REVIEW

Victims and vectors: highly pathogenic avian influenza H5N1 and the ecology of wild birds

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ABSTRACT

The emergence of highly pathogenic avian influenza (HPAI) viruses has raised concerns about the role of wild birds in the spread and persistence of the disease. In 2005, an outbreak of the highly pathogenic subtype H5N1 killed more than 6,000 wild waterbirds at Qinghai Lake, China. Outbreaks have continued to periodically occur in wild birds at Qinghai Lake and elsewhere in Central China and Mongolia. This region has few poultry but is a major migration and breeding area for waterbirds in the Central Asian Flyway, although relatively little is known about migratory movements of different species and connectivity of their wetland habitats. The scientific debate has focused on the role of waterbirds in the epidemiology, maintenance and spread of HPAI H5N1: to what extent are they victims affected by the disease, or vectors that have a role in disease transmission? In this review, we summarise the current knowledge of wild bird involvement in the ecology of HPAI H5N1. Specifically, we present details on: (1) origin of HPAI H5N1; (2) waterbirds as LPAI reservoirs and evolution into HPAI; (3) the role of waterbirds in virus spread and persistence; (4) key biogeographic regions of outbreak; and (5) applying an ecological research perspective to studying AIVs in wild waterbirds and their ecosystems.

Keywords: *waterfowl, Anatidae, satellite telemetry, host ecology, Central Asian Flyway*

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1. INTRODUCTION

Emerging and re-emerging infectious diseases are global problems of great concern to human as well as animal health (Daszak *et al.*, 2000; Chomel, 2007). In many cases, human activities have been responsible for providing conditions for the emergence of disease originating from wildlife reservoirs (Lebarbenchon *et al.*, 2008). Human-mediated changes include habitat fragmentation, modification or destruction (Pimm *et al.*, 1995) and globalisation of the marketplace resulting in the frequent movement of humans and products of trade across international boundaries (McMichael *et al.*, 1999). These human activities, coupled with the increasingly global human population, growing demand for food, and intensification of farming practices have been responsible for the emergence of Human Immunodeficiency Virus (HIV-AIDS) in Africa, West Nile Virus and Lyme Disease in North America (Daszak *et al.*, 2000) and Avian Influenza Viruses (hereafter AIVs) in Eurasia. Of these emerging diseases, AIVs have been identified as a significant threat to humans, poultry and wild birds due to their global distribution and pandemic potential (de Jong *et al.*, 1997; Webster *et al.*, 2006; Peiris *et al.*, 2007).

AIVs are classified as type A influenza viruses and express one of 16 different hemagglutinin (HA) proteins (H1-H16) and one of nine different neuraminidase (NA) proteins (N1-N9) on their surface (Webster *et al.*, 1992). All of the 16 HA and nine NA antigenic subtypes have been documented in aquatic wild birds (Webster *et al.*, 1992). Waterbird families, particularly the Anatidae (ducks, swans and geese) and Charadriidae (shorebirds) and to a lesser extent the Laridae (gulls) and Sternidae (terns) have been identified as the major reservoirs for AIVs in nature (Stallknecht and Shane, 1988). Within their natural host, AIVs are thought to have evolved into the perfect host-parasite relationship (Figure 1). Infections are typically asymptomatic and rarely prove fatal but are associated with intense viral shedding in faeces to maximise opportunities for transmission to susceptible individuals (Webster *et al.*, 1978, 1992).

Avian influenza viruses are broadly classified into low pathogenic (LPAI) and highly pathogenic (HPAI) based on their ability to cause mortality in chickens (Alexander, 2000). Most naturally occurring forms, for which wild birds are the reservoir, are LPAI with the sporadic emergence of HPAI documented in artificial systems where wild birds and poultry are placed in close association (Alexander, 2000; Webster and Govorkova, 2006). HPAI viruses have caused a substantial number of deaths in domestic and wild birds, several mammalian species, as well as in humans (Webster *et al.*, 2006; FAO, 2009). Over the past decade, HPAI H5N1 in particular, has emerged as a significant zoonotic pathogen averaging a 60% case-fatality rate in affected humans and the potential to cause a global pandemic should human-to-human transmission capability evolve in this subtype (Li *et al.*, 2004; Peiris *et al.*, 2007).

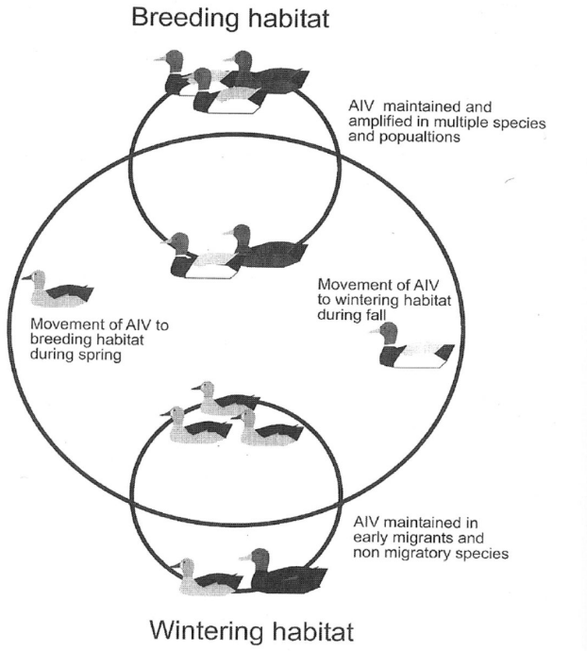

Figure 1 Migration leads to cycling of AIV between breeding and wintering habitat (Swayne, 2008).

The persistence and spread of HPAI H5N1 is primarily linked with the movement of poultry or poultry products (Gauthier-Clerc *et al.*, 2007). However, in 2005 an outbreak of HPAI H5N1 occurred among migratory waterbirds congregating at Qinghai Lake, in west-central China (Chen *et al.*, 2005; Liu *et al.*, 2005). The subsequent rapid emergence of HPAI H5N1 in Russia, Western Europe, the Middle East and northern Africa has implicated migratory waterbirds as vectors (Normile, 2005; Normile, 2006; Olsen *et al.*, 2006; FAO, 2007). While some evidence suggests migratory waterbirds are involved in the long-distance movement of HPAI H5N1, the extent or mechanism of their involvement is not well understood (Stallknecht and Brown, 2008). At present, their role in HPAI H5N1 transmission remains controversial, due to questions about the distance infected birds may migrate (van Gils *et al.*, 2007; Weber and Stilianakis, 2007; Gaidet *et al.*, in press) and despite sampling of more than 500,000 healthy individuals, no wild bird reservoir has been identified (FAO, 2009). Consequently, the role of migratory waterbirds as hosts for HPAI H5N1 needs to be better understood

to ensure control strategies target and curb the critical stages of the virus' transmission cycle.

In this review, we summarise current knowledge and identify gaps in understanding of the role of migratory waterbirds in the ecology and epidemiology of HPAI H5N1. Specifically, we present details on: (1) the origin of HPAI H5N1; (2) waterbirds as reservoirs for LPAI and transition of LPAI to HPAI; (3) the spread and persistence of HPAI H5N1; (4) key biogeographic regions involving outbreaks including Poyang Lake and Qinghai Lake in China; and (5) applying an ecological research perspective for studying AIVs in waterbirds and their ecosystems.

2. EMERGENCE OF HPAI H5N1

The precursors to HPAI H5N1 existed in the form of several LPAI subtypes. LPAI H5N1 was first reported in domestic geese in Guangdong, China in 1996 (Xu *et al.*, 1999). LPAI subtypes, including LPAI H5N1, were subsequently introduced into Hong Kong through poultry in 'wet' or live poultry markets (Webby and Webster, 2001). Wet market conditions enabled reassortment of LPAI H5N1 with viruses in domestic quail (subtype H9N2), a goose-like virus (H5N1), and virus from domestic ducks (H6N1) that led to the emergence of HPAI H5N1 in 1997 (Webby and Webster, 2001; Chen *et al.*, 2004; Webster *et al.*, 2006). This virus rapidly became widespread in wet markets and infected humans in Hong Kong, and by December 2007 had resulted in 18 cases of which six were fatal (Sims and Brown, 2008). This HPAI H5N1 genotype was eradicated by extensive culling of poultry in Hong Kong and has not been subsequently detected (Webster and Govorkova, 2006).

New HPAI H5N1 genotypes have emerged several times in different countries that maintained active poultry trade with southern China since 2000 (Chen *et al.*, 2004; Webster *et al.*, 2006; Duan *et al.*, 2007). Southern China has been regarded as labelled the 'epicentre' of avian influenza (Shortridge and Stuart-Harris, 1982) and continues to play a role as the source of HPAI H5N1 (Webby and Webster, 2001; Smith *et al.*, 2006a). HPAI H5N1 genotype 'Z' initiated a new wave of transmission across Southeast Asia (Li *et al.*, 2004; Smith *et al.*, 2006a). Wild ducks from southern China are regarded as crucial for the persistence of genotype Z that is now endemic in poultry populations (Chen *et al.*, 2004). Variants of the Z genotype are found in Indonesia, Cambodia, Laos, Vietnam, China, Thailand and Malaysia, and genotype 'V' has been found in Japan and Korea (Li *et al.*, 2004).

Recently, variants of the Z genotype have demonstrated increasing pathogenicity and an expanding host range beyond poultry and wild birds (Figure 2). This genotype shows a unique ability to infect mammals without reassortment in an intermediate host such as swine (de Jong and Hien, 2006), as evidenced by cases in humans, pigs and carnivorous species (Cardona *et al.*, 2008). Despite its success in colonising a broad range of hosts, HPAI H5N1 is currently unable to replicate effectively within the respiratory tract of mammals, precluding transmission between humans and at present, preventing a global pandemic (Smith *et al.*, 2006b).

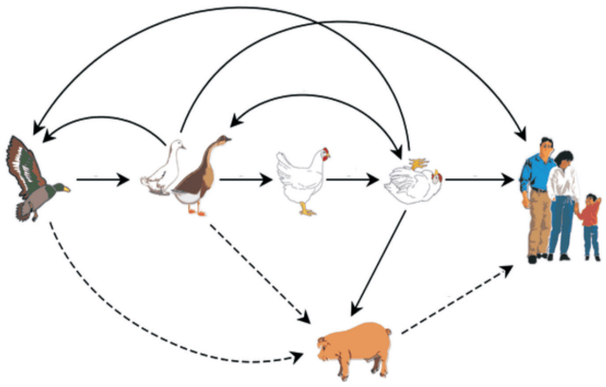

Figure 2 Emergence of H5N1 influenza virus (after Webster *et al.*, 2006). A low pathogenic H5 influenza virus is believed to have spread from waterfowl to domestic ducks and geese, then to domestic chickens. In chickens, the H5 virus became highly pathogenic before it was transferred back to domestic ducks and geese. The resulting lethal virus spread to domestic poultry farms, humans, and occasionally to pigs. (Solid lines, transmission demonstrated; dotted lines, transmission postulated but not demonstrated).

3. WILD BIRDS AS RESERVOIRS FOR LPAI

Over 105 species of wild birds in 26 different families have yielded infections of LPAI viruses, with most host species belonging to either the Anatidae (ducks, swans and geese) or Charadriidae (shorebirds) (Olsen *et al.*, 2006; Stallknecht and Brown, 2008). Evidence suggests that these two families act as optimal hosts for LPAIs in wild systems owing to their preference for freshwater habitat and ability to perform long-distance migration (Hinshaw *et al.*, 1985; Stallknecht and Shane, 1988).

The aquatic habitats of Anatidae and Charadriidae species may favour efficient faecal–oral transmission of LPAI, the main route of infection for the pathogen (Webster *et al.*, 1992). AIVs replicate in the epithelial cells of the host's intestinal tract and are shed from the cloaca in high titre concentrations (Webster *et al.*, 1978). Moreover, species which adopt surface feeding strategies, such as dabbling ducks, may be at higher risk of infection compared with species that forage at

deeper depths, such as diving ducks. Field studies demonstrate that AIV infection occurs with highest prevalence among waterfowl species such as the Mallard (*Anas platyrhynchos*), Common teal (*Anas crecca*), Northern pintail (*Anas acuta*) (Hinshaw *et al.*, 1980b; Kocan *et al.*, 1980; Hinshaw *et al.*, 1985; Rundstadler *et al.*, 2007) and the Northern shoveler (*Anas clypeata*) (Hill *et al.*, 2010). Non-foraging attributes may also favour transmission including flocking or sexual behaviour (Garamszegi and Moller, 2007) as well as guild and microhabitat preferences.

Anatidae and Charadriidae act as ideal hosts for AIVs, because long-distance migration is ubiquitous in these taxa. Anatidae have received a greater research focus because surveillance studies show that they have a higher prevalence than Charadriidae (Iverson *et al.*, 2008). The annual movement of waterfowl between breeding sites at high latitudes and wintering sites at low latitudes facilitates the spread of AIVs over large geographic areas (Hinshaw *et al.*, 1985; Garamszegi and Moller, 2007), and migratory birds have even been referred to as ''shuttles'' for LPAI (Wang *et al.*, 2008). Migration also involves aggregations at stopover sites which increases intra- and inter-specific contact, promoting transmission. Waterfowl congregating at high densities at breeding and stopover sites feed intensively prior to migration thereby contributing to faecal–oral transmission.

4. EVOLUTION FROM LPAI TO HPAI

Chickens and turkeys are regarded as the recipient host in which evolution from LPAI to HPAI occurs. It has been proposed that high pathogenicity arises due to insertion of amino acids at the HA0 cleavage site, a process that occurs more readily in chicken and turkey hosts (Alexander, 2003). This is supported by the large number of outbreaks that have involved gallinaceous poultry, with more than 6,500 cases reported worldwide between 2003 and 2009 (FAO, 2009). HPAI induces mortality in poultry due to replication of the virus within the host cells, which damages the vital organs and tissues (Rott, 1992). Genetic analysis has demonstrated that subtypes H5 and H7 are more prone to changing into highly pathogenic forms (Shortridge *et al.*, 1998; Spackman *et al.*, 2003), but other subtypes such as H9 can also be involved.

