ecología – H5N1 – Influenza aviar – Influenza aviar altamente patógena – Transmisión.

References

1. Alexander D.J. (2000). – A review of avian influenza in different bird species. *Vet. Microbiol.*, **74** (1-2), 3-13.

2. Alexander D.J. (2001). – Orthomyxoviridae – avian influenza. *In* Poultry diseases (F.E.A. Jordan, ed.). W.B. Saunders, London, 281-290.

3. Alexander D.J. (2007). – An overview of the epidemiology of avian influenza. *Vaccine*, **25** (30), 5637-5644. E-pub.: 9 November 2006.

4. Anderson R.M. & May R.M. (1986). – The invasion, persistence and spread of infectious diseases within animal and plant communities. *Philos. Trans. roy. Soc. Lond., B, biol. Sci.*, **314** (1167), 533-570.

5. Artois M. (2003). – Wildlife infectious disease control in Europe. *J. Mt. Ecol.*, **7** (Suppl.), S89-97.

6. Artois M. & Aubert M. (1985). – Modélisation de la rage : le point de vue de biologistes. *In* Pasteur et la rage. *Infos tech. Serv. vét.*, **280**, S282.

7. Artois M., Bengis R., Delahay R.J., Duchêne M.J., Duff P., Ferroglio E., Gortazar C., Hutchings M. *et al.* (2009). – Wildlife disease surveillance and monitoring. *In* Management of disease in wild mammals (R.J. Delahay, G.C. Smith & M. Hutchings, eds). Springer, Berlin, 187-213.

8. Artois M., Delahay R., Guberti V. & Cheeseman C. (2001). – Control of infectious diseases of wildlife in Europe. *Vet. J.*, **162** (2), 141-152.

9. Arzel C., Elmberg J. & Guillemain M. (2006). – Ecology of spring-migrating Anatidae: a review. *J. Ornithol.*, **147** (2), 167-184.

10. Austin F.J. & Webster R.G. (1993). – Evidence of ortho- and paramyxoviruses in fauna from Antarctica. *J. Wildl. Dis.*, **29** (4), 568-571.

11. Bean W.J., Schell M., Katz J., Kawaoka Y., Naeve C., Gorman O. & Webster R.G. (1992). – Evolution of the H3 influenza virus hemagglutinin from human and nonhuman hosts. *J. Virol.*, **66** (2), 1129-1138.

12. Becker W.B. (1966). – The isolation and classification of Tern virus: influenza A-tern South Africa-1961. *J. Hyg. (London)*, **64** (3), 309-320.

13. Bentz P.-G. (1985). – Studies on some urban mallard *Anas platyrhynchos* populations in Scandinavia. Part I: causes of death, mortality and longevity among Malmö mallards as shown by ringing recoveries. *Fauna norv. Ser. C, Cinclus.*, **8**, 44-56.

14. Berthold P. (1993). – Bird migration. A general survey. Oxford University Press, Oxford, 1-239.

15. Blancou J. (2006). – Les 'Pestes aviaires' et leur très longue histoire. *Bull. Soc. des Sciences méd. du Grand-Duché de Luxembourg*, 109-130.

16. Blancou J., Artois M., Gilot-Fromont E., Kaden V., Smith G.C., Hutchings M., Chambers M.A., Houghton S. & Delahay R.J. (2009). – Options for the control of disease: targeting the infectious or parasitic agent. *In* Management of disease in wild mammals (R.J. Delahay, G.C. Smith & M. Hutchings, eds). Springer, Berlin, in press.

17. Boere G.C. & Stroud D.A. (2007). – The flyway concept: what it is and what it isn't. *In* Waterbirds around the world (G.C. Boere, C.A. Galbraith & D.A. Stroud, eds). The Stationery Office, Scotland Ltd., Edinburgh. Available at: www.jncc.gov.uk (accessed on 6 March 2009).

18. Boon A.C., Sandbulte M.R., Seiler P., Webby R.J., Songserm T., Guan Y. & Webster R.G. (2007). – Role of terrestrial wild birds in ecology of influenza A virus (H5N1). *Emerg. infect. Dis.*, **13** (11), 1720-1724.

19. Boyce W.M., Sandrock C., Kreuder-Johnson C., Kelly T. & Cardona C. (2008). – Avian influenza viruses in wild birds: a moving target. *Comp. Immunol. Microbiol. infect. Dis.* (in press). E-pub.: 2 May 2008.

20. Bragstad K., Jørgensen P., Handberg K., Hammer A.S., Kabell S. & Fomsgaard A. (2007). – First introduction of highly pathogenic H5N1 avian influenza A viruses in wild and domestic birds in Denmark, Northern Europe. *Virol. J.*, **4**, 43.

21. Brown J.D., Goekjian G., Poulson R., Valeika S. & Stallknecht D.E. (2008). – Avian influenza virus in water: infectivity is dependent on pH, salinity and temperature. *Vet. Microbiol.* (in press). E-pub.: 6 November 2008.

