Chapter 10
Epidemiology of Highly Pathogenic Avian Influenza Virus Strain Type H5N1

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Introduction

Highly pathogenic avian influenza (HPAI) is a severe disease of poultry. It is highly transmissible with a flock mortality rate approaching 100% in vulnerable species (Capua et al. 2007a). Due to the potentially disastrous impact the disease can have on affected poultry sectors, HPAI has received huge attention and is classified as a notifiable disease by the World Organisation for Animal Health (OIE).

Among the family *Orthomyxoviridae*, only viruses of the influenza A genus are known to infect birds. Most virus strain types have been isolated in water birds which are considered to be the main hosts (Webster et al. 1992). When avian influenza viruses spill over into susceptible domestic poultry species (e.g. chickens and turkeys) they can cause a mild disease described as low pathogenic avian influenza (LPAI) (Capua et al. 2004; Osterhaus et al. 2008); however, some subtypes (namely H5 and H7) can undergo mutations into a highly pathogenic form (Alexander et al. 1993; Garcia et al. 1996; Rohm et al. 1995). Only 25 HPAI epidemics have been recorded in poultry since 1959. Despite the sporadic nature of outbreaks, there appears to be a trend for increasing frequency over the past two decades, as well as a trend for increasing economic impact per outbreak (Alexander et al. 2009).

Since 2003, 63 countries have reported outbreaks of HPAI strain type H5N1 in domestic and wild birds (OIE 2010a), and the disease is now endemic in poultry populations in several countries in Asia and in Egypt. HPAI virus (HPAIV) H5N1 has also shown potential for cross-species transmission, including infection of humans. HPAI therefore remains of concern for public health, particularly with respect to its pandemic potential (Ferguson et al. 2004).

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In this review, we present the main epidemiological characteristics of the ongoing HPAI H5N1 pandemic in poultry.

Worldwide Spread and Continuing Evolution of HPAIV H5N1

1996–2003: Emergence of HPAIV H5N1

The first HPAI H5N1 outbreak is thought to have occurred in a commercial goose farm in Guangdong province, China, in 1996 (Chen et al. 2004; Xu et al. 1999; Alexander 2007a). This strain was likely to have been introduced from wild birds as a LPAI virus and undergone subsequent mutation (Vijaykrishna et al. 2008). The resulting HPAIV was the first of a lineage that has generated multiple genetic re-assortants and is the precursor of all subsequent HPAIV H5N1. A H5N1 variant caused an outbreak in a Hong Kong farm in April 1997, as well as demonstrating potential for human infection and resulting in the first public attention of the potential importance of this strain (de Jong et al. 1997; Subbarao et al. 1998). The same virus re-emerged in Hong Kong in December 1997 and was found to be highly prevalent in live bird markets (LBMs) (Shortridge 1999), where it may have circulated for several months in the absence of effective surveillance (Guan et al. 2009). The first outbreaks in Hong Kong were controlled through the slaughter of all poultry on the island, although additional outbreaks were reported in 2001 and 2002 (Sims et al. 2003a, b).

From 1999 to 2003, multiple genotypes of H5N1 viruses were isolated in domestic waterfowl in Southern China and in birds imported from China to Hong Kong for slaughter (Chen et al. 2004; Sims et al. 2003a, b; Cauthen et al. 2000; Guan et al. 2002; Li et al. 2004; Martin et al. 2006; Wang et al. 2008a). H5N1 viruses were also isolated from duck meat imported from China to South Korea (Tumpey et al. 2002) and Japan (Mase et al. 2005). In Viet Nam, two Ha Noi LBMs were found to be silently infected with H5N1 viruses in 2001 (Nguyen et al. 2005). Moreover, two human cases were reported in Hong Kong in 2003 from a family with recent travel history to China (Peiris et al. 2004).

These findings suggest that H5N1 viruses circulated extensively in southern China following emergence (Sims et al. 2003a; Guan et al. 2002; Martin et al. 2006; Duan et al. 2008). Moreover, the wide genetic variation displayed by isolates indicates that the pool of AI viruses in the region is large and that a high number of H5N1 genotypes have emerged over a relatively short time period. Southern China, and more specifically Guangdong province (Wallace et al. 2007), therefore appears to be the prime source of emergent HPAIV H5N1, which have subsequently spread at both a regional and an international scale (Wallace et al. 2007; Chen et al. 2006). Among the multiple re-assortants, the genotype *Z* has become the dominant genotype since 2002 (Li et al. 2004; Duan et al. 2008). This virus, first detected in Guangxi, China, in 2001 (Guan et al. 2009), is more virulent than its predecessors and infects a wider range of species (Eagles et al. 2009).

2003: Major Outbreak Waves in Southeast Asia

From November 2003 to February 2004, eight countries (Viet Nam, Thailand, Indonesia, South Korea, Japan, Cambodia, Laos and China) reported outbreaks of HPAI H5N1 to the OIE (Li et al. 2004). However, the virus is very likely to have circulated in several of these countries, including Viet Nam and Indonesia, for several months before official notification (Vijaykrishna et al. 2008). Thailand and Viet Nam were particularly affected, with the spread of infection to most provinces by the end of January 2004. A second wave of outbreaks started in June 2004 and culminated in Thailand in late 2004 and in Viet Nam in early 2005 (Guan et al. 2009). Malaysia reported its first cases in August 2004, but subsequently eradicated the disease through mass culling (Martin et al. 2006).

