

Epidemiology of low pathogenic avian influenza viruses in wild birds

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Summary

Although extensive data are available on low pathogenic avian influenza (LPAI) virus surveillance in wild birds in North America and Europe, data are scarce for other parts of the world, and our understanding of LPAI virus ecology in the natural reservoir is still far from complete. The outbreak of highly pathogenic avian influenza (HPAI) of the H5N1 subtype in the eastern hemisphere has put an increased focus on the role of wild birds in influenza virus transmission. Here, the authors review the current knowledge of the (molecular) epidemiology, genetics and evolution of LPAI viruses in wild birds, and identify some important gaps in current knowledge.

Keywords

Evolution – Influenza A virus – Low pathogenic avian influenza – Surveillance – Wild bird.

Introduction

It is generally accepted that wild birds represent the principal natural reservoir of low pathogenic avian influenza viruses (LPAIV) (76). This concept stems from the notion that LPAI viruses are present in wild birds throughout the world (10, 21, 44, 58, 65), and that these viruses may, on occasion, be transmitted directly or indirectly to other hosts such as domestic birds, pigs, horses, mink, marine mammals and humans (7, 76). Much of our current knowledge of LPAI infections of wild birds has come from long-term surveillance studies, performed in various parts of the world (22, 27, 28, 34, 39, 43, 56, 67). Most of the earliest studies focused on the collection of faecal samples or cloacal swabs from wild birds, followed by attempts to isolate the virus by inoculation of samples into embryonated chicken eggs in the laboratory (e.g. 34). Recently, many laboratories have implemented molecular diagnostic tests, such as conventional or real-time reverse transcriptase polymerase chain reaction (RT-PCR) methods targeting highly conserved gene segments of LPAIV, to complement these classical diagnostic methods

(e.g. 39). These molecular methods allow the rapid identification of specimens positive for influenza A virus among large collections of samples, and these can be used subsequently for targeted virus isolation attempts (12, 82, 83). The PCR-based methods can be more sensitive than classical approaches (13, 61). Because of the introduction of high-throughput molecular methods in the laboratories, and as a result of the recently increased interest in (highly pathogenic) avian influenza, influenza virus surveillance activities have expanded considerably. Surveillance studies have increased in size, geographical and temporal coverage, and the types of samples under investigation (samples from both the respiratory and the intestinal tracts). As a result, the increased attention on avian influenza offers unique opportunities to increase our knowledge of LPAIV epidemiology in wild birds.

Low pathogenic avian influenza viruses have been isolated from over 100 wild bird species belonging to more than 25 families (44, 76). Although many wild bird species may occasionally harbour LPAIV, birds of wetlands and aquatic environments such as the Anseriformes (particularly

ducks, geese and swans) and Charadriiformes (particularly gulls, terns and waders) appear to constitute the major natural reservoirs of LPAIV (76). Anseriformes and Charadriiformes are distributed around the globe, except for the most arid regions (8). Many Anseriformes and Charadriiformes migrate over long distances and have the potential to distribute LPAIV between countries or continents. Birds breeding in one geographical region often follow similar migratory flyways, but these major flyways are simplifications because there are numerous exceptions where populations behave differently from the common patterns (8, 39, 70). Within the continents and along the major flyways, migration connects many bird populations in time and space at common breeding areas, staging sites, during migration, or at shared wintering areas. As a result, virus-infected birds can transmit LPAIV to other populations that subsequently may bring them to new areas. The transmission of viruses and their geographical spread is dependent on the ecology of the migrating hosts. For instance, most migrating birds rarely fly the full distance between breeding and non-breeding areas without stopping. Rather, birds make frequent stopovers during migration, and spend more time eating and preparing for migration than actively performing flights (1). Many species aggregate at favourable stopover or wintering sites, resulting in high local bird densities. Such sites may be important for transmission of LPAIV between wild birds and between different bird species.

Antigenic variation of low pathogenic avian influenza viruses in wild birds

Low pathogenic avian influenza viruses can be classified on the basis of the antigenic properties of two glycoproteins expressed on the surface of the virus particles: haemagglutinin (HA) and neuraminidase (NA). In wild birds throughout the world, LPAIV representing 16 HA (H1 to H16) and 9 NA (N1 to N9) antigenic subtypes have been detected, and these are found in the majority of possible combinations (also called subtypes, e.g. H1N1, H16N3) (14, 82). Most of the HA and NA subtypes have been found throughout the world, although some (e.g. H14, H15) appear to be detected only infrequently, or only in particular areas (29, 34, 52). Most combinations of HA and NA have been detected in wild birds, although some are relatively rare; for instance the H8 subtype is detected only sporadically, and primarily in combination with N4 (34, 39). The antigenic subtypes were originally classified on the basis of double immunodiffusion assays with hyperimmune animal sera (82), but other tests such as the haemagglutination inhibition assay and neuraminidase inhibition assay may be used for routine

antigenic characterisation of LPAIV (12, 77, 83). Nowadays, several laboratories rely on molecular diagnostic tests and sequencing to determine LPAIV subtypes (12, 23, 63, 69, 77, 83).

