WATERBIRDS

(e.g., Olsen ¸ . 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 ¸ . (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

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

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 ¸ . 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 ¸ . 2004). Virologists have documented a detailed database of such histories in a variety of domestic species. Extensive reassortment also occurs within host species (Hatchette ¸ . 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 ¸ . 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 ¸ . 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

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 bi-

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 ( ) 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 . (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 . 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 . (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 . 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

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 ¸ . 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 ¸ . 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 ¸ . 2004; Chen ¸ . 2004; Webster 2004; Webster ¸ . 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 ¸ . 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 ¸ . 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 f 12.1352 0 TD 0.7598

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 ¸ . 2004; Chen ¸ . 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 popula-

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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