The viruses circulating in Viet Nam, Thailand, Malaysia, Cambodia and Laos at this time belonged to the clade 1 and were derived from viruses previously identified in Yunnan province, China. In contrast, viruses circulating in Indonesia belonged to clade 2.1, believed to have originated in Hunan province, China (Guan et al. 2009; Wang et al. 2008b). China thus appears to have been the epicentre of disease spread, with countries in Indochina acting as a sink (Wallace et al. 2007). However, within these countries, geographic diversity was already emerging with viruses circulating in northern Viet Nam more closely related to those in Thailand, and viruses in the Mekong region related to those in Cambodia (Smith et al. 2006a).

2005–2006: Westerly Virus Spread

The death of more than 6,000 wild birds due to HPAIV H5N1 in May 2005 in Qinghai Lake, Western China, prompted fears of the possibility of wider spread of the virus through bird migration (Liu et al. 2005; Chen et al. 2005). This was the first occurrence of the clade 2.2 H5N1 variant, whose lineage rapidly spread from China to Europe, Africa and the Middle East (Guan et al. 2009).

Soon after the first detection of the virus in wild birds in China, infection was found in wild birds in Mongolia (Gilbert et al. 2006a). In July 2005, several outbreaks were reported across Siberia (Feare 2007), and from October wild bird deaths were detected around the Caspian and Black seas. Poultry outbreaks of HPAI H5N1 were subsequently reported in Crimea (Feare 2007). Although HPAI H5N1 viruses had been detected in Europe before 2006, previous occurrences had been scarce and isolated (Van Borm et al. 2005; Alexander 2007b). From February 2006, dead birds, mainly mute swans, were found in several countries, including Austria, Croatia, Denmark, France, Germany, Greece, Scotland, Sweden and Switzerland. Outbreaks in domestic poultry were also detected in some of these countries but were generally rapidly controlled, with transmission between premises remaining limited (Brown 2010).

Whilst limited disease spread was occurring in Europe, the clade 2.2 variant was also spreading within domestic poultry populations in the Middle East, particularly Iran, Azerbaijan, Afghanistan, Pakistan, as well as into Africa (Feare 2007; Brown 2010), first affecting Nigeria in January 2006 (Cattoli et al. 2009), and Egypt, Niger, Cameroon, Burkina Faso, Sudan, Cote d'Ivoire and Djibouti shortly after. A total of 1,024 outbreaks were reported in Egypt from February to December 2006, either in commercial or backyard flocks (Aly et al. 2008). The disease spread further in Africa in 2007, with Ghana, Togo and Benin all reporting outbreaks (Cattoli et al. 2009).

Phylogenetic studies suggest that there have been several independent introductions of H5N1 viruses into Europe (Salzberg et al. 2007; Gall-Recule et al. 2008; Starick et al. 2008) and Africa (Ducatez et al. 2006, 2007; Fasina et al. 2009). However, all the viruses have been closely related, and viruses circulating in Russia in 2005 have been proposed as putative progenitors of this Euro-African lineage (Cattoli et al. 2009; Ducatez et al. 2007).

From 2007: Virus Maintenance and Genetic Diversification

Although outbreaks are now reported less frequently than during the first outbreak waves in 2003–2006, HPAI H5N1 has become endemic in several regions of Asia and Africa. LBM surveys in southern China from January 2004 to June 2006 (Chen et al. 2006; Smith et al. 2006a) and from 2007 to 2009 (Jiang et al. 2010) demonstrated that H5N1 viruses continue to circulate in a variety of poultry species. A new variant has also emerged during this period: the Fujian-like variant (clade 2.3.4), related to genotype V. Since 2005, this variant has gradually replaced the previously prevailing sub-lineage in China (Duan et al. 2008; Smith et al. 2006a; Li et al. 2010a), as well as in northern Viet Nam (Wan et al. 2008; Dung Nguyen et al. 2008) where the disease also appears to be endemic. This variant had also been detected in Laos, Malaysia and Thailand (Smith et al. 2006a; Saito et al. 2008). As well as 2.3.4, several clades continue to circulate in China, including clade 7 and clade 2.3.2 (Jiang et al. 2010). Introduction of clade 7 has also occurred in Myanmar (Saito et al. 2008) and northern Viet Nam (Nguyen et al. 2009). Moreover, clade 2.3.2 has been isolated in several other Asian countries as well as in Europe, in both poultry and wild birds, as a result of a new wave of cross-continental spread from Asia to Europe (Jiang et al. 2010; Boltz et al. 2010; Kim et al. 2010). Since 2001, nine distinct genotypes have been detected in Viet Nam, at least four of which appear to have emerged in the country, others having been introduced (Wan et al. 2008). New variants in Viet Nam appear to be first detected in northern parts, and to then spread to the south (Wan et al. 2008), although clade 1 is still prevalent in southern Viet Nam (Wan et al. 2008; Dung Nguyen et al. 2008). In Indonesia, the disease has been officially declared as endemic since 2006 (OIE 2010a), and outbreaks in poultry are frequent, particularly on the islands of Java, Bali, Sulawesi and Sumatra (Henning et al. 2010). Clade 2.1 is still the predominant

variant in that country (Eagles et al. 2009), although new reassortants with different transmission and evolutionary dynamics appear to continually emerge on Java, the main endemic focus, and subsequently spread to other regions (Lam et al. 2008; Takano et al. 2009). Hence, the co-circulation of multiple sub-lineages and their continuing evolution both in China and in Southeast Asia has led to the generation of new variants that are able to spread widely across the region.

It has been suggested that HPAI H5N1 is unlikely to be endemic in Cambodia, or in Laos (Buchy et al. 2009; Boltz et al. 2006), where outbreaks seem to result from virus reintroduction from neighbouring endemic areas, rather than through perpetuated transmission. In Thailand, the disease appears to have been effectively controlled, with interventions in place to control sporadic emergence. This is also the case in South Korea and Japan (Eagles et al. 2009).