22. Brown J.D., Stallknecht D.E. & Swayne D.E. (2008). – Experimental infection of swans and geese with highly pathogenic avian influenza virus (H5N1) of Asian lineage. *Emerg. infect. Dis.*, **14** (1), 136-142.

23. Brown J.D., Stallknecht D.E., Beck J.R., Suarez D.L. & Swayne D.E. (2006). – Susceptibility of North American ducks and gulls to H5N1 highly pathogenic avian influenza viruses. *Emerg. infect. Dis.*, **12** (11), 1663-1670.

24. Brown J.D., Stallknecht D.E., Valeika S. & Swayne D.E. (2007). – Susceptibility of wood ducks to H5N1 highly pathogenic avian influenza virus. *J. Wildl. Dis.*, **43** (4), 660-667.

25. Butler D. (2006). – Blogger reveals China's migratory goose farms near site of flu outbreak. *Nature*, **441** (7091), 263.

26. Canadian Cooperative Wildlife Health Centre/Working Group on Wildlife Diseases of the World Organisation for Animal Health (2008). – Health risk analysis in wild animal translocations. Available at: www.ccwhc.ca/wildlife_health_topics/risk_analysis/rskguidintro.php (accessed on 28 February 2009).

27. Capua I. & Marangon S. (2003). – The use of vaccination as an option for the control of avian influenza. *In* 71st General Session, International Committee of the World Organisation for Animal Health (OIE), 18-23 May, Paris. OIE, Paris, 1-11.

28. Capua I. & Marangon S. (2007). – Control and prevention of avian influenza in an evolving scenario. *Vaccine*, **25** (30), 5645-5652. E-pub.: 9 November 2006.

29. Cardona C.J., Xing Z., Sandrock C.E. & Davis C.E. (2008). – Avian influenza in birds and mammals. *Comp. Immunol. Microbiol. infect. Dis.* (in press). E-pub.: 15 May 2008.

30. Caughley G. & Sinclair A.R.E. (1994). – Wildlife ecology and management. Blackwell Publishing, Malden, Massachusetts, 1-334.

31. Chen H., Li Y., Li Z., Shi J., Shinya K., Deng G., Qi Q., Tian G. *et al.* (2006). – Properties and dissemination of H5N1 viruses isolated during an influenza outbreak in migratory waterfowl in western China. *J. Virol.*, **80** (12), 5976-5983.

32. Chen H., Smith G.J., Li K.S., Wang J., Fan X.H., Rayner J.M., Vijaykrishna D., Zhang J.X. *et al.* (2006). – Establishment of multiple sublineages of H5N1 influenza virus in Asia: implications for pandemic control. *Proc. natl Acad. Sci. USA*, **103** (8), 1-6. E-pub.: 10 February 2006.

33. Chen H., Smith G.J., Zhang S.Y., Qin K., Wang J., Li K.S., Webster R.G., Peiris J.S. & Guan Y. (2005). – Avian flu: H5N1 virus outbreak in migratory waterfowl. *Nature*, **436** (7048), 191-192.

34. Chen R. & Holmes E.C. (2009). – Frequent inter-species transmission and geographic subdivision in avian influenza viruses from wild birds. *Virology*, **383** (1), 156-161. E-pub.: 8 November 2008.

35. Davidson R.S., Marion G., White P.C. & Hutchings M.R. (2009). – Use of host population reduction to control wildlife infection: rabbits and paratuberculosis. *Epidemiol. Infect.*, **137** (1), 131-138. E-pub.: 18 April 2008.

36. De Jong M., Diekmann O. & Heesterbeek J.A.P. (1995). – How does transmission of infection depend on population size? *In* Epidemic models, their structure and relation to data (D. Mollison, ed.). Cambridge University Press, Cambridge.

37. de Wit E. & Fouchier R.A. (2008). – Emerging influenza. *J. clin. Virol.*, **41** (1), 1-6.

38. Dhama K., Mahendran M. & Tomar S. (2008). – Pathogens transmitted by migratory birds: threat perceptions to poultry health and production. *Int. J. Poult. Sci.*, **7** (6), 516-525.

39. Doctrinal D., Artois M., Sabatier P. & Bicout D. (2006). – Modélisation du risque d'exposition de la volaille aux virus influenza aviaire d'origine sauvage. *Épidémiol. Santé anim.*, **50**, 71-81.

40. Donis R.O., Bean W.J., Kawaoka Y. & Webster R.G. (1989). – Distinct lineages of influenza virus H4 hemagglutinin genes in different regions of the world. *Virology*, **169** (2), 408-417.

41. Ducatez M.F., Tarnagda Z., Tahita M.C., Sow A., De Landtsheer S., Londt B.Z., Brown I.H., Osterhaus A. *et al.* (2007). – Genetic characterization of HPAI (H5N1) viruses from poultry and wild vultures, Burkina Faso. *Emerg. infect. Dis.*, **13** (4), 611-613.

