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Avian Influenza: An Ecological and Evolutionary Perspective for Waterbird Scientists

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Abstract.—Highly pathogenic avian influenza (HPAI) type A of the subtype H5N1 has recently spread widely and rapidly across Eurasia, and even to Africa, with deaths of both wild and domestic birds recorded. There are fears that it may soon spread to the Americas. Media accounts, communications from international bodies and national governments, and even some of the professional research literature attributes the spread, in part, to movements of HP strains by migratory birds. The origin of highly pathogenic strains is attributed to mutations, or to reassortment of virus genes from different host species. In this paper we review these hypotheses in light of knowledge about the ecology and evolution of avian influenza, looked at from the viewpoint of its natural reservoir - waterbirds. Our purpose here is to alert waterbird biologists that they have much to contribute to the science of this globally-important issue. New technologies have revealed that the genome of avian influenza contains much variation beyond that recognizable by classical antibody techniques, and have established avian influenza as a rapidly evolving and diversifying lineage. The extensive genetic variability in the viral genome and extensive reassortment within host species suggests that high pathogenicity could repeatedly and independently evolve from low pathogenic ancestors under appropriate selection pressures, such as those in poultry production systems. This makes infection of wild birds by HPAI lineages evolved in poultry a more likely occurrence than the reverse. The available evidence largely fits this model. We make recommendations that will help reduce the incursion of domestically-evolved avian influenza strains into wild populations of birds. *Received 4 April 2006, accepted 3 July 2006.*

Key words.—Avian influenza, avian flu, ecology, evolution, influenza A, HPAI, LPAI, H5N1, waterbirds.

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Governments, international bodies, and the public around the world are gravely concerned about the potential impact of the avian influenza viruses on human health and on the global economy (Li *et al.* 2004; Chen *et al.* 2005; Ferguson *et al.* 2005). The recent rapid spread of avian influenza viruses, repeated outbreaks of highly pathogenic strains in domestic poultry with accompanying economic costs, cases of direct transfer of the virus from birds to humans, and the apparent high death rate among infected humans have combined to make 'avian influenza', 'highly pathogenic' and 'H5N1' household words, and the subject of much preparatory organization by agencies such as The World Health Organization (WHO) and the Food and Agriculture Organization (FAO). Governments are busily developing surveillance schemes and contingency plans to be able to deal with a pandemic that many claim to be imminent and inevitable. Fauci (2006) provides a current overview.

Migratory birds and waterbirds in general play central roles in this critical issue. It is widely asserted in the press, on websites of governments and international agencies (e.g., WHO), and even in the scientific literature, that highly pathogenic genotypes of H5N1 virus are spread to domestic poultry through contact with wild birds (Li *et al.* 2004; Chen *et al.* 2005). Relatively fewer studies highlight the dearth of evidence linking avian influenza outbreaks to migratory birds

(e.g., Olsen *et al.* 2006). To help in evaluating the role that waterbirds play in the epidemiology of this disease it is essential to investigate these and other claims critically, and to gather more data. Our purpose here is to alert waterbird biologists that they have much to contribute to the science of this globally-important issue. We briefly review the available information on the ecology and evolution of avian influenza. We provide a primer on the structure and function of influenza viruses aimed at giving ornithologists a quick entrée into a rather technical literature, outline what is known of the ecology and evolutionary biology of the virus, and review management practices that could be useful in preventing outbreaks in poultry, wild birds and humans. Our focus is on waterbirds, rather than domestic poultry, humans, or human health.

INFLUENZA VIRUS STRUCTURE AND NOMENCLATURE

Much of what we present here is based on the reviews by Horimoto and Kawaoka (2001) and Earn *et al.* (2002). Influenza viruses belong to the Orthomyxoviridae family of RNA viruses. It occurs naturally in many species of wild aquatic birds, and is maintained in wild populations.

Avian influenza viruses infect the gastrointestinal tract in its natural avian host species, but can infect the respiratory tract and other organ systems. Viral particles are shed in the feces for a time shortly after infection after which viral replication stops, presumably because the immune system has cleared the infection. The virus is transmitted very efficiently via bird-to-bird contact transmission and fecal shedding into the water supply (Webster *et al.* 1992).

The influenza virus has a very small genome with only 8 RNA segments. Six of these code for the proteins HA, NA, NP, PB1, PB2, and PA. The remaining two RNA segments are transcribed to mRNAs and translated in different reading frames to yield two proteins each, M1 and M2, and NS1 respectively. Based on variants of the M1 and NP proteins, influenza viruses are classified into three major 'types': A, B and C (Webster *et al.* 1992, Murphy and Webster 1996, Earn *et al.* 2002). Type A influenza virus occurs in a wide range of birds and mammals, is geographically widespread, and is epidemiologically the most important. It is commonly referred to as avian or bird flu. Type B is restricted to and is an important cause of illness in humans. Type C is not known to cause illness and very little is known about it. Neither Type B nor Type C have ever been isolated from waterbirds or poultry and are not discussed further.

Two proteins (HA, or hemagglutinin; and NA, or neuraminidase) are arrayed on the envelope of influenza A virions, and interact with receptor sites on the exterior of host cell membranes, and play important roles in gaining the virion access to the interior of cells. The complexity of the molecular interactions on the cell membrane restricts the types of cells that a virion can invade successfully, and usually confines a specific viral genotype to one host species, or at least to specific types of receptor sites. (Earn *et al.* 2002)

Prior to the development of sequencing technologies, virologists relied on serology (i.e., identification of the presence of antibodies in blood serum of the host, produced in response to viral surface proteins,) to classify viruses. Sixteen forms of HA and nine forms of NA were eventually described, and their combinations form the basis for the classification of influenza A viruses by 'subtypes'. Hence the H5N1 'subtype' of avian influenza contains the fifth described form of HA and the first described form of NA. (Note that notation format HxNy abbreviates hemagglutinin as 'H' and neuraminidase as 'N'.) Most but not all of the 144 possible pairwise HA-NA combinations have been observed in wild birds. The nomenclature of avian influenza viruses further uses a system that identifies the type (A, B or C), the host species from which it was isolated, the location, the isolate number, and the year. For example, 'A/mallard/Alberta/211/98 (H1N1)' is isolate number 211, a type A, subtype H1N1 virus, from a mallard in Alberta caught in 1998. This isolate (with

34 others) was used by Hatchette *et al.* (2004) in their analysis of avian influenza viruses in wild ducks in Canada.

Sequence data have revealed much variability within each subtype (Li *et al.* 2004; WHO 2005). This variability is genetic, and is found in the RNA sequences coding for all of the virus proteins. This discovery helps explain the phenomenon of 'antigenic drift' (i.e., progressive accumulation of individual mutations resulting in the gradual decline in the intensity of the antibody response), which requires the regular replacement of vaccine strains (e.g., Earn *et al.* 2002), as details of the structure of the HA and NA molecules change and so reduce the ability of the host's immune system to recognize variant subtypes of the virus. Horimoto and Kawaoka (2001) attribute antigenic drift to point mutations and 'immunological pressure'. Influenza viruses commonly change by antigenic drift which means there are small changes that occur all the time to the HA and NA proteins (Webster *et al.* 1992; Earn *et al.* 2002). Type A influenza viruses also can undergo antigenic shifts which are sudden major changes that can create a new viral subtype through genetic reassortment, the exchange of viral segments when one host is infected by two different viral subtypes. How these processes occur in wild birds is not well known, and clarifying the underlying evolutionary processes would help a great deal in understanding the virus and its epidemiology (Webster *et al.* 1992; Murphy and Webster 1996; Earn *et al.* 2002).