The genetic diversity that exists within African virus isolates appears to be due to the prolonged circulation and evolution of viruses in a segregated area rather than due to the reintroduction of new variants (Cattoli et al. 2009; Salzberg et al. 2007). In Nigeria, for example, the co-circulation of multiple sub-lineages led to the emergence of new variants which gradually replaced introduced virus strains (Owoade et al. 2008; Fusaro et al. 2010; Monne et al. 2008). Some new introductions are likely to have occurred, however, as genotypes closely related to those circulating in Europe and the Middle East in 2007 were detected in July 2008 (Fusaro et al. 2009). No outbreak has been reported in Nigeria since 2009 (OIE 2010b). The disease has been formally declared endemic in Egypt (Aly et al. 2008) and surveillance campaigns have highlighted high prevalence in farms and LBMs (Abdelwhab et al. 2010; Hafez et al. 2010). Several sub-lineages have become established, co-circulate and continue to evolve in the country (Arafa et al. 2010; Abdel-Moneim et al. 2009) to the extent that they were reclassified as a new third-order clade, 2.2.1 (Balish et al. 2010).

Outbreaks have regularly occurred in Bangladesh since 2007, suggesting that the disease may now be endemic in the country (Ahmed et al. 2010; Biswas et al. 2008; ProMED-mail 2010a, b). Moreover, several outbreak waves have been reported in India since 2006. The viruses associated with these waves have been of clade 2.2, and outbreaks are considered to be the result of new introductions, as interventions were reported to mitigate the successive outbreaks (Chakrabarti et al. 2009; ProMED-mail 2009, 2010c; Ray et al. 2008; Mishra et al. 2009; Murugkar et al. 2008).

Wild Birds: Putative Disease Spreaders and Reservoir

From 2002 to 2005, reported H5N1 outbreaks in wild birds in Asia (Hong Kong, Japan, South Korea, Thailand, China, Cambodia) (Feare 2007; Ellis et al. 2004; Kwon et al. 2005a; Desvaux et al. 2009) tended to be isolated and limited. They generally involved a small number of fatalities among non-migrant species, and occurred in the vicinity of poultry outbreaks or among captive or semi-captive wild bird species. However, the emergence of new variants responsible for mass mortality

in wild birds in Qinghai Lake, China, in 2005 (Liu et al. 2005; Chen et al. 2005) led to the notion that migratory birds could spread H5N1 viruses beyond Southeast Asia (Olsen et al. 2006).

Virus Spread to Disease-Free Areas

Most virus introductions into Europe in 2005–2006 were probably caused by wild birds (Kilpatrick et al. 2006; Pfeiffer et al. 2006). Indeed, the virus spread to the Caspian and Black sea occurred with the autumn Anatidae migration (Gilbert et al. 2006a), and the subsequent spread to western Europe was very likely due to unusual cold weather that caused wild birds to leave the Caspian and Black sea (Reperant et al. 2010). Moreover, wild birds have been implicated as the cause of disease introduction or reintroduction to several other countries, including Russia (Sharshov et al. 2010), Mongolia (Spackman et al. 2009), Nigeria (Owoade et al. 2008; Fusaro et al. 2010; Gaidet et al. 2008), Egypt (Saad et al. 2007), India (Chakrabarti et al. 2009; Murugkar et al. 2008), Japan (Uchida et al. 2008), and South Korea (Kang et al. 2010). However, the evidence is generally scarce and these conclusions are based on the fact that the timing of poultry outbreaks was associated with bird migration, or in some cases from outbreak investigations. However, and in general, the lack of information and the weakness of surveillance systems in some of the countries involved means it is difficult to rule out other possible causes, such as live bird trade. As such, the relative importance of wild birds in the introduction of disease remains hypothetical and the subject of continued debate. For example, assuming that wild birds are a major virus spreader, outbreaks would be expected in the Philippines, New Zealand, and Australia which are on the flyways of several Asian migratory waterfowl species (Gilbert et al. 2006a; Krauss et al. 2010): this has not yet been the case. However, it was shown experimentally that species migrating to Australia shed lower quantities of viruses than those migrating westward (East et al. 2008). Most surveillance campaigns have either failed to isolate the virus in wild birds or found it only on very rare occasions (e.g. Thailand (Siengsanan et al. 2009), Egypt (Saad et al. 2007), Switzerland (Baumer et al. 2010)), and even more rarely during migration periods (Feare 2010).

An additional argument against the role of wild birds as long distance transporters of H5N1 is that, to date, the majority of infected wild birds have been found either sick or dead (Olsen et al. 2006). In order to carry a virus over long distances, infected wild birds would need to show few or no adverse clinical symptoms (Weber et al. 2007). There have been reports of H5N1 infection in apparently healthy wild birds, particularly ducks (Chen et al. 2006; Starick et al. 2008; Saad et al. 2007; Siengsanan et al. 2009; Feare and Yasue 2006; Globig et al. 2009), as well as some terrestrial birds, such as sparrows (*Passer montanus*) (Kou et al. 2005). Experimental studies have demonstrated that some species, such as mallards (*Anas platyrhynchos*) and pochards (*Aythya ferina*)

(Keawcharoen et al. 2008; Brown et al. 2006), can shed the virus without or with very limited disease signs. Moreover, immunity induced by prior LPAI infection was shown experimentally to prevent overt clinical disease (Fereidouni et al. 2009). However, such studies do not account for the effect of migration on the immunological state of the birds involved. Indeed, the physiological cost of migration is high and the impact of avian influenza viruses on bird fitness will condition their long-distance spread (Weber et al. 2007). Although known to induce no or mild symptoms, LPAI infection was shown to delay migration and increase the frequency of stopovers in free-living mallards (*A. platyrhynchos*) and Bewick's swans (*Cygnus columbianus bewickii*) (van Gils et al. 2007; Latorre-Margalef et al. 2009).

