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Review

Recent expansion of highly pathogenic avian influenza H5N1: a critical review

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Wild birds, particularly waterfowl, are a key element of the viral ecology of avian influenza. Highly pathogenic avian influenza (HPAI) virus, subtype H5N1, was first detected in poultry in November 1996 in southeast China, where it originated. The virus subsequently dispersed throughout most of Asia, and also to Africa and Europe. Despite compelling evidence that the virus has been dispersed widely via human activities that include farming, and marketing of poultry, migratory birds have been widely considered to be the primary source of its global dispersal. Here we present a critical examination of the arguments both for and against the role of migratory birds in the global dispersal of HPAI H5N1. We conclude that, whilst wild birds undoubtedly contribute to the local spread of the virus in the wild, human commercial activities, particularly those associated with poultry, are the major factors that have determined its global dispersal.

The highly pathogenic avian influenza H5N1 (HPAI H5N1) is an influenza A virus. Influenza A viruses are widespread in the animal kingdom, occurring mainly in birds, humans, horses, pigs and sometimes in cetaceans and mustelids. These viruses differ markedly genetically according to their hosts and their geographical origin. Subtypes are defined on the basis of the antigenicity of the haemagglutinin and neuraminidase proteins (Webster *et al.* 1992). The haemagglutinin allows the virus to attach to the surface of a cell, while the neuraminidase allows the virus to be released. Sixteen subtypes of haemagglutinin (H1–H16) and nine subtypes of neuraminidase (N1–N9) have been described (Fouchier *et al.* 2005). In the case of human flu, the haemagglutinins H1, H2 and H3, and neuraminidases N1 and N2 circulate, or circulated, through human populations naturally by evolving their own lineages (Manuguerra 2001). H5, H7 and H9 have also been observed in humans although in these cases humans were not the initial targets. These viruses crossed the species barrier but did not become established in human populations. In contrast, the diversity of influenza A viruses is greater in birds. All 16 haemagglutinins and nine neuraminidases have been isolated from birds (Fouchier *et al.* 2005, Olsen *et al.* 2006), although the subtype H16 has been described only very recently (Fouchier *et al.* 2005, Olsen *et al.* 2006). There are many genotypes of avian influenza viruses (AIV) varying geographically and between years, as well as between species and populations of birds. In most cases, AIV are not contagious to humans, but are actively evolving, and virulence for the animal host varies markedly. Surface antigenic proteins undergo two types of evolution, drift and shift. Shift induces major changes by replacement of gene segments (Webster 1998).

AIV have been isolated in wild birds worldwide, underlining the importance of wild birds in viral epidemiology (Alexander 2000). The role of birds in the dispersal of AIV has been well established for many years, and they are the central element of the viral ecology of avian influenza. Every year in Europe, AIV circulate among domestic and wild birds, just as does the virus of human flu in human populations. AIV have been found in at least 12 orders of birds, including ducks, passerines, waders, gulls, terns, pheasants, and falcons (Stallknecht & Shane 1988,

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Alexander 2000). The majority of species concerned live in aquatic environments. Anseriforms (ducks, geese and swans) have a particularly wide variety of subtypes of viruses (Deibel *et al.* 1985, Webster *et al.* 1992, Alexander 2000). All subtypes have been found in wild ducks and geese, with the exception of H13 and H16, described only in gulls. Among waterbirds, the Mallard *Anas platyrhynchos* is particularly important because virtually all the subtypes have been isolated from it (Munster *et al.* 2005). A study conducted in Italy, from 1992 to 1998, isolated 22 AI subtypes, the subtype H1N1 being the most frequent (De Marco *et al.* 2003, 2004). Fifty per cent of ducks carried antibodies against AIV, which means they had been in contact with the viruses at some time during the previous months or years. Waterbirds are so readily infected by AIV because their environment provides an ideal mode of viral dispersal. The AIV are particularly abundant in the final part of the digestive tract, which explains why they are found mainly in the faeces (Webster *et al.* 1992), and they can remain alive in water for long periods (Ito *et al.* 1995), from few days in water at 35 °C to a month at 4 °C (Stallknecht *et al.* 1990). Faeces spread in the water and viruses can be transmitted to other waterbirds (Webster *et al.* 1992). In a recent study, however, experimental inoculation of HPAI H5N1 of domestic Mallards showed that the digestive tract was not the main site of replication for this subtype; viruses replicated rapidly in the trachea, suggesting an oral transmission path (Sturm-Ramirez *et al.* 2005).

It has been shown in wild ducks in North America that co-infections by two different subtypes of virus circulating simultaneously in the body of the animal were frequent (Sharp *et al.* 1997, Hatchette *et al.* 2004). This phenomenon increases the possibility of genetic re-assortment and, consequently, the emergence of new viral subtypes. This phenomenon has already been described in waders in North America, wild ducks in Japan and Common Guillemots *Uria aalge* in Sweden (Makarova *et al.* 1999, Liu *et al.* 2004, Wallensten *et al.* 2005). These studies involved exchanges of genes between American and European viral lineages, which, though rare, probably happen occasionally where the migratory paths of American and European birds cross.

Knowledge of the epidemiology of AIV in wild birds is still lacking. AIV evolve more slowly than the human Influenza A, perhaps because they are in a state of more stable coevolutionary balance with wild birds than is the case with human Influenza A with humans (Manuguerra 2001). We have little information on the specificity of AIV in relation to bird species, whether there is competition between virus subtypes, if there are seasonal peaks of infection, or if temperate regions of Europe have their own endemic subtypes or are colonized anew every spring by African subtypes, or every autumn by Siberian subtypes. In America, shorebirds mainly carry the viruses north during the spring migration while ducks carry the viruses south during the autumn (Olsen *et al.* 2006). Genetic analysis of viruses from ducks and shorebirds also suggests that their viral genes pools are not separated (Olsen *et al.* 2006).

