Ecology of Avian Influenza Virus in Birds

Douglas Causey[1] and Scott V. Edwards[2]

[1]Department of Biological Sciences, University of Alaska Anchorage, Anchorage; [2]Department of Organismal and Evolutionary Biology, Harvard University, Cambridge, Massachusetts

Avian influenza A virus (an orthomyxovirus) is a zoonotic pathogen with a natural reservoir entirely in birds. The influenza virus genome is an 8-segment single-stranded RNA with high potential for in situ recombination. Two segments code for the hemagglutinin (H) and neuraminidase (N) antigens used for host-cell entry. At present, 16 H and 9 N subtypes are known, for a total of 144 possible different influenza subtypes, each with potentially different host susceptibility. With >10,000 species of birds found in nearly every terrestrial and aquatic habitat, there are few places on earth where birds cannot be found. The avian immune system differs from that of humans in several important features, including asynchronous B and T lymphocyte systems and a polymorphic multigene immune complex, but little is known about the immunogenetics of pathogenic response. Postbreeding dispersal and migration and a naturally high degree of environmental vagility mean that wild birds have the potential to be vectors that transmit highly pathogenic variants great distances from the original sources of infection.

More than 1400 human pathogens are known to medical science, and 65% of them are zoonotic—that is, originating in nonhuman hosts [1, 2]. Less than 5% of all pathogens are viruses, but more than a third of the emerging diseases recorded in the past century are caused by RNA viruses. Most zoonotic viral pathogens are not readily transmitted among humans; instead, humans appear to be so-called "dead-end" hosts, in which the viral infection causes disease but is not successfully transmitted to another host [3]. In most cases, the virus-host system has coevolved sufficiently so that replication can take place only in the specific host associated with a particular virus. Only mutations in the viral genome will enable the virus to replicate and to infect new hosts efficiently or at all [4, 5]. This rarely occurs, and most zoonotic viral pathogens persist in human populations only with continuous infection from their natural hosts, or reservoir [6, 7]. However, viruses composed of single-stranded RNA or DNA, such as influenza viruses, can mutate very quickly, because there is no inhibiting mismatch of base pairs, as there are in viruses with a double-stranded genome.

The genomes of many viruses are linear or closed (e.g., circular), which means that, when the virus completes the replication cycle within the host cell, the genome replicates intact. Some viruses, such as influenza viruses, have segmented genomes—that is, the genetic material is contained in distinct and separate units that are analogous to the chromosomes of more-complex organisms. This enables each of the genetic units not only to mutate independently of the others but also to replicate independently by genetic reassortment if there are different types of the virus within the same host cell. Thus, because of its segmented, single-stranded, RNA-based genome, influenza virus has a very high potential for adaptive change as a human pathogen [8–10].

AVIAN INFLUENZA AND PANDEMIC INFLUENZA

Influenza viruses are single-stranded RNA viruses of the family Orthomyxoviridae, of which 3 types (A, B, and C) are recognized; only influenza A and B viruses occur in highly pathogenic forms. The natural reservoir of influenza A viruses is birds, and, consequently, many are known as avian influenza viruses. These viruses nat-

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Reprints or correspondence: Dr. Douglas Causey, University of Alaska Anchorage, 3211 Providence Dr., Anchorage, Alaska 99508 (afdc@uaa.alaska.edu).

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urally infect the intestinal tract of wild birds and, as expected in coevolved host-parasite systems, usually cause asymptomatic infections in their natural hosts [10]. Influenza A viruses are classified into subtypes determined by the hemagglutinin (H) and neuraminidase (N) antigens used for host-cell entry by the virus during replication. At present, 16 H and 9 N subtypes have been identified. Each virus has 1 H and 1 N subtype in any combination, for a total of 144 possible different influenza virus variants (confusingly, also called "subtypes"). Thus, for example, the influenza pandemic of 1918 was caused by an H1N1 subtype of influenza A virus [9], probably of avian origin, and the current influenza A virus subtype that is of concern is H5N1. Avian influenza viruses normally do not infect species other than bird species but have been found infrequently in a range of other animal species, including marine mammals, domestic animals, and humans.

Reservoirs for all H and N subtypes of avian influenza virus include aquatic birds, particularly waterfowl, in which the vectors multiply in the gastrointestinal tract, producing large amounts of virus usually without producing clinical signs. Infections in poultry or in nonnatural hosts cause a wide spectrum of symptoms, and viruses can be divided into 2 groups according to their pathogenicity [8, 9].

