Chapter 1

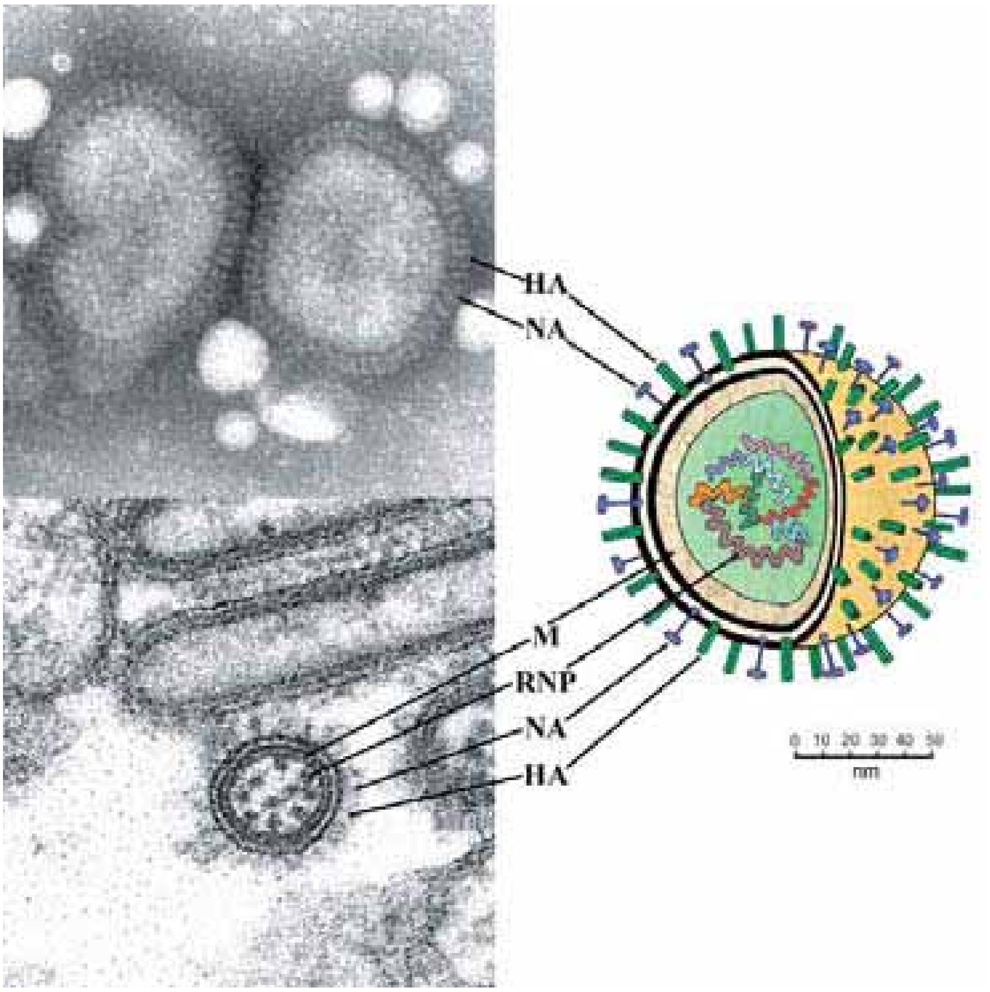
Avian influenza and the H5N1 virus

ECOLOGY AND BIOCHEMICAL PROPERTIES OF AVIAN INFLUENZA VIRUSES

Avian influenza (AI) is an infectious disease of birds caused by type A influenza viruses of the Orthomyxoviridae family (Figure 1.1). These viruses most commonly infect poultry (e.g. domestic chickens, turkeys, ducks, quails and geese) as well as many types of wild birds. Some AI viruses are also known to infect a variety of mammals, including humans.

FIGURE 1.1

Electron micrographs and diagram of an avian influenza virus



HA- haemagglutinin glycoprotein, M- Capsid, NA-neuraminidase glycoprotein, RNP- ribonucleoprotein

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The different AI virus subtypes are distinguished by the haemagglutinin and neuraminidase antigens (glycoproteins) that cover the virus surface (Figure 1.1). Sixteen different haemagglutinin (H1-H16) and nine different neuraminidase (N1-N9) antigens have been characterised and each viral subtype is identified by the particular antigen combination it possesses (e.g. H5N1 or H3N2). All 16 haemagglutinin and nine neuraminidase antigens have been identified in wild bird populations. Genetically, AI viruses are composed of eight distinct ribonucleic acid (RNA) segments.