THE SYMPTOMS OF AVIAN INFLUENZA

Every host species interacts and evolves with its own parasites, including phases of crisis during which the host may have difficulties resisting the parasite and may develop sometimes fatal diseases. In the case of AIV, two phenotypes of virulence have been described, a high virulence and a low virulence. The low pathogenic (LP) subtypes are asymptomatic or lead to benign respiratory symptoms. The highly pathogenic (HP) subtypes are responsible for high levels of mortality in poultry, the so-called 'fowl plague'. At the time of writing, only subtypes H5 and H7 have been shown to be responsible for HP phenotypes (Alexander 2000). The majority of H5 and H7 subtypes are LP but have the potential to become HP. This shift from LP to HP subtypes is achieved by the introduction of basic amino acids into the cleavage site of the polypeptide precursor of the haemagglutinin (Alexander 2000, Olsen *et al.* 2006). During the last 50 years, 25 disease outbreaks due to HP subtypes have been identified in birds, primarily from poultry (Alexander 2000). These outbreaks have generally remained localized in limited geographical areas, except more recently in the United States in 1983, in Mexico in 1994, in Pakistan in 1995 and, of course, since 2003 in Asia, where large epizootics occurred, causing the death of a considerable number of domestic birds and important economic losses. In Europe, Italy was affected in 1997 by a HP H5N2 (Capua *et al.* 1999) and in 1999 by the subtypes HP H7N1 and HP H7N3 (Capua *et al.* 2002, Stegeman *et al.* 2004). Having remained free for 75 years, the Netherlands was affected by a HP H7N7 subtype in February 2003 (Elbers *et al.* 2004).

HP subtypes have rarely been isolated in wild birds. The majority of the described cases concerning wild birds were in those living near infected chickens, the transmission being from domestic birds to wild birds. This was the case of the HPAI H5N1 subtype, which evolved at first in poultry for several years. Before the

Asian HPAI H5N1 outbreak, there had been no evidence of the presence of HPAI in wild birds apart from a Common Tern *Sterna hirundo* in Africa and a few birds found dead near outbreaks in poultry, despite numerous surveys over many years. This is in keeping with the theory that mutation from LPAI to HPAI takes place in gallinaceous poultry species. It is, however, very likely that HP subtypes appear regularly in wild individuals. The fact that epizootics are not detected in the wild populations results probably from an ecology unfavourable to the HP subtypes of the viruses, in particular lower densities of hosts and stronger competition with other LP viruses. This low virulence of subtypes in wild birds is doubtless the consequence of a long coevolution, allowing the viruses to remain in their hosts without destroying them.

Current theories are that the mutation from LP to HP appeared after the introduction in domestic birds. Wild birds constitute a permanent source of gene fragments of LP subtypes, which are sometimes transmitted to domestic birds. How the virus subtypes in domestic populations then evolve depends on poultry rearing practices. When bird densities are low, a very virulent subtype leading to high host mortality may disappear because of the impossibility of transmitting quickly to healthy birds before the death of sick ones. In Asia, densities of domestic birds are especially high. These ecological conditions favour the preservation and the fast transmission of very virulent strains. This scenario seems to apply, for example, to the epizootic of the H5N2 subtype, in 1994, in Mexico (Horimoto *et al*. 1995). LP H5N2 subtypes circulate at present in Europe, simultaneously in wild birds and poultry. They do not raise economic problems as long as they do not evolve towards more virulent phenotypes. During an epizootic of a HP subtype, the situation becomes even more difficult for poultry when the virus acquires the capacity to remain in certain birds without activating symptoms. These healthy carriers can then spread the virus over long periods and infect a large number of birds without being discovered. This is the current situation for the HPAI H5N1 subtype in Asia, where certain domestic ducks are healthy carriers and are not detected, while chickens or turkeys continue to suffer high mortality (Li *et al*. 2004, Sturm-Ramirez *et al*. 2005).

THE ORIGIN OF THE HIGHLY PATHOGENIC H5N1 SUBTYPE

HPAI H5N1 evolved after the end of 1996, at least in populations of domestic birds. Although not detected again for several years, it started to spread again from 2003. It is now endemic in poultry farms in southeast Asia (Chen *et al*. 2006). This subtype thus arises from domestic farms where conditions allow it to remain and propagate. Wild birds contacted this subtype only later.

The term H5N1 in reality covers numerous different genotypes which are continually evolving and whose virulence varies. In autumn 2005, a LP H5N1 subtype was discovered in Italy in a wild duck (ProMED 2005a). HPAI H5N1 subtypes circulating at present from Asia to Europe are very contagious and cause high mortality in poultry. Their peculiarity is that they are sometimes able to cross the species barrier and lead to mortality in mammals, notably humans. Domestic carnivores have died probably after ingestion of contaminated birds. Cases were reported in cats as early as 2004 in Thailand (Tiensin *et al*. 2005), but also by experimental inoculation (Kuiken *et al*. 2004), before new cases were detected in Germany and Austria at the beginning of 2006. A study conducted in Thailand in an infected zone showed that 25% of dogs and 7% of cats carried antibodies indicating that they had been infected (Butler 2006a). Wild carnivores can also be infected; the Owston's Civet *Chrotogale owstoni*, a globally threatened viverrid, in Viet Nam (Roberton *et al*. 2006), a Marten *Martes foina* in Germany on the island of Rügen and a mink *Mustela* sp. in Sweden (WHO 2006) have all been shown to have died as a result of AIV infection. The virus has also been passed to wild mammals in captivity, including Ferret *Mustela putorius furo*, Tiger *Panthera tigris* and Leopard *Panthera pardus* (Thanawongnuwech *et al*. 2005, WHO 2006). In October 2004, infection of tigers in Srinacha zoo in the east of Thailand, apparently by ingestion of contaminated chicken meat (tigers were fed whole carcasses of infected chickens), led to the euthanasia of 147 tigers (Tiensin *et al*. 2005). A current hypothesis is that cats can transmit the virus to other cats (Kuiken *et al*. 2004, 2006), but there is still doubt as to the existence of this transmission route in the wild. This is, however, of great importance because it suggests that the virus may now be able to pass from one mammal to another. The HPAI H5N1 subtype is not the only subtype that can be transmitted from birds to mammals. Harbour Seals *Phoca vitulina* died as a result of an H7N7 subtype in the United States in 1980 (Lang *et al*. 1981) and farmed mink as a result of an H10N4 subtype in Sweden in 1984 (Englund 2000). Cases of transmission from domestic birds to humans have been reported for H7N7, H7N2, H7N3