ECOLOGY OF AVIAN INFLUENZA

Most knowledge of avian influenza has come from virology, veterinary science, and medicine, and most is written from the perspectives of either domestic animal or human health. The available information is extensive and detailed, but little is known about the ecology of avian influenza viruses in natural populations. A similar state of affairs is demonstrated by the recently published 'Birds of Two Worlds' (Greenberg and Marra 2005), among whose 33 chapters exploring the biology of migratory birds only one (Ricklefs *et al.* 2005) is devoted to the study of avian disease. Integration of these disparate bodies of knowledge is essential, and waterbird biologists have much to learn if they are to inform policy-making on the treatment, prevention and response to avian influenza outbreaks in poultry, or to the expected human pandemic.

All known influenza A virus subtypes have been documented in waterbirds (reviewed by Webster *et al.* 1992; Murphy and Webster 1996; Ito and Kawaoka 1998; Alexander 2000; Webby and Webster 2001; Fouchier *et al.* 2005) and many of these form long-term host-virus associations in the wild (Webby and Webster 2001; Webster and Hulse 2004). The virus has also been isolated from a wide range of wildlife (birds of many species, American mink *Mustela vison*, harbor seals *Phoca vitulina*, whales (Cetacea) and domestic (pigs *Sus scropha*, chickens *Gallus gallus*, turkeys *Meleagris gallopavo*, ducks (Anatidae), domestic horses *Equus caballus*, humans *Homo sapiens*) avian and mammalian hosts (Webster *et al.* 1992; Webby and Webster 2001; Webster *et al.* 2006). The earliest documentation of wild infections of influenza A viruses dates to the 1960s, with infections in ducks and seabirds (Slemons *et al.* 1974; Webster *et al.* 1992; Webby and Webster 2001; Slemons *et al.* 2003; Laver 2004), but there can be little doubt that wild aquatic birds have a long evolutionary association with the virus. Its widespread occurrence in gulls, shearwaters, other seabirds, shorebirds and ducks has led to the recognition of waterbirds, in general, as the primary natural reservoir of the virus (Hinshaw 1980; Webster *et al.* 1992; Webby and Webster 2001; Hatchette *et al.* 2004). Not all subtypes are equally successful in establishing stable associations, because hosts vary in susceptibility and in the efficiency of transmission (Sturm-Ramirez *et al.* 2004).

Studies published on the ecology of avian influenza in wild birds in North America (e.g., Slemons *et al.* 1974; Stallknecht and Shane 1988; Webster *et al.*1992; Stallknecht 1997; Webby and Webster 2003; Krause *et al.* 2004; Webster and Hulse 2005) have made attempts to bridge this interdisciplinary gap. Since 1974 thousands of swab samples collected from migratory waterfowl and shore-

birds in Alberta and (since 1985) in Delaware Bay, provide the first and most extensive insight into patterns of occurrence of avian influenza viruses in nature.

Influenza A viruses are common in southward migrating waterfowl (~25%) and much less prevalent in northward migrants (0.3%). Post-breeding pre-migratory staging areas are thought to be important locations for disease transmission (Hinshaw *et al.* 1980; Ito *et al.* 1995; Hanson *et al.* 2002), because there is a high prevalence of infection in juvenile ducks, evident from heavy fecal shedding of the virus and increased abundance and densities of a variety of wild bird species co-mingling. The prevalence of Influenza A viruses declines in the course of southward migration, and individuals on southern wintering areas have much lower prevalence of the virus, indicating a loss of viral infection during migration. Influenza A viruses isolated from wintering and resident waterfowl in Texas (southern North America) include greater diversity and rarer subtypes, indicating long-term changes in viral lineages in the wild (e.g., Hanson *et al.* 2005). Ducks returning northward in spring have much lower viral titers than southward migrants, but in high enough titers to re-establish the virus in their northern breeding grounds (Webster *et al.* 1992; Ito *et al.* 1995; Kraus *et al.* 2004). It is possible that avian influenza viruses could survive the winter and re-infect birds arriving on breeding areas (Webster *et al.* 1992). Persistence of the virus in the environment (e.g., in water) depends on factors such as pH, temperature, salinity and other physicochemical variables (Stallknecht *et al.* 1990). The virus is hardy, and can remain infective outside an avian host for up to 35 days in fecal matter in cold, moist conditions (at 4°C), though less long in warmer conditions (at 20°C).

Certain subtypes seemingly dominate in particular migratory flyways and their prevalence varies from year to year (Hinshaw *et al.* 1985; Ito *et al.* 1995; Hanson *et al.* 2002; Hatchette *et al.* 2004; Krauss *et al.* 2004; Hanson *et al.* 2005).

The role of avian species other than waterfowl in perpetuating avian influenza remains unclear (Alexander 2000). Shorebirds (Charadriidae and Scolopacidae) are thought to be important in the dissemination and maintenance of influenza A viruses in the wild (Webster *et al.* 1992; Webster *et al.* 2006), although data are as yet limited. The prevalence of infection in northward migrant shorebirds is higher than in waterfowl (14.2% vs. 0.3%), but the significance of this is not yet known. Studies of viral ecology in migratory waterbirds in Eurasia indicate similar patterns of viral infection relative to North American studies, with interannual variation in the diversity and seasonality of the viral shedding in relation to migration (Otsuki *et al.* 1987; Okazaki *et al.* 2000; Munster *et al.* 2005). The pattern of occurrence of avian influenza in seabirds is less known, although gulls tend to have higher prevalence of influenza in late summer and early fall (Olsen *et al.* 2006). These patterns are likely related to aggregations in breeding colonies and garbage dumps or other feeding areas.

EVOLUTIONARY BIOLOGY OF AVIAN INFLUENZA

The availability of avian influenza RNA sequence data has made phylogenetic analyses possible. These have proven very informative, and have introduced a stronger evolutionary perspective into avian influenza studies. As expected of an RNA virus, avian influenza viruses represent a rapidly evolving and diversifying lineage; aquatic birds are indeed the ancestral hosts of avian influenza and shorebirds, ducks and gulls share ancestral genes of several avian influenza subtypes (Widjaja *et al.* 2004).

Influenza A genes are evolving, but phylogenetic trees are often not fully congruent with each other, because the viral genes that mingle in new hosts may contain diverse reassortants from different host species. For example, in the 1990s a reassortant influenza A (H3N2) virus lineage established itself in USA swine, with genes whose closest and most recent known ancestors were from human, bird and swine hosts (Zhou *et al.* 1999). Such reassortant viruses are often referred to as combinations of e.g., 'swine flu', 'human flu', or 'bird flu'. In this context 'swine', 'human' or 'bird' refer to the host of the most

recent known ancestor of at least one of the genes in the reassortant descendant virus. The viruses that caused the 1957 and 1968 human influenza pandemics were reassortants of human and bird flus, while all the genes in the virus that caused the 1918 pandemic were descended directly from birds (i.e., the virus was not 'reassortant'; Taubenberger *et al.* 2005). By more distant ancestry, all influenza genes are 'bird flu' genes.

In the 1990s USA swine-flu epidemic, the newly-evolved virus co-existed in the domestic pig population with 'classical' H1N1 swine virus (which itself derived from an earlier reassortment). Subsequently, further reassortments generated other novel strains of influenza A (see Hachette *et al.* 2004). Virologists have documented a detailed database of such histories in a variety of domestic species. Extensive reassortment also occurs within host species (Hatchette *et al.* 2004). Nevertheless, the trees reveal that viral lineages in different hosts maintain phylogenetic distinctiveness, likely because shifts to new hosts are comparatively rare.