Within poultry, the evolution from LPAI to HPAI takes place via one of two possible genetic processes. Mutation (or genetic drift) results from insertion, deletion or substitution of single nucleotides during transcription of the genome (Stech *et al.*, 1999) owing to poor site-fidelity of the RNA polymerase (Holland *et al.*, 1982). This produces a number of different viral subtypes each with varying fitness levels for the host environment. Those with high fitness replicate and transmit efficiently while those that are mala-dapted are quickly eliminated from the viral gene pool (Suarez, 2000). Alternatively, reassortment (or genetic shift) occurs when a single host is infected with two viral subtypes, facilitating exchange of genetic segments between the two viruses. This process can change the antigenic nature of the virus, enabling it to 'sidestep' the immune system of the host and spread through populations (Webster *et al.*, 1992; Webby and Webster, 2001).

Genetic reassortment has been proposed as the primary mode by which LPAI evolves into HPAI subtypes within poultry hosts (Hatchette *et al.*, 2004; Macken *et al.*, 2006; Dugan *et al.*, 2008). The segmented-nature of the AIV genome, composed of eight single-stranded RNA fragments, is likely to favour the exchange of entire segments between viruses co-infecting the same poultry host. Reassortment is ubiquitous among waterfowl (Hinshaw *et al.*, 1980a) and a recent study on the viral gene pool in wild Anseriformes indicated that recombinants accounted for 26% of LPAI isolates (Dugan *et al.*, 2008). The importance of re-assortment in the evolution of HPAI can be seen by examining the past three human pandemics that took place in 1918, 1957 and 1968 – all of which were caused by recombinants of human and avian influenzas (Webster *et al.*, 1992).

5. SIGNIFICANCE OF WILD BIRD–POULTRY INTERACTIONS

A key factor integral to the evolution of LPAI into HPAI is the interaction between wild birds and poultry (Figure 3). Wet markets have been identified as a primary source of HPAI H5N1 in China (Liu *et al.*, 2003; Wang *et al.*, 2006; Yu *et al.*, 2007) and Thailand (Amonsin *et al.*, 2008) and were the original site of the 1997 outbreak in Hong Kong (Shortridge *et al.*, 1998). Wet markets typically house multiple bird species drawn from populations in different geographic regions together at high densities, which coupled with the daily introduction of new birds is conducive to the exchange of AIV subtypes between hosts (Webster, 2004; Cardona *et al.*, 2009). Low hygiene standards and poor biosecurity in wet markets may promote the faecal–oral transmission of AIVs, or respiratory transmission in the case of HPAI H5N1. Over-crowded and inadequate housing of birds can compromise the immune system, making

Figure 3 The wild bird–poultry interface (**a**) domestic and wild ducks housed together ready for slaughter at a wet market; (**b**) transport of ducks for sale at a wet market; (**c**) farming of domestic ducks on a natural wetland; and (**d**) ducks foraging in rice paddies. (Photos courtesy of United Nations System Influenza Coordination, www.flickr.com/photos/unsic/).

domestic and wild birds susceptible to AIV exposure, resulting in infection.

Domestic ducks and geese are raised in backyard or free-ranging farms throughout Asia which promotes contact with wild birds, allowing evolution of HPAI subtypes (Songserm *et al.*, 2006). Backyard poultry includes several species of chickens, ducks, guinea fowl, and geese that are fed on grain but also actively forage around houses and within rice fields, wetlands and other water bodies (Figure 3). Backyard poultry practices are widespread in Asia with an estimated 60–80% of households keeping some form of poultry (FAO, 2001) and the lack of biosecurity allows wild and domestic birds to come into contact. In particular, free-ranging domestic ducks have proven to be a significant risk factor in the outbreak of HPAI H5N1, because they may carry the virus asymptomatically (Hulse-Post *et al.*, 2005). Within Thailand, hot spots of HPAI H5N1 correspond closely with the density of free-ranging ducks (Gilbert *et al.*, 2006a; Songserm *et al.*, 2006; Gilbert *et al.*, 2008). Also, domestication of ducks and geese that originate from wild populations (*see* 9.1 Poyang Lake) may contribute to the appearance of HPAI subtypes.

Rice fields provide important grazing opportunities for wild waterbirds inhabiting adjacent wetlands, potentially introducing LPAIs into domestic poultry (Muzaffar *et al.*, in press). In Southeast Asia, poultry production systems are often integrated with rice agriculture–flocks are brought to post-harvest fields to feed on waste grains, and faeces from birds are used as fertiliser (Devendra and Thomas, 2002). With loss of natural wetlands, many wild waterfowl species feed in and around rice fields, thereby increasing the chances of intermixing between domestic and wild birds (Gilbert *et al.*, 2007) and contact between wild bird species. This intermixing becomes exacerbated during the dry season when the inundated paddies provide important artificial wetlands (Gilbert *et al.*, 2007) that concentrate waterbirds.

Figure 4 Species of concern in areas where HPAI H5N1 has been reported: (**a**) and (**b**) black-necked cranes congregate in the Lhasa Valley, the overwintering site for 75% of the global population (Qinghai-Tibetan Plateau: People's Daily Online - http://english.people.com.cn/); and (**c**) and (**d**) bar-headed geese congregating on Qinghai Lake, China. (Photos courtesy of J. Takekawa, USGS Western Ecological Research Center).

Finally, 'bridge species' or animals that may carry the virus between waterbirds and poultry in the case of HPAI H5N1, may have a role in the emergence of HPAI (Kou *et al.*, 2005; FAO, 2007). These wild birds live in close proximity to human settlements and may act as a link between wetlands occupied by waterbirds and poultry at farms, backyard settings and markets (FAO, 2007). Some wild bird taxa such as corvids, sparrows, and pigeons tolerate human-modified environments and show resistance against HPAI viruses (Perkins and Swayne, 2003). Challenge studies with HPAI H5N1 revealed that pigeons show few clinical symptoms of the virus upon infection (Boon *et al.*, 2007; Yu *et al.*, 2007), and thus, may act as 'silent carriers' within human-modified environments. In rural landscapes, wild birds such as egrets are ubiquitous along canals and agricultural ponds and may also serve as a bridge between domestic and wild birds.

6. WATERBIRDS AS VECTORS

Poultry production practices, migratory bird movements, legal and illegal wildlife and domestic bird trade, and human travel have all been suggested as possible mechanisms of AIV spread, however, most scientific debate has focused on poultry and migratory birds as the likely vectors. The circulation of HPAI H5N1 was limited to poultry in Southeast Asia for nearly a decade before the rapid westward advance of the virus in late 2005. This event was immediately preceded by the outbreak of HPAI H5N1 at Qinghai Lake during April 2005 (Chen *et al.*, 2005; Liu *et al.*, 2005; Lei *et al.*, 2007). The outbreak killed more than 6,000 waterbirds and remains the largest mass die-off in wild birds due to HPAI H5N1 infection. Qinghai Lake is renowned for migrating and breeding waterbirds, and poultry are rarely found in this arid steppe region (*see* 9.2 Qinghai Lake), suggesting that migratory birds may have introduced the virus (Chen *et al.*, 2005; Lei *et al.*, 2007; Prosser *et al.*, submitted). Two additional large scale wild bird mortality events have been reported in Russia. The first in July 2005 at Lake Chany, Russia where more than 5,000 wild birds perished including pochard, mallard, and common teal. The second event occurred in the summer of 2006 at Tuva Republic, Russia where

more than 3100 Great crested grebes (*Podiceps cristatus*) died (FAO, 2009). These large outbreaks, combined with the rapid, long-distance movement of HPAI H5N1 through Eurasia has prompted concern about the role of migratory birds as vectors (Olsen *et al.*, 2006). In addition, wild bird outbreaks of smaller scale have continued to occur along the Central Asian Flyway (Table 1).

The spread of HPAI H5N1 has also been correlated with the transport of poultry. Poultry originating from China is traded along the trans-Siberian railway, with Novosibirsk in Russia acting as a major hub for transportation in this region (Gauthier-Clerc *et al.*, 2007). Novosibirsk has been proposed as the focal point for the virus's westward radiation from Russia to Croatia during late 2005 (Wallace *et al.*, 2007), and transportation routes originating in the city closely correspond with the trajectory of the virus (Gauthier-Clerc *et al.*, 2007). Phylogenetic analysis of AIV supports movement of poultry as the cause for introduction of HPAI H5N1 into the African continent with its first appearance in Nigeria during February 2006 (Kilpatrick *et al.*, 2006; Fasina *et al.*, 2009). Inadequate biosecurity, high density of urban poultry farms and the growth in live poultry markets have been cited as reasons for rapid spread of the virus into west African countries including Niger, Burkino Faso, Cameroon, Cote D'Ivoire, Ghana and Togo (Sims and Brown, 2008; Fasina *et al.*, 2009).

Alternatively, there are epidemiological features of the spread of HPAI H5N1 that argue against human trade of poultry as the sole disseminator of the virus. Initial outbreaks in Turkey, Romania and Ukraine during October 2005 were situated close to wetlands that act as important wintering grounds for migratory ducks (Gilbert *et al.*, 2006b). Gilbert *et al.* (2006b) demonstrated a significant correlation between the migratory pathway of ducks originating from Siberia and spread of the virus from Russia to the Black Sea. The genetic relatedness of the HPAI H5N1 subtypes occurring in this region and Qinghai Lake also supports introduction of the virus by migratory birds (Lvov *et al.*, 2006). In addition, between January and May 2006, member states of the European Union reported the incidence of HPAI H5N1 in 741 dead wild birds from over 60 species (Pittman *et al.*, 2007). The majority of birds were waterfowl including mute and whooper swans and to a lesser extent ducks (Table 1). The outbreak may have been prompted by prolonged freezing weather conditions around the Black Sea which forced resident and migratory birds to seek refuge in western Europe (Pittman *et al.*, 2007). Infection of poultry was restricted to isolated cases in Sweden and Germany (Alexander, 2007) and occurred after the outbreak in wild birds, reinforcing that trade of poultry was not involved (Kilpatrick *et al.*, 2006).

6.1 Can infected birds migrate?

The lack of recorded cases of HPAI viruses persisting for prolonged periods in wild birds is the main argument against the involvement of migratory birds in the spread of HPAI H5N1 over large distances (Feare, 2007; Weber and Stilianakis, 2007). In wild birds, the virus has mostly been identified from sick or dead birds implying that they are physiologically unable to fly long distance and disseminate HPAI H5N1. Field studies have also shown that waterfowl infected with LPAI have diminished body condition (van Gils *et al.*, 2007) and hampered foraging and migratory performance (Latorre-Margalef *et al.*, 2008).

However, experimental studies (Table 2) clearly demonstrate that responses to HPAI H5N1 vary widely across species of migratory waterfowl (Brown *et al.*, 2006; Brown *et al.*, 2008; Keawcharoen *et al.*, 2008). Challenge of six swan and goose species produced a range of clinical responses to HPAI H5N1, with the Black swan (*Cygnus atratus*) dying 2–3 days after infection, compared to the Bar-headed goose (*Anser indicus*) that either died up to 8 days post-infection or survived (Brown *et al.*, 2008). During this asymptomatic period, bar-headed geese can potentially fly over 700 km, therefore acting as effective disseminators of the virus over long distances (Gaidet *et al.*, in press). Ducks such as the mallard have earned the moniker – the ''Trojan Horse'' of HPAI H5N1 (Hulse-Post *et al.*, 2005) due to their unique ability to remain asymptomatic while shedding abundant quantities of the virus in laboratory studies (Keawcharoen *et al.*, 2008).

These experimental findings highlight that species and individuals may contribute differently to the spread of HPAI H5N1. However, birds held in a laboratory benefit from regular food supply and stable environmental conditions and thus do not experience the same survival pressures as their wild counterparts. Whether responses to HPAI H5N1 infection in lab-reared birds accurately reflect responses in wild birds is a key question (Feare, 2007). Cases of healthy HPAI H5N1-infected mallards from the field (Chen *et al.*, 2006a; Lvov *et al.*, 2006) show that wild birds can indeed survive infection. Other HPAI-infected species in healthy condition have also been reported, albeit in low numbers ($n = 18$) compared to the hundreds of thousands of wild birds sampled as part of international surveillance efforts (Table 3).