42. Dudley J.P. (2006). – Bird flu outbreak in United Kingdom reveals global vulnerabilities. *BioScience*, **56** (3), 182-183.

43. Earn D.J., Dushoff J. & Levin S.A. (2002). – Ecology and evolution of the flu. *Trends Ecol. Evol.*, **17**, 334-340.

44. Ellis T.M., Bousfield R.B., Bissett L.A., Dyrting K.C., Luk G.S., Tsim S.T., Sturm-Ramirez K.M., Webster R.G. *et al.* (2004). – Investigation of outbreaks of highly pathogenic H5N1 avian influenza in waterfowl and wild birds in Hong Kong in late 2002. *Avian Pathol.*, **33** (5), 492-505.

45. European Union (2006). – New FluBird: network for early warning of influenza viruses in migratory birds in Europe. Available at: www.new-flubird.eu (accessed on 28 February 2009).

46. European Union Directorate General for Health and Consumer Affairs (SANCO) (2006). – Ornithological data relevant to the spread of avian influenza in Europe (phase 2). SANCO, Brussels. Available at: http://ec.europa.eu/environment/nature/conservation/wildbirds/birdflue/docs/spread_avian_influenza.pdf (accessed on 24 March 2009).

47. Ferguson N.M., Cummings D.A., Cauchemez S., Fraser C., Riley S., Meeyai A., Iamsirithaworn S. & Burke D.S. (2005). – Strategies for containing an emerging influenza pandemic in Southeast Asia. *Nature*, **437** (7056), 209-214. E-pub.: 3 August 2005.

48. Ferguson N.M., Cummings D.A., Fraser C., Cajka J.C., Cooley P.C. & Burke D.S. (2006). – Strategies for mitigating an influenza pandemic. *Nature*, **442** (7101), 448-452. E-pub.: 26 April 2006.

49. Ferguson N.M., Galvani A.P. & Bush R.M. (2003). – Ecological and immunological determinants of influenza evolution. *Nature*, **422** (6930), 428-433.

50. Fouchier R.A., Munster V.J., Keawcharoen J., Osterhaus A.D. & Kuiken T. (2007). – Virology of avian influenza in relation to wild birds. *J. Wildl. Dis.*, **43**, S7-14.

51. Fouchier R.A., Munster V., Wallensten A., Bestebroer T.M., Herfst S., Smith D., Rimmelzwaan G.F., Olsen B. & Osterhaus A.D. (2005). – Characterization of a novel influenza A virus hemagglutinin subtype (H16) obtained from black-headed gulls. *J. Virol.*, **79** (5), 2814-2822.

52. Garamszegi L.Z. & Møller A.P. (2007). – Prevalence of avian influenza and host ecology. *Proc. roy. Soc. Lond., B, biol. Sci.*, **274** (1621), 2003-2012.

53. Gauthier-Clerc M., Lebarbenchon C. & Thomas F. (2007). – Recent expansion of highly pathogenic avian influenza H5N1: a critical review. *Ibis (London)*, **149**, 202-214.

54. Ghedin E., Sengamalay N.A., Shumway M., Zaborsky J., Feldblyum T., Subbu V., Spiro D.J., Sitz J. *et al.* (2005). – Large-scale sequencing of human influenza reveals the dynamic nature of viral genome evolution. *Nature*, **437** (7062), 1162-1166. E-pub.: 5 October 2005.

55. Gilbert M., Chaitaweesub P., Parakamawongsa T., Premashthira S., Tiensin T., Kalpravidh W., Wagner H. & Slingenbergh J. (2006). – Free-grazing ducks and highly pathogenic avian influenza, Thailand. *Emerg. infect. Dis.*, **12** (2), 227-234.

56. Gilmour J.S. & Munro R. (1991). – Wildlife disease: management or masterly inactivity? *J. nat. Hist.*, **25**, 537-541.

57. Globig A., Starick E. & Werner O. (2006). – Influenza virus infections in migrating waterfowl: results of a two-year study in Germany [in German]. *Berl. Münch. tierärztl. Wochenschr.*, **119** (3-4), 132-139.

58. Globig A., Staubach C., Beer M., Koeppen U., Fiedler W., Wilking H., Starick E., Teifke J.P. *et al.* (2008). – Epidemiological and ornithological aspects of outbreaks of highly pathogenic avian influenza virus H5N1 of Asian lineage in wild birds in Germany, 2006 and 2007. *Transboundary emerg. Dis.* (in press).

59. Gog J.R. & Grenfell B.T. (2002). – Dynamics and selection of many-strain pathogens. *Proc. natl Acad. Sci. USA*, **99** (26), 17209-17214. E-pub.: 12 December 2002.