and H9N2 (Lin *et al*. 2000). In 2003 in Holland, the H7N7 subtype caused the death of a veterinarian and several dozen cases of conjunctivitis. In Hong Kong in 1999, two children infected by the H9N2 subtype developed respiratory disorders (Lin *et al*. 2000). It is possible that LP AIV are regularly transmitted to humans who are frequently in contact with birds. In Europe, millions of wild birds are shot, cooked and consumed by hunters and their dogs every year. A recent study in Iowa, United States, showed transmission of H11N9 virus from wild birds to hunters and wildlife professionals (Gill *et al*. 2006).

There are several hypotheses, based on genetic analyses, to explain the origin of the HPAI H5N1 subtype. The progenitor HPAI H5N1 was discovered in 1996 in domestic geese in the province of Guangdong, southern China (Chen *et al*. 2006). The lack of precise and long-term studies in domestic and wild birds does not allow us to reconstitute exactly the phylogenetic origin of this subtype. It seems to have come from certain ancestral segments of LP subtypes from wild birds. It shows segments of genes similar to those found in Hong Kong in an H9N2 subtype of the Japanese Quail *Coturnix coturnix japonica*, and in an H6N1 subtype of a Green-winged Teal *Anas carolinenis* (Webster *et al*. 2006). This last subtype itself shows similarities to an H6N5 subtype isolated in a shearwater in Australia in 1973 (Hoffmann *et al*. 2000). The domestic goose virus would have been dispersed from the region of Guangdong to Hong Kong through the commercial movements of poultry. Domestic chickens would then have become a new host in 1997. From the year 2000, it seems that domestic ducks frequently became hosts of the virus without expressing the associated disease. An experiment shows that domestic ducks can excrete the virus for more than 2 weeks (Li *et al*. 2004). This would have allowed the virus to extend, thanks to trade, over a vast zone without being discovered. This geographical expansion ended in the genetic differentiation of the virus (Chen *et al*. 2006). In 2001, seven different genotypes were identified in poultry in Hong Kong and in the province of the Guangdong, and in five other provinces in 2002. The major epizootic started between December 2003 and January 2004 in chickens (more susceptible than domestic ducks), with episodes being reported almost simultaneously in eight countries in southeast Asia. This outbreak was not due to a unique genotype, but to multiple genotypes, which had gradually diverged genetically. The geographical extension and the genetic evolution of the virus since 1996 had probably taken place without any link with the wild birds.

THE HPAI H5N1 SUBTYPE IN WILD BIRDS

Until 2005, the number of cases of wild birds reported as having contracted the HPAI H5N1 was still small compared with the geographical distribution of the virus (Chen *et al*. 2006). In spite of searches for the virus in wild birds in Asia in 2003 and 2004, very few individuals were found to be positive. In 2004, in Hong Kong, the sampling of 2200 wild birds showed only negative results (Sabirovic *et al*. 2005). The only wild birds in Asia found sick were victims of the virus circulating in domestic birds (FAO 2005). Sporadic cases were identified. In South Korea, for example, three Magpies *Pica pica* were found dead in March 2004, near a farm with infected chickens (Kwon *et al*. 2005). In Hong Kong, the virus was found in Tree Sparrow *Passer montanus*, Peregrine Falcon *Falco peregrinus* and Grey Heron *Ardea cinerea* (FAO 2005, Kou *et al*. 2005). Wild species held in captivity were also affected, as in a zoo in Cambodia (FAO 2005). On 18 October 2004, two smuggled Thai Eagles *Spizaetus nipalensis* were seized at customs at Brussels international airport after a flight from Bangkok (Van Borm *et al*. 2005). The birds were found to be infected with the HPAI H5N1 subtype. The cause of their infection is not known, but it is likely that they had been fed with carcasses of infected chickens. Overall, HPAI H5N1 is recorded as having caused mortality in more than 60 species of wild birds (Ellis *et al*. 2004, Olsen *et al*. 2006).