Table 1 Key events of HPAI H5N1 in wild birds in natural habitat (adapted from ProMED-mail: <http://www.promedmail.org/pls/otn/f?p=2400:1200:151681116305603>; Rappole and Hubalek, 2006; and Office International des Epizooties (OIE): <http://www.oie.int/downld/AVIAN%20INFLUENZA/ A2004_AI.php>). Waterfowl species are indicated by italics

Date	Country	Species (number)	Event description
17 Dec 2002	Hong Kong	Grey heron (1), Tree sparrow (1)	Lok Ma Chau: 1st dead bird at wetland mitigation pond
06 Jan 2003	Hong Kong	Black-headed gull (1)	Lantau Island: dead bird
11 Jan 2004	South Korea	Korean magpie (3)	Yangju, Kyunggi Province: 1st dead birds
19 Jan 2004	Hong Kong	Peregrine falcon (1)	Gold Coast: dead bird
14 Dec 2004	Thailand	Asian open-bill stork (2), Little cormorant (1), Red-collared dove (1), Scaly-breasted munia (1), Black drongo (1)	Nakhon Sawan, Saraburi and Lop Buri provinces: first dead birds (all native species)
30 Apr 2005–29 Jun 2005	China	*Bar-headed goose* (3,282), Great cormorant (1,302), Great black-headed gull (929), Brown-headed gull (570), *Ruddy shelduck* (145)	Qinghai Lake: 6184 birds in first major epizootic
23 Jul 2005–31 Aug 2005	Russia	Great crested grebe, *Gadwall*, *Mallard*, *Ruddy shelduck*, *Mute swan*, Coot, *Common pochard*	Chany Lake, Novosibirsk: 1st dead birds near poultry outbreaks
10 Aug 2005	Mongolia	*Bar-headed goose* (89), *Whooper swan*, *Mute swan*, *Eurasian wigeon*, *Ruddy shelduck*	Erhel Lake, Hovsgol Province: 1st dead birds
04 Oct 2005–20 Oct 2005	Romania	*Lesser white-fronted goose*, Great blue heron, Grey heron, *Mute swan*, Common moorhen (137)	Maliuc village, Tulcea: 1st dead wild birds
19 Oct 2005–30 Apr 2005	Croatia	*Mute swan* (15)	Zdenci municipality, freshwater fish farm: 1st dead birds
11 Nov 2005	Kuwait	Greater flamingo (1)	Coaal beach: 1st dead wild bird
07 Dec 2005–12 Dec 2005	Russia	*Mute swan*	Kalmykia Republic and Arakhan Region: dead wild birds
10 Jan 2006–31 Mar 2006	Hong Kong	Oriental magpie robin (2), Little egret, Large-billed crow, Crested myna, White-rumped munia, Japanese white eye	New Territories: dead wild birds in rural area
08 Feb 2006	Bulgaria	*Mute swan* (1)	Danube Delta: 1st dead wild bird
09 Feb 2006	Greece	*Mute swan* (3), *Red-breasted goose* (1)	Thermaikos Gulf and Skyros Island: 1st dead swans and goose
11 Feb 2006	Italy	*Mute swan* (1)	Puglia, Sicily, and Calabria: 1st dead wild bird
11 Feb 2006–03 Mar 2006	Slovenia	*Northern pintail* (1), *Mute swan* (1)	1 dead wild birds, 48 total
13 Feb 2006	Iran	*Whooper swan* (1)	1 dead wild bird
17 Feb 2006–31 Mar 2006	Germany	*Coot* (1), Grey heron (1), *Whooper swan*, *Mute swan*, *Mallard*, *Canada goose*, *Tufted duck*, Northern goshawk, White stork, *Greylag goose*, Common kestrel, Common pochard, Common buzzard, Peregrine falcon	Ruegen Island, Baltic Sea followed by mainland administrative divisions of Brandenburg, Baden Württemberg, Bavaria, Mecklenburg-Weern Pomerania and Schleswig-Holein: 1 dead wild birds, 125 total
17 Feb 2006–13 Mar 2006	France	*Mute swan* (4), *common pochard* (1), great crested grebe	Marlieux, Birieux, Villars les Dombes, Versailleux and Lapeyrouse, Chanoz Chatenay and Divonne les Bains: 1st dead wild birds
13 Feb 2006–24 Feb 2006	Hong Kong	Magpies (2), Large-billed crow (1), House crow (1), White-backed munia (1), Munia spp. (1)	Kowloon, Hong Kong islands, Mongkok: dead birds, 14 total
18 Feb 2006–30 Apr 2006	Austria	*Mute swan* (1)	Graz-Umgebung Di. reservoir: 1st dead wild bird
20 Feb 2006	Bosnia-Herzegovina	*Mute swan* (15)	Srednjobosanski Canton, Plivsko Lake: 1st dead wild birds
20 Feb 2006	Slovakia	*Smew* (1), Peregrine falcon (1)	Near Aurian-Hungarian border: 1st dead wild birds
21 Feb 2006	Hungary	*Mute swan* (3), Black-headed gull, *Mallard*	Southern region: 1st dead wild birds
23 Feb 2006	Georgia	*Mute swan* (10)	Adjara village, small lake: 1st dead wild birds

Table 1 Key events of HPAI H5N1 in wild birds in natural habitat (adapted from ProMED-mail: <http://www.promedmail.org/pls/otn/f?p=2400:1200:151681116305603>; Rappole and Hubalek, 2006; and Office International des Epizooties (OIE): <http://www.oie.int/downld/AVIAN%20INFLUENZA/ A2004_AI.php>). Waterfowl species are indicated by italics (*continued*)

Date	Country	Species (number)	Event description
24 Feb 2006–15 Mar 2006	Sweden	*Tufted duck* (25), *Greater scaup* (3), *Mute swan* (2), Eagle owl (2), *Common merganser* (2), Herring gull (1), *Mallard* (1), *Smew* (1), Common buzzard (1)	Gotland, Kalmar, Blekinge and Södermanland counties: 1st dead wild birds
26 Feb 2006–03 Apr 2006	Switzerland	*Common pochard* (1), Coot (1), *Goosander* (1), *Tufted duck* (1), *Mute swan*, *Mallard*	Jet d'eau Pier, Geneva and eckborn: 1st dead wild birds
01 Mar 2006–27 Mar 2006	Poland	*Mute swan* (2), *Goosander*, hawk	Kotrzyn and Torun near Viula River: 1st dead wild birds, 10 birds total
12 Mar 2006–31 May 2006	Denmark	*Tufted duck* (26), *Mute swan* (4), *Whooper swan* (3), Peregrine falcon (1), *Greylag goose* (1), Great crested grebe (1), Rough-legged buzzard (1), Eurasian magpie (1), Common buzzard (5)	Bornholm, Fredriksborg, Funen, Southern Jutland, Weern Zealand and orroem: 1st dead wild birds
20 Mar 2006–31 May 2006	Czech Republic	*Mute swan* (1)	Ceske Budejovice near Vtala River: 1st dead wild bird
30 Apr 2006	United Kingdom	*Whooper swan* (1)	Fife, Scotland: 1st dead wild bird
19 Apr 2006	China	*Bar-headed goose* (399), *Ruddy shelduck*	Nagqu Prefecture, Tibetan Autonomous Region; Golog Tibetan Autonomous Prefecture, Qinghai: dead birds
15 Jun 2006–31 Jul 2006	Russia	*Geese* (1622)	Ubsu-Nur Lake, Ovyursky Dirict, Siberia (bordering Mongolia)
07 Jul 2006	Spain	Scaly-breasted munia (6), Great crested grebe (1)	Alava Province: 1st dead wild bird
04 Jan 2007	Japan	Hodgson's hawk eagle (1)	Kyushu, Sagara village: 1st dead wild bird
15 Jan 2007	Hong Kong	Crested goshawk (1)	Shek Kip Mei Health Centre: dead bird
17 Feb 2007	Hong Kong	Common kestrel (1)	Pak Tin Eate in Sham Shui Po: dead bird
26 Jun 2007–31 Jul 2007	Germany	*Mute swan* (5), *Canada goose* (1), Black-necked grebe (1)	Nuremburg and Thuringia near Saxony: dead birds found in urban pond, 38 total
29 Jun 2007	Czech Republic	*Mute swan* (1)	Lednice, south Moravia: dead wild bird
03 Jul 2007	France	*Mute swan* (3)	Assenoncourt, Moselle: dead young birds
12 Dec 2007	Poland	Crane (2), White stork (1), *Mute swan* (1)	Krzykaly village, northern province: dead wild birds, bird recovery center
06 Feb 2008	United Kingdom	*Mute swan* (3)	Abbotsbury, Dorset coast near Chesil Beach: dead swans at wild bird reserve
28 Jan 2008	Hong Kong	Black-crowned night heron (1)	Ocean Park (theme park), Southern Hong Kong: dead wild bird
31 Mar 2008	Switzerland	*Common pochard* (1)	Healthy infected wild bird, Lake Sempach, Lucerne
21 Apr 2008	Japan	*Swan* (6)	Lake Towada, Akita Prefecture and Lake Saroma, E. Hokkaido: dead and ill wild swans
07 Feb 2009	Hong Kong	Peregrine falcon (2), Cattle egret (1), Grey heron (1)	Villages of Sha Lo Wan, Sham Wat Wan, San Shek Wan, Yau Kam Tau, Tai O, Lantau Island, Butterfly Beach and Mai Po Reserve: wild and domestic dead birds
08 May 2009	China	Great crested grebe (121)	Genggahu Lake, Hainan Prefecture, Qinghai Province
22 May 2009	Mongolia	*Whooper swan* (9)	Doitiin Tsagaan Lake, Ugii-nuur soum District
11 Jun 2009	Russia	'wild birds' (58)	Ubsu-Nur Lake, Ovursky District, Siberia
31 Jul 2009	Mongolia	*Bar-headed goose*, *Ruddy shelduck*, *Common goldeneye*, *Whooper swan*, Mongolian gull (171)	Doroo Tsagaan Lake, Tsetserleg soum District

Table 2 Pathologic response of birds exposed to HPAI H5N1 by intranasal inoculation

Taxonomic group	Species	HPAI H5N1 genotype	Morbidity (% sick)	Mortality (% dead)	Shedding duration (days)	Viral titre shed[a]	Reference
Ducks	Blue winged teal	A/duckmeat/Anyang/010%	0% (0/3)	0% (0/3)	1–2	2.0	Brown *et al.*, 2006
	Redhead		0% (0/3)	0% (0/3)	4	4.0	
	Mallard		0% (0/3)	0% (0/3)	1–2	2.1	
	Northern pintail		0% (0/3)	0% (0/3)	1–4	1.1	
	Wood duck		67% (2/3)	33% (1/3)	7	5.0	
Passerines	European starling	A/duck/Thailand/144/2005	0% (0/3)	0% (0/3)	–	3.8	Boon *et al.*, 2007
	Carneaux pigeon		0% (0/3)	0% (0/3)	–	<1	
	House sparrow		100% (3/3)	100% (3/3)	–	4.7	
Geese	Bar-headed goose	A/whooper swan/Mongolia/222/2005	100% (5/5)	40% (2/5)	5–8	5.10	Brown *et al.*, 2008
	Cackling goose		100% (4/4)	75% (3/4)	4–6	5.25	
	Black swan		100% (5/5)	100% (5/5)	2–3	6.46	
	Trumpeter swan		100% (5/5)	100% (5/5)	4–6	6.14	
	Whooper swan		100% (4/4)	100% (4/4)	4–6	6.30	
	Mute swan		100% (5/5)	100% (5/5)	3–7	5.58	
Galliformes	White leghorn chicken	A chicken/Hong Kong/220/97	100% (8/8)	100% (8/8)	–	–	Perkins and Swayne, 2003
	Plymouth rock chicken		100% (8/8)	100% (8/8)	–	–	
	Japanese quail		100% (8/8)	100% (8/8)	–	–	
	Bobwhite quail		100% (8/8)	100% (8/8)	–	–	
	Broad-breasted white turkey		100% (6/6)	100% (6/6)	–	–	
	Pearl guineafowl		100% (8/8)	100% (8/8)	–	–	
	Ringneck pheasant		100% (8/8)	100% (8/8)	–	–	

[a]Average maximum titre (EID_{50}/mL) isolated from the oropharynx.

Table 3 Wild birds in healthy condition detected with natural infections of highly pathogenic avian influenza

Host species (no. individuals)	Location	Sampling site	Date of outbreak	Reference
'Wild ducks' (6)	Poyang Lake, China	Wetland	January 2005	Chen *et al.*, 2006
Great crested grebe (1)	Siberia, Russia	Wetland	July 2005	Lvov *et al.*, 2006
Common teal (1)	Port Said, Egypt	Live bird market	January 2006	Saad *et al.*, 2007
Bar-headed goose (1)	Qinghai Province, China	Wetland	April 2006	Lei *et al.*, 2007
White-faced whistling duck (1), Spur-winged goose (1)	Hadejia-Hguru Wetlands, Nigeria	Wetland	February 2007	Gaidet *et al.*, 2008a
Common pochard (1)	Lake Sempach, Switzerland	Wetland	March 2008	FAO, 2009
Mallard (3), Coot (3)	Yenicam, Konacik and Aybeder, Turkey	Hunting ground/wetland	January–February 2008	Newman *et al.*, 2008
Mallard (1)	Starnberg, Germany	Hunting ground	January 2009	FAO, 2009

7. WILD BIRDS AS VICTIMS

The spread of HPAI H5N1 among waterbirds is likely to have conservation implications because of the high mortality rate in some species. The virus has proven fatal for at least 12 of the 27 avian orders (Roberton *et al.*, 2006). HPAI H5N1 also has an increasing host range with at least 66 avian species reported to have naturally-occurring infections in wild habitat and an additional 33 in captivity (USGS National Wildlife Health Center, 2009). Four host species are listed under the International Union for the Conservation of Nature (IUCN) Red List: the endangered Red-breasted goose (*Branta ruficollis*), endangered Swan goose (*Anser cygnoides*), vulnerable Houbara bustard (*Chlamydotis undulata macqueenii*), and vulnerable Black-necked crane (*Grus nigricollis*).

Species that are dependent on wetland habitat in countries where HPAI H5N1 outbreaks occur may be adversely impacted by the virus. Qinghai Lake attracts migratory birds from Asia, Siberia, New Zealand and Australia and die-offs were observed in at least eight species including the Bar-headed goose, Brown-headed gull (*Larus brunnicephalus*), Great black-headed or Pallas's gull (*Larus icthyaetus*), Great cormorant (*Phalacrocorax carbo*), Whooper swan (*Cygnus cygnus*), Ruddy shelduck (*Tadorna ferruginea*), and Pochard (Chen *et al.*, 2006b). The outbreak is believed to have significantly reduced the global population of bar-headed geese (Figure 4) by 10% (Liu *et al.*, 2005), although their total population size has not been quantified. For other gregarious species that are IUCN-listed such as the Black-necked crane (Figure 4) and Siberian crane (*Grus leucogeranus*), use of wintering or breeding grounds in countries where HPAI H5N1 is recurring may present a challenge to conserving their populations (Kirby *et al.*, 2008). For example, Poyang Lake in central China supports 95% of the global population of Siberian cranes during the winter (Li and Mundkur, 2007) and has also been the site of recurring outbreaks (Chen *et al.*, 2006b).

