VIROLOGY OF AVIAN INFLUENZA IN RELATION TO WILD BIRDS

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ABSTRACT: The outbreak of HPAI H5N1 influenza virus in Asia, which spread to Russia, the Middle East, Europe, and Africa, has put increased focus on the role of wild birds in the persistence of influenza viruses. The ecology, epidemiology, genetics, and evolution of influenza viruses cannot be fully understood without taking into account the ecology of their hosts. Here, we review current knowledge on global patterns of low pathogenic influenza virus infections in wild birds, discuss these patterns in the context of host ecology and behavior, and identify gaps in the current knowledge. Potentially important differences between low pathogenic influenza viruses and highly pathogenic H5N1 viruses in wild birds are discussed, in particular with respect to pathogenesis, virus secretion, and host range.

Key words: Ecology, epidemiology, HPAI, influenza A virus, LPAI, wild birds.

Influenza A viruses infect many species, including humans, pigs, horses, mink, felids, marine mammals, and a wide range of domestic birds, but wildfowl and shorebirds form the virus reservoir in nature (Webster et al., 1992). Influenza viruses are classified based on two glycoproteins expressed on virus particles; the hemagglutinin (HA) and neuraminidase (NA) (Webster et al., 1992). In wild birds and poultry, influenza viruses representing 16 HA and 9 NA antigenic subtypes are circulating (Fouchier et al., 2005), in numerous combinations (e.g., H1N1, H16N3). The HA protein is initially synthesized as a single polypeptide (HA0) that is cleaved into HA1 and HA2 subunits by proteases. The HA mediates binding of the virus to host cells and fusion with endosomal membranes (Webster et al., 1992). H5 and H7 influenza viruses may become highly pathogenic following introduction into poultry and cause outbreaks of highly pathogenic avian influenza (HPAI), in contrast to other virus subtypes.

The switch from a low pathogenic avian influenza (LPAI) virus phenotype, common in wild birds and poultry, to the HPAI virus phenotype is achieved by the introduction of basic amino acid residues into the HA0 cleavage site, facilitating systemic virus replication due to enhanced HA cleavability outside the respiratory and intestinal tracts. HPAI isolates have been obtained primarily from commercially raised poultry (Alexander, 2000). In the last decade, HPAI outbreaks have occurred frequently, caused by influenza viruses of subtype H5N1 in Southeast Asia (ongoing since 1997); H5N2 in Mexico (1994), Italy (1997), and Texas (2004); H7N1 in Italy (1999); H7N3 in Australia (1994), Pakistan (1994), Chile (2002), and Canada (2003); H7N4 in Australia (1997); and H7N7 in The Netherlands (2003) (Alexander, 2000; Munster et al., 2005).

LPAI viruses have been isolated from at least 105 wild bird species of 26 different families (Table 1) (Olsen et al., 2006). All subtypes have been detected in avian species, and relatively few in other species. Many wild bird species may harbor influenza viruses, but birds of wetlands and aquatic environments such as the Anseriformes (particularly ducks, geese, and swans) and Charadriiformes (particularly gulls, terns, and waders) constitute the major natural virus reservoir (Webster et al., 1992). Anseriformes and Charadriiformes are distributed globally, except for the most arid regions of the world (Del Hoyo et al., 1996). In birds, influenza...
viruses preferentially infect cells lining the intestinal tract and are excreted in high concentrations in feces. It was shown that influenza viruses remain infectious in lake water up to 4 days at 22°C and more than 30 days at 0°C, and the relatively high virus prevalence in birds living in aquatic environments may in part be due to efficient transmission through the fecal–oral route via surface waters (Webster et al., 1992).

Migration is a common strategy for birds occupying seasonal habitats, and may range from short local movements to intercontinental migrations. Migratory birds can carry pathogens, provided these do not significantly affect the bird’s health status. Many Anseriformes and Charadriiformes are known to perform regular long-distance migrations (Del Hoyo et al., 1996) thereby potentially distributing influenza viruses between countries or even continents. It is important to realize that the transmission of the viruses and their geographical spread are dependent on the ecology of the migrating hosts. For instance, migrating birds rarely fly the full distance between breeding and nonbreeding areas without stopping over and “refueling” along the way. Rather, birds make frequent stopovers during migration, and spend more time eating and preparing for migration than actively performing flights (Alerstam and Lindstrom, 1990). Many species aggregate at favorable stopover or wintering sites, resulting in high local densities. Such sites may be important for transmission of influenza viruses between wild and captive birds, and between different species.