The trees also reveal parallel evolutionary events. For example, 'swine flu' independently evolved in Eurasia and America. Trees reveal distinct American and Eurasian lineages for several influenza virus A genes. A low pathogenic strain of H5N1 has been detected in healthy wild birds in both Eurasia and in North America (CFIA 2005), and is very different from the highly pathogenic Asian strain. These lineages are evolving independently, and while the Eurasian form is highly pathogenic (causing severe disease in chickens, referred to as Highly Pathogenic Avian Influenza, HPAI), the North American form is low pathogenic (not causing any clinical signs of illness in chickens, referred to as Low Pathogenic Avian Influenza, LPAI). The clear separation of the trees is remarkable, because it seems inevitable that there must be some contact on Arctic breeding grounds between migrants of Old and New World origin. Geographical segregation is evident even within the recent phylogeny of H5N1 in China (Chen *et al.* 2006). Comparison of a large number of samples from both wild and domestic birds reveals that the current Eurasian H5N1 avian influenza virus originated in China at least a decade ago, and that it has evolved into distinct lineages associated with particular geographic regions. The mechanisms maintaining the separation (Kraus *et al.* 2004) are obviously of great current interest with the potential spread of Eurasian H5N1 to America.

EVOLUTION OF HIGH AND LOW PATHOGENICITY

Many infections are defeated by a host's immune system before they can establish, but those that manage to defeat or evade the host's immune defenses vary greatly in the intensity of illness subsequently induced in the host—i.e., their 'virulence'. They may cause few or no symptoms, may make the host very ill, or may even kill it. Avian influenza shows a particularly wide range of virulence. The terms 'low pathogenic' (LP) and 'highly pathogenic' (HP) originated in poultry science to describe forms of the virus that have mild and lethal effects on commercial poultry flocks (Webster *et al.* 1992; Webster *et al.* 1978; Webster *et al.* 2006).

Why does virulence vary so widely? There is much literature to suggest that low virulence is the natural state of affairs between diseases and their hosts. Statements such as 'Influenza viruses in aquatic birds appear to be approaching or have reached an optimal state of adaptation . . .' (Horimoto and Kawaoka 2001), or 'viral genes have achieved maximal fitness in their natural avian host as compared with other species' (Hatchette *et al.* 2004) are evidently based on the low pathogenicity of avian influenza in wild birds. Modern evolutionary biology, in contrast, views disease virulence as an evolved trait that changes according to circumstance. Some circumstances (discussed below) select for high virulence, others for low virulence. This work shows that selection can even favor pathogens that kill their hosts, so long as by doing so the pathogen gains a fitness advantage over less-virulent forms. In fact, the life, health or death of the host is of evolutionary interest to the disease agent only in so far as its own fitness is affected (Ewald 1994; Ewald and De Leo 2002).

A complete understanding of the virulence level of a disease requires knowledge of both the mechanisms that give a pathogen its virulence ('proximate' explanations) as well as the selective factors favoring high or low virulence ('ultimate' explanations). Sherman (1988) details how these 'levels of explanation' should never be confused, and resolves a number of controversies in the biological literature by showing that the alternative hypotheses are not true competitors, because one concerns mechanisms while the other concerns selective factors. It is essential to distinguish carefully between explanations for virulence based on mechanisms, and those based on selective factors. The literature on influenza is abundant for the former, and sparse for the latter.

Many details are known about some of the mechanisms that give influenza A its virulence. Attention has focused on HA, because it is intimately involved with gaining access to the cell, and because historically it was (along with NA) visible to virologists, who prior to the development of sequencing technologies had to rely on serology. However, it seems improbable that virulence depends on HA alone: all of the virus's genes are likely to be involved in its ability to infect a cell, elude the host's defenses, and pirate systems and materials for replication (i.e., its virulence). Finlay and McFadden (2006) provide an overview of the diversity and complexity of these processes. Nevertheless HA is very important, and a key factor determining whether a particular form of HA can invade a cell is the detailed structure of the 'cleavage site', the area of the HA molecule at which enzymes of the host cell (proteases) cut it into HA1 and HA2. This is the first step of the process whereby the envelope of the virus fuses with the cell membrane so that the virion's contents can be launched into the cell's interior (see Webster and Husle 2004). Small differences in the RNA sequence coding for HA affect the three-dimensional structure of HA and so affect this process, which is being actively studied (e.g., Noda *et al.* 2006). Horimoto and Kawaoka (2001) provide a summary accessible to non-specialists (such as most readers of Waterbirds).

In contrast with the extensive knowledge of mechanisms, hardly any work in the medical or virological literature on influenza A virus has considered 'ultimate' explanations for virulence—i.e., the factors that give selective advantage to variants with different levels of pathogenicity. Much literature seems to suggest that all that prevents highly pathogenic forms of the virus from emerging is the acquisition of the necessary mutations or reassortments to defeat host defenses. This is certainly the view promulgated in the popular media and even some of the scientific literature. While it is undoubtedly true that the necessary variation must be present before high virulence can evolve, this would on its own not be sufficient for highly virulent forms to spread: the circumstances favoring high virulence must also be present. As there appears to be tremendous variation in the subtypes of avian influenza present in a host population at any time (Hatchette *et al.* 2004), as well as extensive genetic variation within each subtype, it would seem that a viral population could rapidly evolve higher or lower virulence as the ecological conditions selecting for the level of virulence change.

The most important of these conditions is the ease of transmission to new hosts (Ewald 1994; Ewald and De Leo 2002). There is a fundamental trade-off between virulence (making the host sick) and transmission (infecting new hosts). Ewald (1994) outlines how both the mode of transmission and the availability of potential new hosts affect the evolved level of virulence. For example, vector-borne diseases (transmitted by mosquitoes, ticks etc.) or diseases transmitted by fomites (physical objects such as instruments, clothing etc.) generally evolve higher virulence than non-vector borne diseases, because the pathogen travels to new hosts without being reliant on the host to contact potential new hosts. Hence, extreme illness ('prostration') of the host does not necessarily impair and may even aid vector-borne or fomite-borne transmission. Increased virulence is also favoured by easy access to new hosts, especially if they are immunologically naïve, and by the frequent introduction of new, naïve hosts.

These considerations lead to a number of predictions. First, the level of virulence of avian influenza in wild and domestic birds should differ greatly. Wild birds, especially if migratory, must be able to move great distances, and as described above, wild populations have repeated exposure to a great many subtypes of the virus. Consequently, avian influenza viruses should evolve and maintain low virulence in wild birds, as is indeed seen (Webster *et al.* 1992). In contrast, many domestic poultry are housed in high-density, commercially mass-produced situations, and are all similarly immunologically naïve. Moreover, the disease may be borne from farm to farm by fomites or 'cultural' vectors (Ewald 1994) such as garbage trucks, farm workers, or in poultry trade. High pathogenicity is thus selected for (or possibly not selected against) by the conditions prevailing in some commercial poultry production situations, namely dense clusters of immunologically naïve hosts, with the potential for 'cultural' vectors such as vehicles, cages and farm workers transporting the virus between such clusters. Indeed, one could hardly imagine a better-designed environment for the evolution of high virulence in a pathogen than the current worldwide network of industrial poultry farms.