A particular AI virus subtype may include a variety of similar but distinct strains (the term "clades" is often used to describe these sub-populations), based on genetic sequences and the clustering – or not – of the isolates. The different strains originate either through genetic mutation as the virus replicates or via recombination (exchange of parts of a segment) or reassortment (exchange of a full segment) of genetic material between different viruses infecting a common host. Specific viral strains (e.g. A/bar-headed goose/Qinghai/5/2005 H5N1) are identified by: 1) influenza type; 2) host species from which the strain was isolated; 3) geographic location; 4) laboratory strain designation; 5) year of isolation[1]; and 6) viral subtype.

AI viruses are classified as low pathogenic (LPAI) or highly pathogenic (HPAI) depending on their virulence in domestic chickens (Figure 1.2). Most AI poultry infections are caused by LPAI strains that may produce a mild disease manifested by a variety of respiratory, enteric or reproductive signs (depending on the strain). Clinical signs may include decreases in activity, food consumption or egg production, coughing and sneezing, ruffled feathers, diarrhoea and/or tremors. Often, few visible clinical signs are noted and some LPAI outbreaks may go entirely undetected unless there is specific laboratory testing for the presence of the virus. Quality assured vaccines, when well applied and used in conjunction with other disease control measures (such as improved hygiene and care, and movement management), are effective in preventing the introduction of AI viruses and their spread within and among domestic flocks.

AI viruses are transmitted via direct contact with an infected bird or indirectly via close exposure to materials contaminated with infected faeces or possibly respiratory secretions. However, AI viruses have limited ability to survive outside the host where persistence in the environment is highly dependent on moisture, temperature and salinity. AI viruses can, however, persist for years in ice in high latitude lakes and have been shown to persist for over one month in other cool, moist habitats. In fact, the viruses are most often encountered in wetland habitats frequented by waterbird species, including Anatidae (ducks, geese and swans) and Charadriidae (shorebirds), which are the most common wild avian hosts of AI viruses.

In wild birds, LPAI infection can affect foraging and migratory performance (van Gils *et al*. 2007), but most infected birds show no obvious clinical signs of disease. Common AI strains and their wild host populations have developed an evolutionary equilibrium over time whereby the virus does not cause serious disease or mortality. Periodically, wild birds, particularly ducks and geese, have been identified as the source of virus introductions to poultry. Reassortment or recombination between LPAI viruses in a common host can, but

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[1] Year of isolation does not necessarily correspond to its first appearance.

FIGURE 1.2

Chicken with H5N1 highly pathogenic avian influenza

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does not necessarily, lead to more pronounced virulence. In addition, during viral replication while circulating in domestic flocks, AI viruses also undergo frequent mutations which can give rise to new biological characteristics (i.e. from LPAI to a more virulent or "highly pathogenic" avian influenza or HPAI viruses). Emergent HPAI strains are often more contagious (depending on the density of susceptible hosts) and typically virulent in gallinaceous species, resulting in disease outbreaks with up to 100 percent mortality in unprotected poultry flocks; these are popularly known as "bird flu" or "fowl plague" outbreaks. Though culling of domestic poultry is the most effective means of containing the disease when a

HPAI outbreak occurs, it does depend on early detection and reporting. A compensation plan can often lead to transparency, early reporting, and offset socio-economic losses.

To date, all HPAI outbreaks in poultry have been caused by H5 or H7 strains, but these strains have rarely been found in wild bird populations. However, over the last few years a particularly virulent strain of the H5N1 AI virus has shown the ability to infect poultry and numerous wild birds, as well as wild and domestic cats (Felidae), weasels (Mustelidae), domestic dogs (Canidae) and other mammals, including humans.

The emergence of the zoonotic H5N1 HPAI virus has caused considerable concern among medical and veterinary experts, public health officials, wildlife biologists, wildlife conservationists and, after considerable media attention, the general public. The H5N1 virus that emerged in Asia in late 2003 is particularly alarming because of its high virulence in poultry, ability to infect a variety of hosts, and potential to spread quickly over large geographic areas, presumably via commercial poultry and wild bird trade, and possibly migratory waterbird routes.