60. Guberti V. & Newman S.H. (2007). – Guidelines on wild bird surveillance for highly pathogenic avian influenza H5N1 virus. *J. Wildl. Dis.*, **43**, S29-34.

61. Guberti V., Scremin M., Busani L., Bonfanti L. & Terregino C. (2007). – A simulation model for low-pathogenicity avian influenza viruses in dabbling ducks in Europe. *Avian Dis.*, **51** (1 Suppl.), 275-278.

62. Hars J., Ruette S., Benmergui M., Fouque C., Fournier J.-Y., Legouge A., Cherbonnel M., Daniel B. *et al.* (2008). – The epidemiology of the highly pathogenic H5N1 avian influenza in mute swan (*Cygnus olor*) and other Anatidae in the Dombes region (France), 2006. *J. Wildl. Dis.*, **44** (4), 811-823.

63. Haydon D.T., Cleaveland S., Taylor L.H. & Laurenson M.K. (2002). – Identifying reservoirs of infection: a conceptual and practical challenge. *Emerg. infect. Dis.*, **8** (12), 1468-1473.

64. Hinshaw V.S., Webster R.G. & Turner B. (1980). – The perpetuation of orthomyxoviruses and paramyxoviruses in Canadian waterfowl. *Can. J. Microbiol.*, **26** (5), 622-629.

65. Hinshaw V.S., Wood J.M., Webster R.G., Deibel R. & Turner B. (1985). – Circulation of influenza viruses and paramyxoviruses in waterfowl originating from two different areas of North America. *Bull. WHO*, **63** (4), 711-719.

66. Kaden V., Renner C., Rothe A., Lange E., Hänel A. & Gossger K. (2003). – Evaluation of the oral immunisation of wild boar against classical swine fever in Baden-Württemberg. *Berl. Münch. tierärztl. Wochenschr.*, **116** (9-10), 362-367.

67. Kaleta E.F., Hergarten G. & Yilmaz A. (2005). – Avian influenza A viruses in birds – an ecological, ornithological and virological view. *Dtsch. tierärztl. Wochenschr.*, **112** (12), 448-456.

68. Kalthoff D., Breithaupt A., Teifke J.P., Globig A., Harder T., Mettenleiter T.C. & Beer M. (2008). – Experimental infection of mute swans with highly pathogenic avian influenza virus H5N1. *Emerg. infect. Dis.*, **14**, 1267-1270.

69. Karesh W.B. & Cook R.A. (2005). – The human–animal link. *Foreign Affairs*, **84**, 38-50.

70. Karesh W.B., Cook R.A., Gilbert M. & Newcomb J. (2007). – Implications of wildlife trade on the movement of avian influenza and other infectious diseases. *J. Wildl. Dis.*, **43**, S55-59.

71. Kawaoka Y., Chambers T.M., Sladen W.L. & Webster R.G. (1988). – Is the gene pool of influenza viruses in shorebirds and gulls different from that in wild ducks? *Virology*, **163** (1), 247-250.

72. Keawcharoen J., van Riel D., van Amerongen G., Bestebroer T., Beyer W.E., van Lavieren R., Osterhaus A.D., Fouchier R.A. & Kuiken T. (2008). – Wild ducks as long-distance vectors of highly pathogenic avian influenza virus (H5N1). *Emerg. infect. Dis.*, **14** (4), 600-607.

73. Kelly T.R., Hawkins M.G., Sandrock C.E. & Boyce W.M. (2008). – A review of highly pathogenic avian influenza in birds, with an emphasis on Asian H5N1 and recommendations for prevention and control. *J. avian Med. Surg.*, **22**, 1-16.

74. Komar N. & Olsen B. (2008). – Avian influenza virus (H5N1) mortality surveillance. *Emerg. infect. Dis.*, **14** (7), 1176-1178.

75. Krauss S., Obert C.A., Franks J., Walker D., Jones K., Seiler P., Niles L., Pryor S.P. *et al.* (2007). – Influenza in migratory birds and evidence of limited intercontinental virus exchange. *PLoS Pathog.*, **3** (11), e167.

76. Krauss S., Walker D., Pryor S.P., Niles L., Chenghong L., Hinshaw V.S. & Webster R.G. (2004). – Influenza A viruses of migrating wild aquatic birds in North America. *Vector-borne zoonotic Dis.*, **4** (3), 177-189.

77. Kwon Y.K., Joh S.J., Kim M.C., Lee Y.J., Choi J.G., Lee E.K., Wee S.H., Sung H.W. *et al.* (2005). – Highly pathogenic avian influenza in magpies (*Pica pica sericea*) in South Korea. *J. Wildl. Dis.*, **41** (3), 618-623.

78. Lang A.S., Kelly A. & Runstadler J.A. (2008). – Prevalence and diversity of avian influenza viruses in environmental reservoirs. *J. gen. Virol.*, **89** (Pt 2), 509-519.