Genetic variation of low pathogenic avian influenza viruses in wild birds

Genetically, LPAIV can be divided into two lineages, the Eurasian and American virus lineages. This is probably the result of long-term ecological and geographical separation of the wild birds, and hence the viruses, of the eastern and western hemispheres (53, 66). For each of the eight gene segments of LPAIV, this distinction between Eurasian and American lineages can be made (9, 33, 42). However, bird populations of North America and Eurasia are not completely separated; several waterfowl and shorebird species cross between hemispheres during migration or have breeding ranges that include both north-eastern Russia and north-western North America (8). Most shorebird species with breeding grounds in the Russian Far East winter in Southeast Asia and Australia, but some species winter along the west coast of the Americas. Similarly, most species with breeding grounds in Alaska winter in the Americas, but some may winter in Asia (16, 81). The overlap in distribution of ducks is not as profound as that of shorebirds, but species such as the Northern Pintail (*Anas acuta*) are common in both North America and Eurasia (8) and could also provide an intercontinental bridge for LPAIV. Indeed, LPAIV carrying a mix of genes from the American and Eurasian lineages have been isolated, indicating that allopatric speciation is only partial (32, 33, 36, 38, 71, 72). Nevertheless, the detection of LPAIV with genes of the American lineage in Eurasia and vice versa is still a relatively rare phenomenon, and the partial geographical isolation of LPAIV hosts thus seems sufficient to facilitate divergent evolution of separate gene pools (9, 33, 42).

Evolution of LPAIV in wild birds is relatively slow compared with evolution in mammals (76), but is certainly not negligible. Within both the American and Eurasian genetic lineages, multiple sublineages of viral genes cocirculate, but there are generally no consistent temporal or spatial correlations (9, 33, 42, 62). Recently, some evidence was obtained for the circulation of a unique phylogenetic lineage of viruses in South America (50). It is possible that, with the ongoing high-throughput sequencing efforts for LPAIV, signals of temporal, spatial and host-related variation in LPAIV evolution may become evident.

Genetic data from duck and shorebird LPAIV isolates from the Americas suggest an active interplay between these host species (62). Although certain HA subtypes are reported to be more prevalent in either shorebirds or ducks in North America (28, 34), this does not seem to have resulted in differences in the genetic composition of LPAIV obtained from these two reservoirs. This contrasts with the LPAIV of the H13 and H16 subtypes, which are predominantly isolated from gulls and terns and have evolved into a separate genetic lineage of LPAIV, the gull lineage. The gene segments of gull H13 and H16 viruses are genetically distinct from those of LPAIV from other wild birds, which suggests that they have been isolated for sufficient time to allow genetic differentiation (14, 28). This concurs with the observation that gull influenza viruses do not readily infect ducks when they are inoculated experimentally (76).

Genome reassortment

The segmented nature of the influenza virus genome enables evolution by a process known as genetic reassortment, the mixing of genes from two or more influenza viruses. Recent studies indicate that genetic 'sublineages' do not persist, but frequently reassort with other viruses (19, 37). As a result, LPAIV of a particular subtype do not necessarily have the same genetic make-up, even within a single year or a single host species. The high prevalence of LPAIV in some wild bird species and the detection of concomitant infections in individual birds (9, 55) support the notion that reassortment occurs frequently in nature. The ongoing full genome sequencing projects (41) are starting to provide information on the frequency of reassortment in the wild bird reservoir. An analysis of 167 full virus genomes revealed a high proportion of mixed subtype infections. Moreover, high rates of genome reassortment were reported, leading to the conclusion that LPAIV have transient 'genome constellations' (9).