Extensive surveillance studies of wild ducks in the Northern Hemisphere have revealed high prevalence of influenza virus primarily in juvenile birds, with a peak in virus recovery in early fall prior to southbound migration. In North America, the prevalence falls from ~60% in ducks sampled at marshalling sites close to the Canadian breeding areas in early fall, to 0.4–2% at the wintering grounds in the southern USA and ~0.25% upon the ducks’ return to the breeding grounds in spring (Krauss et al., 2004).

Similar patterns have been observed in Northern Europe, but influenza virus detection during spring migration can be significantly higher, up to 6.5%. Surveillance on the nesting grounds of ducks in Siberia prior to winter migration revealed the presence of influenza viruses in up to 8% of birds (Okazaki et al., 2000). The prevalence throughout the year raises the possibility that influenza virus can be perpetuated in ducks alone. This contrasts with earlier hypotheses, in which additional host species or preservation of

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**Table 1. Prevalence of influenza A virus in wild birds.**

<table>
<thead>
<tr>
<th>Family</th>
<th>Species</th>
<th>Sampled (N)</th>
<th>Positive (N)</th>
<th>Prevalence (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ducks</td>
<td>36</td>
<td>34,503</td>
<td>3,275</td>
<td>9.5</td>
</tr>
<tr>
<td>Geese</td>
<td>8</td>
<td>4,806</td>
<td>47</td>
<td>1.0</td>
</tr>
<tr>
<td>Swans</td>
<td>3</td>
<td>5,009</td>
<td>94</td>
<td>1.9</td>
</tr>
<tr>
<td>Gulls</td>
<td>9</td>
<td>14,505</td>
<td>199</td>
<td>1.4</td>
</tr>
<tr>
<td>Terns</td>
<td>9</td>
<td>2,521</td>
<td>24</td>
<td>0.9</td>
</tr>
<tr>
<td>Waders</td>
<td>10</td>
<td>2,637</td>
<td>21</td>
<td>0.8</td>
</tr>
<tr>
<td>Rails</td>
<td>3</td>
<td>1,962</td>
<td>27</td>
<td>1.4</td>
</tr>
<tr>
<td>Petrels</td>
<td>5</td>
<td>1,416</td>
<td>4</td>
<td>0.3</td>
</tr>
<tr>
<td>Cormorants</td>
<td>1</td>
<td>4,500</td>
<td>18</td>
<td>0.4</td>
</tr>
</tbody>
</table>

*a* See Olsen et al. (2006) for details.

*b* Dabbling ducks (28,955) and diving ducks (1,011) influenza virus prevalence of 10.1% and 1.6%, respectively.
infectious influenza viruses in frozen lakes over the winter would play a role in the perpetuation of avian influenza viruses (Webster et al., 1992).

All HA and NA subtypes, with the exception of H13–H16, are circulating in wild ducks in North America and Northern Europe. In a 26-year longitudinal study performed in Canada, influenza viruses of subtypes H3, H4, and H6 were isolated from ducks most frequently; H1, H2, H7, H10, and H11 less frequently; and H5, H8, H9, and H12 only sporadically (Krauss et al., 2004). In other North American and European studies, influenza viruses of subtypes H3, H4, and H6 also were detected frequently, and the detection of other virus subtypes was not significantly different (Webster et al., 1992; Munster et al., 2005). Thus the prevalence of influenza virus in general, and the distribution of subtypes, may vary between different surveillance studies depending on species, time, and place. In the Canadian studies, cyclic patterns of influenza virus subtypes have been reported; peaks in virus isolation of a particular HA subtype were followed 1–2 years later by reduced rates of isolation of this subtype. This observation is of particular interest but awaits confirmation in other surveillance studies. Cyclic patterns have been described for measles and whooping cough in humans, and are determined in part by spatial factors, herd immunity, and population age structure (Rohani et al., 1999). Cycling of influenza virus in wild birds could provide similar new insights into the ecology of influenza viruses in their natural hosts.