79. Latorre-Margalef N., Gunnarsson G., Munster V.J., Fouchier R.A., Osterhaus A.D., Elmberg J., Olsen B., Wallensten A. *et al.* (2009). – Effects of influenza A virus infection on migrating mallard ducks. *Proc. roy. Soc. Lond., B, biol. Sci.*, **276** (1659), 1029-1036.

80. Lin Y.P., Shu L.L., Wright S., Bean W.J., Sharp G.B., Shortridge K.F. & Webster R.G. (1994). – Analysis of the influenza virus gene pool of avian species from southern China. *Virology*, **198** (2), 557-566.

81. Littin K.E. & Mellor D.J. (2005). – Strategic animal welfare issues: ethical and animal welfare issues arising from the killing of wildlife for disease control and environmental reasons. *In* Animal welfare: global issues, trends and challenges (A.C.D. Bayvel, S.A. Rahman & A. Gavinelli, eds). *Rev. sci. tech. Off. int. Epiz.*, **24** (2), 767-782.

82. Liu C.-M., Lin S.-H., Chen Y.-C., Lin K.C., Wu T.-S. & King C.-C. (2007). – Temperature drops and the onset of severe avian influenza A H5N1 virus outbreaks. *PLoS ONE*, **2** (2), e191.

83. Liu J., Xiao H., Lei F., Zhu Q., Qin K., Zhang X.W., Zhang X.L., Zhao D. *et al.* (2005). – Highly pathogenic H5N1 influenza virus infection in migratory birds. *Science*, **309** (5738), 1206. E-pub.: 6 July 2005.

84. Liu J.H., Okazaki K., Bai G.R., Shi W.M., Mweene A. & Kida H. (2004). – Interregional transmission of the internal protein genes of H2 influenza virus in migratory ducks from North America to Eurasia. *Virus Genes*, **29** (1), 81-86.

85. Liu R., Duvvuri V.R. & Wu J. (2008). – Spread pattern formation of H5N1 – avian influenza and its implications for control strategies. *Math. Model. nat. Phenom.*, **3** (7), 161-179.

86. Longini I.M. Jr, Nizam A., Xu S., Ungchusak K., Hanshaoworakul W., Cummings D.A. & Halloran M.E. (2005). – Containing pandemic influenza at the source. *Science*, **309** (5737), 1083-1087. E-pub.: 3 August 2005.

87. McDonald R.A., Delahay R.J., Carter S.P., Smith G.C. & Cheeseman C.L. (2008). – Perturbing implications of wildlife ecology for disease control. *Trends Ecol. Evol.*, **23** (2), 53-56. E-pub.: 11 January 2008.

88. Makarova N.V., Kaverin N.V., Krauss S., Senne D. & Webster R.G. (1999). – Transmission of Eurasian avian H2 influenza virus to shorebirds in North America. *J. gen. Virol.*, **80** (Pt 12), 3167-3171.

89. Martin V., De Simone L. & Lubroth J. (2007). – Geographic information systems applied to the international surveillance and control of transboundary animal diseases, a focus on highly pathogenic avian influenza. *Vet. ital.*, **43**, 437-450.

90. Morgan I.R. & Westbury H.A. (1981). – Virological studies of Adelie penguins (*Pygoscelis adeliae*) in Antarctica. *Avian Dis.*, **25** (4), 1019-1026.

91. Munster V.J., Baas C., Lexmond P., Waldenström J., Wallensten A., Fransson T., Rimmelzwaan G.F., Beyer W.E. *et al.* (2007). – Spatial, temporal, and species variation in prevalence of influenza A viruses in wild migratory birds. *PLoS Pathog.*, **3** (5), e61.

92. Munster V.J., Wallensten A., Baas C., Rimmelzwaan G.F., Schutten M., Olsen B., Osterhaus A.D. & Fouchier R.A. (2005). – Mallards and highly pathogenic avian influenza ancestral viruses, Northern Europe. *Emerg. infect. Dis.*, **11** (10), 1545-1551.

93. O'Brien D.J., Schmitt S.M., Fierke J.S., Hogle S.A., Winterstein S.R., Cooley T.M., Moritz W.E., Diegel K.L. *et al.* (2002). – Epidemiology of *Mycobacterium bovis* in free-ranging white-tailed deer, Michigan, USA, 1995-2000. *Prev. vet. Med.*, **54** (1), 47-63.

94. Olsen B., Munster V.J., Wallensten A., Waldenström J., Osterhaus A.D. & Fouchier R.A. (2006). – Global patterns of influenza A virus in wild birds. *Science*, **312** (5772), 384-388.

95. Osterhaus A., Munster V.J. & Fouchier R. (2008). – Epidemiology of avian influenza. *In* Avian influenza (H.D. Klenk, M.N. Matrosovich, J. Stech, eds). Karger, Basel, 1-10.