The length of the asymptomatic infectious period may allow wild bird species to take part in short-distance disease spread (Kalthoff et al. 2008; Brown et al. 2008) as was probably the case for mute swans in Europe. Hence, whilst some birds, and in particular the dabbling ducks, may have a putative role in long-distance disease spread, there remains considerable uncertainty in the role of wild birds in all but the short-distance spread of HPAIV H5N1.

Farm-to-Farm Virus Spread and Virus Maintenance in Wild Birds

Opportunities for free-range poultry and wild birds to mix are numerous: terrestrial wild birds are likely to mix with scavenging poultry and the transformation of wetland areas into rice fields may have increased the rate of contacts between wild and domestic waterfowl (Artois et al. 2009). Access of wild birds to food and watering sources for poultry may also allow indirect transmission. On a local scale, wild birds could therefore transmit the infection to domestic birds and play a role in the spread of virus between farms. Indeed, presence of wild birds in feed troughs or in poultry confinement areas was identified as a risk factor for farm infection in case-control studies in Hong Kong, Viet Nam and Bangladesh (Henning et al. 2009a; Kung et al. 2007; Biswas et al. 2009a). Moreover, the risk of infection was higher in the vicinity of wetlands or water bodies in China, Bangladesh, Thailand and Romania (Biswas et al. 2009a; Ward et al. 2008; Paul et al. 2010a; Fang et al. 2008).

Outbreaks in wild birds are often associated with outbreaks on poultry farms (e.g. South Korea (Lee et al. 2008), Japan (Uchida et al. 2008), Russia (Feare 2007), Pakistan, India, Czech Republic, Poland and Ukraine (Feare 2010)). Virus isolates from both poultry and wild bird populations are often closely related phylogenetically, indicating that viruses were transmitted from one population to another, although the direction of transmission (i.e. from wild birds to poultry or vice versa) cannot be determined with certitude (Lee et al. 2008). In Thailand (Siengsanan et al. 2009), infected wild birds, mostly peri-domestic and commensal species, were rarely found in the areas where the disease was reported in poultry and are therefore unlikely to play a significant role in the epidemiology of the disease in that country. Moreover, experimental studies have shown that

for terrestrial (i.e. non-aquatic) wild birds (e.g. sparrows), the intra-species transmission rates or rate of transmission to chickens is relatively low (Boon et al. 2007; Forrest et al. 2010).

Although the isolation of H5N1 viruses in wild birds is generally rare, it has been suggested that sample sizes used are often insufficiently large to detect very low prevalence (Fereidouni et al. 2009). It has been assumed that H5N1 viruses could be maintained at low prevalence levels in small subpopulation pockets of certain wild bird species (Haase et al. 2010), particularly ducks (Krauss et al. 2010), and such populations could then act as a virus reservoir for poultry. With regard to LPAI viruses, environmental contamination is very likely to play a role in virus maintenance, allowing transmission between wild bird populations that do not share the same site temporally (Brown et al. 2007, 2008). These viruses are mainly transmitted via the faecal-oral route (Webster et al. 1992) and high virus titres are released into the environment where they remain for long periods, particularly in surface water (Brown et al. 2007; Stallknecht et al. 2010). Indeed, viruses shed in the Arctic during one breeding season may remain infectious at the return of migrating birds for the following season (Ito et al. 1995). The isolation of LPAI viruses in wintering sites (Gaidet et al. 2007; Stallknecht and Shane 1988) supports the hypothesis that viruses could be also perpetuated in migratory bird populations throughout the year. In contrast, the maintenance of H5N1 viruses among wild bird populations is uncertain. H5N1 viruses do not persist as long as LPAI viruses in water (Brown et al. 2007). Moreover, these viruses are predominantly shed by the respiratory tract by Anseriformes species (Keawcharoen et al. 2008; Brown et al. 2006), and hence the transmission of H5N1 viruses requires high contact rates, and could thus be maladapted to natural ecosystems, where the contact rates vary between seasons and species.

Trade and Live Bird Markets

Disease Introduction into Disease-Free Areas

In the last 20 years, poultry production has increased at a huge rate in Asia. For example, between 1985 and 2005, the production of chicken and duck meat in China increased by almost 7 times (Gilbert et al. 2007). Increases in poultry production have also led to increases in both local and international trade in poultry products, including both legal and illegal activities. The commercial movement of live birds and poultry products may therefore have played a major role in the virus spread within and beyond Asia (Sims et al. 2005). Indeed, cross-border trade is very likely to have been responsible for the initial spread of the virus from southern China to Southeast Asia, as well as the continued introduction of new variants into the latter region (Wang et al. 2008b; Wan et al. 2008). A large number of spent hens and ducklings are known to move daily from China to Viet Nam

(personal observations). The isolation of clade 7 viruses from poultry seized at the border between China and Viet Nam, and subsequently in Vietnamese LBMs, demonstrates how cross-border trade can lead to the introduction of new variants into Viet Nam, and how the local market chain can spread introduced viruses locally (Nguyen et al. 2009; Davis et al. 2010). The close similarity between viruses isolated in northern Viet Nam, Thailand and Malaysia is thought to be due to commercial movements of birds, as legal and illegal trade is well developed in the region (Smith et al. 2006b). Likewise, trade into and across Laos is also the most likely cause of virus introduction into that country. The well-established poultry trade, particularly of ducks, from southern Viet Nam to Cambodia (Van Kerkhove et al. 2009) may have caused multiple introductions of H5N1 viruses into Cambodia, and therefore explain the high degree of homology between viruses isolated in both regions (Buchy et al. 2009; Smith et al. 2006b). Virus spread from northern to southern Viet Nam is also believed to occur via the poultry trade (Wan et al. 2008).