It is generally agreed that wild birds serve as the reservoir for LPAI viruses, but the reservoir for the current H5N1 HPAI strains has not yet been identified despite disease sampling from hundreds of thousands of wild healthy migratory and resident birds, including peri-domestic species. The frequent interactions between large numbers of domestic poultry and wild waterbirds in openly grazed rice fields in parts of Southeast Asia and Africa are likely to be sustaining the H5N1 HPAI virus in both the domestic poultry and wildlife sectors.

Fortunately, there is no evidence to date, to indicate that the H5N1 HPAI virus has initiated sustained human-to-human transmission. All evidence suggests close contact with infected domestic birds or their faeces as the principal source for all H5N1 infections in humans. However, there is concern that a mutated or recombinant form of the virus could emerge and acquire improved transmissibility among humans, in which case there is the real potential for a global influenza pandemic.

HISTORY OF THE H5N1 AVIAN INFLUENZA VIRUS

The highly pathogenic strain of the H5N1 AI virus was first isolated and characterised in a domestic goose in the southern Guangdong province of China in 1996 (Table 1.1). The following year, the first H5N1 HPAI outbreak occurred in domestic poultry in Hong Kong, resulting in the culling of over 1.5 million chickens in an effort to contain and eliminate the disease. This outbreak also led to the infection of 18 people (with six fatalities) in what would be the first documented human deaths from the H5N1 virus.

The next outbreak in humans was not detected until February 2003 when two fatal cases of influenza from the H5N1 strain were documented in members of a Hong Kong family that had recently travelled to mainland China. A third member of the family died of severe respiratory disease while in China, but no samples were taken to confirm if the H5N1 virus was responsible.

Suspected H5N1 HPAI virus outbreaks reappeared in Southeast Asia as early as the middle of 2003, but confirmed infections were not reported again until December 2003-January 2004, when captive tigers (*Panthera tigris*) and leopards (*Panthera pardus*) fed on chicken carcasses were diagnosed with the virus in a zoo in Thailand. Soon after, H5N1 HPAI virus outbreaks swept through domestic poultry in eight East and Southeast Asian

TABLE 1.1

Important events in the discovery, detection and spread of the H5N1 highly pathogenic avian influenza virus (January 1996-September 2007)