96. Parmley E.J., Bastien N., Booth T.F., Bowes V., Buck P.A., Breault A., Caswell D., Daoust P.Y. *et al.* (2008). – Wild bird influenza survey, Canada, 2005. *Emerg. infect. Dis.*, **14** (1), 84-87.

97. Pasick J., Berhane Y., Embury-Hyatt C., Copps J., Kehler H., Handel K., Babiuk S., Hooper-McGrevy K. *et al.* (2007). – Susceptibility of Canada geese (*Branta canadensis*) to highly pathogenic avian influenza virus (H5N1). *Emerg. infect. Dis.*, **13** (12), 1821-1827.

98. Pastoret P.P. & Brochier B. (1999). – Epidemiology and control of fox rabies in Europe. *Vaccine*, **17** (13-14), 1750-1754.

99. Perkins J.M. & Swayne D.E. (2003). – Varied pathogenicity of a Hong Kong-origin H5N1 avian influenza virus in four passerine species and budgerigars. *Vet. Pathol.*, **40** (1), 14-24.

100. Pfeiffer D.U. (2007). – Assessment of H5N1 HPAI risk and the importance of wild birds. *J. Wildl. Dis.*, **43**, S47-50.

101. Philippa J.D., Munster V.J., Bolhuis H., Bestebroer T.M., Schaftenaar W., Beyer W.E., Fouchier R.A., Kuiken T. & Osterhaus A.D. (2005). – Highly pathogenic avian influenza (H7N7): vaccination of zoo birds and transmission to non-poultry species. *Vaccine*, **23** (50), 5743-5750. E-pub.: 19 September 2005.

102. Rappole J.H. & Hubálek Z. (2006). – Birds and influenza H5N1 virus movement to and within North America. *Emerg. infect. Dis.*, **12** (10), 1486-1492.

103. Reid A.H., Fanning T.G., Slemons R.D., Janczewski T.A., Dean J. & Taubenberger J.K. (2003). – Relationship of pre-1918 avian influenza HA and NP sequences to subsequent avian influenza strains. *Avian Dis.*, **47** (3 Suppl.), 921-925.

104. Rose K., Newman S., Uhart M. & Lubroth J. (2006). – Wild bird highly pathogenic avian influenza surveillance. Sample collection from healthy, sick and dead birds. Food and Agriculture Organization of the United Nations (FAO) Animal Production and Health Proc., 1810-0732, No. 4. FAO, Rome, 1-56.

105. Sabirovic M., Wilesmith J., Hall S., Coulson N. & Landeg F. (2006). – Outbreaks of HPAI H5N1 virus in Europe during 2005/2006. International Animal Health Division, Department of Environment, Food and Rural Affairs, London.

106. Schäfer J.R., Kawaoka Y., Bean W.J., Suss J., Senne D. & Webster R.G. (1993). – Origin of the pandemic 1957 H2 influenza A virus and the persistence of its possible progenitors in the avian reservoir. *Virology*, **194** (2), 781-788.

107. Scott A., Zepeda C., Garber L., Smith J., Swayne D., Rhorer A., Kellar J., Shimshony A., Batho H. & Caporale V. (2006). – The concept of compartmentalisation. *Rev. sci. tech. Off. int. Epiz.*, **25** (3), 873-879.

108. Seo S.H., Peiris M. & Webster R.G. (2002). – Protective cross-reactive cellular immunity to lethal A/goose/Guangdong/1/96-like H5N1 influenza virus is correlated with the proportion of pulmonary CD8(+) T cells expressing gamma interferon. *J. Virol.*, **76** (10), 4886-4890.

109. Sharkey K.J., Bowers R.G., Morgan K.L., Robinson S.E. & Christley R.M. (2008). – Epidemiological consequences of an incursion of highly pathogenic H5N1 avian influenza into the British poultry flock. *Proc. roy. Soc. Lond., B, biol. Sci.*, **275** (1630), 19-28.

110. Sharp G.B., Kawaoka Y., Wright S.M., Turner B., Hinshaw V. & Webster R.G. (1993). – Wild ducks are the reservoir for only a limited number of influenza A subtypes. *Epidemiol. Infect.*, **110** (1), 161-176.

111. Shortridge K.F. (1992). – Pandemic influenza: a zoonosis? *Semin. respir. Infect.*, **7** (1), 11-25.

112. Simon C., Doctrinal D. & Bicout D. (2006). – Risque de contact entre oiseaux sauvages et domestiques dans la région de la Dombes, France. *Épidémiol. Santé anim.*, **50**, 27-39.

113. Sims L.D., Domenech J., Benigno C., Kahn S., Kamata A., Lubroth J., Martin V. & Roeder P. (2005). – Origin and evolution of highly pathogenic H5N1 avian influenza in Asia. *Vet. Rec.*, **157** (6), 159-164.