The spread of HPAI H5N1 within captive populations may also have conservation implications, particularly as these animals increasingly represent valuable genetic stock for populations diminishing in the wild. Infection with the virus has been recorded in a growing number of bird species in open zoos (Ellis *et al.*, 2004), sanctuaries (Desvaux *et al.*, 2009) and other captive populations (Khan *et al.*, 2009). Captive conditions may provide opportunities for HPAI H5N1 to expand its host range due to the artificially high concentration and assemblage of species. This is demonstrated by the large number of species that died due to contact with the virus at

Kowloon Park and Penfold Park in Hong Kong in 2002, including 16 species of ducks, five species of geese, four species of swans and two species of flamingos (Ellis *et al.*, 2004). Similarly large numbers of species were infected by HPAI H5N1 at Phom Tamao Wildlife Rescue Center, Cambodia where a total of 86 birds died, representing eight taxonomic orders and 12 families (Desvaux *et al.*, 2009). Conservation listed species may be the most susceptible to outbreak in captivity, due to the typically low diversity of the major histocompatibility complex (MHC), a gene that plays a central role in disease resistance (Hughes, 1991). Outbreaks in tigers (*Panthera tigris*) at a zoo in Thailand highlights that endangered mammals may suffer diminished populations due to infection with HPAI H5N1 (Thanawongnuwech *et al.*, 2005).

Outbreaks of HPAI H5N1 reduce the abundance of birds in both wild and captive environments, but little is known about the population-level impacts of the virus. For example, does HPAI H5N1-induced mortality impact the overall productivity of a population? The behaviour of the virus bears similarities to avian pox (*Poxvirus avium*) in Hawaiian forest birds (van Riper *et al.*, 1986) and avian cholera (*Pasteurella multocida*) in Lesser snow geese (*Chen caerulescens caerulescens*; Samuel *et al.*, 1999) that likely have a regulatory effect on host populations. Similar to HPAI H5N1, these pathogens show an annual cycle of outbreak and follow a density-dependent pattern of infection. Unlike avian pox and avian cholera that use wild birds as a primary reservoir, HPAI H5N1 is not endemic in wild birds. By contrast, HPAI H5N1 appears to be self-limiting in wild bird populations and requires spillover from poultry, the host in which reassortment to HPAI subtypes occurs (Webster *et al.*, 2006).

8. ENVIRONMENTAL PERSISTENCE, IMMUNITY AND CIRCULATION

8.1 Environmental persistence

Despite evidence that waterbirds may act as vectors for HPAI H5N1, there is limited data on the circulation and persistence of the virus between waterbirds. The remarkably low number of healthy, HPAI H5N1-infected wild birds identified to date indicates a lack of a proven wild bird reservoir. As a consequence, waterbird infections most likely result from repeated spillover from poultry or environmental transmission, and less likely because the virus is endemic in wild populations. Three factors are necessary to ensure the persistence of AIV among wild birds: (1) virus shed at sufficient titres for an adequate period of time; (2) stability of the virus in the environment; and (3) titres sufficient for infection of the next host (Stallknecht and Brown, 2008).

A possible factor limiting circulation between waterbirds is the changing viral shedding pattern of HPAI H5N1. A number of studies (Brown *et al.*, 2006; Zhou *et al.*, 2006; Ellstrom *et al.*, 2008; Keawcharoen *et al.*, 2008; Munster *et al.*, 2009) report higher titres of the virus from the oropharynx compared to the cloaca, and for a longer duration, evidence that the primary site of replication for HPAI H5N1 has shifted to the respiratory tract from the intestinal tract (Sturm-Ramirez *et al.*, 2005). These modifications in the replication site are likely to have resulted from adaptation of HPAI H5N1 to poultry inhabiting indoor environments and selection for air-borne transmission (Weber and Stilianakis, 2008). Chickens are regarded as the ''route modulator'' in which crossover of the replication sites is likely to have occurred (Mararova *et al.*, 2003). Viral shedding from the oral cavity may promote transmission between poultry in a wet market or in a crowded breeding colony, but is unlikely to be as efficient as faecal shedding in dispersing viral particles in the aquatic habitats occupied by waterfowl.

Experimental studies demonstrate that LPAI viruses are more environmentally robust than HPAI viruses (Brown *et al.*, 2007). Two HPAI H5N1 genotypes (one from Mongolia and another from Anyang, China) were tested for persistence in water at different temperatures and salinities (Brown *et al.*, 2007). These genotypes were estimated to persist for periods of 82–158 days at 17°C, and for shorter periods (maximum of 30 days for the Anyang genotype) in water. These values were lower than the persistence estimates for LPAI viruses at the same temperature (Stallknecht *et al.*, 1990; Brown *et al.*, 2007). Yet, the duration had marked overlap with some wild LPAI subtypes, indicating that some of these HPAI H5N1 genotypes may persist long enough in nature (Brown *et al.*, 2007) to become part of the endemic cycles of viruses. To date there have been no successful attempts to isolate HPAI H5N1 from environmental samples, suggesting a low probability that HPAI H5N1 can persist in the aquatic habitat of wild birds.

Outbreaks of the HPAI H5N1 at Qinghai Lake in 2005–2006 and Poyang Lake in 2006 demonstrate that dense congregations of birds may counteract barriers to HPAI H5N1 transmission. Large numbers of wild bird species gather in waterbodies, particularly during pre-migration staging (Li and Mundkur, 2007) with peak infection rates in ducks reported during this period (Olsen *et al.*, 2006). By contrast, gulls and geese are densely clustered at breeding sites, and

prevalence peaks occur at these times (Olsen *et al.*, 2006). Interactions between different species of birds at feeding, stopover and wintering sites permits the constant host-switching by viruses in waterbird complexes (Webster *et al.*, 1992). This is supported by genetic studies that show partitioning of AIV subtypes according to geographic location rather than host species, suggesting that common feeding or breeding sites allow the virus to readily infect many species (Chen and Holmes, 2009). Outbreaks in wild birds need to be studied in greater detail to establish the extent to which HPAI H5N1 genotypes have become entrenched in wild bird cycles.

8.2 Immune status and condition

A number of immunological and condition factors may be involved in triggering HPAI H5N1 outbreak in waterbird populations. Cold temperatures increase the survival of both H5 and H7 subtypes in the external environment (Brown *et al.*, 2007). The success of the virus to transmit in low temperatures is also likely to arise from the effect of cold weather on the host and associated suppression of the immune system. Analysis of HPAI H5N1 activity during 2005–2006 indicated that 27 outbreaks were preceded by a drop in temperature due to Siberian cold air mass dominating Eurasia (Liu *et al.*, 2007; Ottaviani *et al.*, in revision). Liu *et al.* (2007) proposed that this dramatic weather fluctuation may have stressed birds already physically depleted from performing migration, making them susceptible to HPAI H5N1 infection. Furthermore, this drop in temperature may have resulted in crowding of wild birds into remaining unfrozen habitat, further contributing to stress and also facilitating density dependent virus transmission (Ottaviani *et al.*, in revision).

Low precipitation has also been identified as a predictor of HPAI H5N1 outbreak among wild birds. In particular, below average rainfall has been correlated with risk of outbreak in mainland China (Fang *et al.*, 2008). The reduction in available wetland caused by less precipitation may explain this phenomenon. Human activities have also reduced or degraded wetlands used by wild birds due to the continuing demand for agricultural lands (Long *et al.*, 2007). Subsequently, the high concentration of waterbirds in fewer, smaller wetlands is likely to heighten opportunities for contact with the virus, prompting HPAI H5N1 outbreaks (Fang *et al.*, 2008). Crowding of birds in the limited wetlands available may elicit a stress response, compromising the ability of the immune system to fight viral infection.

The act of migration is also likely to make waterbirds more vulnerable to HPAI H5N1 infection. Migration is an energetically costly activity that reduces the body condition of birds as flight muscle mass is run down (Weber and Stilianakis, 2007). Due to the high energy demands of migration, there is a trade-off against physiological functions including the immune system (Moller and Erritzoe, 1998). Waterfowl that undertake migration may therefore experience immune suppression and have little resistance against invasion of pathogens. This is demonstrated by a study of hematozoan parasites in which migratory distance of waterfowl and parasite species richness were positively correlated (Figuerola and Green, 2000). Authors have speculated that the poor condition of birds arriving after long-distance migration may have been a factor in the outbreak of HPAI H5N1 at Qinghai during 2005 (Chen *et al.*, 2006a), but a wind-tunnel experiment showed that immunity was not diminished by long distance flights in shorebirds (Hasselquist *et al.*, 2007).

A recent experimental study of mute swans illustrates that some individuals of apparently susceptible species survive infections with HPAI H5N1 genotypes (Kalthoff *et al.*, 2008). Naïve mute swans died shortly after infection, but those with prior exposure to AIVs were able to survive without apparent symptoms (Kalthoff *et al.*, 2008). Moreover, prior infection with low pathogenic H5N2 provides homosubtype immunity against HPAI H5N1 in waterfowl (Fereidouni *et al.*, 2009). This is consistent with studies that have found young, immunologically-naïve birds to be more susceptible to fatal HPAI H5N1 infection than adults that have acquired resistance (Pantin-Jackwood and Swayne, 2007; Kalthoff *et al.*, 2008). In addition, older birds may be more resistant against AIVs because maturation of host cells can impede viral replication (Pantin-Jackwood and Swayne, 2007). At a later age (after individuals pass their peak in breeding) this trend changes and birds show reduced immunity with increasing age in the wild (Holmes and Austad, 2004).

9. AN ECOLOGICAL RESEARCH PERSPECTIVE

In order to unravel the role of waterbirds in the epidemiology of the disease, improved understanding of their migratory movements, geographic range and habitat use in zones of infection are urgently needed. Movement data may also provide insights into the extent to which habitat use of waterfowl and poultry overlap, and the spatial and temporal correlation between waterfowl migration and outbreaks in poultry or humans. Here we briefly describe our ecological approach in China, widely believed to be the epicentre of HPAI H5N1 (Webby and Webster, 2001), to better understand mechanisms of disease

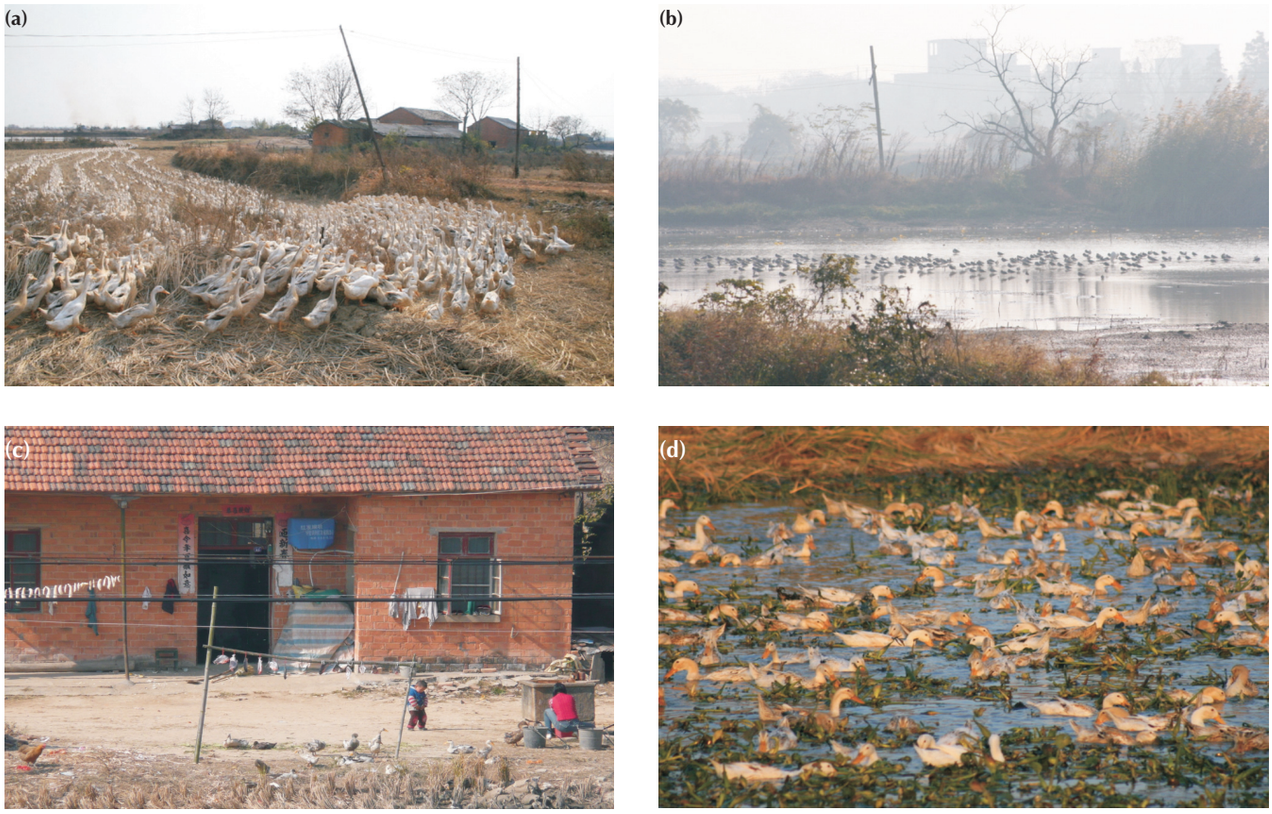

Figure 5 Poyang Lake and surroundings: (**a**) domestic ducks being herded from the lake; (**b**) wild birds inhabiting the lake surrounded by dwellings; (**c**) backyard poultry farming; and (**d**) domestic ducks foraging in the lake. (Photos courtesy of J. Takekawa, USGS Western Ecological Research Center).

spread potentially involving wild birds. This approach aimed to improve surveillance of wild birds on the basis of: (1) focused-studies in areas where HPAI H5N1 has become entrenched and re-occurs annually; and (2) broad-scale studies across Asian flyways to develop better understanding of waterfowl movements in relation to the spread of the HPAI H5N1.