Although migratory birds have been implicated, illegal cross-border trade from infected neighbouring countries, such as Bangladesh, is also a probable route for multiple virus introductions to India (Chakrabarti et al. 2009; Murugkar et al. 2008).

Larger scale poultry movements can also occur, for example legal and illegal trade of live poultry from China (ProMED-mail 2006) to Nigeria were known to be frequent (Cecchi et al. 2008). Phylogenetic studies indicate that the patterns of virus evolution and geographical strain distribution in Africa are coherent with poultry trade patterns (Cattoli et al. 2009). Hence, although the movement of infected wild birds cannot be ruled out, poultry trade is very likely to have had a role in the introduction of the virus into Africa.

Wild bird migration is considered to have been the main route for the introduction of the virus to Europe (Kilpatrick et al. 2006); however, the first two reports of H5N1 infection in Europe (Alexander 2007b; van den Berg et al. 2008), and a subsequent report in 2007 (Irvine et al. 2007), were associated with trade. Trade, rather than bird migration, is thought to have caused the westerly virus spread across Russia in 2005.

Farm-to-Farm Virus Transmission and Maintenance

Trade is also likely to be an important mechanism by which HPAI can spread from farm to farm (Sims 2007). Five out of seven outbreak waves in Viet Nam occurred around the celebration of the Tet, during which poultry trade activities increase drastically (Pfeiffer et al. 2007; Minh et al. 2009). Traders, or poultry collectors, have been particularly implicated in the farm-to-farm virus spread given that they may move between a potentially large number of farms in the course of a single day (Van Kerkhove et al. 2009), and come into direct contact with birds on each of these. In the absence of effective sanitation and disinfection, traders themselves, their equipment and their vehicles may act as important mechanical transmitters of

infection. Indeed, farms visited by traders were at higher risk of infection in Hong Kong (Kung et al. 2007) and Thailand (Paul et al. 2010a) whilst farms that always used the same trader or who prevented the entry of traders were at lower risk in Bangladesh (Biswas et al. 2009b) and Nigeria (Métras et al. 2009), respectively. In northern Viet Nam, it was observed that traders tended to link communes with similar infection status, suggesting that they may have had a role in the spread of the virus between them (Soares Magalhaes et al. 2010).

LBMs are likely to play a particularly important role in the spread of HPAI. Retail marketing of live poultry was the main source of exposure to infection on chicken farms in Hong Kong (Kung et al. 2007). During the 2008 epidemic in South Korea, the virus was suspected to have spread throughout the country via LBMs and then to poultry farms (Kim et al. 2010). Investigations in Bangladesh identified egg trays and contaminated vehicles from LBMs as the cause of 47% of farm outbreaks (Biswas et al. 2008).

As well as trade activities that allow transmission at a local scale, the movement of poultry and poultry products along major transport routes is also likely to contribute to virus spread. Proximity to major roads, highways or big cities, and density of roads have been identified as risk factors for HPAI H5N1 in China (Fang et al. 2008), Thailand (Paul et al. 2010b), Bangladesh (Loth et al. 2010), Viet Nam (Pfeiffer et al. 2007), Indonesia (Yupiana et al. 2010) and Romania (Ward et al. 2008). In Nigeria, a high proportion of cases were located in proximity to main roads (Rivas et al. 2010). For countries where the road network is poorly developed, most commercial poultry movements occur on a small number of main roads, with LBMs located along these, with big cities, like Phnom Penh in Cambodia (Van Kerkhove et al. 2009), attracting a huge proportion of the commercial flow, and as such a large number of LBMs.

Farms supplying LBMs are typically either backyard or small-scale commercial farms, with a low level of biosecurity (Van Kerkhove et al. 2009; Tiensin et al. 2005; Soares Magalhaes et al. 2007). Importantly, it has been observed in Viet Nam, Bangladesh and Indonesia that farmers facing an outbreak may attempt to sell apparently healthy or even sick poultry in order to minimise economic losses. Such practices will inevitably increase the probability that infected birds will be introduced into the market chain (Biswas et al. 2009a; Yupiana et al. 2010; Phan Dang et al. 2007).

The major impact of live animal markets in virus spread has been described for severe acute respiratory syndrome (SARS) (Guan et al. 2003) and foot and mouth disease (FMD) (Ferguson et al. 2001; Ortiz-Pelaez et al. 2006). Due to the high density of hosts, LBMs offer conditions for virus amplification, re-assortment and cross-species transmission (Webster 2004). The diversity and abundance of LPAI viruses within LBMs has long been known (Shortridge et al. 1977; Senne et al. 1992), and is important in East Asian markets (Guan et al. 2002; Chen et al. 2006, 2009; Amonsin et al. 2008; Choi et al. 2005; Liu et al. 2003; Ge et al. 2009; Lee et al. 2010). H5N1 viruses have also been identified in these markets where they may circulate silently: whilst HPAI outbreaks were not reported in Viet Nam until 2003, H5N1 virus was identified in two LBMs around Ha Noi in 2001 (Nguyen et al. 2005). Moreover, during the H5N1 epidemics which affected

Hong Kong in 1997, the prevalence of the infection in chickens in LBMs reached 19.5% (Shortridge 1999). Since 2003, H5N1 viruses have been isolated from LBMs in both epidemic and endemic areas, such as China (Jiang et al. 2010; Chen et al. 2009), Thailand (Amonsin et al. 2008), Indonesia (Santhia et al. 2009), South Korea (Kang et al. 2009), Bangladesh (Biswas et al. 2008) and Nigeria (Joannis et al. 2008). A survey in Egypt in 2009 found that 12.4% (71/573) of sampled LBMs were infected (Abdelwhab et al. 2010). The environment of 47% markets sampled in 2007–2008 in Indonesia was found to be contaminated (Indriani et al. 2010).