Migratory birds had first been claimed as the primary cause of the spread of this virus in Asia in 2004 (Bangkok Post 2004). In the *New York Times*, a spokesman for the World Health Organization stated that 'Migratory birds are what carry the diseases' (Bradsher 2004). The assessment of the situation concerning wild birds brought the World Organization for Animal Health (OIE) and the Food and Agriculture Organization of the United Nations (FAO) to conclude, in their report of the conference of Paris of 7 and 8 April 2005, that only localized infections, and these limited to resident wild birds, were proved and that the dispersal of the virus by migratory birds was not established. But this perception changed quickly during the ensuing weeks. From the middle of 2005, in Russia and in Europe, migratory wild birds were again thought to be the main cause of the dispersal of HPAI H5N1 outside Asia. This claim was based on the discovery in May 2005 that hundreds of wild birds had died on Lake Quinghaihu, in the province of Qinghai, on the high Asian plateau (Chen *et al*. 2005, Liu *et al*. 2005). In July 2005, the

virus crossed the Russian border. According to official Russian reports, wild birds were discovered to have died from July onwards along the expansion route of the virus. In December 2005, the assessment for Russia stated that 62 sites of infection had been recorded, distributed across ten regions (OIE 2006a). In only two of these sites were important mortalities of wild birds mentioned, totalling 840 birds, mainly swans. These cases were reported late in the year, at the end of November in the Astrakhan region and in mid-December in the Kalmykia republic. In October 2005, 137 swans died in the Tulcea region, in Romania. In Turkey, at the end of January 2006, only two pigeons, one Turtle Dove *Streptopelia turtur*, a cormorant *Phalacrocorax* sp. and a wild duck were reported to have died in the infected zones. The most westerly point was reached at the end of October in Croatia, where swans were found dead. However, no species of wild bird is known to migrate routinely from China to Croatia. During this phase of expansion of the virus westward, official reports systematically indicated that the mode of distribution of the virus must be by way of migratory wild birds (OIE 2006a).

New massive sampling campaigns of wild birds took place in Asia in 2005 and 2006. In Hong Kong, only a Chinese Squacco Heron *Ardeola bacchus* was detected carrying the virus in 2005 among 9000 wild birds sampled. At the beginning of 2006, surveillance of dead birds discovered a Magpie Robin *Copsychus saularis* carrying the virus, among 1600 analyses, confirming the rarity of the virus in wild birds (OIE 2006a). A study based on samples taken from 13 100 'migratory ducks' and 'migratory geese' between December 2002 and March 2005 in the zone infected by the virus in eastern China showed that, until 2004, none of these wild birds was infected by HPAI H5N1 (Chen *et al*. 2006). The next winter, between January and March 2005, of 4674 'apparently healthy migratory ducks', six (0.1%) were carrying the HPAI H5N1. Three species of ducks were caught – Mallard, Falcated Teal *Anas falcata* and Spot-billed Duck *Anas poecilorhyncha* – but the authors do not indicate which species were infected. The Falcated Teal is a migrant on the coast of China. Part of the Spot-billed Duck population is sedentary, and some of the Mallards have a domestic origin. The data in this study could provide the strongest evidence for dispersal by migratory birds, but lack of precision in identifying the species infected prevents any conclusions being drawn (Feare & Yasué 2006). Yasué *et al*. (2006) highlighted this frequent problem. Essential information on the sampling methodology and wild bird population is often missing while laboratory methods are reported in great detail. A lack of better ecological data can lead to unwarranted assumptions and conclusions that affect public perception and management measures.

By 31 January 2006, none of the sampling campaigns in the European Union (EU) had detected HPAI H5N1, either in wild or in domestic birds. Nevertheless, millions of birds wintering in the EU crossed contaminated zones in Russia during the autumn. From February 2006, the virus began to spread further west in Europe, affecting numerous EU countries. This time, the great majority of reported cases concerned dead wild birds. Most belonged to resident waterbird species frequenting relatively deep water, including Mute Swan *Cygnus olor*, Great Crested Grebes *Podiceps cristatus* and Grey Herons. In France and in Scandinavia, Common Pochard *Aythya ferina* and Tufted Duck *A. fuligula* were also discovered. The majority of individuals of these two species are migrants. The species in which the virus was most frequently discovered was the Mute Swan, as in France, Italy, Poland, Hungary and Croatia. This species is generally sedentary in western Europe but migratory further east. Dead Whooper Swans *Cygnus cygnus* were found in Germany, Denmark and Scotland. Bird predators or scavengers were also listed among the victims: Peregrine Falcon, Goshawk *Accipiter gentilis* and Common Buzzard *Buteo buteo*. So too were mammals that predate birds: domestic cats on the island of Rügen in Germany and in Austria, and a marten in Germany. This expansion of the disease was caused by hard-weather movements of wild birds during a period of freezing winter conditions around the Black Sea.

Even though investigations in Europe have not yet detected the existence of healthy wild birds carrying the HPAI H5N1, their existence is highly likely, particularly in wild ducks of the genus *Anas*. The virus has by now circulated for several years and is endemic in a large part of southeast Asia (Chen *et al*. 2006). The number of domestic breeds infected by the virus as well as their wide geographical distribution increases the probability of contact between wild birds and the virus. However, it is intriguing that the number of wild birds contaminated by the virus seems so small, and that the virus apparently passes from domestic birds to wild birds only with difficulty. In 2004, it was demonstrated that domestic Mallard ducks, which belong to the genus *Anas*, could be healthy carriers of the virus and play a central role in

the production and the preservation of HPAI H5N1 (Li *et al.* 2004, Sturm-Ramirez *et al.* 2005). There is no reason why wild Mallards should not possess the same capacities. The official reports, on cases of mortality by HPAI H5N1 in wild birds, often lack precision concerning the infected species. In fact, very few wild ducks of the genus *Anas* have been found dead, even though they are the most abundant waterbirds in winter, whilst among the other Anatidae, ducks of the genus *Aythya* such as Common Pochard, Tufted Duck and Greater Scaup *A. marila* have been reported repeatedly, as well as geese and swans. Despite tens of thousands of faecal samples obtained from live *Anas* ducks in Europe or Africa, the virus has not been found. It seems that the faecal–oral route is not the main transmission path of HPAI H5N1 (Sturm-Ramirez *et al.* 2005), and the lack of tracheal sampling may explain the lack of detection in wild birds.