ORIGINS OF OTHER LETHAL HP VIRUSES

An alternative route to high pathogenicity is associated with the transfer of the disease to new species. A pathogen that is well-adapted to an ancestral host species may be maladapted to a new host species, behaving far more lethally than in its ancestral species (Rossiter 2001; Gordon *et al.* 2005). Well-documented examples include the transfer in the 1890s of rinderpest from cattle to African ungulates (Rossiter 2001), the transfer of Parvovirus in 1977 from cats (harmless) to dogs (lethal) (Barker and Parrish 2001), and the transfer of Simian Immunodeficiency Virus (SIV) from primates (low virulence) to humans in the 1950s, where it has evolved into Human Immunodeficiency Virus (HIV) (deadly) (Gordon *et al.* 2005).

Such cross-species transfers with associated changes in virulence have been recorded for avian influenza. Influenza A in harbor seals (*Phoca vitulina*) was first documented in 1979 and killed about 25% of a wintering aggregation in Cape Cod, Massachusetts (Van Campen and Early 2001). The likely source of the virus was shorebirds, since all isolates were genetically similar to avian isolates. Currently, most infections within seal populations are not fatal, suggesting the subsequent evolution of lower virulence in this host. In marine mammals, the ability of influenza A viruses to kill is often attributed to the cumulative effects of additional stressors, such as concurrent bacterial infections (Van Campen and Early 2001).

Influenza viruses were first recorded in domestic pigs during the 1918 influenza pandemic (Webster *et al.* 1992). H1N1, H1N2 and H3N2 subtypes persist in pig populations in North America (Karasin *et al.* 2000) today, but are not associated with mortality at this time. The European pig populations primarily had the variants of the H1N1 subtype until H3N2 was introduced in the 1980s (Webster *et al.* 1992). Transmission back and forth from humans to pigs has occurred on an infrequent basis and the potential for pigs to serve as reservoirs that could generate pandemic human influenza is also recognized (Webster *et al.* 1992; Karasin *et al.* 2000).

PROCESSES SPREADING VIRULENT AVIAN INFLUENZA A

Together, these considerations identify three main possibilities for the local origin and geographic spread of HPAI in poultry: (1) An existing HPAI infection might be spread from one farm to another by fomites/environmental conditions/cultural vectors such as inspectors, veterinarians, workers, vehicles, trade in eggs, birds, or feathers, or even by wind or water if the farms are in close proximity; (2) Wild bird viruses mixing with domestic bird viruses could transfer infections to poultry flocks. The virus could cause asymptomatic or mild disease or it could prove highly lethal. (3) LPAI could evolve into HPAI in domestic poultry in response to one of more of the conditions identified above. The LPAI ancestor virus may be

present in the flock, or may have been deposited there by wild birds. Fauci (2006; see his Figure 2) theorized that HPAI genotypes of the virus are derived from LPAI spread by wild water birds, and become highly pathogenic by 'progressive mutation following passage from one susceptible [chicken] to the next.'. We include his model in this category even though he did not explicitly identify the process as an evolutionary one, because the 'successive passages' of the virus through hosts must exert selection on viruses. Note that these are not mutually exclusive, and more than one or indeed even all three processes could in theory be involved.

What is the evidence for each of these processes? Without question, local and perhaps even long-distance spread by 'cultural' vectors is implicated in transporting HPAI viruses. Examples of cultural vectors are vehicles, implements and workers that spread the virus from farm to farm locally, as in the 2004 LPAI H7N3 outbreak in British Columbia. Longer distance spread of the virus is possible in local and international trade. The virus could be carried on crating, on eggs, on feathers, or by birds. It seems to us that the rapid spread of H5N1 across Eurasia can be easily explained by the cultural vector hypothesis.

It seems less likely that migratory waterbirds are involved in maintaining and spreading HPAI. Horimoto and Kawaoka (2001) state that 'Virulent strains of influenza A have never been collected from apparently healthy waterfowl, with the exception of pathogenic isolates that were collected from ducks and geese near a chicken influenza outbreak'. Recently, a rare occurrence of HPAI in wild birds was documented. In summer 2005, some 1,500 bar-headed geese (*Anser indicus*) and other waterbirds breeding at Qinghai Lake in central China died of an HPAI. The strain proved lethal to experimentally infected chickens and mice. In their report, Liu *et al.* (2005) speculated that the lethal viruses might be emerging from reassortment of genomes in domestic fowl whose LPAI ancestors originated in wild birds overwintering in Southeast Asia. Subsequent work (Chen *et al.* 2005) showed that the virus was most closely related to a form isolated from poultry in southern China. The high mortality of the bar-headed geese supports the hypothesis that ecological conditions in the wild select against highly pathogenic forms of the virus, in accord with the 'virulence-transmission trade-off' hypothesis.

Chen *et al.* (2006) reported the presence of HPAI H5N1 in two apparently healthy migratory ducks from Poyang Lake in Jiangxi, China. Isolates from Poyang Lake were also most closely related to the Qinghai Lake isolates, suggesting that the virus has been carried a distance of ~1700 km by migratory birds. The Poyang lake isolates also retained high pathogenicity in chickens, which may implicate migratory birds in spreading the virus. The isolation of HPAI H5N1 from Mongolia, Siberian Russia, Romania, and Turkey without any clear link to poultry operations have led some to suggest that migratory birds are involved in the spread of the virus. This idea has been vigorously debated in the scientific literature (reviewed by Olsen *et al.* 2006) and even if migratory birds are associated with certain outbreaks, they are unlikely to be major factors spreading the virus Asia and Europe and into Africa, particularly since there are no data on whether infected birds could survive the long flights and shed the virus (Chen *et al.* 2006, Olsen *et al.* 2006). Recently, the discovery of a facility to breed bar-headed geese near Qinghai Lake has further weakened the notion that migratory birds may be important contributors to the spread of the virus (Butler 2006). Chen *et al.* (2006) conclude that "the establishment of regional virus sublineages suggests that H5N1 virus is perpetuated in poultry largely through the movement of poultry and poultry products rather than by continued reintroduction of viruses by migrating birds". Further work is required to test whether the virus in wild birds originated in domestic birds or vice versa and to clarify how that information could apply to the current spread of the disease across Eurasia. In contrast with wild birds, derivation of HPAI genotypes from LPAI predecessors in poultry has been described several times in the literature. Kawaoka *et al.* (1987) and Röhm *et al.* (1996) describe outbreaks of highly pathogenic avi-

an influenza in poultry. The putative LPAI avian ancestors were non-pathogenic to their original wild bird hosts (e.g., tern and swan in Röhm *et al.* 1996), and while circulating in poultry subsequently acquired the extra amino acids at specific cleavage sites that gave rise to a highly pathogenic variant in poultry.

As with wild birds, diverse subtypes of influenza A have been reported from the poultry industry and live bird markets (Panigrahy *et al.* 2002; Webster 2004). Prior to the outbreak of HPAI H5N2 in poultry in several of the United States in 1983 (which caused great economic losses), the virus had been present for a considerable period (as much as 8 years) as a LPAI strain before manifesting as HPAI. In the outbreak of HPAI H7N3 in poultry in British Columbia (February 2004), the virus had been detected a few days earlier in LPAI and had rapidly mutated into the HPAI form. The subsequent 'shift' to HPAI resulted in the depopulation of millions of chickens, turkeys and other domestic poultry to limit the spread of the virus (CFIA 2004; Kermode-Scott 2004). Repeated outbreaks of HPAI H5N1 in Asia during 1997-present have wreaked havoc in the poultry industries of China, Thailand, Cambodia, Laos, Vietnam, Malaysia, Indonesia, Korea and Japan. Phylogenetic work reveals that the virus has been present and evolving for at least ten years, first in the LPAI form, and now in the HPAI form.