114. Situngkir H. (2004). – Epidemiology through cellular automata: case of study avian influenza in Indonesia. arXiv:nlin/0403035v1.

115. Stallknecht D.E. & Brown J.D. (2007). – Wild birds and the epidemiology of avian influenza. *J. Wildl. Dis.*, **43**, S15-20.

116. Stallknecht D.E. & Shane S.M. (1988). – Host range of avian influenza virus in free-living birds. *Vet. Res. Commun.*, **12** (2-3), 125-141.

117. 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.

118. Stöhr K. & Meslin F.M. (1996). – Progress and setbacks in the oral immunisation of foxes against rabies in Europe. *Vet. Rec.*, **139** (2), 32-35.

119. Sturm-Ramirez K.M., Hulse-Post D.J., Govorkova E.A., Humberd J., Seiler P., Puthavathana P., Buranathai C., Nguyen T.D. *et al.* (2005). – Are ducks contributing to the endemicity of highly pathogenic H5N1 influenza virus in Asia? *J. Virol.*, **79** (17), 11269-11279.

120. Süss J., Schäfer J., Sinnecker H. & Webster R.G. (1994). – Influenza virus subtypes in aquatic birds of eastern Germany. *Arch. Virol.*, **135** (1-2), 101-114.

121. Swinton J., Woolhouse M.E., Begon M.E., Dobson A.P., Ferroglio E., Grenfell B.T., Guberti V., Hails R.S. *et al.* (2002). – Microparasite transmission and persistence. *In* The ecology of wildlife diseases (P.J. Hudson, A. Rizzoli, B.T. Grenfell, H. Hans & A.P. Dobson, eds). Oxford University Press, Oxford, 83-101.

122. Tanimura N., Tsukamoto K., Okamatsu M., Mase M., Imada T., Nakamura K., Kubo M., Yamaguchi S. *et al.* (2006). – Pathology of fatal highly pathogenic H5N1 avian influenza virus infection in large-billed crows (*Corvus macrorhynchos*) during the 2004 outbreak in Japan. *Vet. Pathol.*, **43** (4), 500-509.

123. Taylor P.D., Day T., Nagy D., Wild G., André J.-B. & Gardner A. (2006). – The evolutionary consequences of plasticity in host-pathogen interactions. *Theoret. Popul. Biol.*, **69** (3), 323-331. E-pub.: 15 February 2006.

124. Teifke J.P., Klopfleisch R., Globig A., Starick E., Hoffmann B., Wolf P.U., Beer M., Mettenleiter T.C. & Harder T.C. (2007). – Pathology of natural infections by H5N1 highly pathogenic avian influenza virus in mute (*Cygnus olor*) and whooper (*Cygnus cygnus*) swans. *Vet. Pathol.*, **44** (2), 137-143.

125. Thrusfield M. (2007). – Veterinary epidemiology, 3rd Ed. Blackwell Publishing, Oxford, 1-624.

126. Toma B., Dufour B., Sanaa M., Bénet J.J., Shaw A., Moutou F. & Louza A. (2001). – Épidémiologie appliquée à la lutte collective contre les maladies animales transmissibles majeures, 2nd Ed. Association pour l'Étude de l'Épidémiologie des Maladies Animales, Maisons-Alfort, France, 1-696.

127. United States Department of Agriculture/Animal and Plant Health Inspection Service (2007). – Further guidance on biosecurity and disease prevention and control for nonconfinement poultry production operations. Available at: www.aphis.usda.gov/newsroom/hot_issues/avian_influenza/contents/printable_version/non-confinement_poultry-ai_guidance4-18-06.pdf (accessed on 28 February 2009).

128. Van den Berg T., Lambrecht B., Marché S., Steensels M., Van Borm S. & Bublot M. (2008). – Influenza vaccines and vaccination strategies in birds. *Comp. Immunol. Microbiol. infect. Dis.*, **31** (2-3), 121-165. E-pub.: 24 September 2007.

129. Van Gils J.A., Munster V.J., Radersma R., Liefhebber D., Fouchier R.A. & Klaassen M. (2007). – Hampered foraging and migratory performance in swans infected with low-pathogenic avian influenza A virus. *PLoS ONE*, **2** (1), e184.

130. Von Hoyningen-Huene V. & Scholtissek C. (1983). – Low genetic mixing between avian influenza viruses of different geographic regions. *Arch. Virol.*, **76** (1), 63-67.

131. Wahlgren J., Waldenström J., Sahlin S., Haemig P.D., Fouchier R.A., Osterhaus A.D., Pinhassi J., Bonnedahl J. *et al.* (2008). – Gene segment reassortment between American and Asian lineages of avian influenza virus from waterfowl in the Beringia area. *Vector-borne zoonotic Dis.*, **8** (6), 783-790.

132. Wallensten A. (2006). – Influenza A virus in wild birds. Thesis (medical dissertation). Linköping University, Linköping, Sweden.