9.1 Poyang Lake and HPAI H5N1

Poyang Lake, located 50 km northeast of Nanchang in Jiangxi Province, is the largest freshwater lake in China with a total area of about 224 km^2 (Shankman and Liang, 2003). Over 100,000 birds winter in Poyang Lake, many of which are long-distance migrants, the most notable being the approximately 3,000 Siberian Cranes, representing almost 95% of the global population (Kanai *et al.*, 2002). Among the 300 bird species recorded, a significant proportion are migratory Anatidae including Whooper swan, Greater white-fronted goose (*Anser albifrons*), Greylag goose (*Anser anser*), Chinese spotbill (*Anas poecilorhyncha zonorhyncha*), Eurasian wigeon (*Anas penelope*), and Mallard (Li and Mundkur, 2007).

The human population of Poyang Lake is about 10 million and extensive dikes have been built to protect farmlands from periodic flooding (Shankman and Liang, 2003). About 14 million domestic poultry, particularly ducks and geese, form an important source of protein in the region (Figure 5). Grazing of poultry within Poyang Lake and in surrounding cropland maximises overlap between wild bird and domestic poultry, thereby increasing chances of transmission of AIVs. In addition, a relatively recent phenomenon in the Poyang Lake region over the past decade includes rearing wild waterfowl in captivity. Migratory swan geese (*Anser cygnoides*) as well as nonmigratory Chinese spotbills are raised in captivity for sale in markets. Many of these farm-raised waterfowl are allowed to feed or fly in the wetlands, creating the direct interaction of farmed and free-ranging wild birds.

The current situation surrounding Poyang Lake and other regions in southern China, where large numbers of domestic ducks and geese are raised in areas with little or no biosafety (FAO, 2001) is conducive to the emergence of novel HPAI subtypes and spillover into wild bird populations. The highly crowded conditions in which ducks and geese are kept and raised also

Figure 6 Qinghai Lake and surroundings: (**a**) the closed inland basin surrounded by mountains; (**b**) bar-headed geese that congregate during the summer; (**c**) sheep foraging on the shoreline and a flock of northern pintail ducks; (**d**) characteristic grassland vegetation. (Photos courtesy of J. Takekawa, USGS Western Ecological Research Center).

provide ample opportunity for reassortment if more than one LPAI infection is present in individuals. This may explain the frequent emergence of different genotypes of HPAI H5N1 within poultry in China, especially since 2003 (Chen *et al.*, 2006b; Webster *et al.*, 2006). Grazing opportunities in adjacent wetlands and rice fields with wild waterbirds could help introduce new LPAIs from the wild into domestic poultry. Introductions of new AIVs from domestic to wild birds are possible, especially since wild birds are greatly outnumbered by domestic ducks. Maximum wild bird counts in Poyang Lake exceeded 270,000 individuals in 2004 (Li and Mundkur, 2007) but were far fewer than the 14 million domestic poultry.

Chen *et al.* (2006b) documented the two HPAI H5N1 genotypes ('Z' and 'V') from Poyang Lake between October 2004 and March 2005. Although they identified the species sampled (Mallard, Falcated teal, *Anas falcata*, and Chinese spotbill), they only reported positive results pooled together into 'migratory ducks' which prevented reaching conclusions on how the different species (with very different ecology and behaviour) may have been involved in the spread of the virus. This lack of detail in identifying migratory waterfowl to species level precludes analyses of ecological aspects of the disease and has been noted by others (Muzaffar *et al.*, 2006; Yasue *et al.*, 2006; Feare, 2007).

9.2 Qinghai Lake and HPAI H5N1

The first evidence of a large HPAI H5N1 epizootic in wild waterbirds since the emergence of HPAI H5N1 was the outbreak at Qinghai Lake reported in 2005. Qinghai Lake is located at the northeast of the Qinghai-Tibet Plateau about 280 km west of Xining in Qinghai Province, China (Liu *et al.*, 2004). It is situated at an elevation of 3,193 m above sea level and is the largest saltwater lake in China with an area of about 526 km^2 (Figure 6). Human activities, particularly the husbandry of yaks and other livestock in the region, has degraded the surrounding grasslands through overgrazing (Liu *et al.*, 2004). Three small island complexes within the lake constitute breeding areas for Bar-headed geese, Brown-headed gulls, Pallas's gulls and Great cormorants. The largest breeding area, Egg Island is a peninsula sheltered by a hill from westerly winds with a freshwater spring that supports dense colonial nesting populations of both Bar-headed geese and gulls.

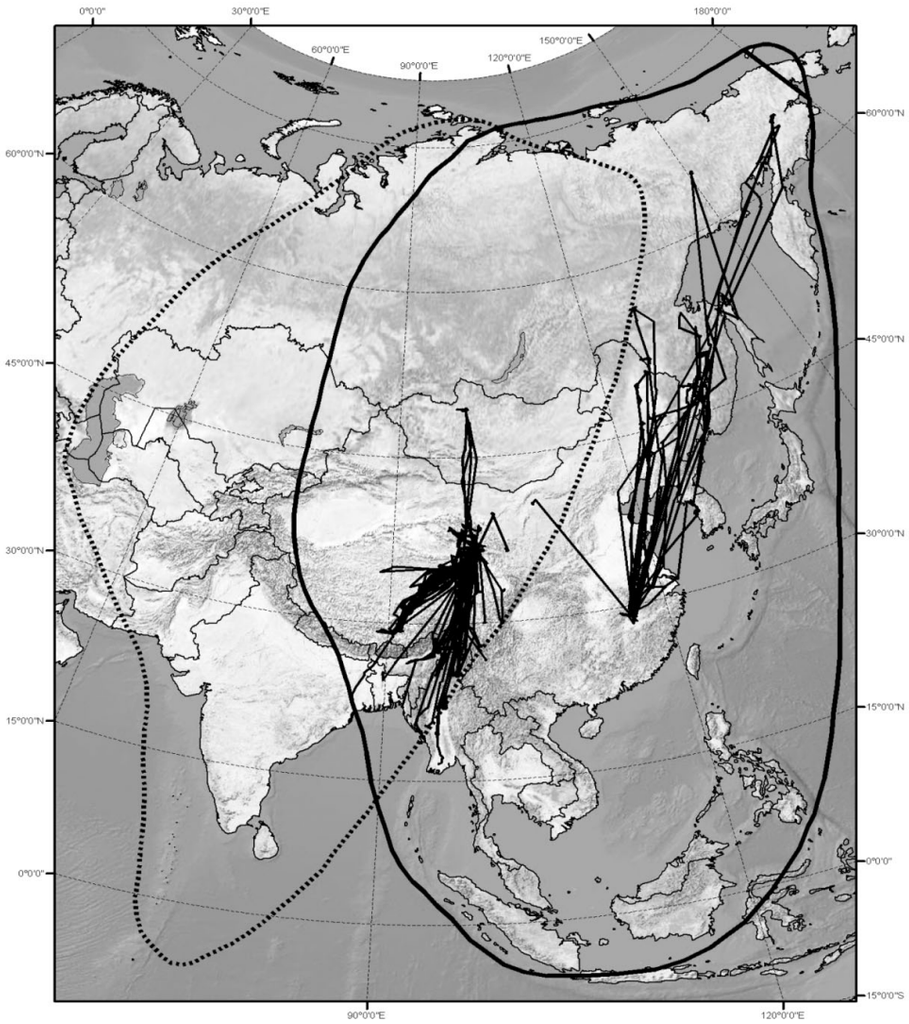

Figure 7 Proposed outlines for the Central Asian (dashed line) and East Asian (solid line) Flyways for waterfowl (Mundkur and Miyabayashi, 1998), and movement paths for >100 waterfowl marked with satellite transmitters.

Over 6,000 waterbirds (including 3,282 Bar-headed geese, 929 Pallas's gulls, 570 Brown-headed gulls, 1,302 Great cormorants, and 145 Ruddy shelducks) died between May and June 2005 from HPAI H5N1 infections (Chen *et al.*, 2005, 2006a; Liu *et al.*, 2005; Olsen *et al.*, 2006). This marked a contrast to all previous cases of HPAI H5N1 in wild birds in East Asia, which were likely spillover from poultry outbreaks (Chen *et al.*, 2005; Gauthier-Clerc *et al.*, 2007). The outbreak started during early May when the first Bar-headed geese began breeding and some individuals were infected and died (Chen *et al.*, 2006a). A peak in infections occurred within the next few days leading to more deaths of Bar-headed geese. Subsequent peaks in deaths occurred diffusely from mid-May and early June, with records of concomitant infections in other species. The outbreak ended by 29 June, after which no more dead birds were recorded (Chen *et al.*, 2006a). The arrival of infected Bar-headed geese has been questioned as the means of possible HPAI H5N1 introduction, since many were already present near the breeding colonies by mid-March.

The isolates from Qinghai Lake were of the 'Z' and 'V' genotypes that were genetically very closely related to those from Poyang Lake (Chen *et al.*, 2006a), suggesting an indirect link between the two sites via migratory birds (Chen *et al.*, 2006a; Wallace *et al.*, 2007). We did not find any direct connectivity of these wetlands from our movement data synthesised from more than 100 waterbirds captured and marked with satellite transmitters at Poyang Lake and Qinghai Lake in 2006–2008 (Figure 7). Our study species included Bar-headed geese and Ruddy shelducks, both infected by HPAI H5N1 at Qinghai Lake during 2005, as well as seven duck species at Poyang Lake. Re-emergence of HPAI H5N1 at Qinghai Lake in 2006 identified six strains derived from those found

in 2005, including one most similar to a duck in Novosibirsk, Russia (Wang *et al.*, 2008). Two competing hypotheses emerge to explain these events: the virus may either be re-introduced by wild birds during their northward spring migration (Prosser *et al.*, submitted) or result from local circulation of viruses at Qinghai Lake, possibly persisting in the springs and freshwater wetlands surrounding the saline lake.

9.3 Migratory flyways and movements of wild waterbirds

While studies have often concluded that the trajectory of HPAI H5N1 closely corresponds with poultry transport routes (Feare, 2007; Gauthier-Clerc *et al.*, 2007), a paucity of data on the temporal and spatial movements of waterfowl prevents the same argument being put forward for wild birds. Compared to shorebirds, for which information on some flyways exist (Boere and Stroud, 2006), the migratory movements of waterfowl have not been well-researched in Asia, with the exception of recent efforts to better understand wild birds and their potential to spread HPAI H5N1 (Muzaffar *et al.*, 2008; Newman *et al.*, 2009; Prosser *et al.*, 2009; Takekawa *et al.*, 2010). In addition, lack of detailed movement data detracts from HPAI H5N1 modelling studies that rely on the limited and coarse-scale wild bird movement data currently available (Peterson and Williams, 2008; Kilpatrick *et al.*, 2006; Gilbert *et al.*, 2006b).

Qinghai Lake falls in an area of overlap between the Central Asian Flyway and the East Asian-Australasian or East Asian Flyway (Li and Mundkur, 2007). A great diversity of ducks, geese and shorebirds use these flyways for migration. Bar-headed geese, for instance, use both the Central Asian and East Asian Flyways, with breeding populations occurring from Lhasa, Tibet to as far northeast as Qinghai Lake and western Mongolia (Javed *et al.*, 2000; Li and Mundkur, 2007) while wintering in northern India, coastal Bangladesh, south-central Thailand and southern China (Miyabayashi and Mundkur, 1999; Javed *et al.*, 2000). There is evidence to indicate that populations using each flyway may be distinct keeping them allopatric during part of the year. Most species of ducks and geese in the two flyways have different migratory populations (Boere and Stroud, 2006), and this could be a reflection of altered migratory routes in response to environmental conditions such as drier, cooler temperatures and low food availability (*e.g.* Miller *et al.*, 2005).

Our ongoing studies on bird migration based on satellite telemetry of a number of duck and geese species show migration routes are separated along the two flyways with birds in the Central Asian Flyway distinct from the East Asian Flyway (Figure 7). Bar-headed geese (Takekawa *et al.*, 2009) and Ruddy shelducks (author's unpublished data), marked in Qinghai Lake spent the non-breeding season in India, Bangladesh, and Myanmar; whereas duck species marked at Poyang Lake were located in northern China, eastern Mongolia and Russia during the breeding season (Takekawa *et al.*, 2010). The separation of the two major flyways seems to preclude a direct migratory link between Poyang Lake and Qinghai Lake from the perspective of HPAI H5N1 spread. It is possible that other species or populations migrate directly between areas, or that waterbirds at other wetlands link these two regions through shared migratory stopovers.

9.4 Informing surveillance and sampling

Our knowledge of persistence, transmission and dispersal of AIVs in wild bird populations depends largely on results from surveillance sampling. Owing to the low probability of detection, the absence of an apparent wild bird reservoir according to current sampling regimes, does not conclusively prove that it does not exist (Flint, 2007). In addition, surveillance sampling often does not take into account the ecology of wild birds (Guberti and Newman, 2007). For example, the assumption of random sampling is often undermined in surveillance studies as wild bird populations are not distributed homogeneously, either spatially or temporally, due to the cycle of migration (Figure 1). It is widely-accepted that AIVs readily infect bird species that share breeding or feeding grounds (Chen and Holmes, 2009) and therefore presence of AIVs may be strongly related to intermixing of populations along migratory flyways. In addition, other traits that influence AIV prevalence such as species, age, flocking and sexual behaviours (Garamszegi and Moller, 2007) or foraging methods (Hill *et al.*, in press) differ markedly between host populations.