The Asian context of poultry farms may be significant in the evolution of HPAI H5N1 (Webster 2004). Live-animal markets or wet markets occur throughout Asia, where a diversity of live domestic and wild geese, chickens, quail, passerine birds, mammals, reptiles and live fish are sold. Poultry are generally kept separated from, but certainly not far from, a wide range of other animals, making these markets ideal places for cross-infection, and the exchange, acquisition and evolution of viral genes (Li *et al.* 2004; Chen *et al.* 2004; Webster 2004; Webster *et al.* 2006). HPAI H5N1 was first detected in Hong Kong in 1997 and was widespread in poultry markets because of co-housing of a diversity of live animals (Webster *et al.* 2006). The precursors of this HPAI H5N1 were detected in geese in live poultry markets in Guangdong, China (1996) where they caused a small number of deaths (Webster *et al.* 2002). This virus however, spread through poultry acquiring gene segments from quail and ducks before becoming a widespread goose virus in the outbreak of 1997 (Webby and Webster 2001). Subsequent to depopulation of all domestic poultry during this outbreak, reassorted subtypes of H5N1 continued to arise from goose and duck reservoirs (Webster *et al.* 2002; Li *et al.* 2004). The virus spread to exotic felids, domestic cats, ferrets and mice (Webster *et al.* 2006). One form, referred to as the Z genotype (Li *et al.* 2004) became dominant and swept through poultry farms in the region resulting in the culling of millions of domestic birds. Experimental evidence shows that ducks and other poultry may harbor HPAI H5N1 and can be asymptomatic (Chen *et al.* 2005; Hulse-Post *et al.* 2005; Li *et al.* 2005), suggesting that they are involved in silently amplifying the virus in poultry populations. Clearly, poultry have played and continue to play a central role in the emergence of HPAI H5N1.

In our view, the weight of evidence supports the model that the widely-dispersed occurrences of HPAI recorded over recent decades in commercial poultry (see Horimoto and Kawaoka 2001) represent multiple independent evolutionary events leading to high virulence within commercial flocks, likely with local spreading by cultural vectors. Phylogenetic comparisons (e.g., Chen *et al.* 2005) will be especially valuable in testing this model. Additionally, serological surveys of healthy domestic terrestrial and aquatic poultry throughout Asia would help reveal the status of HPAI H5N1 in wild populations and the role of domestic ducks in the spread and persistence of H5N1 viruses (Hulse-Post *et al.* 2005).

MANAGEMENT OF HPAI OUTBREAKS: THE HUMAN-DOMESTIC ANIMAL-WILDLIFE INTERFACE

The emergence of globally-significant infectious diseases is contingent upon the exposure and mixture of diseases of domestic and wild origin (Daszak *et al.* 2000), and the

spread of HPAI viruses appears closely associated with human activity (Li *et al.* 2004). Most management needs to therefore take place at human-domestic animal-wildlife interfaces (Daszak *et al.* 2000). Containment of HPAI outbreaks has so far involved culling of infected poultry and disinfection of enclosures (e.g., Chen *et al.* 2004; Webster and Hulse 2005). Here, we propose management intervention in three major areas: i) diseases within poultry; ii) disease overlap between poultry and wild birds; iii) disease within the international wildlife trade.

Diseases Within Poultry

Global trade in poultry is enormous, representing an estimated global consumption of 81.8 million tons in 2006 (FAO 2006). Worldwide, many large industrial operations produce and ship hundreds of thousands of birds per year, and HP avian influenza represents an enormous economic hazard. For owners of small flocks in developing nations, an HPAI epidemic can wipe out a livelihood. A good understanding of the four routes (vectors, spread by wild birds, evolution from LP ancestors, zoonosis) whereby HPAI strains originate and spread is therefore of great importance.

The role played by vectors is addressed by improving biosecurity. The recent isolation of H7N3 from poultry farms in British Columbia resulted in the development of new guidelines and the better enforcement of existing guidelines to help eliminate the virus from poultry farms in Canada (CFIA 2004). (British Columbia poultry growers insist on voluntary compliance with these stricter regulations, even though the elimination of this epidemic took almost four months, required the destruction of millions of chicken, turkeys and other poultry, and was very costly.) The Canadian *Health of Animals Act* and the *Health of Animals Regulations* establish the protocol for isolation, disinfection and destruction of animals at the event of a disease outbreak (CFIA 2004). Other Canadian regulations such as the *Feeds Act Regulations* and *Meat Inspection Act and Regulations* ensure that meat and other materials in poultry are free of disease agents, and govern the proper disposal and disinfection of wastes and offal. Compensation for Destroyed Animals Regulations allows the owners to claim losses incurred as a result of an outbreak. Similar programs and regulations exist in the US and in Europe to help ensure biosecurity in the poultry industry. Along with monitoring and surveillance, these regulations will help to reduce the circulation of viruses within and between poultry farms, and thus limiting the opportunity such mixing of viruses creates for moving from LPAI to HPAI.

Better regulations are also needed for the global trade in poultry, to remove the opportunity for infected (but asymptomatic) poultry to spread the disease (Panigrahy *et al.* 2002). The idea that highly pathogenic strains are spread to poultry by wild birds underlies additional regulations in some European countries requiring poultry to be housed indoors. We have argued that wild birds pose less risk than other methods of virus introduction. In spite of these and other regulations, we feel that occasional outbreaks of influenza in poultry will continue, indicating that there are gaps in disease prevention measures that need to be investigated. No regulations anywhere in the world that we are aware of address the allowable density of poultry, or address any of the other factors discussed above from the point of view of selection for high pathogenicity.

Disease Transfer Between Poultry and Wild Birds

It is widely supposed that wild and migratory birds spread HP strains of avian influenza to poultry, but there is ample opportunity and published evidence for the reverse process. Domestic poultry in Asia are still largely reared in backyards or in outdoor enclosures allowing exposure to wildlife and their viruses (Chen *et al.* 2004; Li *et al.* 2004). Wild birds often feed in close proximity to or in mixed flocks with domestic birds (e.g., co-feeding of domestic ducks and waterfowl in wetland complexes, Chen *et al.* 2005; Liu *et al.* 2005). Contact between wild birds and poultry is limited at most large-scale poultry farms in

North America and Europe, where management at this interface is probably the most important action for preventing and controlling outbreaks of Avian Influenza in both poultry and humans (Tracey *et al.* 2004; Chen *et al.* 2004; Normile 2005).

Limiting such contact is important to shield wild birds from HPAI evolved in domestic poultry and to shield poultry from wild birds, since wild bird LPAI can evolve into HPAI in poultry. Most such transfers are likely to be pathogenic for wild bird populations, and will quickly extinguish themselves, but they may be highly damaging in the process of doing so (e.g., Olsen *et al.* 2006). The 1500 bar-headed geese killed by HPAI at Qinghai Lake in China in 2005 represented approximately 3% of the entire world population of this threatened species (Miyabayashi and Mundkur 1999).

There are several ways for domestic viruses to find their way into wild birds. Small-scale farms may discard their dead animals by feeding them to wild birds (raptors and crows) and since dead birds may harbor (and thereby transmit) viable viruses to a range of wildlife (van Borm *et al.* 2005; Enserink and Kaiser 2005), this activity urgently needs careful evaluation as a potential source of outbreaks. We recommend an immediate cessation of the unprotected disposal from poultry operations of any carcasses, offal and fecal matter that might be exposed to wild birds.