Following multiple outbreaks of HPAI H5N1 in Hong Kong between 1997 and 2003, control strategies were implemented across the LBM chain (Sims et al. 2003a, b; Guan et al. 2007). These interventions appear to have been successful as only one outbreak has been reported on the island since 2003 (ProMED-mail 2008). Among control measures, the provision of rest days that allow markets to be emptied and disinfected, have been associated with a significant decrease in the rate of isolation of LPAI viruses in Hong Kong retail markets (Kung et al. 2003; Lau et al. 2007). Similar observations have been noted in the United States where surveys highlighted that rest days, frequent cleaning and disinfection decrease the risk that the market is positive for LPAI (Bulaga et al. 2003; Garber et al. 2007; Yee et al. 2008; Trock et al. 2008). These observations suggest that the level of infection in markets is not simply the result of multiple introductions of infected birds, but the consequence of virus re-circulation and amplification within them. In Indonesia, the H5N1 virus isolation rate was higher among poultry sampled in LBMs than in farms, also suggesting that the virus may be amplified in the market chain (Santhia et al. 2009).

Thus, LBMs may play a key role in the epidemiology of avian influenza viruses; acting as a network "hub", they may be responsible for sustaining endemic infection within the poultry sector. Poultry that are purchased alive, birds that return unsold, or the movement of people or equipment contaminated with virus at such markets may play an important role in the onward spread of the disease.

Ducks: Silent Viral Vectors and Potential Reservoir

Asymptomatic Infection

It has been noted that the susceptibility of ducks to H5N1 has varied since these viruses first emerged. Until 2002, infected ducks tended to show mild or no clinical signs following infection (Shortridge et al. 1998; Alexander 2000; Perkins et al. 2002). In contrast, H5N1 viruses isolated in Hong Kong in 2002 were pathogenic for wild waterfowl (Ellis et al. 2004; Sturm-Ramirez et al. 2004) whilst new variants that have emerged since 2003 have tended to have lower pathogenicity.

Such viruses are excreted in high titres for extended periods of up to 17 days, often in the absence of clinical signs (Hulse-Post et al. 2005). Indeed, viruses have

been isolated from healthy ducks in Laos (Boltz et al. 2006), and serological surveys in Viet Nam (Takakuwa et al. 2010) found H5 and N1 inhibiting antibodies in unvaccinated birds suggesting that ducks had been infected and survived, although no outbreak was reported. In Thailand in 2005, free-range duck flocks shed viruses for 5–10 days before being culled with few or no disease signs (Songserm et al. 2006a). Moreover, H5N1 viruses were reported to have circulated silently in a commercial duck flock in Germany (Harder et al. 2009).

There appears to be some variability in the pathogenicity of H5N1 viruses for ducks by H5N1 strain (Londt et al. 2008; Saito et al. 2009; Sturm-Ramirez et al. 2005; Tian et al. 2005; Middleton et al. 2007; Vascellari et al. 2007; Bingham et al. 2009). In an experimental setting, Saito et al. (2009) found that the mortality rate could vary from 50 to 75% according to the strain. During outbreaks in South Korea in 2008, the morbidity and mortality rates of an infected duck farm were 60 and 50%, respectively (Kim et al. 2010). In contrast, mortality in waterfowl in Egypt appeared to be lower than 30% (Abdel-Moneim et al. 2009). Even when the infection is lethal, virus shedding tends to persist longer in ducks than in chickens. Saito et al. (2009) showed that the mean death time (MDT) varied between 4.8 and 6.3 days in ducks. Moreover, susceptibility may vary with duck breed (Saito et al. 2009) and age (Londt et al. 2010): infection that was always lethal for 8-week-old ducks was mild in 12-week-old ducks. During the 2003–2004 epidemic in South Korea, morbidity and mortality were lower in adult birds than in younger ones, and infection in a duck breeder farm was only detected due to the identification of symptoms in ducklings (Kwon et al. 2005b). This suggests that long-life duck flocks, such as breeder and layer flocks, are at highest risk of amplifying and silently spreading the virus.

Domestic waterfowl may act as asymptomatic virus carriers, and therefore act as a potential virus reservoir for more susceptible species, such as chickens. The presence of these so-called “Trojan horses” complicates the control of the disease.

Farm-to-Farm Spread and Maintenance

The density of duck flocks in a region has been shown to be a risk factor for infection with HPAI H5N1 (Pfeiffer et al. 2007; Paul et al. 2010b; Gilbert et al. 2006b; Tiensin et al. 2009). At the farm level, the number of ducks present and interactions with ducks from other flocks have been also identified as risk factors for disease (Henning et al. 2009a; Biswas et al. 2009a, b; Paul et al. 2010a). The presence of ponds and water bodies, which may act as an interface between domestic and wild waterfowl and between neighbouring waterfowl flocks, also increases the risk of infection (Biswas et al. 2009a; Ward et al. 2008; Paul et al. 2010a; Fang et al. 2008). Water bodies may therefore act as a meeting point where H5N1 viruses can be transmitted directly or indirectly between ducks from different flocks when they congregate at these places.

With the exception of Thailand, which has restructured its duck raising system since 2004, duck flocks are rarely kept in strict confinement in Southeast Asia (Songserm et al. 2006a; Burgos et al. 2008a, b). Flocks are generally free-ranging or have open access to ponds. Biosecurity measures are difficult to implement in such systems, and the same inadequate biosecurity measures that allow HPAIV H5N1 to enter a flock may allow the virus to spread onwards in the event of an outbreak.