Effective research into HPAI H5N1 requires an interdisciplinary approach with fields such as virology, epidemiology, pathology, geospatial science, veterinary science and ecology playing roles. Experts from these disciplines have been responsible for genetically characterising and determining the phylogenetic relationships among HPAI H5N1 genotypes, investigating the clinical effects of the virus and creating models to predict the distribution and spread of HPAI H5N1. Yet further information is needed on the avian hosts that carry HPAI H5N1 – both domestic and wild birds, to gain a complete understanding of the epidemio-ecology of

the virus. In particular, the debate over the agent of HPAI H5N1 dispersal highlights a lack of information in Asian flyways on species dissimilarities, geographic variation of species groups and subpopulations, and seasonal or temporal differences in migratory movements.

For example, phylogeographic studies have applied analyses of genetic similarities to show intriguing relationships among areas (Janies *et al.*, 2007; Wallace *et al.*, 2007). Their results suggest potential movements of waterbirds – from Qinghai Lake to Novosibirsk and back toward Mongolia – that has little support in the ecological literature. Instead, recent findings connect the migratory pathway of ruddy sheldducks and bar-headed geese from Qinghai Lake, China to breeding areas in central Mongolia, corresponding with the northwest spread of HPAI H5N1 during 2005 (Prosser *et al.*, 2009). Advances in technology for ecology studies such as stable isotope mapping and satellite telemetry tracking of individual birds offer tools to acquire fine-scale data on the spatial ecology of waterfowl (Peterson and Williams, 2008). Through the use of satellite telemetry, a growing number of studies are defining the detailed movement patterns of waterfowl that inhabit HPAI H5N1-infected areas such as Asia and Africa. Recent GPS-tracking studies of Garganey (*Anas querquedula*) reveal that the species undertakes a migratory route encompassing Moscow, the Danube delta and northern Nigeria, corresponding spatially with the introduction of the virus from Eurasia into Africa (Gaidet *et al.*, 2008b).

10. CONCLUDING REMARKS

The role of wild birds in the persistence and geographic spread of HPAI H5N1 continues to be debated. At present, HPAI H5N1 is entrenched in poultry in Indonesia and Egypt, and continues to periodically re-emerge in Vietnam, China, Thailand, Bangladesh, and India. Conditions that increase the interface between poultry and wild birds such as wet markets and duck-rice agriculture exist throughout much of Southeast Asia constituting a risk for spillover of HPAI H5N1 to wild birds. While domestic ducks aid in the persistence of HPAI H5N1 in these regions, wild birds are likely responsible for the northward spread of the virus to Central China, Mongolia and Russia via the Central Asian Flyway (Gilbert *et al.*, in review). Determining transmission routes between poultry and wild birds is a challenge and techniques that combine approaches from different disciplines is key to improving our understanding of this and other emerging infectious diseases of significance to human, domestic animal and wildlife health.

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REFERENCES

Alexander, D.J. (2000) A review of avian influenza in different bird species. *Vet. Microbiol.*, **74**, 3–13.

Alexander, D.J. (2003) Should we change the definition of avian influenza for eradication purposes? *Avian Dis.*, **47**, 976–981.

Alexander, D.J. (2007) Summary of avian influenza activity in Europe, Asia, Africa, and Australasia, 2002–2006. *Avian Dis.*, **51**, 161–166.

Amonsin, A., Choatrakol, C., Lapkuntod, J., Tantilertcharoen, R., Thanawongnuwech, R., Suradhat, S., Suwannakarn, K., Theamboonlers, A. and Poovorawan, Y. (2008) Influenza virus (H5N1) in live bird markets and food markets, Thailand. *Emerg. Infect. Dis.*, **14**, 1739–1742.

Boere, G. and Stroud, D. (2006) The flyway concept: what it is and what it isn't. In: Boere, G., Galbraith, C.A., Stroud, D. and Bridge, L.K. (eds.). *Waterbirds Around The World: A Global Overview Of The Conservation, Management And Research Of The World's Waterbird Flyways*, The Stationary Office, Edinburgh.

Boon, A.C., Sandbulte, M.R., Seiler, P., Webby, R.J., Songserm, T., Guan, Y. and Webster, R.G. (2007) Role of terrestrial wild birds in ecology of influenza A virus (H5N1). *Emerg. Infect. Dis.*, **13**, 1720–1724.

Brown, J.D., Stallknecht, D.E., Beck, J.R., Suarez, D.L. and Swayne, D.E. (2006) Susceptibility of North American ducks and gulls to H5N1 highly pathogenic avian influenza viruses. *Emerg. Infect. Dis.*, **12**, 1663–1670.

Brown, J.D., Stallknecht, D.E. and Swayne, D.E. (2008) Experimental infection of swans and geese with highly pathogenic avian influenza virus (H5N1) of Asian lineage. *Emerg. Infect. Dis.*, **14**, 136–42.

Brown, J.D., Swayne, D.E., Cooper, R.J., Burns, R.E. and Stallknecht, D.E. (2007) Persistence of H5 and H7 avian influenza viruses in water. *Avian Dis.*, **51**, 285–289.

Cardona, C., Yee, K. and Carpenter, T. (2009) Are live bird markets reservoirs of avian influenza? *Poult. Sci.*, **88**, 856–859.

Cardona, C.J., Xing, Z., Sandrock, C.E. and Davis, C.E. (2008) Avian influenza in birds and mammals. *Comp. Immunol. Microbiol. Infect. Dis.*, **32**, 255–273.

Chen, H., Deng, G., Li, Z., Tian, G., Li, Y., Jiao, P., Zhang, L., Liu, Z., Webster, R.G. and Yu, K. (2004) The evolution of H5N1 influenza viruses in ducks in southern China. *Proc. Natl. Acad. Sci. USA*, **101**, 10452–10457.

Chen, H., Li, Y., Li, Z., Shi, J., Shinya, K., Deng, G., Qi, Q., Tian, G., Fan, S., Zhao, H., Sun, Y. and Kawaoka, Y. (2006a) Properties and dissemination of H5N1 viruses isolated during an influenza outbreak in migratory waterfowl in western China. *J. Virol.*, **80**, 5976–5983.

Chen, H., Smith, G.J., Li, K.S., Wang, J., Fan, X.H., Rayner, J.M., Vijaykrishna, D., Zhang, J.X., Zhang, L.J., Guo, C.T., Cheung, C.L., Xu, K.M., Duan, L., Huang, K., Qin, K., Leung, Y.H., Wu, W.L., Lu, H.R., Chen, Y., Xia, N.S., Naipospos, T.S., Yuen, K.Y., Hassan, S.S., Bahri, S., Nguyen, T.D., Webster, R.G., Peiris, J.S. and Guan, Y. (2006b) Establishment of multiple sublineages of H5N1 influenza virus in Asia: implications for pandemic control. *Proc. Natl. Acad. Sci. USA*, **103**, 2845–2850.

Chen, H., Smith, G.J., Zhang, S.Y., Qin, K., Wang, J., Li, K.S., Webster, R.G., Peiris, J.S. and Guan, Y. (2005) Avian flu: H5N1 virus outbreak in migratory waterfowl. *Nature*, **436**, 191–192.

Chen, R. and Holmes, E.C. (2009) Frequent inter-species transmission and geographic subdivision in avian influenza viruses from wild birds. *Virology*, **383**, 156–161.

Chomel, B. (2007) Wildlife, exotic pets and emerging zoonoses. *Emerg. Infect. Dis.*, **13**, 6–11.

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

de Jong, J.C., Claas, E.C., Osterhaus, A.D., Webster, R.G. and Lim, W.L. (1997) A pandemic warning? *Nature*, **389**, 554.

de Jong, M.D. and Hien, T.T. (2006) Avian influenza A (H5N1). *J. Clin. Virol.*, **35**, 2–13.

Desvaux, S., Marx, N., Ong, S., Gaidet, N., Hunt, M., Manguerra, J.-C., Sorn, S., Peiris, M., Van der Werf, S. and Reynes, J.-M. (2009) Highly pathogenic avian influenza virus (H5N1) outbreak in captive wild birds and cats, Cambodia. *Emerg. Infect. Dis.*, **15**, 475–478.

Devendra, C. and Thomas, D. (2002) Crop-animal interactions in mixed farming systems in Asia. *Agr. Syst.*, **71**, 27–40.

Duan, L., Campitelli, L., Fan, X.H., Leung, Y.H., Vijaykrishna, D., Zhang, J.X., Donatelli, I., Delogu, M., Li, K.S., Foni, E., Chiapponi, C., Wu, W.L., Kai, H., Webster, R.G., Shortridge, K.F., Peiris, J.S., Smith, G.J., Chen, H. and Guan, Y. (2007) Characterization of low-pathogenic H5 subtype influenza viruses from Eurasia: implications for the origin of highly pathogenic H5N1 viruses. *J. Virol.*, **81**, 7529–7539.

Dugan, V.G., Chen, R., Spiro, D.J., Sengamalay, N., Zaborsky, J., Ghedin, E., Nolting, J., Swayne, D.E., Runstadler, J.A., Happ, G.M., Senne, D., Wang, R., Slemons, R.D., Holmes, E.C. and Taubenberger, J.K. (2008) The evolutionary genetics and emergence of avian influenza viruses in wild birds. *PLOS Pathog.*, **4**, e1000076.

Ellis, T.M., Bousfield, R.B., Bissett, L.A., Dyrting, K.C., Luk, G.S., Tsim, S.T., Sturm-Ramirez, K., Webster, R.G., Guan, Y. and Malik Peiris, J.S. (2004) Investigation of outbreaks of highly pathogenic H5N1 avian influenza in waterfowl and wild birds in Hong Kong in late 2002. *Avian Pathol.*, **33**, 492–505.

Ellstrom, P., Latorre-Margalef, N., Griekspoor, P., Waldenstrom, J. and Olofsson, J. (2008) Sampling for low-pathogenic avian influenza A virus in wild mallard ducks: Oropharyngeal versus cloacal sampling. *Vaccine*, **26**, 4414–4416.

Fang, L.Q., de Vlas, S.J., Liang, S., Looman, C.W., Gong, P., Xu, B., Yan, L., Yang, H., Richardus, J.H. and Cao, W.C. (2008) Environmental factors contributing to the spread of H5N1 avian influenza in mainland China. *PLoS ONE*, **3**, e2268.

FAO (2001) Farming systems and poverty: improving farmers' livelihoods in a changing world. Food and Agricultural Organization of the United Nations and World Bank, Rome.

FAO (2007) Wild birds and avian influenza: an introduction to applied field research and disease sampling techniques. FAO Animal Production and Health Manual No. 5 (D. Whitworth, S.H. Newman, T. Mundkur, and P. Harris, eds.), Rome.

FAO (2009) EMPRES-i global animal health information system of FAO's Emergency Prevention Programme for Transboundary Animal Diseases. <http://empres-i.fao.org/empres-i/home>. Food and Agriculture Organization of the United Nations, Animal Health Service, Animal Production and Health Division. Rome, Italy.

Fasina, F.O., Bisschop, S.P., Joannis, T.M., Lombin, L.H. and Abolnik, C. (2009) Molecular characterization and epidemiology of the highly pathogenic avian influenza H5N1 in Nigeria. *Epidemiol. Infect.*, **137**, 456–463.

Feare, C.J. (2007) The role of wild birds in the spread of HPAI H5N1. *Avian Dis.*, **51**, 440–447.

Fereidouni, S.R., Starick, E., Beer, M., Wilking, H., Kalthoff, D., Grund, C., Hauslaigner, R., Breithaupt, A., Lange, E. and Harder, T.C. (2009) Highly pathogenic avian influenza virus infection of mallards with homo- and heterosubtypic immunity induced by low pathogenic avian influenza viruses. *PLoS ONE*, **4**, e6706.

Figuerola, J. and Green, A.J. (2000) Haematozoan parasites and migratory behaviour in waterfowl. *Evol. Ecol.*, **14**, 143–153.

Flint, P.L. (2007) Applying the scientific method when assessing the influence of migratory birds on the dispersal of H5N1. *Virol. J.*, **4**, 132.

Gaidet, N., Cappelle, J., Newman, S.H., Takekawa, J.Y., Prosser, D., Iverson, S., Douglas, D.C., Perry, W.M. and Mundkur, T. Potential for rapid spread of HPAI H5N1 by wildfowl: dispersal rates and distances determined from satellite telemetry. *J. Appl. Ecol.* (in press).

Gaidet, N., Cattoli, G., Hammoumi, S., Newman, S.H., Hagemeijer, W., Takekawa, J.Y., Cappelle, J., Dodman, T., Joannis, T., Gil, P., Monne, I., Fusaro, A., Capua, I., Manu, S., Micheloni, P., Ottosson, U., Mshelbwala, J.H., Lubroth, J., Domenech, J. and Monicat, F. (2008a) Evidence of infection by H5N2 highly pathogenic avian influenza viruses in healthy wild waterfowl. *PLoS Pathog.*, **4**, e1000127.

Gaidet, N., Newman, S.H., Hagemeijer, W., Dodman, T., Cappelle, J., Hammoumi, S., De Simone, L. and Takekawa, J.Y. (2008b) Duck migration and past influenza A (H5N1) outbreak areas. *Emerg. Infect. Dis.*, **14**, 1164–1166.

Garamszegi, L.Z. and Moller, A.P. (2007) Prevalence of avian influenza and host ecology. *Proc. Biol. Sci.*, **274**, 2003–2012.

Gauthier-Clerc, M., Lebarbenchon, C. and Thomas, F. (2007) Recent expansion of highly pathogenic avian influenza H5N1: a critical review. *Ibis*, **149**, 202–214.

Gilbert, M., Chaitaweesub, P., Parakamawongsa, T., Premashthira, S., Tiensin, T., Kalpravidh, W., Wagner, H. and Slingenbergh, J. (2006a) Free-grazing ducks and highly pathogenic avian influenza, Thailand. *Emerg. Infect. Dis.*, **12**, 227–234.