THE HYPOTHESIS OF DISPERSAL BY MIGRATING BIRDS

At the beginning of 2005, the virus was still confined to southeast Asia. Cases were reported from Indonesia to China, including Cambodia, Thailand and Vietnam. On the other hand, Pakistan, India, Bangladesh and Burma were not infected. In spring 2005, wild birds were found dead from the virus in the centre of China, in the zone of Lake Quinghaihu (Chen *et al.* 2005, Liu *et al.* 2005). The birds were mainly Bar-headed Geese *Anser indicus*, Brown-headed Gulls *Larus brunnicephalus* and Great Black-headed Gulls *Larus ichthyaetus*. The OIE indicated that the first case was detected on 4 May, and deduced a primary infection by 15 April (OIE 2006a). The above three species reproduce in spring and in summer on the high plateau of Central Asia, in China and Mongolia, and spend the winter further south, in India and on coasts and lakes of South Asia. The Bar-headed Geese winter in India, Bangladesh, Nepal and Pakistan from October, and depart northwards at the end of March. In April 2005, these birds thus migrated from the uninfected south to Central Asia, and it is seems extremely unlikely that they carried the virus to Lake Quinghaihu. However, by February 2004, the virus was well established in China around an east–west major highway, from Shanghai on the east coast to the city of Urumqi in the province of Xinjiang in the northwest, by way of Liujlapu, Jinguyan, and Zhonghe (FAO 2006a). Quinghaihu Lake is situated to the west of Zhonghe. In spring 2005, migratory birds thus arrived in this area, which had already been infected for more than a year. If Bar-headed Geese brought the virus to Lake Quinghaihu during their spring migration, it is necessary to postulate that they contracted the virus during 2004 in China, and would thus have been likely to contaminate India during the autumn or winter of 2004/05. It thus seems far more likely that these migratory birds were the victims of the H5N1 by arriving in spring 2005 on breeding areas, which were already infected by the virus. The wild bird mortality at Quinghaihu was an important basis for claims of the role of migratory birds in spreading H5N1 because the lake was considered as remote. However, a year later in May 2006, it was revealed that the main species affected, the Bar-headed Goose, was artificially reared near the lake, raising the possibility that farmed birds were the source of the outbreak (Butler 2006b).

If migratory wildfowl were a key agent of the dispersal of H5N1, then it seems likely that the spring migrations of 2004 and 2005 would have infected large areas of central Asia, eastern Russia and Siberia. Similarly, autumn migration in 2005 of birds that had nested in Siberia and central Asia would have spread the virus further to India, Pakistan, Bangladesh, the Middle East, east Africa, Australia and New Zealand. For example, Bar-headed Geese, Brown-headed Gulls and Great Black-headed Gull, the species found dead in China, all have India as a key wintering area.

In summer Siberia hosts breeding birds which have wintered in Europe, Africa, the Middle East, India, southeast Asia, Australia and North America, in particular passerines, waders, ducks and geese. So, in summer 2005 Siberia should have been a place of exchange of the HPAI H5N1 virus if migratory birds were a key agent of dispersal. During autumn 2005, birds migrating south would in turn have dispersed the virus back to the areas of winter origin of these species. During their migrations, wildfowl would also be likely to contaminate stopover areas en route. A new wave of contamination, on a wide front from Europe to Central Asia, would then have begun in spring 2006.

THE HYPOTHESIS OF DISPERSAL BY HUMAN ACTIVITY

During the previous epizootics of HP subtypes of H5 and H7, it was shown that the expansion of these viruses was due to human activities, in particular movements of poultry or their products (Webster 1998, Alexander 2000). Does this same mechanism explain the dispersal of HPAI H5N1?

Figure 1. Map showing the trans-Siberian railways.

The first virus entered the EU in October 2004 in two illegally imported Thai Eagles (Van Borm *et al*. 2005). On 16 September 2005, a similar case occurred. A Pionus Parrot *Pionus* sp. native to Surinam, kept in quarantine with Mesias *Leiothrix* sp. officially stated to be from Taiwan, was found dead and declared infected by the virus. It appeared later that Mesias, and not the parrot, were infected by the virus, but the samples had been mixed by the UK authorities (Defra 2005). Nevertheless, Taiwan was free of the virus at this time. Against these detected cases, how many may have been imported and gone unnoticed? Millions of wild birds (Broad *et al*. 2003), mostly passerines for aviculture, are imported into Europe both legally and illegally from Asia. For example on 20 October 2005, the Taiwanese authorities discovered 1000 exotic birds contaminated by the virus HPAI H5N1 in a container forwarded illegally for China (ProMED 2005b).

The virus started its westward expansion across Eurasia in July 2005 in Novosibirsk, Russia. The westward road and railway links from areas of infection in China provide the most obvious routes for the initial spread of the virus if the main agent of dispersal was human movement of birds. Novosibirsk is a road and railroad hub connected to Mongolia, China and Kazakhstan. The trans-Siberian railway passes through this city and continues to the west, passing Omsk (Fig. 1). The northern route passes Tiumen and continues to Moscow. The southern route through the Urals passes Kurgan and Tcheliabinsk, by Samara and to the south of Moscow towards Tambov and Tula. From Samara, major main highways run southwards to Volgograd and Astrakhan, and westwards to Rostov and Crimea. The chronology and the routes of the expansion of the HPAI H5N1 follow exactly this trajectory. The first diagnostic dates by administrative regions of Russia are on 22 July in the region of Novosibirsk, on 23 July in the Territory of the Altai, on 27 July in the region of Omsk, on 28 July in the region of Tiumen, on 4 August in the region of Kurgan, on 13 August in the region of Tcheliabinsk, on 19 October in the region of Tula, on 24 October in the region of Tambov, on 22 November in the region of Astrakhan, and on 12 December in the republic of Kalmykia along the Caspian Sea (OIE 2006a). In the hypothesis of an expansion by human activities, it was predictable that the virus would continue on this trajectory. If we assume spread along major human transport routes and continue according to the axes of virus dispersal in the year 2005, the virus would spread from Turkey and Russia southwards into Armenia, Azerbaijan, Iran, Iraq, Syria, Egypt and eastwards into Africa, and westwards to Poland, Byelorussia, the Balkans, and then into western Europe. From western China the virus would spread to Tajikistan, Afghanistan, Pakistan, Nepal and India, and from Thailand towards Burma, Bangladesh and India. In this scenario, the virus is most likely to be stopped, or at least slowed, at the borders of countries with stricter controls by customs and more organized veterinarian services, as is the case in the EU. Export of the virus may not only occur via movements of live