Modes of virus transmission in wild birds

In wild birds, LPAIV are thought to preferentially infect cells lining the intestinal tract (59, 79). As a result, virus can be excreted in high concentrations in bird faeces. It has been shown that influenza viruses can remain infectious in lake water for prolonged periods: up to four days at 22°C and for more than 30 days at 0°C (64, 79). The relatively high virus prevalence in birds living in aquatic environments may in part be due to this relative stability in surface waters, facilitating efficient transmission via the faecal-oral route (76, 78). Preservation of the viability of

LPAIV in frozen surface waters has been suggested to contribute to LPAIV epidemiology, by enabling the virus to infect birds after their return to the breeding grounds in spring (27). Whether and how much the conservation of LPAI viruses in frozen surface waters contributes to LPAIV epidemiology still remains largely unknown.

Although it has long been known that significant replication of LPAIV can be observed in the respiratory tract of wild birds (31), this notion has recently received renewed attention in part owing to studies on HPAI viruses (5, 11, 30). While transmission via the faecal-oral route may be the primary mode of LPAIV transmission in many bird species, transmission via respiratory secretions may also occur, and may be relevant for particular bird species (for instance land-based birds) (11). In addition, faecal-cloacal transmission (via 'cloacal drinking') has been postulated to represent a third potential route of transmission (2). More studies are needed to identify the most efficient routes of transmission for different wild bird families.

Low pathogenic avian influenza viruses in ducks

Numerous surveillance studies of wild ducks in the northern hemisphere have revealed higher LPAIV prevalence in juvenile, presumably immunologically naive, birds with a peak in early autumn, when the birds start migrating south. In North America, the prevalence falls from more than 60% in early autumn, in ducks sampled at marshalling sites close to the Canadian breeding areas, to 0.4% to 2% at the wintering grounds in the southern United States of America (USA), and ~0.25% when the ducks return to the northern breeding grounds in spring (76). Similar patterns have been observed in Northern Europe, although LPAIV detection during the spring migration can be significantly higher (39, 73, 74). Surveillance of the nesting grounds of ducks in Alaska and Siberia before winter migration revealed the presence of LPAIV in 2.5% to 8% of birds (26, 43). Such year-round prevalence raises the possibility that LPAIV may persist in duck populations around the year, and thus they may constitute a primary reservoir of LPAIV (44).

All HA and NA subtypes, with the exception of H13 to H16, circulate in wild ducks in North America and Northern Europe. In a 26-year longitudinal study performed in Canada, LPAIV of subtypes H3, H4 and H6 were isolated from ducks most frequently, and other subtypes less frequently (34). Although in other North American and European studies LPAIV of subtypes H3, H4 and H6 were also detected frequently, the detection of other virus subtypes was not significantly different (18,

39). Thus, the prevalence of LPAIV in general, as well as the specific distribution of subtypes, may vary between different surveillance studies depending on species, time and place.

In Canadian studies, cyclic patterns of LPAIV subtypes were reported: peaks in virus isolation of an HA subtype were followed 1 to 2 years later by reduced rates of isolation of this subtype (34). This observation awaits confirmation in other long-term surveillance studies. Cycling of LPAIV subtypes in wild birds could provide new insights into the ecology of LPAIV in their natural hosts, potentially related to immunity.

Surveillance of LPAIV in ducks has been performed in Japan since the late 1970s (45, 46, 47, 48, 57, 68). As in other studies, LPAIV prevalence and the subtypes isolated varied between years and locations. The prevalence of LPAIV in wild birds elsewhere in Asia is largely unknown, but several studies have been conducted in live bird markets, in which most HA and NA subtypes were found in poultry (24, 35, 40). It is plausible that the circulation of LPAIV subtypes in poultry at least partially reflects that in wild birds, but no direct connection has yet been established.

Dabbling ducks of the *Anas* genus – with Mallards (*Anas platyrhynchos*) the most extensively studied species – have been found to be infected with LPAIV more frequently than other birds (34, 39, 49). Dabbling ducks feed primarily on surface waters and may switch between breeding grounds between years ('abmigration'), which is in part attributable to mate choice (8). This behaviour could provide an opportunity for LPAIV to be transmitted between different host subpopulations.