The active feeding of wildlife to entertain aesthetic and conservation needs is widespread and highly controversial (Orams 2002), yet very few empirical studies have evaluated the impacts of such activities on wildlife populations. Whereas positive impacts, such as increase in populations are noted, negative impacts, such as dependence on artificial food sources, habituation to human contact and the spread of diseases are rarely discussed (Smith *et al.* 1999; Orams 2002). The feeding of dead chickens to Bald Eagles at Sheffield Mills in King's County (Nova Scotia) serves as a good example (Flemming 1998). Wintering Bald Eagles congregate here to scavenge on dead chickens provided from local farms. This has served to mend hard feelings between farmers and eagles (traditionally regarded as harmful to livestock) and has become an income-generating venture. Chickens that have died of *unknown* causes (perhaps including avian pathogens) are fed to the eagles. This is cause for concern, because raptors (and other generalist wild birds such as gulls) are evidently able to carry avian influenza viruses but are capable of remaining asymptomatic (e.g., Manvell *et al.* 2000; van Borm *et al.* 2005) during which time transmission to other species may occur. Further evaluation and management intervention is needed in this area. There will never be a real consensus on the benefits of feeding wildlife (Orams 2002) and the crucial management objective will be monitoring and regulating the practices to ensure that the dissemination of diseases is minimized.

Diseases Within the International Wildlife Trade

The pet trade has become a billion dollar industry and both the legal and illegal movement of birds and other wildlife pose a significant threat with regard to exotic disease spread (van Borm 2005). That exotic diseases can spread rapidly once introduced by the pet trade was illustrated vividly by the entry and spread of West Nile Virus into the Americas (Campbell *et al.* 2002), which caused human mortality as well as extensive mortality and morbidity in many wild bird species. Avian influenza A viruses have been isolated from cage birds of different kinds in the international pet trade, and subtypes such as H9N2 and highly pathogenic H5N1 have been recorded (Masaji *et al.* 2001; van Borm *et al.* 2005).

Caged birds and their ability to amplify the virus have not been studied. It is known that infected birds may remain asymptomatic as was documented in two crested hawk eagles, *Spizaetus nipalensis* smuggled for the falconry trade (van Borm *et al.* 2005). Cage bird markets, both legal and illegal, potentially assist the spread of HP avian influenza viruses. The isolation of H5N1 from exotic birds in quarantine or confiscated by customs officials in Europe has resulted in a temporary ban on

the importation of exotic birds into the European Union. The European Union Wild Bird Declaration (2005), signed by 226 non-government organizations, further urged the EU to ban permanently the import of exotic birds into the EU. The US and Canada have both banned the import of birds from Asia.

CONCLUSIONS

Understanding the evolution of LPAI to HPAI viruses, as well as the origin and spread of HPAI has become urgently important. Models for HPAI origin and spread most frequently promulgated in the media and official publications appear incomplete, or flawed. These shortcomings in our knowledge of this serious disease could have disastrous consequences for the protection of human health, the global economy, and for domestic poultry operations, in both developed and developing nations, and—the point of this paper—for populations of wild birds.

Much of the current discussion on the origin of HPAI appears devoid of evolutionary thinking. Often the origin of HPAI genotypes is attributed to the acquisition of 'mutations', while the role of ecological conditions that select for high or low virulence is ignored. Conditions in modern large-scale poultry production seem ideal for the evolution of high virulence, while those faced by free-living migratory birds favor low virulence. Consequently, the global poultry production system with its extensive trade in poultry and poultry products appears the most likely source for the repeated evolution of highly pathogenic strains from LPAI ancestors. HPAI outbreaks seem attributable to this process, and to local and even distant spread of these strains by trade and vectors. It appears unlikely to us that HPAI originates in wild birds, or even that wild birds can spread HPAI very rapidly.

One of our main conclusions is that wild birds need protection from these HP strains. We recommend more research on, and surveillance of, disease evolution and transmission in domestic poultry. Measures aimed at improving on-farm biosecurity are also essential. In particular, the proper disposal and disinfection of wastes and offal seems paramount in preventing spread of viruses within the poultry industry. The global trade, legal and illegal, in exotic birds and poultry needs careful surveillance and better enforcement of existing laws. Finally, the unprotected disposal from poultry operations of any carcasses, offal and fecal matter to which wild birds might be exposed should be halted.

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LITERATURE CITED

Alexander, D. J. 2000. A review of avian influenza in different bird species. Veterinary Microbiology 74: 3-13.

Barker, I. K. and C. R. Parrish. 2001. Parvovirus infections. Pages 131-146 *in* E. S. Williams and I. K.Barker, (Eds.). Infectious Diseases of Wild Mammals. Iowa State University Press/Ames.

Butler, D. 2006. Blogger reveals China's migratory goose farms near site of flu outbreak. Nature 441: 263.

Campbell, G. L., A. A. Marfin, R. S. Lanciotti, and D. J. Gubler. 2002. West Nile virus. The Lancet Infectious Diseases 2: 519-529.

CFIA. 2004. Comprehensive Report on the 2004 outbreak of high Pathogenicity Avian Influenza (H7N3) in the Frazer Valley of British Columbia, Canada. Canadian Food Inspection Agency, Technical Report.

CFIA. 2005. Wild Bird Survey Avian Influenza H5 Subtype Analysis. Canadian Food Inspection Agency. http://www.inspection.gc.ca/english/anima/heasan/disemala/avflu/2005wildsauv/surtype.shtml

Chen, H., G. Deng, Z. Li, G. Tian, Y. Li, P. Jiao, L. Zhang, Z. Liu, R. G. Webster and K. Yu. 2004. The evolution of H5N1 influenza viruses in ducks in southern China. Proceedings of the New York Academy of Sciences 101: 10453-10457.

Chen, H., G. J. D. Smith, S. Y. Zhang, K. Qin, J. Wang, K. S. Li, R. G. Webster, J. S. M. Peiris, and Y. Guan. 2005. H5N1 virus outbreak in migratory waterfowl. Nature 436: 191-192.

Chen, H., G. J. D. Smith, K. S. Li, J. Wang, X. H. Fan, J. M. Rayner, D. Vijaykrishna, J. X. Zhang, L. J. Zhang, C. T. Guo, C. L. Cheung, K. M. Xu, L. Duan, K. Huang, K. Qin, Y. H. C. Leung, W. L. Wu, H. R. Lu, Y. Chen, N. S. Xia, T. S. P. Naipospos, K. Y. Yuen, S. S. Hassan, S. Bahri, T. D. Nguyen, R. G. Webster, J. S. M. Peiris, and Y. Guan. 2006. Establishment of multiple sublineages of H5N1 influenza virus in Asia: Implications for pandemic control. Proceedings of the National Academy of Sciences 103: 2845-2850.

Daszak, P., A. A. Cunningham and A. D. Hyatt. 2000. Emerging infectious diseases of wildlife: threats to biodiversity and human health. Science 287: 443-449.

Earn, D. J. D., J. Dushoff and S. A. Levin. 2002. Ecology and evolution of the flu. Trends in Ecology and Evolution 17: 334-340.

Enserink, M. and J. Kaiser. 2005. Avian flu finds new mammal hosts. Science 305: 1385.

Ewald, P. W. 1994. Evolution of Infectious Disease. Oxford University Press, Oxford.

Ewald, P. W. and G. De Leo. 2002. Alternative transmission modes and the evolution of virulence. Pages 10-25 *in* Adaptive Dynamics of Infectious Diseases: In Pursuit of Virulence Management (U. Dieckmann, J. A. J. Metz, M. W. Sabelis and K. Sigmund,. Eds.). Cambridge University Press, Cambridge.