poultry. The transport of droppings or waste to fertilize fish farms in Asia, eastern Europe and Africa could also be a major route of dispersal (Melville & Shortridge 2006, Feare 2007). In the Qinghai Province, the region of the wild bird outbreaks in May 2005, such fisheries have been developed by the United Nations Development Programme and the FAO (FAO 1990).

Migratory birds do not recognize borders, yet the virus remained restricted to China and southeast Asia for some years. The virus was in northwest China from the beginning of 2004. It spread quickly towards Europe once the Russian border was crossed. In January 2006, the virus was detected in the impoverished Kurdish regions of Anatolia in eastern Turkey, but subsequent epidemiological inquiries showed that the virus was already very widespread in Turkey, in particular along the main highway connecting Ankara to Anatolia. Trade in low-value poultry, collected by truck and resold throughout Turkey to low-income farmers, allowed the virus to spread quickly (Rosenthal 2006). The announcement of the presence of the virus in Nigeria, on 6 February 2006 (the first infection would have been roughly 1 month before, on about 10 January), further reinforced belief that migratory birds were the main agents of dispersal for the virus. The fact that the outbreak was very remote from European and Asian outbreaks and did not fit a progressive dispersal of the virus strengthened this view. However, the outbreak proved to originate on an industrial farm of 46 000 laying hens in Jaji in the State of Kaduna, hundreds of kilometres from wetlands with wintering wildfowl. This first African outbreak was situated in one of the zones of most extensive poultry industry in Africa and thus, on the commercial hypothesis of viral dispersal, one of the zones most at risk. The Nigerian Minister of Agriculture declared that the commercial trade had probably brought the virus because, in spite of bans on such movements, the importation of poultry from contaminated zones had continued throughout 2005 (Birdlife International 2006). Commercial aviculture requires chicks that are just a few days old, but Nigeria has to import them because it does not have either adequate temperatures or technologies to produce these for itself. A recent study showed that the HPAI H5N1 was introduced into Nigeria on at least three separate occasions (CIDRAP 2006, Ducatez *et al.* 2006), and although it did not draw conclusions about the means of introduction, if migratory birds were the source, it seems surprising that other wintering areas of European and Asian wildfowl in Africa were not contaminated.

A scenario of virus expansion identical to that in southeast Asia in 2004 now ensued in Africa. Thanks to the poultry trade, the virus quickly spread throughout Nigeria and reached adjacent countries, on 13 February in the south of Niger, on 21 February in the north of Cameroon in domestic duck farms and in March in guineafowl *Numida* sp. farms in central Burkina Faso.

This commercial scenario is the one that explained the expansion and the maintenance of the virus in southeast Asia until 2004, via the legal and illegal trade in poultry (Gilbert *et al.* 2006a). In their study conducted in the east of China, Chen *et al.* (2006) isolated the virus in 0.1% of wild ducks and geese and 1.8% of domestic ducks and geese. Phylogenetic studies allowed the authors to show that southern China was the epicentre of the disease, from where the virus was repeatedly introduced into neighbouring countries such as Viet Nam (Nguyen *et al.* 2005), by way of the poultry trade, thus creating new genetic lineages of the virus, which are co-circulating now (Chen *et al.* 2006). Even long-distance dispersion of the HPAI H5N1 by live and dead poultry has been highlighted (Melville & Shortridge 2006). It was introduced from Lanzhou into Lhasa in Tibet, 1500 km away, by live chickens. Duck meat infected by the virus has been discovered in Korea and Japan imported from China. Neither is Europe protected from illegal exchanges. On 11 January 2006, the German Minister of Agriculture imposed strict customs controls, notably on goods coming from infected countries. From the first day of this operation, 200 kg of meat and live poultry were seized by German customs in Frankfurt. On 19 February 2006, 21 tonnes of poultry imported illegally from China were seized, crossing the province of Alicante, Spain, in trucks (Thinkspain 2006). Multiple sources of contagion associated with human movements are possible.

THE SITUATION IN 2006

It is clear that the expansion of the virus HPAI H5N1 did not simply follow migratory routes. From February 2004, the virus was present in northwest China, not far from the Russian Altai and border of Kazakhstan (Fig. 2; FAO 2006a). The virus started its progress westward again in summer 2005 by crossing the Russian border. In July, the virus progressed along the southern border of Russia near the border with Kazakhstan between Novosibirsk and Omsk, then during the two first weeks of August on the