133. Wallensten A., Munster V.J., Elmberg J., Osterhaus A.D., Fouchier R. & Olsen B. (2005). – Multiple gene segment reassortment between Eurasian and American lineages of influenza A virus (H6N2) in guillemot (*Uria aalge*). *Arch. Virol.*, **150**, 1685-1692.

134. Wallensten A., Munster V.J., Karlsson M., Lundkvist A., Brytting M., Stervander M., Osterhaus A.D., Fouchier R.A. & Olsen B. (2006). – High prevalence of influenza A virus in ducks caught during spring migration through Sweden. *Vaccine*, **24** (44-46), 6734-6735. E-pub.: 6 June 2006.

135. Wallensten A., Munster V.J., Latorre-Margalef N., Brytting M., Elmberg J., Fouchier R., Fransson T., Haemig P.D. *et al.* (2007). – Surveillance of influenza A virus in migratory waterfowl in northern Europe. *Emerg. infect. Dis.*, **13**, 404-411.

136. Weber T.P. & Stilianakis N.I. (2007). – Ecologic immunology of avian influenza (H5N1) in migratory birds. *Emerg. infect. Dis.*, **13** (8), 1139-1143.

137. Webster R.G. (1998). – Influenza: an emerging disease. *Emerg. infect. Dis.*, **4** (3), 436-441.

138. Webster R.G. & Rott R. (1987). – Influenza virus A pathogenicity: the pivotal role of hemagglutinin. *Cell*, **50** (5), 665-666.

139. 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.

140. Webster R.G., Krauss S., Hulse-Post D. & Sturm-Ramirez K. (2007). – Evolution of influenza A viruses in wild birds. *J. Wildl. Dis.*, **43**, S1-6.

141. Webster R.G., Peiris M., Chen H. & Guan Y. (2006). – H5N1 outbreaks and enzootic influenza. *Emerg. infect. Dis.*, **12** (1), 3-8.

142. Whitworth D., Newman S., Mundkur T. & Harris P. (2007). – Wild birds and avian influenza: an introduction to applied field research and disease sampling techniques. Food and Agriculture Organization of the United Nations (FAO) Animal Production and Health Manual. FAO, Rome.

143. Widjaja L., Krauss S.L., Webby R.J., Xie T. & Webster R.G. (2004). – Matrix gene of influenza A viruses isolated from wild aquatic birds: ecology and emergence of influenza A viruses. *J. Virol.*, **78** (16), 8771-8779.

144. Wobeser G.A. (2007). – Disease in wild animals. Investigation and management, 2nd Ed. Springer-Verlag, Berlin, 1-393.

145. Woodroffe R., Donnelly C.A., Cox D.R., Bourne F.J., Cheeseman C.L., Delahay R.J., Gettinby G., McInerney J.P. & Morrison W.I. (2006). – Effects of culling on badger *Meles meles* spatial organization: implications for the control of bovine tuberculosis. *J. appl. Ecol.*, **43** (1), 1-10.

146. Woolhouse M.E., Haydon D.T. & Antia R. (2005). – Emerging pathogens: the epidemiology and evolution of species jumps. *Trends Ecol. Evol.*, **20** (5), 238-244.

147. World Organisation for Animal Health (OIE) (2007). – General guidelines for animal health surveillance. *In* Terrestrial Animal Health Code, Appendix 3.8.1. Available at: www.oie.int/eng/normes/mcode/en_chapitre_3.8.1.htm #rubrique_surveillance-principes_generaux (accessed on 12 December 2008).

148. World Organisation for Animal Health (OIE) (2008). – World Animal Health Information Database (WAHID) Interface. Available at: www.oie.int/wahis/public.php?page= home (accessed on 12 December 2008).

149. Wright P.F. & Webster R.G. (2001). – Orthomyxoviruses. *In* Fields virology (D.M. Knipe & P.M. Howley, eds.), 4th Ed. Lippincott Williams & Wilkins, Philadelphia, Pennsylvania, 1533-1579.

150. Yee K.S., Carpenter T.E. & Cardona C.J. (2008). – Epidemiology of H5N1 avian influenza. *Comp. Immunol., Microbiol. infect. Dis.* (in press).

151. Zepeda C. (2007). – Highly pathogenic avian influenza in domestic poultry and wild birds: a risk analysis framework. *J. Wildl. Dis.*, **43**, S51-54.

152. Zhang G. (2007). – Surveillance of influenza A virus in environmental ice and water samples. Bowling Green State University, Bowling Green, Ohio, 128.

153. Zohari S., Gyarmati P., Thorén P., Czifra G., Bröjer C., Belák S. & Berg M. (2008). – Genetic characterization of the NS gene indicates co-circulation of two sub-lineages of highly pathogenic avian influenza virus of H5N1 subtype in Northern Europe in 2006. *Virus Genes*, **36** (1), 117-125. E-pub.: 3 January 2008.