FAO 2006. Escalating bird flu crisis jeopardizes global poultry trade prospects. Food and Agriculture Organization of the United Nations.
<http://www.fao.org/newsroom/en/news/2006/1000240/index.html>

Fauci, A. S. 2006. Emerging and re-emerging infectious diseases: influenza as a prototype of the host-pathogen balancing act. Cell 124: 665-670.

Ferguson, N. M., C. Fraser, C. A. Donnely, A. C. Ghani and R. M. Anderson. 2005. Public health risk from the avian H5N1 influenza epidemic. Science 304: 968-969.

Flemming, R. 1998. The return of the Bald Eagle. East Coast Birds Magazine. <http://museum.gov.ns.ca/mnh/nature/nsbirds/sitemap.htm>

Finlay, B. B. and G. McFadden. 2006. Anti-immunology: evasion of the host immune system by bacterial and viral pathogens. Cell 124: 767-782.

Fouchier, R. A. M., V. J. Munster, A. Wallensten, T. M. Bestebroer, S. Herfst and D. J. Smith. 2005. Characterization of a novel influenza A virus hemagglutinin subtype (H16) obtained from black-headed gulls. Journal of Virology 79: 2814-22.

Gordon, S., I. Pandrea, R. Dunham, C. Apetrei and G. Silvestri. 2005. The call of the wild: what can be learned from studies of SIV infection of natural hosts? The 2005 HIV Sequence Compendum 2-29. <http://www.hiv.lanl.gov/content/hiv-db/HTML/2005compendium.html>

Greenberg, R. and P. Marra (Eds). 2005. Birds of Two Worlds: The Ecology and Evolution of Temperate-Tropical Migration. Johns Hopkins University Press.

Hanson, B. A., A. D. E. Stallknecht, D. E. Swayne, L. A. Lewis and D. A. Senne. 2002. Avian Influenza Viruses in Minnesota Ducks During 1998-2000. Avian Diseases 47: 867-871.

Hanson, B. A., D. E. Swayne, D. S. Lobpries, J. Hurst and D. E. Stallknecht. 2005. Avian Influenza viruses and paramyxoviruses in wintering and resident ducks in Texas. Journal of Wildlife Diseases 41: 624-628.

Hatchette, T. F., D. Walker, C. Johnson, A. Baker, S. P. Pryor and R. G. Webster. 2004. Influenza A viruses in feral Canadian ducks: extensive reassortment in nature. Journal of General Virology 85: 2327-2337.

Hinshaw, V. S., R. G. Webster and B. Turner. 1980. The perpetuation of orthomyxoviruses and paramyxoviruses in Canadian waterfowl. Canadian Journal of Microbiology 26: 662-629.

Hinshaw, V. S., J. M. Wood, R. G. Webster, R. Deibel and B. Turner. 1985. Circulation of influenza viruses and paramyxoviruses in waterfowl: comparison of different migratory flyways in North America. Bulletin of the World Health Organization 63: 711-719.

Hulse-Post, D. J., K. M. Sturm-Ramirez, J. Humberd, P. Seiler, E. A. Govorkova, S. Krauss, C. Scholtissek, P. Puthavathana, C. Buranathai, T. D. Nguyen, H. T. Long, T. S. P. Naipospos, H. Chen, T. M. Ellis, Y. Guan, J. S. M. Peiris and R. G. Webster. 2005. Role of domestic ducks in the propagation and biological evolution of highly pathogenic H5N1 influenza viruses in Asia. Proceedings of National Academy of Sciences 102: 10682-10687.

Horimoto, T. and Y. Kawaoka. 2001. Pandemic Threat Posed by Avian Influenza A Viruses. Clinical Microbiology Reviews. 14: 129-149.

Ito, T., K. Okazaki, Y. Kawaoka, A. Takada, R. G. Webster and H. Kida. 1995. Perpetuation of influenza A viruses in Alaskan waterfowl reservoirs. Archives of Virology 140: 1163-1172.

Ito, T. and Y. Kawaoka. 1998. Avian Influenza. Pages 3120-3128 *in* Textbook of Influenza (K.G. Nicholson, R. G. Webster and A. J. Hay, Eds.). Blackwell Science, Oxford.

Karasin, A. I., I. H. Brown, S. Carman and C. W. Olsen. 2000. Isolation and characterization of H4N6 avian influenza viruses from pigs with pneumonia in Canada. Journal of Virology 74:9322-9327

Kawaoka, Y, A. Nestorowicz, D. J. Alexander and R. G. Webster. 1987. Molecular analyses of the hemagglutinin genes of H5 influenza viruses: origin of a virulent turkey strain. Virology 158: 218-227.

Kermode-Scott, B. 2004. WHO confirms avian flu infections in Canada. British Medical Journal 328: 913.

Kraus, S., D. Walker, S. P. Pryor, L. Niles, L. Chenghong, V. S. Hinshaw and R. G. Webster. 2004. Influenza A Viruses of Migrating Wild Aquatic Birds in North America. Vector-Borne and Zoonotic Diseases 4: 177-189.

Laver, G. 2004. From the Great Barrier Reef to a "cure" for the flu. Perspectives in Biology and Medicine 47: 590-96.

Li, K. S., Y. Guan, J. Wang, G. J. D. Smith, K. M. Xu, L. Duan, A. P. Rahardjo, P. Puthavathana, C. Buranathai, T. D. Nguyen, A. T. S. Estoepangestie, A. Chaisingh, P. Auewarakul, H. T. Long, N. T. H. Hanh, R. J. Webby, L. L. M. Poon, H. Chen, K. F. Shortridge, K. Y. Yuen and R. G. Webster. 2004. Genesis of a highly pathogenic and potentially pandemic H5N1 influenza virus in eastern Asia. Nature 430: 209-213.

Liu, J., H. Xiao, F. Lei, Q. Zhu, K. Qin, X. -W. Zhang, X. -L. Zhang, D. Zhao, G. Wang, Y. Feng, J. Ma, W. Liu, J. Wang and G. F. Gao. 2005. Highly pathogenic H5N1 influenza virus infection in migratory birds. Science 309: 1206.

Masaji, M., T. Imada, Y. Sanada, M. Etoh, N. Sanada, K. Tsukamoto, Y. Kawaoka and S. Yamaguchi. 2001. Im-

ported parakeets harbor H9N2 Influenza A Viruses that are genetically closely related to those transmitted to humans in Hong Kong. Journal of Virology 75: 3490-3494.

Manvell, R. J., P. McKinney, U. Wernery and K. Frost. 2000. Isolation of a highly pathogenic influenza A virus of subtype H7N3 from a peregrine falcon (*Falco peregrinus*). Avian Pathology 29:635-637.

Miyabayashi, Y. and T. Mundkur. 1999. Atlas of the Key sites for Anatidae in the East Asian Flyway. Wetlands International, Tokyo and Kuala Lumpur.

Munster, V. J., A. Wallensten, C. Baas, G. F. Rimmelzwaan, M. Schutten, B. Olsen, A. D. M. E. Osterhaus and R. A. M. Fouchier. 2005. Mallards and Highly Pathogenic Avian Influenza Ancestral Viruses, Northern Europe. Emerging Infectious Diseases 11:10 available from <http://www.cdc.gov/ncidod/EID/vol11no10/05-0546.htm>

Murphy, B. R. and R. G. Webster. 1996. Orthomyxoviruses. Pages 1397-1446 *in* Virology (B. N. Fields, D. M. Knipe and P. M. Howley, Eds.). Lippincott-Raven Publishers, New York, U.S.A.