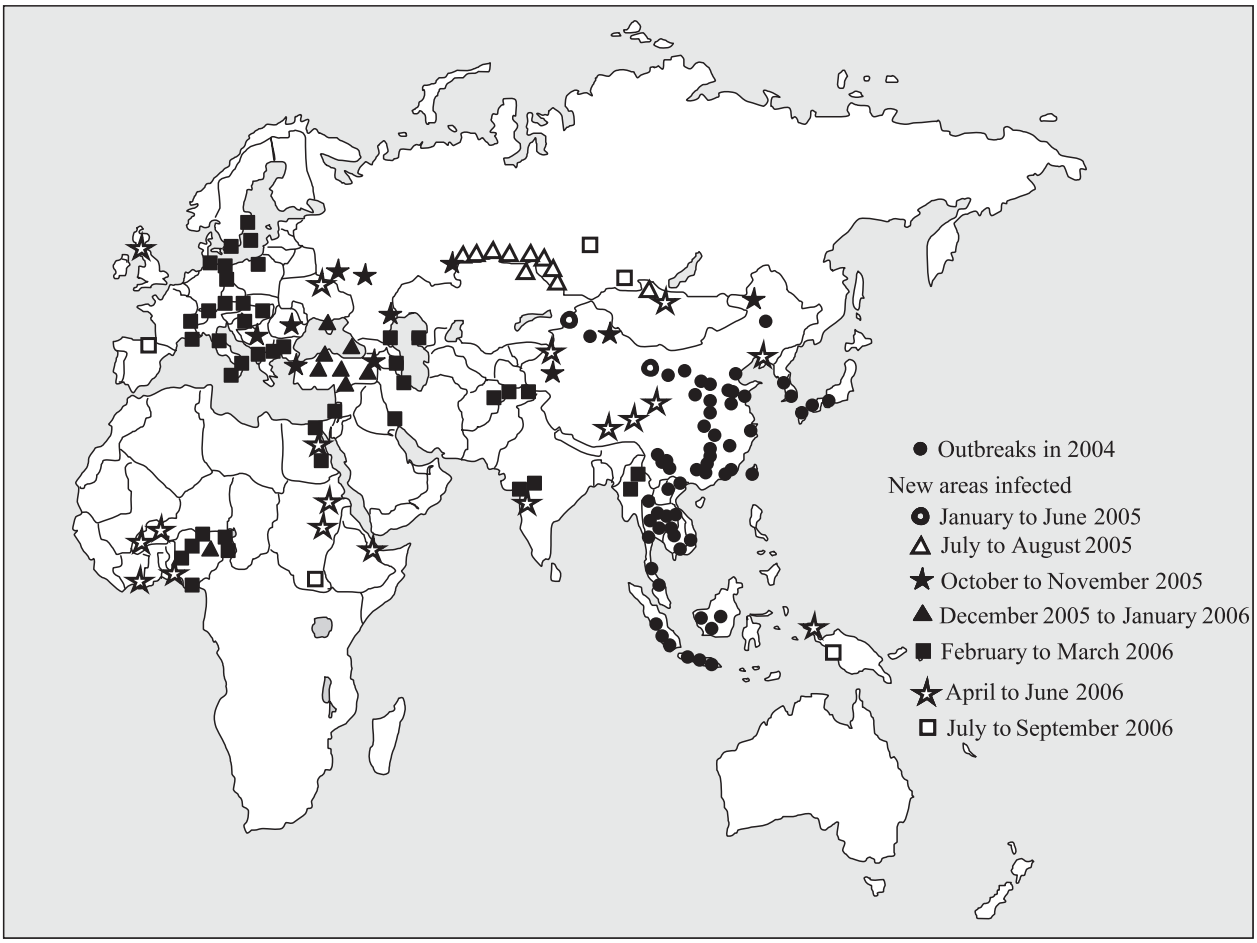

Figure 2. Map showing the outbreaks in 2004 and new areas infected in 2005 and 2006. Sources: Emergency Prevention System (EMPRES) for Transboundary Animal and Plant Pests (http://www.fao.org) and World Organization for Animal Health (http://www.oie.int).

other side of the border in Kazakhstan, and at the end of August in the southeastern Urals around Tcheliabinsk (OIE 2006a). In October, it reached the south of Moscow, Romania, Croatia and the western edge of Turkey. In December, the first cases were reported on the Crimean Peninsula in Ukraine and in eastern Turkey. In January, new outbreaks were detected right across Turkey. At the same time, from October to January, outbreaks continued to be detected in the original areas, in Thailand, in Viet Nam, in China and in Indonesia. The virus thus gradually spread according to an east–west linear axis during summer and autumn 2005, from China to western Russia, then along two north–south axes around the Black Sea, one from Crimea to Romania and western Turkey, and the other from Astrakhan to eastern Turkey. By the beginning of January 2006, no case had yet been reported in India, in Pakistan, Bangladesh, the Middle East, Africa, America or Australia, although the southwards passage of migratory birds had ended and they were beginning to start their northward migration, which takes place from February to June according to species.

The summer of 2005 marked a new epidemiological phase with the fast progression of the virus westwards to the Balkans (Fig. 1). In numerous countries, the epidemiological data are not reliable or precise enough to allow us to understand the causes of the expansion, but the trajectory of the virus does not correspond with the main migration routes of wild birds. Gilbert *et al.* (2006b) concluded that the spread of the virus is consistent in space and time with the autumn migration of the Anatidae family. Their study remains, however, correlative and should be balanced by other explanations not investigated such as important trade from Russia to the Black Sea.

Two new stages in the progression of the virus took place at the beginning of 2006: the arrival of the virus in sub-Saharan Africa in Nigeria and a new phase of progression westwards in Europe. Indeed, numerous sporadic outbreaks appeared in Europe from February to March; at first in southern Italy, in Greece, in Serbia-Montenegro, in Hungary, then in the centre of Italy, Slovenia, Austria, northern Germany, eastern France, then Slovakia, the Czech Republic, the south of France, the south of Germany, Albania, Poland, Switzerland, Denmark, the south of Sweden and finally Scotland. This stage, involving almost exclusively mortality in wild birds, corresponds very well with a movement of wild birds westwards, not during a migration season, but pushed by a very cold spell in eastern Europe, and the sporadic contamination of water across a broad geographical front. The spread does not correspond to the usual routes of migrants and it is still not possible to determine which bird species were responsible for the spread of the virus because the mortality was very largely observed in resident species. After this phase, it is remarkable that outbreaks remained localized and did not extend like an epizootic around each initial outbreak. February and March are major periods of migration, in particular for ducks, towards their northern breeding areas. In France, outbreaks remained localized in some ponds in the south near Dombes and one pond in Bouches-du-Rhône, whilst wild birds circulated between these lakes, other regions and other countries.