Influenza virus surveillance of ducks also has been performed in Japan since the late 1970s. As in other studies, influenza virus prevalence and isolated subtypes varied between years and locations. The prevalence of influenza virus in wild birds elsewhere in Asia is largely unknown, but several studies have been conducted in live bird markets in the area where most HA and NA subtypes were found in poultry (Webster et al., 1992; Nguyen et al., 2005). It is plausible that the circulation of the virus subtypes in poultry at least partially reflects that in wild birds.

Dabbling ducks of the genus Anas, with mallards (Anas platyrhynchos) being the most extensively studied species, are more frequently found infected with influenza viruses than other birds, including diving ducks (Table 1). Differences in viral prevalence between ecological guilds of ducks are likely in part related to behavior. Dabbling ducks feed primarily on food in surface waters, while diving ducks forage at deeper depths and more often in marine habitats (Del Hoyo et al., 1996). Dabbling ducks display the propensity of abmigration, the switching of breeding grounds between years, which is in part due to mate choice (Alerstam and Lindstrom, 1990; Del Hoyo et al., 1996). This behavior could provide an opportunity for influenza viruses to be transmitted between different host subpopulations. In dabbling ducks, influenza virus infection generally causes no major clinical signs, and experimental infections indicate that animals only produce a transient, low-level humoral immune response that may be sufficient to provide partial protection against homologous reinfection, but unlikely to confer protection against heterologous reinfections (Kida et al., 1980). Different influenza virus subtypes also can infect ducks concomitantly, creating the opportunity for gene reassortment (Sharp et al., 1997).

Little is known about the prevalence of influenza viruses in wild ducks in the Southern Hemisphere or potential transmission between the hemispheres. There is little connectivity between Anatidae species of the Northern and Southern Hemispheres and most species stay year-round within each breeding continent. The blue-winged teal (Anas discors) is one of the few North American species that most abundantly has a wintering distribution including parts of South
America (Del Hoyo et al., 1996). There are several duck species that could serve as influenza virus hosts in South America (Del Hoyo et al., 1996), but surveillance data are not available. For only 6 of 39 Anatidae species breeding in Eurasia, at least part of the population winters south of the Sahara Desert in Africa; for example, the garganey (Anas querquedula) and the northern pintail (Anas acuta) each have African winter populations >1,000,000 (Scott and Rose, 1996). Similar to South America, none of the 22 Anatidae species that breed in sub-Saharan Africa spend the nonbreeding season outside the continent. However, there are several species with large, widespread populations in Africa (Liu et al., 2005), and some that perform movements within Africa (Brown et al., 1982). Potential areas for mixing of Eurasian and African ducks are in West Africa, near the Senegal and Niger rivers, the inland deltas of Nigeria and Mali, and Lake Chad (Scott and Rose, 1996), and influenza viruses in African Anatidae populations can thus be linked to Eurasia through migrating species. Anatidae of Oceania are mainly resident, and do not perform regular seasonal migrations (Del Hoyo et al., 1996).

The first recorded isolation of influenza virus from wild birds was from a common tern (Sterna hirundu) in 1961. This HPAI H5N3 virus was responsible for an outbreak in South Africa where at least 1,600 of these birds died (Alexander, 2000). The most frequently detected influenza virus subtype in gulls is H13, a subtype rarely found in other birds. Recently, a novel virus subtype (H16), which is related to H13, was described in black-headed gulls (Larus ridibundus) in Sweden (Fouchier et al., 2005). The genes of H13 and H16 viruses are genetically distinct from those of influenza viruses from other hosts, suggesting they have been genetically isolated during sufficient time to invoke genetic differentiation (Fouchier et al., 2005). This is in agreement with the observation that gull influenza viruses do not readily infect ducks upon experimental inoculation (Webster et al., 1992). Although other influenza virus subtypes also are occasionally detected in terns and gulls, it is plausible that the viruses that are genetically indistinguishable from viruses of other avian hosts are most likely not endemic in gulls and terns. Influenza viruses can be detected in a small proportion of gulls, with the highest virus prevalence reported in late summer and early fall (Fouchier et al., 2005). Most gull species breed in colonies (Del Hoyo et al., 1996), with adults and juveniles crowded in a small space, creating good opportunities for virus spread. This situation contrasts that in dabbling ducks that do not breed in dense colonies (Del Hoyo et al., 1996), and epizootics could be more easily initiated when birds congregate in large numbers during molt, migration, or wintering.