Gilbert, M., Xiao, X., Chaitaweesub, P., Kalpravidh, W., Premashthira, S., Boles, S. and Slingenbergh, J. (2007) Avian influenza, domestic ducks and rice agriculture in Thailand. *Agric. Ecosyst. Environ.*, **119**, 409–415.

Gilbert, M., Xiao, X., Domenech, J., Lubroth, J., Martin, V. and Slingenbergh, J. (2006b) Anatidae migration in the western Palearctic and spread of highly pathogenic avian influenza H5N1 virus. *Emerg. Infect. Dis.*, **12**, 1650–1656.

Gilbert, M., Xiao, X., Pfeiffer, D.U., Epprecht, M., Boles, S., Czarnecki, C., Chaitaweesub, P., Kalpravidh, W., Minh, P.Q., Otte, M.J., Martin, V. and Slingenbergh, J. (2008) Mapping H5N1 highly pathogenic avian influenza risk in Southeast Asia. *Proc. Natl. Acad. Sci. USA*, **105**, 4769–4774.

Gilbert, M., Xiao, X., Takekawa, J.Y., Loth, L., Prosser, D.J., Biradar, C., Balachandran, S., Rao, M.V.S., Mundkur, T., Yan, B., Xing, Z., Hou, Y., Batbayar, N., Natsagdorj, T., Bishop, C.M., Butler, P.J., Wikelski, M., Hogerwerf, L., Slingenbergh, J. and Newman, S.H. (in review) Linking persistence of HPAI H5N1 in poultry with episodic long-distance spread by migratory waterfowl. *Emerg. Infect. Dis.*

Guberti, V. and Newman, S.H. (2007) Guidelines on wild bird surveillance for highly pathogenic avian influenza H5N1 virus. *J. Wildl. Dis.*, **43**, 29–34.

Hasselquist, D., Lindstrom, A., Jenni-Eiermann, S., Koolhaas, A. and Piersma, T. (2007) Long flights do not influence immune responses of a long-distance migrant bird: a wind-tunnel experiment. *J. Exp. Biol.*, **210**, 1123–1131.

Hatchette, T.F., Walker, D., Johnson, C., Baker, A. and Pryor, S. (2004) Influenza A viruses in feral Canadian ducks: extensive reassortment in nature. *J. Gen. Virol.*, **85**, 2327–2337.

Hill, N.J., Takekawa, J.Y., Cardona, C.J., Ackerman, J.T., Schultz, A.K., Spragens, K.A. and Boyce, W.M. (2010) Waterfowl ecology and avian influenza in California: do host traits inform us about viral occurrence? *Avian Dis.*, **53**, 426–432.

Hinshaw, V.S., Bean, W.J., Webster, R.G. and Sriram, G. (1980a) Genetic reassortment of influenza A viruses in the intestinal tract of ducks. *Virology*, **102**, 412–419.

Hinshaw, V.S., Webster, R.G. and Turner, B. (1980b) The perpetuation of orthomyxoviruses and paramyxoviruses in Canadian waterfowl. *Can. J. Microbiol.*, **26**, 622–629.

Hinshaw, V.S., Wood, J.M., Webster, R.G., Deibel, R. and Turner, B. (1985) Circulation of influenza viruses and paramyxoviruses in waterfowl originating from two different areas of North America. *B. World Health Organ.*, **63**, 711–719.

Holland, J., Spindler, K., Horodyski, F., Grabau, E., Nichol, S. and VandePol, S. (1982) Rapid evolution of RNA genomes. *Science*, **215**, 1577–1585.

Holmes, D. and Austad, S. (2004) Declining immunity with age in the wild. *Sci. Aging Knowledge Environ.*, **2004**, 22.

Hughes, A.L. (1991) MHC polymorphism and the design of captive breeding programs. *Conserv. Biol.*, **5**, 249–251.

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

Iverson, S.A., Takekawa, J.Y., Schwarzbach, S.E., Cardona, C.J., Warnock, N., Bishop, M.A., Schirato, G.A., Paroulek, S., Ackerman, J.T., Ip, H.S. and Boyce, W. (2008) Low prevalence of avian influenza virus in shorebirds on the Pacific Coast of North America. *Waterbirds*, **31**, 602–610.

Janies, D., Hill, A.W., Guralnick, R., Habib, F., Waltari, E. and Wheeler, W.C. (2007) Genomic analysis and geographic visualization of the spread of avian influenza (H5N1). *Syst. Biol.*, **56**, 321–329.

Javed, S., Takekawa, J.Y., Douglas, D.C., Rahmani, A.R., Kanai, Y., Nagendran, M., Choudhury, B.C. and Sharma, S. (2000) Tracking the spring migration of a bar-headed goose (*Anser indicus*) across the Himalaya with satellite telemetry. *Global Environ. Res.*, **2**, 195–205.

Kalthoff, D., Breithaupt, A., Teifke, J.P., Globig, A., Harder, T., Mettenleiter, T.C. and Beer, M. (2008) Highly pathogenic avian influenza virus (H5N1) in experimentally infected adult mute swans. *Emerg. Infect. Dis.*, **14**, 1267–1270.

Kanai, Y., Uetaa, M., Germogenov, N., Nagendran, M., Mitad, N. and Higuchie, H. (2002) Migration routes and important resting areas of Siberian cranes (*Grus leucogeranus*) between northeastern Siberia and China as revealed by satellite tracking. *Biol. Conserv.*, **106**, 339–346.

Keawcharoen, J., van Riel, D., van Amerongen, G., Bestebroer, T., Beyer, W.E., van Lavieren, R., Osterhaus, A.D., Fouchier, R.A. and Kuiken, T. (2008) Wild ducks as long-distance vectors of highly pathogenic avian influenza virus (H5N1). *Emerg. Infect. Dis.*, **14**, 600–607.

Khan, O.A., Shuaib, M.A., Rhman, S.S., Ismail, M.M., Hammad, Y.A., Baky, M.H., Fusaro, A., Salviato, A. and Cattoli, G. (2009) Isolation and identification of highly pathogenic avian influenza H5N1 virus from Houbara bustards (*Chlamydotis undulata macqueenii*) and contact falcons. *Avian Pathol.*, **38**, 35–39.

Kilpatrick, A.M., Chmura, A.A., Gibbons, D.W., Fleischer, R.C., Marra, P.P. and Daszak, P. (2006) Predicting the global spread of H5N1 avian influenza. *Proc. Natl. Acad. Sci. USA*, **103**, 19368–19373.

Kirby, J.S., Stattersfield, A.J., Butchart, S.H.M., Evans, M.I., Grimmett, R.F.A., Jones, V.R., O'Sullivan, J., Tucker, G.M. and Newton, I. (2008) Key conservation issues for migratory land- and waterbird species on the world's major flyways. *Bird Conserv. Int.*, **18**, S40–S73.

Kocan, A.A., Hinshaw, V.S. and Daubney, G.A. (1980) Influenza A viruses isolated from migrating ducks in Oklahoma. *J. Wildl. Dis.*, **16**, 281–285.

Kou, Z., Lei, F.M., Yu, J., Fan, Z.J., Yin, Z.H., Jia, C.X., Xiong, K.J., Sun, Y.H., Zhang, X.W., Wu, X.M., Gao, X.B. and Li, T.X. (2005) New genotype of avian influenza H5N1 viruses isolated from tree sparrows in China. *J. Virol.*, **79**, 15460–15466.

Latorre-Margalef, N., Gunnarsson, G., Munster, V.J., Fouchier, R.A.M., Osterhaus, A., Elmberg, J., Olsen, B., Wallensten, A., Haemig, P.D., Fransson, T., Brudin, L. and Waldenstrom, J. (2008) Effects of influenza A virus infection on migrating mallard ducks. *Proc. R. Soc. B*, **276**, 1029–1036.

Lebarbenchon, C., Brown, S.P., Poulin, R., Gauthier-Clerc, M. and Thomas, F. (2008) Evolution of pathogens in a man-made world. *Mol. Ecol.*, **17**, 475–484.

Lei, F., Tang, S., Zhao, D., Zhang, X., Kou, Z., Li, Y., Zhang, Z., Yin, Z., Chen, S., Li, S., Zhang, D., Yan, B. and Li, T. (2007) Characterization of H5N1 influenza viruses isolated from migratory birds in Qinghai Province of China in 2006. *Avian Dis.*, **51**, 568–572.

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

Li, Z.W.D. and Mundkur, T. (2007) *Numbers and Distribution of Waterbirds and Wetlands in the Asia-Pacific Region: Results of the Asian Waterbird Census 2002–2004*. Wetlands International, Kuala Lumpur.

Liu, C.M., Lin, S.H., Chen, Y.C., Lin, K.C., Wu, T.S. and King, C.C. (2007) Temperature drops and the onset of severe avian influenza A H5N1 virus outbreaks. *PLoS ONE*, **2**, e191.

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

Liu, M., He, S., Walker, D., Zhou, N., Perez, D.R., Mo, B., Li, F., Huang, X., Webster, R.G. and Webby, R.J. (2003) The influenza virus gene pool in a poultry market in south central China. *Virology*, **305**, 267–275.

Liu, Y., Zhai, Y., Gao, J. and Ni, S. (2004) Assessment of grassland degradation near Lake Qinghai, West China, using Landsat TM and *in situ* reflectance spectra data. *Int. J. Remote Sens.*, **24**, 4177–4189.

Long, P.R., Szekely, T., Kershaw, M. and O'Connell, M. (2007) Ecological factors and human threats both drive wildfowl population declines. *Anim. Conserv.*, **10**, 183–191.

Lvov, D.M., Schelkanov, M.I.U., Deriabin, P.G., Grebennikova, T.V., Prilipov, A.G., Nepoklonov, E.A., Onishchenko, G.G., Vlasov, N.A., Aliper, T.I., Zaberezhnyi, A.D., Kireyev, D.E., Krasheninnikov, O.P., Kiryukhin, S.T., Burtseva, E.I. and Slepushkin, A.N. (2006) Isolation of influenza A/H5N1 virus strains from poultry and wild birds in west Siberia during epizooty (July 2005) and their depositing to the state collection of viruses (August, 2005). *Vopr. Virusol.*, **51**, 11–14.

Macken, C., Webby, R.J. and Bruno, W. (2006) Genotype turnover by reassortment of replication complex genes from avian influenza A virus. *J. Gen. Virol.*, **87**, 2803–2815.

Mararova, N.V., Ozaki, H., Kida, H., Webster, R.G. and Perez, D.R. (2003) Replication and transmission of influenza viruses in Japanese quail. *Virology*, **310**, 8–15.

McMichael, A.J., Bolin, B., Costanza, R., Daily, G.C., Folke, C., Lindahl-Keissling, K., Lindgren, E. and Niklasson, B. (1999) Globalization and the sustainability of human health: an ecological perspective. *Bioscience*, **49**, 205–210.

Miller, M.R., Takekawa, J.Y., Fleskes, J.P., Orthmeyer, D.L., Casazza, M.L. and Perry, W.M. (2005) Spring migration of Northern Pintails from California's Central Valley wintering area tracked with satellite telemetry: routes, timing, and destinations. *Can. J. Zool.*, **83**, 1314–1332.

Miyabayashi, Y. and Mundkur, T. (1999) Atlas of Key Sites for Anatidae in the East Asian Flyway. <http://www.jawgp.org/anet/aaa1999/aaaendx.htm>. Wetlands International–Japan, Tokyo, and Wetlands International–Asia Pacific, Kuala Lumpur.

Moller, A.P. and Erritzoe, J. (1998) Host immune defence and migration in birds. *Evol. Ecol.*, **12**, 945–953.

Munster, V.J., Baas, C., Lexmond, P., Bestebroer, T.M., Guldemeester, J., Beyer, W.E., de Wit, E., Schutten, M., Rimmelzwaan, G.F., Osterhaus, A.D. and Fouchier, R.A. (2009) Practical considerations for high-throughput influenza A virus surveillance studies of wild birds by use of molecular diagnostic tests. *J. Clin. Microbiol.*, **47**, 666–673.

Muzaffar, S.B., Takekawa, J., Prosser, D.J., Douglas, D.C., Yan, B., Xing, Z., Hou, Y., Palm, E.C. and Newman, S.H. (2008) Seasonal movements and migration of Pallas's gulls *Larus ichthyaetus* from Qinghai Lake, China. *Forktail*, **24**, 100–107.

Muzaffar, S.B., Takekawa, J.Y., Prosser, D.J., Newman, S.H. and Xiao, X. (in press) Rice production systems and avian influenza: interaction between mixed-farming systems, poultry and wild birds. *Waterbirds*.

Muzaffar, S.B., Ydenberg, R.C. and Jones, I.L. (2006) Avian influenza: an ecological and evolutionary perspective for waterbird scientists. *Waterbirds*, **29**, 243–406.

Newman, S.H., Honhold, N., Erciyas, K. and Sanz-Alvarez, J. (2008) *Investigation of the role of wild birds in highly pathogenic avian influenza outbreaks in Turkey between January and February 2008*: Unpublished mission report. Food & Agriculture Organization of the United Nations, Crisis Mangement Centre–Animal Health Division–EMPRES, Rome.

Newman, S.H., Iverson, S.A., Takekawa, J.Y., Gilbert, M., Prosser, D.J., Batbayar, N., Natsagdorj, T. and Douglas, D.C. (2009) Migration of whooper swans and outbreaks of highly pathogenic avian influenza H5N1 virus in eastern Asia. *PLoS One*, **4**, e5729.

Normile, D. (2005) Avian influenza. Are wild birds to blame? *Science*, **310**, 426–428.

Normile, D. (2006) Avian influenza. Evidence points to migratory birds in H5N1 spread. *Science*, **311**, 1225.