Until recently, the prevalence of LPAI viruses in wild ducks in the southern hemisphere and potential transmission between the hemispheres were largely unknown. There is little connectivity between northern and southern Anatidae species, 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 has a winter distribution that includes South America (8). There are several other duck species that could serve as hosts for LPAIV in South America (8), but surveillance data are still very limited (50, 60). Similarly, only 6 of 39 Anatidae species breeding in Eurasia winter with at least part of the population south of the Sahara desert in Africa; for example, the Garganey (*Anas querquedula*) and the Northern Pintail (*Anas acuta*) each have African winter populations in excess of one million birds (54). As in South America, none of the 22 Anatidae species that breed in sub-Saharan Africa spends the nonbreeding season outside the continent. However, there are several species with

large, widespread populations in Africa, and some migrate within Africa (6, 54). Potential areas for mixing of Eurasian and African ducks are in West Africa, near the Senegal and Niger Rivers, the floodplains of the Niger River in Nigeria and Mali, and Lake Chad (54). A recent surveillance study in several African countries indeed demonstrated the presence of LPAIV in Eurasian and African duck species (15). The LPAIV in African Anatidae populations thus probably can be linked to Eurasia through migrating species. Anatidae of Oceania are mainly resident and do not perform regular seasonal migrations (8).

Infection with LPAIV generally causes no major clinical signs in dabbling ducks, and experimental infections indicate that animals only produce a transient, low-level humoral immune response, which may be sufficient to provide partial protection against reinfection with viruses of the same subtype, but is less likely to confer protection against heterologous reinfections (31). To understand the ecology of LPAIV in wild birds better, a more thorough understanding of the (cross-) protective immune response induced upon LPAIV infection is needed.

Low pathogenic avian influenza viruses in gulls and terns

The first recorded isolation of influenza virus from wild birds was from a Common Tern (*Sterna hirundo*) in 1961. This HPAI H5N3 virus was responsible for an outbreak in South Africa in which at least 1,300 of these birds died (4). The most frequently detected LPAIV subtype in gulls is H13, a subtype rarely found in other birds (20). Recently, a 'novel' virus subtype (H16), related to H13, was described in Black-headed Gulls (*Larus ridibundus*) in Sweden (14). Similar viruses have now been detected elsewhere (9, 33). The LPAIV can be detected in a small proportion of gulls, with the highest virus prevalence reported in summer and early autumn, when the birds leave their nests. Most gull species breed in colonies (8), with adults and juveniles crowded into a small space. This situation contrasts with that in dabbling ducks, which do not breed in dense colonies (8), and epizootics could be more easily initiated when birds congregate in large numbers during migration. Although other LPAIV subtypes are also occasionally detected in terns and gulls (44), it is possible that these are not endemic in these species. A recent study in Argentina demonstrated the presence of H13 LPAIV in gulls in South America. Genetically, this H13 virus was distinct from those circulating in gulls elsewhere, potentially as the result of some geographical isolation (50). Gulls and terns in Africa have also been reported to harbour LPAIV, but the subtypes have not been described (15).

Low pathogenic avian influenza viruses in waders

Waders in the Charadriidae and Scolopacidae families are adapted to either marine or freshwater wetland areas and often live side-by-side with ducks (51). Long-term LPAIV surveillance studies are still sparse for many parts of the world, but data from North America suggest a distinct role of these birds in the perpetuation of certain virus subtypes. The LPAIV of subtypes H1 to H12 have been isolated from birds migrating through the eastern USA, with a high prevalence of certain HA subtypes (H1, H2, H5, H7, H9 to H12) and a larger variety of HA/NA combinations compared with LPAIV isolated from ducks in Canada, suggesting that waders maintain a wider spectrum of viruses. Moreover, the seasonal prevalence of LPAIV in waders was found to be reversed compared with that in ducks, with higher virus prevalence (~14%) during the spring migration (34). This has led to the hypothesis that different families of wetland birds are involved in perpetuation of LPAIV, and it suggests a role for waders in carrying the virus north to the duck breeding grounds in spring. Recent genetic analyses have not revealed striking differences between LPAIV from ducks and waders in the Americas, suggesting that these viral gene pools are not separated (9, 33, 42, 62, 80). Moreover, Hanson and colleagues recently published a large multi-year surveillance study on Charadriiformes in the Americas (17) which suggested that LPAIV infections among shorebirds may be localised, species specific, and highly variable with respect to virus subtypes. It is thus plausible that the Delaware Bay area, where many initial shorebird studies were performed, has provided data that may not be translated directly to shorebirds in other parts of the world. For instance, studies in waders in Northern Europe have failed to produce similar results to those for Delaware Bay (39). In a recent study in Australia, H4N8 and H11N9 LPAIV were detected in Red-necked Stints (*Calidris ruficollis*) and Sharp-tailed Sandpipers (*Calidris acuminata*), respectively (25). Because many wader species of the northern hemisphere are long-distance intercontinental migrants (70) they have the potential to distribute LPAIV around the globe. While it is clear that waders are permissive to LPAIV, to what extent they contribute to LPAI virus epidemiology remains less clear.