At the beginning of 2006, the virus continued its progressive extension in poultry, but not wild birds, along several axes: one from Turkey and the south of Astrakhan towards Azerbaijan, Iraq, Iran, Israel, Jordan, the Gaza Strip and Egypt; another from southern Russia and China towards Kazakhstan, Afghanistan, Iran and northwestern India; and a third from Nigeria towards Niger, Cameroon and Burkina Faso (OIE 2006a).

CONCLUSIONS

The phenology and geographical pattern of expansion of the HPAI H5N1 does not correspond to the pattern of bird migration. First, it took several months for the virus to spread from China to the Balkans. Migratory birds such as ducks and waders travel several hundred kilometres in a single day. If migrating birds mainly dispersed the virus, the virus should also spread by large jumps of thousands of kilometres, throughout the migratory stopping places of Asia and Africa. The observed expansion has rather been by a progressive expansion from isolated outbreaks, the geographical pattern of which corresponds well with major routes and patterns of human commerce. Secondly, from July 2005 onwards, if migratory birds were a main agent of dispersal, one would have expected massive mortalities of wild birds, both in the breeding areas and along all migratory routes, as bird populations would have been encountering this virus for the first time. However, only sporadic cases were observed. The cases in Western Europe after the cold spell on the Black Sea showed that the virus can spread through infected wild birds travelling short distances (Feare 2007), but no evidence for long-distance transmission during seasonal migration has yet been found (Feare 2007). Analysing 52 introduction events into countries, Kilpatrick *et al.* (2006) concluded that both poultry and the trade in wild birds represent a larger risk than migratory birds for the introduction of HPAI H5N1 to the Americas.

In summary, although it remains possible that a migratory bird can spread the virus HPAI H5N1 and contaminate poultry, the evidence overwhelmingly supports the hypothesis that human movements of domestic poultry have been the main agent of global dispersal of the virus to date. The occurrence of an outbreak at a commercial turkey farm in Suffolk, England, in February 2007 fits this wider pattern.

In spite of the absence of evidence that migratory birds play a major role in the dispersal of the virus, many statements to this effect were made by international institutions, non-governmental organizations and media, and a debate between epidemiologists and ecologists followed (e.g. Normile 2005, 2006a, 2006b, Fergus *et al.* 2006). However, from autumn 2005 it was largely presented as fact that migratory birds were the main potential agent of global dispersal (e.g. Derenne & Bricaire 2005, FAO 2005), even as evidence emerged in Asia that spread was mainly mediated by human activities (Melville & Shortridge 2004). OIE reports (e.g. OIE 2005, 2006a, 2006c) indicated that the source of outbreaks was contact with migratory birds, but offered no evidence to support this assertion and contributed to the inappropriate emphasis on migratory birds, thus reducing the probability that alternative mechanisms such as poultry movements were fully considered in individual cases. In spite of the declarations of the Nigerian Minister of Agriculture on the probability of the introduction of the virus via the poultry trade (Euro Surveillance 2006), the FAO continued to implicate migratory birds, thus denying problems associated

with commercial exchanges. The natural globalization of the exchanges of migratory birds seemed to hide the globalization – without strict health control – of the exchanges of poultry as the accepted mechanism for disease spread. By May 2006, an international conference in Rome had recognized that the virus was mainly spread through the poultry trade, both legal and illegal, but OIE and FAO media releases (FAO 2006b, OIE 2006b) continued to focus on the possible contribution of spread by wild birds. Given that a key part of the remit of the FAO is to develop international agricultural trade, reticence to accept that this trade is the main agent of global dispersal of HPAI H5N1 is perhaps unsurprising.

The OIE have pleaded for a resolution of the debate over the cause of spread by acknowledging that the solution lies in the control of the epizootic disease, notably by training farmers, the development of necessary veterinary infrastructure and, especially, the provision of financial resources necessary for the countries affected. For its part, the World Health Organization asked for urgent preparation of plans to fight any pandemic and warned of the risk of human mortality, which might run to hundreds of millions. Because of the currently poor controls for animals, there is strong probability that the HPAI H5N1 subtype will become endemic near Europe. However, comparisons between countries show that it is possible to control the epizootic disease. In Taiwan, Japan and South Korea, the virus has disappeared. Control of trade and strong veterinarian surveillance were the keys to this success. At the same time, migratory birds continue to pass through these countries in spring and in autumn. Conversely, in China, Viet Nam and Cambodia, where means were not implemented to control the epizootic disease, the virus is now endemic.

Fear of an immediate economic impact on poultry as a result of the dispersal of HP subtypes by migratory birds could lead to keeping of poultry indoors. Yet the current, major impact of the virus H5N1 has been economic losses and the destabilization of food-producing farms of Asia where very high densities of animals and increased stress factors are particularly favourable for the maintenance and transmission of virulent agents, in particular subtypes of highly pathogenic influenza. Paradoxically, the H5N1 virus coupled with a fear of transmission by wild birds could lead to a reversion to battery farming, which increases risk of outbreaks, rather than maintaining the current trend to better animal welfare resulting from free-range agriculture. All the evidence suggests that maintaining these trends whilst controlling disease through strong veterinary scrutiny and control of trade is more likely to be a successful strategy.

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