The practice of grazing ducks on rice paddies may be a critical factor in the maintenance and spread of H5N1 viruses in Southeast Asia (Henning et al. 2009a, b; Paul et al. 2010a, b; Pfeiffer et al. 2007; Yupiana et al. 2010; Gilbert et al. 2006b, 2008). Young ducks may scavenge for insects and snails during the rice growing period (Minh et al. 2010), while adult ducks are allowed to scavenge on the fields for periods ranging from 2 weeks to 2 months after harvest (Henning et al. 2009a). This husbandry practice involves the frequent movements of flocks from one field to another (Gilbert et al. 2006a), sometimes over long distances (Songserm et al. 2006a; Minh et al. 2010). Hence, rice paddies offer an opportunity for domestic ducks to infect ducks from other flocks, either through direct contacts or indirectly by contaminating the field. Moreover, rice paddies may also provide an interface with wild bird populations. At night, several duck flocks may be housed in common shelters within villages, which may contribute to virus spread between free-grazing duck flocks, as well as wider spread to village poultry. In Thailand, both the first and second waves of outbreaks affected areas with a high density of free-grazing ducks, but these were followed by outbreaks in high chicken density areas (Songserm et al. 2006a) suggesting free-grazing ducks were involved in the dissemination of the virus to the chicken population. The occurrence of most Vietnamese outbreaks around the Tet festival coincides with an increase in poultry trade, as well as the period during which ducks are brought to rice paddies (Pfeiffer et al. 2007).

Impact of Farming Systems and Practices on Virus Spread

Farming Systems

Some studies have found that high poultry density areas or areas with commercial farms were at lower risk of HPAI outbreaks (Biswas et al. 2009a; Yupiana et al. 2010; Henning et al. 2009b). Indeed, small-scale farms have generally appeared to be more susceptible to infection than large-scale industrial farms, probably due to the fact that larger farms applied better husbandry practices, better biosecurity, and were more likely to vaccinate (Sims et al. 2005).

The high proportion of poultry kept in backyard flocks in H5N1 endemic countries such as Egypt, Viet Nam and Cambodia (Burgos et al. 2008a, b; Hosny et al. 2006) has raised the concern that this type of poultry husbandry may contribute to virus maintenance (Peiris et al. 2007; Iqbal 2009). Backyard

flocks were also suspected to have played a role in the spread of the virus in Nigeria (Joannis et al. 2008).

Backyard flocks are typically maintained as low input systems, and are allowed to range freely for most of the day. Although they are primarily produced for household consumption, backyard birds may be sold to mobile traders or directly at LBMs. Levels of biosecurity in this sector are extremely low, or non-existent, and as such the risk of infection may be very high (FAO et al. 2004). In Egypt, for example, a survey carried out in 2007 found H5N1 viruses in 30% of sampled backyard flocks (Hafez et al. 2010). Moreover, access to veterinary services is often limited in this sector and background disease burdens are often high, even in the absence of HPAI. Hence backyard poultry owners may not recognise HPAI H5N1 as an immediate threat (Cardona et al. 2010) and outbreaks caused by H5N1 viruses may remain unreported to veterinary services and interventions subsequently delayed.

In Laos and Cambodia, where almost all poultry are reared in backyards, the mechanisms contributing to virus maintenance are uncertain. It is thought that the poultry density is too low to enable the virus to be maintained and therefore that the sporadic outbreaks that occur in these countries are a consequence of repeated virus introductions, in particular from southern Viet Nam (Buchy et al. 2009). In Thailand, subdistricts with backyard flocks were at lower risk of infection than subdistricts with commercial flocks (Tiensin et al. 2009).

Small-scale commercial farms are very likely to play an important role in the spread of HPAI within affected areas. Such farms may contain several hundred or even thousands of birds within a single shed. Levels of biosecurity are generally low and contacts with poultry production stakeholders may be numerous (e.g. traders, feed sellers) (Sims et al. 2005). Hence, the risk of virus introduction, amplification and then spread to other farms is high. Most outbreaks notified in Viet Nam from 2004 to 2007 occurred in farms with 50–3,000 birds (Burgos et al. 2008a). Henning et al. (2009b) found that medium poultry density, which probably represents small commercial farms, was associated with an increase in the risk of infection in Viet Nam.

Fighting cocks were associated with a higher risk of infection in Thailand, but this association was weak (Paul et al. 2010b; Gilbert et al. 2006b; Tiensin et al. 2009). The presence of quail flocks in an area has also been identified as a risk factor for HPAI, although the epidemiological significance of this finding is unknown (Tiensin et al. 2009).

Husbandry Practices

Several husbandry practices that are not associated with trade have been identified as risk factors for HPAI disease outbreaks. Owners living off the farm or having visitors entering the premises were associated with higher risk of infection (Henning et al. 2009a; Kung et al. 2007), and highlight the potential role of humans

as mechanical transmitters of virus. Increased risks associated with such indirect contact are also likely to occur through shared equipment, as shown for other avian influenza viruses (Capua et al. 2000, 2007b; Wee et al. 2006; Nishiguchi et al. 2007; Thomas et al. 2005). Likewise, contact with wild animals, rodents and even flies may allow H5N1 to spread from farm to farm (Biswas et al. 2009a, b; Barbazan et al. 2008; Butler 2006; Kuiken et al. 2006; Sawabe et al. 2006). Thai subdistricts with poultry slaughterhouses were also at higher risk, probably due to the regular movement of vehicles and cages to and from slaughterhouses, which may have acted as a virus dissemination point (Tiensin et al. 2009).