Waders in the Charadriidae and Scolopacidae families are adapted to marine and wetland areas and often live side-by-side with ducks. Long-term influenza virus surveillance studies are still sparse, but data from North America suggest a distinct role of these birds in the perpetuation of certain virus subtypes (Krauss et al., 2004). Influenza viruses of subtypes H1–H12 have been isolated in birds migrating through the eastern USA, with a high prevalence of certain HA subtypes (H1, H2, H5, H7, and H9–H12) and a larger variety of HA/NA combinations as compared to ducks in Canada, suggesting that waders maintain a wider spectrum of viruses. Moreover, the seasonal prevalence of influenza viruses in waders seems to be reversed as compared to ducks, with high virus prevalence (14%) during spring migration (Krauss et al., 2004). This led to the hypothesis that different families of wetland birds are involved in perpetuation of influenza virus, where waders may carry the virus north to the duck breeding grounds in spring.

Although this may be a plausible hypothesis based on the North American
data, studies in waders in Northern Europe failed to produce similar results, suggesting that these birds are not critical hosts for influenza virus perpetuation in Europe. Recent genetic analyses have not revealed striking differences between influenza viruses from ducks and waders in the Americas, suggesting that these viral gene pools are not separated (Widjaja et al., 2004; Spackman et al., 2005). Many wader species of the Northern Hemisphere are long-distance intercontinental migrants (van de Kam et al., 2004), and may therefore have the potential to distribute influenza viruses around the globe.

As stated above, influenza viruses can be found in numerous other species, but it is unclear in which of these species influenza viruses are endemic and in which the virus is merely a temporal pathogen. Species in which influenza viruses are endemic share the same habitat at least part of the year with species such as geese, swans, rails, petrels, and cormorants in which influenza viruses are frequently detected. In these birds, as well as numerous other species, influenza virus prevalence seems to be lower as compared to dabbling ducks, but it should be noted that studies on these species are still limited. It is possible that in some of these species the prevalence of influenza viruses is comparable to that in ducks, but that peak prevalence has been missed due to seasonality or location. As for ducks, gulls, and waders, the behavior and ecology may be an important determinant to function as host species. For instance, geese are mainly herbivorous and often congregate in large flocks for grazing in pastures and agricultural fields, especially during the nonbreeding season. Such flocks may reach numbers of tens of thousands of birds in optimal areas, and often contain several species. Colonial breeding occurs in some goose species, but most nest solitarily or occur in loose groups with little interaction among pairs. Given that wild geese and ducks are the ancestors of today's domestic goose and duck species and that these domestic animals in parts of the world are frequently kept alongside chickens, wild geese and ducks may form the bridge for influenza viruses between wild and domestic birds and should thus both be monitored more intensively.

In 1997 an HPAI outbreak caused by H5N1 influenza virus occurred in the live bird markets of Hong Kong, which also resulted in the first reported case of human infection and fatality attributable directly to avian influenza virus (de Jong et al., 1997). The HPAI H5N1 virus reappeared in 2002 in waterfowl at two parks in Hong Kong, and also was detected in other captive and wild birds (Ellis et al., 2004). It resurfaced in 2004, devastating the poultry industry in a large part of Southeast Asia. In 2005, the virus was isolated during an outbreak among migratory birds in Qinghai Lake, China, affecting large numbers of wild birds (Liu et al., 2005). This single epizootic caused an estimated 10% decrease of the global population of bar-headed geese (Anser indicus), highlighting the potential devastating effects on vulnerable wildlife.