Noda, T., H. Sagara, A. Yen, A. Takada, H. Kida, R. H. Cheng and Y. Kawaoka. 2006. Architecture of ribonucleoprotein complexes in influenza A virus particles. Nature 439: 490-492.

Normile, D. 2005. Ducks may magnify threat of avian flu virus. Science 306: 953.

Okazaki, K., A. Takada, T. Ito, M. Imai, H. Takakuwa, M. Hatta, H. Ozaki, T. Tanizaki, T. Nagano, A. Ninomiya, V. A. Demenev, M. M. Tyaptirganov, T. D. Karatayeva, S. S. Yamnikova, D. K. Lvov and H. Kida. 2000. Precursor genes of future pandemic influenza viruses are perpetuated in ducks nesting in Siberia. Archives of Virology 145: 885-893.

Olsen, B., V. J. Munster, A. Wallensten, J. Waldenstrom, A. D. M. E. Osterhaus, R. A. M. Fouchier. 2006. Global patterns of influenza A virus in wild birds. Science 312 385-388.

Orams, M. B. 2002. Feeding wildlife as a tourism attraction: a review of issues and impacts. Tourism Management 23: 281-293.

Otsuki, K., H. Kairya, K. Matsuo, S. Sugiyama, K. Hoshina, T. Yoshikane, A. Matsumoto and M. Tsubokura. 1987. Isolation of influenza A viruses from migratory waterfowls in San-in District of Japan in the winter of 1984-1985. Japanese Journal of Veterinary Science 49: 721-723.

Panigrahy, B., D. A. Senne and J. C. Pedersen. 2002. Avian Influenza Virus Subtypes Inside and Outside the Live Bird Markets, 1993-2000: A Spatial and Temporal Relationship. Avian Diseases 46:298-307.

Ricklefs, R. E., S. M. Fallon, S. C. Latta, B. L. Swanson and E. Bermingham. 2005. Migrants and their parasites. Pages 210-221 *in* Birds of Two Worlds: The Ecology and Evolution of Migration (R. Greenberg and P. P. Marra, Eds.). Johns Hopkins University Press, Baltimore.

Röhm, C., J. Süss, V. Pohle and R. G. Webster. 1996. Different hemagglutinin cleavage site variants of H7N7 in an influenza outbreak in chickens in Leipzig, Germany. Virology 218: 253-257.

Rossiter, P. 2001. Rinderpest. Pages 37-44 *in* Infectious Diseases of Wild Mammals, E. S. Williams and I. K. Barker, Eds.).Iowa State University Press/Ames.

Sherman, P. W. 1988. The levels of analysis. Animal Behavior 36: 616-619.

Slemons, R. D., D. C. Johnson, J. S. Osborn and F. Hayes. 1974. Type A influenza viruses isolated from wild free-flying ducks in California. Avian Diseases 18: 119-125.

Slemons R. D., W. R. Hansen, K. A. Converse and D. A. Senne. 2003. Type A Influenza Virus Surveillance in Fee-Flying, Nonmigratory Ducks Residing on the Eastern Shore of Maryland. Avian Diseases 47: 1107-1110.

Smith, A. E., S. R. Craven and P. D. Curtis. 1999. Managing Canada geese in urban environments. Jack Berryman Institute Publication 16, and Cornell University Cooperative Extension, Ithaca, New York.

Stallknecht, D. E. 1997. Ecology and Epidemiology of Avian Influenza Viruses in Wild Bird Populations: Waterfowl, Shorebirds, Pelicans, Cormorants, etc. Pages 61-69 *in* Proceedings of the 4th International Symposium on Avian Influenza, U.S. Animal Health Association, Athens, GA.

Stallknecht D. E. and S. M. Shane. 1988. Host range of avian influenza virus in free-living birds. Veterinary Research Communications 12: 125-141.

Stallknecht, D. E., S. M. Shane, M. T. Kearney and P. J. Zwank. 1990. Persistence of avian influenza viruses in water. Avian Diseases 34: 406-411.

Statistics Canada. 2004. Production of poultry by provinces. <http://www40.statcan.ca/l01/cst01/prim55a.htm>

Sturm-Ramirez, K. M., T. Ellis, B. Bousefield, L. Bissett, K. Dyrting, J. E. Rehg, L. Poon, Y. Guan, M. Peiris and R. G. Webster. 2004. Reemerging H5N1 influenza viruses in Hong Kong in 2002 are highly pathogenic to ducks. Journal of Virology 78: 4892-4901.

Tracey, J. P., R. Woods, D. Roshier, P. West and G. R. Saunders. 2004. The role of wild birds in the transmission of avian influenza for Australia: an ecological perspective. Emu 104: 109-124.

van Borm, S. V., I. Thomas, G. Hanquet, G., B. Lambrecht, M. Boschmans, G. Dupont, M. Decaestecker, R. Snacken and T. van den Berg. 2005. Highly pathogenic H5N1 influenza virus in smuggled Thai eagles, Belgium. Emerging Infectious Diseases 11: 702-705.

Van Campen, H. and G. Early. 2001. Orthomyxovirus and Paramyxovirus infections. Pages 271-279 *in* Infectious Diseases of Wild Mammals (E. S. Williams and I. K. Barker, Eds.). Iowa State University Press/Ames.

Webby, R. J. and R. G. Webster. 2001. Emergence of influenza A viruses. Philosophical Transactions of the Royal Society of London B. 356: 1817-1828.

Webby, R. J. and R. G. Webster. 2003. Are We Ready for Pandemic Influenza? Science 302: 1519-1522.

Webster, R. G. 2004. Wet markets—a continuing source of severe acute respiratory syndrome and influenza? The Lancet 363: 234-36.

Webster, R. G., M. Yakhno, V. S. Hinshaw, W. J. Bean, W. J. and K. G. Murti. 1978. Intestinal influenza: replication and characterization of influenza viruses in ducks. Virology 84: 268-278.

Webster, R. G., W. J. Bean, O. T. Gorman, T. M. Chambers and Y. Kawaoka. 1992. Evolution and ecology of influenza A viruses. Microbiological Reviews 56: 152-179.

Webster, R. G., M. Peiris, H. Chen and Y. Guan. 2006. H5N1 outbreaks and enzootic influenza. Emerging Infectious Diseases 12: 3-8.

Webster, R. G. and D. Hulse. 2004. Microbial adaptation and change: avian influenza. Revue Scientifique et Technique (Paris) Off. int. Epiz., 2004, 23: 453-465.

Webster, R. G. and D. Hulse. 2005. Controlling avian flu at the source. Nature 435: 415-416.

Webster, R. G., Y. Guan, M. Peiris, D. Walker, S. Krauss, N. N. Zhou, E. A. Govorkova, T. M. Ellis, K. C. Dyrting, T. Sit, D. R. Perez and K. F. Shortridge. 2002. Characterization of H5N1 Influenza Viruses that continue to circulate in geese in Southeastern China. Journal of Virology 76: 118-126

Widjaja, L., S. L. Krauss, R. J. Webby, T. Xie and R. G. Webster. 2004. Matrix gene of influenza a viruses isolated from wild aquatic birds: ecology and emergence of influenza a viruses. Journal of Virology 16:8771-9.

World Health Organization. 2005. Evolution of H5N1 Avian Influenza viruses in Asia. Emerging Infectious Diseases 11: 1515-1521.

Zhou N. N., D. A. Senne, J. S. Landgraf, S. L. Swenson, G. Erickson, K. Rossow, L. Liu, K. Yoon, S. Krauss and R. G. Webster. 1999. Genetic reassortment of avian, swine, and human influenza A virusesin American pigs. Journal of Virology 73, 8851-8856.

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