Vaccinated flocks had a lower risk of infection in Viet Nam (Henning et al. 2009a). However, in the absence of strict sanitation and disinfection, the movement of vaccinators from farm to farm may actually allow the spread of the virus through mechanical transmission. Indeed, activities associated with the first Vietnamese vaccination campaign may have been responsible for an outbreak wave (Pfeiffer et al. 2007), and vaccinators are likely to play a role in virus dissemination in Egypt (Peyre et al. 2009).

Extension of the Mammalian Host Range

Mammals, Other Than Humans

Although reports of LPAI transmission from birds to mammals are rare, H5N1 viruses have shown a great capacity for xenospecific transmission (Reperant et al. 2009). Among carnivores, various field species have been infected, including tigers (*Panthera tigris*) and leopards (*Panthera pardus*) in Thailand (Keawcharoen et al. 2004) and probably in Cambodia (Desvaux et al. 2009). The susceptibility of domestic dogs and cats has been highlighted by isolation of viruses (Songserm et al. 2006b–c; Leschnik et al. 2007), as well as through serological surveys (Butler 2006). Other carnivore species are also susceptible (Reperant et al. 2009). The role any mammalian species has in the epidemiology of this disease is uncertain. Cats coming into contact with domestic birds, and potentially their droppings, may develop severe disease and excrete virus from the respiratory and digestive tracts (Kuiken et al. 2006). Hence, as well as allowing transmission of H5N1 between cats (Kuiken et al. 2004; Ayyalasomayajula et al. 2008; Rimmelzwaan et al. 2006), such active secretion might suggest a role for these animals in the spread of disease between domestic poultry, although this remains highly speculative (Kuiken et al. 2006).

Although rodents are likely to be exposed to the H5N1 virus through contact with poultry, no cases of natural infection have been reported so far. Mice inoculated experimentally are susceptible and widely used as a model for human infection; however, experimentally inoculated rats appeared to be resistant to the infection (Perkins and Swayne 2003).

Pigs are susceptible to both human and avian influenza viruses and may therefore act as a "mixing vessel" (Ito et al. 1998) for the generation of pandemic viruses through re-assortment, as was recently observed with the emergence of the H1N1 virus (Smith et al. 2009). Surveys carried out in Viet Nam (Choi et al. 2004) suggested that pig susceptibility to H5N1 viruses is low, and this has been confirmed through experimental infection (Lipatov et al. 2008).

Humans are the only primates for which outbreaks have been reported. Macaques have been infected experimentally to serve as a primate model and were found to be susceptible (Rimmelzwaan et al. 2001, 2003).

Humans

To our knowledge, only three HPAIV subtypes have been transmitted to humans: one individual was found infected by the Canadian H7N3 isolate in 2003; in The Netherlands, 89 people were infected by H7N7 viruses, of which one person died (Katz et al. 2009); and as of 31 August 2010, 505 humans have been found to be infected with H5N1, of which 300 have died (WHO 2010). Transmission of H5N1 viruses to humans was first identified in 1997 when a 3-year-old boy died in Hong Kong (Claas et al. 1998). In the following months, 17 additional human cases were reported (Sims et al. 2003a). Viet Nam, Indonesia and Egypt have 79% of all human cases reported to the World Health Organisation. Although some human cases may be the result of human-to-human transmission, especially between family members (Kandun et al. 2006; Gilsdorf et al. 2006; Olsen et al. 2005; Ungchusak et al. 2005; Brankston et al. 2007), this transmission route appears to be very rare. The majority of human cases are thought to have arisen from direct or indirect contact with infected poultry or their products (Wang et al. 2008a; Gambotto et al. 2008).

Pandemic viruses that arose in the last century have all shown an avian origin. Indeed, the H1N1 strain responsible for the 1918–1919 pandemic which caused between 40 and 50 million human deaths (Webster et al. 1992) was entirely derived from an avian virus that adapted to humans. The 1957 and 1968 pandemics, caused by H2N2 and H3N2 viruses, respectively, resulted from re-assortment between viruses of human and avian origins (Kawaoka et al. 1989), and the 2009 H1N1 pandemic was caused by the re-assortment between human, avian and swine viruses (Smith et al. 2009). Moreover, recent re-assortants between H5N1 and human H3N2 viruses have shown high virulence and demonstrated the potential for H5N1 viruses to recombine with strains circulating in the human population (Li et al. 2010b). Therefore, although H5N1 viruses do not seem to transmit easily to humans, the continuing circulation of H5N1 viruses in the poultry population and its high case fatality rate in humans still raises a great concern about the potential emergence of a highly lethal pandemic strain.

Conclusion

Since its emergence in 1996, HPAIV H5N1 has spread across three continents. This sporadic, large-scale spread appears to originate from a small number of areas of virus persistence, particularly in Asia. Such pockets of infection exist within specific agro-ecological niches in which virus persistence and intense genetic diversification allows the continuous emergence of new strains. Trade of live birds and poultry products is likely to be a major pathway for virus dissemination within and beyond these endemic areas, whilst LBMs are likely to contribute to virus persistence. Duck rearing and associated practices create conditions for undetected virus amplification within flocks, and the high level of contacts between duck flocks facilitates the spread of the virus. Moreover, although they are likely to play only a limited role in disease dynamics, wild birds can potentially take part in virus dissemination at both a local and a continental scale.

Due to the multiple features that allow HPAI viruses to persist in some agro-ecosystems, the design of control strategies needs to take into account the local epidemiological patterns and characteristics of these production systems. These policies need to target the pocket of infection as a whole, and thus have to be coordinated at a regional level, as agro-ecosystems generally overlap several countries. Otherwise, attempts to control the virus circulation are likely to fail. This is particularly important as the continuous emergence of new HPAIV strains, and their co-circulation with swine and human influenza viruses, is a major concern for public health.

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