Low pathogenic avian influenza viruses in other wild birds

The LPAIV can be found in numerous other bird species (39), but it is unclear in which of these species LPAIV are

endemic and in which the virus is a transient pathogen. Species in which LPAIV are endemic share the same habitat for at least part of the year with other species in which LPAIV are frequently detected, including geese, swans, rails, petrels and cormorants. In these birds and others, LPAIV prevalence seems to be lower than in dabbling ducks, but it should be noted that studies on these species are limited, and it is possible that peak prevalence has been missed because of its seasonal nature or location, or that the virus is not easily detected in cloacal samples.

As for ducks, gulls and waders, their behaviour and ecology may be an important determinant of their role 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 non-breeding season. Such flocks may contain up to tens of thousands of birds and often include several species. Colonial breeding occurs in some goose species, but most are solitary nesters or nest in loose groups with little interaction between pairs. Given that wild geese and ducks are the ancestors of today's domestic goose and duck species and that these domestic animals are frequently kept alongside chickens in various parts of the world, wild geese and ducks may form the bridge for transmission of LPAIV between wild and domestic birds.

Discussion

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 LPAIV in wild bird populations is limited. Serological evidence indicates that LPAIV occasionally circulate in Antarctica (3, 75), and it is reasonable to assume that LPAIV are distributed globally, wherever competent host species are present. It is possible that some subtypes are rare or are not detected annually in current surveillance studies. Simply because of the limitations of our studies, we are currently biased towards species that are easy to sample during migration or wintering. Second, to understand the global patterns of LPAIV in wild birds, it will be crucial to integrate virus and host ecology with long-term surveillance studies to provide more insight on the year-round perpetuation of LPAIV in wild birds. Possible intercontinental contacts among ducks and shorebirds in areas where migrating birds from the northern and southern latitudes mix are of particular interest. Can LPAIV be perpetuated in ducks alone, or does the interface between ducks and shorebirds, as seems to occur in North America, also occur on other continents? With high-throughput sequencing technology, it should be possible to gain more insight into the genetic variability and evolution of LPAIV in wild birds and to integrate this

information with epidemiology and virus/host ecology. Transmission routes remain largely unknown for many wild bird species. Similarly, the effect of immunity induced by exposure to LPAIV on subsequent reinfection of wild birds remains an important area for further research. The current increased interest in avian influenza provides opportunities not only to increase our knowledge of HPAI but also that of LPAI viruses in wild birds.

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Épidémiologie des virus de l'influenza aviaire faiblement pathogène dans l'avifaune

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Résumé

Contrairement à l'Amérique du Nord et à l'Europe, où la présence dans l'avifaune du virus de l'influenza aviaire faiblement pathogène (IAFP) fait l'objet d'une surveillance très poussée, dans d'autres parties du monde les données sur le sujet sont rares, et de manière générale l'écologie du virus et de son réservoir naturel est encore mal connue. Le foyer survenu en Asie, dû au sous-type H5N1 du virus de l'influenza aviaire hautement pathogène (IAHP) a mis l'accent sur l'importance de l'avifaune dans la transmission du virus de l'influenza. Après avoir fait le point sur l'état actuel des connaissances relatives à l'épidémiologie, à la génétique et à l'évolution moléculaires des virus de l'IAFP dans l'avifaune, les auteurs indiquent les lacunes qui subsistent dans ces domaines.

Mots-clés

Avifaune – Évolution – Influenza aviaire faiblement pathogène – Surveillance – Virus de l'influenza A.

Epidemiología de la influenza aviar de baja patogenicidad en aves silvestres

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Resumen

La vigilancia de los virus de la influenza aviar de baja patogenicidad en aves silvestres de Norteamérica y Europa ha sido ampliamente documentada, pero la información sobre otras regiones del mundo, así como los conocimientos sobre la ecología de esos virus en los reservorios naturales, son muy incompletos. Tras el foco de influenza aviar altamente patógena del subtipo H5N1 registrado en Asia se prestó mayor interés al papel de las aves silvestres en la transmisión de los virus de la enfermedad. En este artículo se pasa revista a los conocimientos actuales sobre la epidemiología, genética y evolución moleculares de los virus de la influenza aviar de baja patogenicidad en aves silvestres, así como a algunas de las importantes lagunas que persisten en ese ámbito.

Palabras clave

Ave silvestre – Evolución – Influenza aviar de baja patogenicidad – Vigilancia – Virus de la influenza aviar de tipo A.

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