Subsequently, the virus appeared in Mongolia, Kazakhstan, Russia, Turkey, Romania, and Croatia, and wild bird deaths have been reported in several of these countries. Presently, the HPAI H5N1 strain that originated in poultry in Southeast Asia has caused mortality in >60 wild bird species (Ellis et al., 2004; Sturm-Ramirez et al., 2004; Liu et al., 2005). In addition, during the devastating outbreaks in poultry, the H5N1 virus was transmitted to >250 humans, leading to >150 deaths (World Health Organization [WHO], 2007), and was isolated from pigs, cats, tigers, and leopards.

It is most likely that the HPAI H5N1 virus circulated continuously in domestic birds in Southeast Asia since 1997, and as a consequence evolved significantly. Poultry trade and mechanical movement of
Infected materials are likely modes for spreading HPAI (Alexander, 2000). Although numerous wild birds became infected, it is not clear whether they played an active role in the geographic spread of the disease. It has been argued that infected birds would be too severely affected to continue migration, and thus unlikely to spread the HPAI H5N1 virus. Although this may be true for most wild birds, it has been shown that in experimental infection of ducks, some animals survive infection and shed the HPAI H5N1 virus without apparent disease signs (Sturm-Ramirez et al., 2004; Hulse-Post et al., 2005). In addition, many wild birds may be partially immune due to previous exposures to LPAI influenza viruses, as was shown for chickens (Seo et al., 2002).

Finally, recent studies suggest that HPAI viruses may become less pathogenic to ducks upon experimental infection, while retaining high pathogenicity for chickens (Hulse-Post et al., 2005; Sturm-Ramirez et al., 2005). Although wild birds may thus play a role in the current HPAI outbreaks, their role for spread of the HPAI H5N1 virus has yet to be supported by solid evidence.

Despite the relatively intense surveillance studies that have been performed for many years in North America and Eurasia, our understanding of the global distribution of influenza viruses in wild bird populations is still limited. Serological evidence indicates that influenza viruses occasionally even circulate in Antarctica (Austin and Webster, 1993) and it is reasonable to assume that influenza viruses are distributed globally wherever competent host species are present. It is possible that some subtypes are rare or not detected annually in current surveillance studies simply because of the limitations of our studies; we are currently biased toward species that are easy to sample, ubiquitously present, and within reach during migration or wintering. Secondly, integrating virus and host ecology will be crucial to understand the global patterns of influenza virus in wild birds. Long-term surveillance studies are needed to provide more insight into the year-round perpetuation of influenza viruses in wild birds. The possible intercontinental contacts among and between ducks and shorebirds in areas where migrating birds from the northern and southern latitudes mix are of particular interest. Can influenza viruses be perpetuated in ducks alone, or does the interface between ducks and shorebirds as suggested in North America (Krauss et al., 2004), also apply to other continents? With the current potential of high-throughput sequencing technology it should also be possible to gain more insight into the genetic variability and evolution of avian influenza viruses, and integrate this information with epidemiology and virus–host ecology.

The recent HPAI H5N1 outbreaks in Eurasia have identified additional gaps in our knowledge of avian influenza viruses in wild birds. It is clear that influenza virus surveillance of wild birds could provide early warning signals for the introduction of HPAI H5N1 virus in new regions, and provide access to strains for characterization. However, for proper risk assessment studies, we need better understanding of the interface between wild and domestic birds, the possible transmission of influenza viruses between these populations, bird behavior, age structures of populations, and detailed migration routes. It also should be realized that our knowledge of LPAI viruses in wild birds cannot simply be extrapolated to HPAI viruses. For instance, the most important host species may be quite different (Table 1) (Ellis et al., 2004; Sturm-Ramirez et al., 2004; Liu et al., 2005), and so may the route of virus transmission. The current increased interest in influenza virus surveillance in wild and domestic birds provides a unique opportunity to increase our understanding not only of HPAI epidemiology but also of the ecology of LPAI viruses in their natural hosts, at the same time and cost.
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