Some forms of these viruses, known as highly pathogenic avian influenza (HPAI) viruses, can cause severe illness and a mortality rate approaching 100%. However, most strains of these viruses are nonvirulent, do not produce clinical signs, or cause only mild respiratory or reproductive disease. These strains are known as low-pathogenic avian influenza (LPAI) viruses and are commonly isolated from many species of wild birds [11, 12]. HPAI viruses, however, are not maintained by wild bird populations but have been isolated occasionally from wild birds during disease outbreaks among domestic poultry. The ability of LPAI viruses to mutate into HPAI viruses, particularly in poultry, and the diversity of viruses circulating in wild bird populations emphasize the potential importance of wild birds as a primary source of the zoonotic introduction of influenza into human populations [8, 13, 14]. When the human-to-human transmission of influenza virus is efficient, the conditions are set for pandemic spread of the disease [15].

PHYSIOLOGY, IMMUNOLOGY, AND NATURAL HISTORY OF BIRDS

Birds are particularly well adapted to serve as vectors or reservoirs of pathogens. They are diverse: there are ~10,000 species, offering ample variation for the hosting of disease pathogens, and they can be found in or around nearly every terrestrial and aquatic habitat throughout the world. As a group, they are the most vagile land vertebrates. Some species travel many kilometers each day searching for food or mates, and some species migrate thousands of kilometers each year, during their seasonal breeding cycle. There are few places on earth where birds cannot be found.

Birds evolved millions of years before humans and are in a lineage very unrelated to mammals and humans. As a consequence, many aspects of their physiology and ecology differ in significant ways, and some of these features facilitate their potential as sources of zoonotic pathogens. Birds have a higher metabolic rate and a correspondingly higher body temperature (up to 43.5°C) than humans. For many bacterial pathogens, the higher body temperatures seem to preclude disease sequelae; with viruses, these higher temperatures are often associated with more-rapid onset and progression of disease [9]. Because birds have no sweat glands, their cooling mechanism is primarily by respiratory evaporative heat loss, or panting. The respiratory rate of the house sparrow rises from 57 breaths/min at 30°C to 160 breaths/min at 43°C. Bird lungs do not change volume during the respiratory cycle; instead, airflow is in only 1 direction, so that there is no flushing effect, as in mammalian lungs. These characteristics can help promote the infectious cycle of respiratory viruses such as infectious bronchitis virus or avian influenza virus [11, 13].

The avian immune system differs from the mammalian immune system in several important ways; the primary difference is that birds have 2 different immune systems, which appear sequentially during development [11, 16, 17]. The cells that first produce antibodies are the B lymphocytes, which are produced as stem cells in the embryonic liver, yolk sac, and bone marrow in the developing embryo and hatchling chick. These cells move to a unique avian structure called the bursa of fabricius (BF), which programs the cells to produce the needed antibodies. The BF involutes or disappears early during development, at about the time when young birds begin to grow feathers (or fledge). Similar to B lymphocytes, the cells associated with the T lymphocyte system begin as stem cells in bone marrow or other tissue. However, the T lymphocytes are programmed in the thymus rather than in the BF. In many species of birds, the T lymphocyte system does not fully develop until after the birds are fully fledged, which, in some cases, is after the young birds have left to migrate. Thus, for some species, there is a period of lowered immune response between the time that the BF has disappeared and the time that the T lymphocyte system becomes fully functional. Infection by disease pathogens during this time can be very problematic for the young bird, leading to high titers of virus and disease caused by a depressed or immunosuppressed system [16, 18–21]. Birds also possess a major histocompatibility complex (MHC), a polymorphic multigene immune complex that is homologous to the MHC in mammals but with fewer genes and different mechanisms of diversification [17].

The avian gastrointestinal tract has evolved to minimize weight and volume, as an adaptation for flight. The intestines

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are relatively short, the passage time of food is quick, and urine and intestinal contents mix to form a thin slurry that can be easily and continuously ejected. Many disease pathogens start as zoonotic disease agents in the medium of ejected bird feces (e.g., *Chlamydia* [chlamydiosis], *Coccidia* [coccidiosis], and West Nile virus) [22]. We do not really understand the complete physiology of most avian viruses, but influenza virus appears to have a single viral membrane receptor that is responsible for virus types (e.g., for avian influenza virus, the receptor is for the host molecule α-2,6-sialic acid). With regard to the host, many birds have immune receptors similar or identical to mammalian receptors and, thus, are effective zoonotic reservoirs for human disease. Although a moderate amount is known about the response of poultry to influenza virus infection, very little is known about the immunogenetic response of wild birds to emerging pathogens [23].