Olsen, B., Munster, V.J., Wallensten, A., Waldenstrom, J., Osterhaus, A.D. and Fouchier, R.A. (2006) Global patterns of influenza a virus in wild birds. *Science*, **312**, 384–388.

Ottaviani, D., de la Roque, S., Khomenko, S., Gilbert, M., Newman, S.H., Roche, B., Schwabenbauer, K., Pinto, J., Robinson, T. and Slingenbergh, J. (in revision) HPAI H5N1 virus in Europe survives during the winter at the cold limit of the migratory waterfowl distribution. *Ecohealth*.

Pantin-Jackwood, M.J. and Swayne, D.E. (2007) Pathobiology of Asian highly pathogenic avian influenza H5N1 virus infections in ducks. *Avian Dis.*, **51**, 250–259.

Peiris, J.S., de Jong, M.D. and Guan, Y. (2007) Avian influenza virus (H5N1): a threat to human health. *Clin. Microbiol. Rev.*, **20**, 243–267.

Perkins, L.E. and Swayne, D.E. (2003) Comparative susceptibility of selected avian and mammalian species to a Hong Kong-origin H5N1 high-pathogenicity avian influenza virus. *Avian Dis.*, **47**, 956–967.

Peterson, A.T. and Williams, R.A.J. (2008) Risk mapping of highly pathogenic avian influenza distribution and spread. *Ecol. Soc.*, **13**, 15.

Pimm, S.L., Russell, G.J., Gittleman, J.L. and Brooks, T.M. (1995) The future of biodiversity. *Science*, **269**, 347–350.

Pittman, M., Freigofas, R., Piazza, V., Brouw, A., Laddomada, A. and Brown, I.H. (2007) Surveillance, prevention and disease management of avian influenza in the European Union. *J. Wildlife Dis.*, **43**, S64–S70.

Prosser, D.J., Takekawa, J.Y., Newman, S.H., Yan, B., Douglas, D.C., Hou, Y., Xing, Z., Li, T., Li, Y., Zhao, D., Perry, W.M., Palm, E.C. and Zhang, D. (2009) Satellite-marked waterfowl reveal migratory connection between H5N1 outbreak areas in China and Mongolia. *Ibis*, **151**, 568–576.

Rappole, J.H. and Hubalek, Z. (2006) Birds and influenza H5N1 virus movement to and within North America. *Emerg. Infect. Dis.*, **12**, 1486–1492.

Roberton, S.I., Bell, D.J., Smith, G.J., Nicholls, J.M., Chan, K.H., Nguyen, D.T., Tran, P.Q., Streicher, U., Poon, L.L., Chen, H., Horby, P., Guardo, M., Guan, Y. and Peiris, J.S. (2006) Avian influenza H5N1 in viverrids: implications for wildlife health and conservation. *Proc. Biol. Sci.*, **273**, 1729–1732.

Rott, R. (1992) The pathogenic determinant of avian influenza. *Vet. Microbiol.*, **33**, 303–310.

Rundstadler, J.A., Happ, G.M., Slemons, R.D., Sheng, Z.-M., Gundlach, N., Petrula, M., Senne, D., Nolting, J., Evers, D.L., Modrell, A., Huson, H., Hills, S., Rothe, T., Marr, T. and Taubenberger, J.K. (2007) Using RRT-PCR analysis and virus isolation to determine the prevalence of avian influenza virus infections in ducks in Minto Flats State Game Refuge, Alaska, during August 2005. *Arch. Virol.*, **152**, 1901–1910.

Saad, M.D., Ahmed, L.S., Gamal-Eldein, M.A., Fouda, M.K., Khalil, F., Yingst, S.L., Parker, M.A. and Montevillel, M.R. (2007) Possible avian influenza (H5N1) from migratory bird, Egypt. *Emerg. Infect. Dis.*, **13**, 1120–1121.

Samuel, M.D., Takekawa, J.Y., Samelius, G. and Goldberg, D.R. (1999) Avian cholera mortality in lesser snow geese nesting on Banks Island, Northwest Territories. *Wildl. Soc. Bull.*, **27**, 780–787.

Shankman, D. and Liang, Q. (2003) Landscape changes and increasing flood frequency in China's Poyang Lake region. *Prof. Geogr.*, **55**, 434–445.

Shortridge, K.F. and Stuart-Harris, C.H. (1982) An influenza epicentre? *Lancet*, **2**, 812–813.

Shortridge, K.F., Zhou, N.N., Guan, Y., Gao, P., Ito, T., Kawaoka, Y., Kodihalli, S., Krauss, S., Markwell, D., Murti, K.G., Norwood, M., Senne, D., Sims, L., Takada, A. and Webster, R.G. (1998) Characterization of avian H5N1 influenza viruses from poultry in Hong Kong. *Virology*, **252**, 331–342.

Sims, L.D. and Brown, I.H. (2008) Multicontinental epidemic of H5N1 HPAI virus (1996-2007). In: Swayne, D.E. (ed.), *Avian Influenza*, pp. 251–286. Blackwell Publishing, Iowa.

Smith, G.J., Fan, X.H., Wang, J., Li, K.S., Qin, K., Zhang, J.X., Vijaykrishna, D., Cheung, C.L., Huang, K., Rayner, J.M., Peiris, J.S., Chen, H., Webster, R.G. and Guan, Y. (2006a) Emergence and predominance of an H5N1 influenza variant in China. *Proc. Natl. Acad. Sci. USA*, **103**, 16936–16941.

Smith, G.J., Naipospos, T.S., Nguyen, T.D., de Jong, M.D., Vijaykrishna, D., Usman, T.B., Hassan, S.S., Nguyen, T.V., Dao, T.V., Bui, N.A., Leung, Y.H., Cheung, C.L., Rayner, J.M., Zhang, J.X., Zhang, L.J., Poon, L.L., Li, K.S., Nguyen, V.C., Hien, T.T., Farrar, J., Webster, R.G., Chen, H., Peiris, J.S. and Guan, Y. (2006b) Evolution and adaptation of H5N1 influenza virus in avian and human hosts in Indonesia and Vietnam. *Virology*, **350**, 258–268.

Songserm, T., Jam-on, R., Sae-Heng, N., Meemak, N., Hulse-Post, D.J., Sturm-Ramirez, K.M. and Webster, R.G. (2006) Domestic ducks and H5N1 influenza epidemic, Thailand. *Emerg. Infect. Dis.*, **12**, 575–581.

Spackman, E., Senne, D.A., Davison, S. and Suarez, D.L. (2003) Sequence analysis of recent H7 avian influenza viruses associated with three different outbreaks in commercial poultry in the United States. *J. Virol.*, **77**, 13399–13402.

Stallknecht, D.E. and Brown, J.D. (2008) Ecology of avian influenza in wild birds. In: *Avian Influenza*, Swayne, D.E. (ed.), pp. 43–58. Blackwell Publishing, Iowa.

Stallknecht, D.E., Kearney, M.T., Shane, S.M. and Zwank, P.J. (1990) Effects of pH, temperature, and salinity on persistence of avian influenza viruses in water. *Avian Dis.*, **34**, 412–418.

Stallknecht, D.E. and Shane, S.M. (1988) Host range of avian influenza virus in free-living birds. *Vet. Res. Commun.*, **12**, 125–141.

Stech, J., Xiong, X., Scholtissek, C. and Webster, R.G. (1999) Independence of evolutionary and mutational after transmission of avian influenza viruses to swine. *J. Virol.*, **73**, 1878–1884.

Sturm-Ramirez, K.M., Hulse-Post, D.J., Govorkova, E.A., Humberd, J., Seiler, P., Puthavathana, P., Buranathai, C., Nguyen, T.D., Chaisingh, A., Long, H.T., Naipospos, T.S., Chen, H., Ellis, T.M., Guan, Y., Peiris, J.S. and Webster, R.G. (2005) Are ducks contributing to the endemicity of highly pathogenic H5N1 influenza virus in Asia? *J. Virol.*, **79**, 11269–11279.

Suarez, D.L. (2000) Evolution of avian influenza viruses. *Vet. Microbiol.*, **74**, 15–27.

Swayne, D. (2008) Epidemiology of avian influenza in agricultural and other man-made systems. In: *Avian Influenza*, Swayne, D.E. (ed.), pp. 59–86. Blackwell Publishing, Ames, IA.

Takekawa, J.Y., Health, S.R., Douglas, D.C., Perry, W.M., Javed, S., Newman, S.H., Suwal, R.J., Rahamani, A.R., Choudhury, B.C., Prosser, D.J., Yan, B., Hou, Y., Batbayar, N., Natsagdorj, T., Bishop, C.M., Butler, P.J., Frappell, P.B., Milsom, W.K., Scott, G.R., Hawkes, L.A. and Wikelski, M. (2009) Geographic variation in Bar-headed Geese *Anser indicus*: connectivity of wintering areas and breeding grounds across a broad front. *Wildfowl*, **59**, 102–125.

Takekawa, J.Y., Newman, S.H., Xiao, X., Prosser, D.J., Spragens, K.A., Palm, E.C., Yan, B., Li, T., Lei, F., Zhao, D., Douglas, D.C., Muzaffar, S.B. and Ji, W. (2010) Migration of waterfowl in the East Asian Flyway and spatial relationship to HPAI H5N1 outbreaks. *Avian Dis.*, **53**, 466–476.

Thanawongnuwech, R., Amonsin, A., Tantilertcharoen, R., Damrongwatanapokin, S., Theamboonlers, A., Payungporn, S., Nanthapornphiphat, K., Ratanamungklanon, S., Tunak, E., Songserm, T., Vivatthanavanich, V., Lekdumrongsak, T., Kesdangsakonwut, S., Tunhikorn, S. and Poovorawan, Y. (2005) Probable tiger-to-tiger transmission of avian influenza H5N1. *Emerg. Infect. Dis.*, **11**, 699–701.

van Gils, J.A., Munster, V.J., Radersma, R., Liefhebber, D., Fouchier, R.A. and Klaassen, M. (2007) Hampered foraging and migratory performance in swans infected with low-pathogenic avian influenza A virus. *PLoS ONE*, **2**, e184.

van Riper, C., 3rd, van Riper, S.G. and Hansen, W.R. (1986) Epizootiology and effect of avian pox on Hawaiian forest birds. *Auk*, **119**, 929–942.

Wallace, R.G., Hodac, H., Lathrop, R.H. and Fitch, W.M. (2007) A statistical phylogeography of influenza A H5N1. *Proc. Natl. Acad. Sci. USA*, **104**, 4473–4478.

Wang, G., Zhan, D., Li, L., Lei, F., Liu, B., Liu, D., Xiao, H., Feng, Y., Li, J., Yang, B., Yin, Z., Song, X., Zhu, X., Cong, Y., Pu, J., Wang, J., Liu, J., Gao, G.F. and Zhu, Q. (2008) H5N1 avian influenza re-emergence of Lake Qinghai: phylogenetic and antigenic analyses of the newly isolated viruses and roles of migratory birds in virus circulation. *J. Gen. Virol.*, **89**, 697–702.

Wang, M., Di, B., Zhou, D.H., Zheng, B.J., Jing, H., Lin, Y.P., Liu, Y.F., Wu, X.W., Qin, P.Z., Wang, Y.L., Jian, L.Y., Li, X.Z., Xu, J.X., Lu, E.J., Li, T.G. and Xu, J. (2006) Food markets with live birds as source of avian influenza. *Emerg. Infect. Dis.*, **12**, 1773–1775.

Webby, R.J. and Webster, R.G. (2001) Emergence of influenza A viruses. *Phil. Trans. R. Soc. Lond. B Biol. Sci.*, **356**, 1817–1828.

Weber, T.P. and Stilianakis, N.I. (2007) Ecologic immunology of avian influenza (H5N1) in migratory birds. *Emerg. Infect. Dis.*, **13**, 1139–1143.

Weber, T.P. and Stilianakis, N.I. (2008) Inactivation of influenza A viruses in the environment and modes of transmission: a critical review. *J. Infect.*, **57**, 361–373.

Webster, R.G. (2004) Wet markets - a continuing source of severe acute respiratory syndrome and influenza? *Lancet*, **363**, 234–236.

Webster, R.G., Bean, W.J., Gorman, O.T., Chambers, T.M. and Kawaoka, Y. (1992) Evolution and ecology of influenza A viruses. *Microbiol. Rev.*, **56**, 152–179.

Webster, R.G. and Govorkova, E.A. (2006) H5N1 influenza - continuing evolution and spread. *N. Engl. J. Med.*, **355**, 2174–2177.

Webster, R.G., Peiris, M., Chen, H. and Guan, Y. (2006) H5N1 outbreaks and enzootic influenza. *Emerg. Infect. Dis.*, **12**, 3–8.

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

Xu, X., Subbarao, K., Cox, N.J. and Guo, Y. (1999) Genetic characterization of the pathogenic influenza A/Goose/Guangdong/1/96 (H5N1) virus: similarity of its hemagglutinin gene to those of H5N1 viruses from the 1997 outbreaks in Hong Kong. *Virology*, **261**, 15–19.

Yasue, M., Feare, C.J., Bennun, L. and Fiedler, W. (2006) The epidemiology of H5N1 avian influenza in wild birds: why we need better ecological data. *BioScience*, **56**, 923–929.

Yu, Z., Song, Y., Zhou, H., Xu, X., Hu, Q., Wu, H., Zhang, A., Zhou, Y., Chen, J., Dan, H., Luo, Q., Li, X., Chen, H. and Jin, M. (2007) Avian influenza (H5N1) virus in waterfowl and chickens, central China. *Emerg. Infect. Dis.*, **13**, 772–775.

Zhou, J.Y., Shen, H.G., Chen, H.X., Tong, G.Z., Liao, M., Yang, H.C. and Liu, J.X. (2006) Characterization of a highly pathogenic H5N1 influenza virus derived from bar-headed geese in China. *J. Gen. Virol.*, **87**, 1823–1833.

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