Finally, common bird behaviors such as forming large feeding flocks composed of many species and individuals and large aggregations of roosting birds and the presence of large, dense colonies of breeding birds bring significant numbers of birds together. Under these conditions, disease can spread very quickly within social groups [24, 25]. Obviously, the effect is magnified among domesticated birds, since individual birds are often inbred and genetically similar and housed in close quarters that are often unsanitary and contaminated with feces, body fluids, and dead animals.

AVIAN ECOLOGY AND ZOONOTIC DISEASE

A large number and variety of influenza viruses are maintained in wild bird populations [22, 24, 26, 27]. Avian influenza viruses have been isolated from >100 species of wild birds from 15 orders, composed of most of the major families. Influenza virus was first isolated from wild birds in South Africa, from common terns *(Sterna hirundo),* in 1961 [28]. An increase in surveillance during the late 1970s revealed that ducks and geese are important reservoirs, but further work has shown that ecological association with either fresh or marine water was the primary determinant of disease incidence [22, 24]. Passerine, dryland birds can serve as significant reservoirs for influenza viruses [28, 29], but only a few of these influenza virus subtypes have been implicated in disease outbreaks.

Seasonal infection patterns have been detected, with the greatest prevalence occurring during late autumn and winter [14, 18, 19, 30]; these patterns may be associated with immune-system development (described above) or with ecological changes in the environment. The movement and age of birds also appear to be important and are correlated with seasonal effects. For example, a significantly higher prevalence of the virus among juvenile mallards was recorded before migration south for the winter [14]. Direct and indirect contact with waterfowl has been associated with outbreaks of avian influenza among domestic birds and has been suggested as a potential cause of initial infection [30–36].

Water is a likely medium for the transfer of nonvirulent avian influenza virus and partially explains the high prevalence of the virus among waterbirds, shorebirds, and seabirds—species that congregate in large numbers in wetlands. The virus can remain infective in freshwater lakes for 4 days at 22°C and for >30 days at 0°C [36] and for even longer periods in ice or frozen ground [37–41]. Ongoing research by D.C. (unpublished data) and by Rogers et al. [37] has suggested a strong environmental component to the natural history of influenza virus infection. Infected birds on breeding grounds in the high Arctic shed viable virions into the environment through feces, and these virions persist in cold water and then in ice or frozen ground throughout the winter. Birds returning during the spring migration encounter the virus in thawing ponds or ground ice and are reinfected [37]. This scenario strongly associates migrating waterbirds with the presence and persistence of avian influenza virus in wild bird populations.

MIGRATION AND AVIAN INFLUENZA

The role of wild birds in the introduction, maintenance, and transmission of disease is largely dependent on a range of ecological factors, including the distribution and density of susceptible animal hosts [11, 16]. The risks associated with wild birds introducing H5N1 or any other subtype of avian influenza virus are virtually impossible to quantify using current information. There is insufficient knowledge of the epidemiology and transmission dynamics of avian influenza virus, although there are nascent attempts at modeling and predictions of major parameters affecting the bird-virus system [41, 42]. However, thus far, all evidence implies that the migration patterns of birds have served as transmission pathways of avian influenza virus in the past and could have played a role in the spread of the H5N1 subtype of avian influenza virus among domestic birds throughout Eurasia [42]. Large-scale surveillance projects in northern Europe, Scandinavia, and Alaska have been predicated on this assumption [21]; as yet, no migratory bird carrying an H5N1 subtype of human or avian origin has been detected.

INTERACTION BETWEEN WILD BIRDS AND HUMANS

The role of wild birds in HPAI virus transmission is difficult to establish with certainty. The physiology of birds, their behavior, and their potential for long-range transmission of disease by migration implicates them as serious agents of pathogenic disease among humans [42]. The extraordinary threat of multiple circulating subtypes of avian influenza virus lies in the propensity of the virus to reassort into new forms [43–46], whereby "new" subtypes can be formed and then amplified

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through density-dependent dynamics associated with dense aggregations of birds. Such a process has been proposed for the emergence of an H5N1 subtype that can infect (so far) a very small number of humans. Correlative data have yet to be found, much less an understandable biological mechanism for a potential shift from a purely avian virus into one that can infect and replicate within humans.

Migratory birds are similarly implicated as active agents of the transmission and spread of disease. The evidence for this is very weak and circumstantial; moreover, other data indicate that the trafficking of wild animals, unregulated commercial transport of poultry, and subsistence hunting may play a role that is as strong or stronger in the spread of HPAI virus vectors through human populations [47, 48]. As in many disease-outbreak situations involving epizootics or leading to pandemics, we are overtaken by the phenomenon far in advance of understanding the causes.

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