1996	First isolation of H5N1 subtype in a domestic goose in **China** (Guangdong province).
1997	First H5N1 outbreak in domestic poultry and humans in **China** (Hong Kong SAR).
1998-2002	No documented outbreaks in domestic poultry and humans. Dec 2002: H5N1 kills a variety of captive ducks and other birds in two bird collections in **China** (Hong Kong SAR).
2003	Feb: H5N1 virus reappears with two human cases in a family in **China** (Hong Kong SAR). Mar-Jul: Suspected but undocumented H5N1 outbreaks in **Southeast Asia**. Dec-Jan 2004: Virus kills two captive big cat species (tiger and leopard) in a **Thailand** zoo after being fed chicken carcasses. Dec: First wave of widespread H5N1 outbreaks begins in **Asia** with infections reported on three poultry farms in the **Republic of Korea**.
2004	Jan-Feb: First H5N1 poultry outbreaks in **Viet Nam**, **Thailand**, **Japan**, **Cambodia**, **Lao PDR**, **Indonesia** and **China**, with first human cases reported in **Viet Nam** and **Thailand**. First domestic cat reported infected in **Thailand**. Jun-Aug: Second wave of H5N1 poultry outbreaks begins in **Southeast Asia**, with first cases recorded in **Malaysia**. Jul: Research indicates H5N1 can be lethal in certain wild waterbirds species. Oct: First report of H5N1 in Europe from two Crested Hawk-eagles (*Spizaetus nipalensis*) smuggled into **Belgium** from **Thailand**. Oct: Virus kills 41 captive tigers in a Thailand zoo after being fed chicken carcasses. Dec: Third wave of H5N1 outbreaks begins in **Southeast Asia**.
2005	Apr-May: H5N1 responsible for deaths of over 6,000 migratory birds (Bar-headed Goose, Pallas's Gull, Brown-headed Gull, Ruddy Shelduck, Great Cormorant and other species) at Qinghai Lake, **China**. Jul-Aug: First H5N1 outbreaks detected in **Russia** (**Siberia**), **Kazakhstan**, **Mongolia** and **China** (Tibetan Plateau and Xinjiang) with reports of dead migratory wild birds in the vicinity of some poultry outbreaks, with the exception of Mongolia. Oct: H5N1 outbreaks in **Turkey**, **Croatia** and **Romania** signalled the first detection of the virus in Europe in domestic poultry and wild birds and heralded its spread into 26 European countries by July 2006. Nov: First report in the Persian Gulf states of a single captive Greater Flamingo (*Phoenicopterus roseus*) in **Kuwait**.
2006	Jan-Feb: First human cases of H5N1 outside Southeast Asia - **Turkey** and **Iraq**. Feb: H5N1 detected in commercial poultry in Africa in **Nigeria** and **Egypt**, where the virus spread to eight countries by May. Feb-Jul: Scattered carcasses of H5N1 infected wild birds reported in most **European Union** countries, including **Austria**, **Czech Republic**, **Denmark**, **France**, **Germany**, **Greece**, **Italy**, **Poland**, **Spain**, **Sweden** and the **United Kingdom**, and in **Switzerland**. Apr-Jun: Reports of H5N1 deaths in Barheaded Geese and other birds around Qinghai Lake, **China** Mar: First H5N1 outbreak in humans associated with handling infected dead wild swan in **Azerbaijan**. (To date, this is the only wild bird to human infection)
2007	Jan-Jun: H5N1 detected in poultry in **Ghana** and **Togo** in Africa and **Kuwait** and in captive falcons and poultry in **Saudi Arabia** in West Asia. Jan: H5N1 detected on a commercial turkey farm in the **United Kingdom** and commercial goose farms in **Hungary**. Apr: First outbreak of H5N1 detected in poultry in **Bangladesh**. Jun-Jul: H5N1 detected in over 200 dead wild birds from three countries (**Czech Republic**, **France** and **Germany**) with two of them (**Czech Republic** and **Germany**) experiencing a concurrent outbreak in domestic poultry.

countries (Cambodia, Taiwan Province of China, Indonesia, Japan, the Republic of Korea, the Lao People's Democratic Republic, Thailand and Viet Nam). This wave of outbreaks resulted in the culling of at least 45 million domestic poultry and at least 35 human cases (24 fatal) in Viet Nam and Thailand (up to March 2004).

Subsequent H5N1 HPAI outbreaks in poultry in the northern summer of 2004 and northern winter of 2004/05 remained confined to Southeast Asia, but human cases spread beyond Viet Nam and Thailand to include Cambodia, Indonesia and China. Most human cases involved contact with infected poultry or contaminated materials, but some possible cases of limited human-to-human transmission could not be ruled out.

Wild birds were not known to be implicated in the initial H5N1 HPAI outbreaks as the disease emerged in Asian poultry in 2003/04, although there was limited surveillance of wild birds being undertaken at that time. However, in May 2005, an H5N1 virus mortalilty event killed over 6,000 waterbirds (mainly Bar-headed Geese [*Anser indicus*], Great Cormorants [*Phalacrocorax carbo*], Pallas's Gulls [*Larus ichthyaetus*], Brownheaded Gulls [*L. brunnicephalus*] and Ruddy Shelducks [*Tadorna ferruginea*]) at the Qinghai Lake National Nature Reserve in northwest China. Estimates indicate that between 5-10 percent of the entire world's population of Bar-headed Geese were killed during this event. This was the second documented mortality event of wild birds as the result of an AI virus. The only previous event occurred in 1961 when many Common Terns (*Sterna hirundo*) were killed during an H5N3 AI mortality incident in South Africa.

The H5N1 AI-related mortality event at Qinghai Lake and subsequent outbreaks or mortality events in China, Siberia, Kazakhstan and Mongolia (Figure 1.3) in July and August 2005 signalled a significant geographic expansion of the disease. The pattern of disease spread has been suggested as evidence of the possible role of migratory waterbirds in disease transmission, although poultry and wild bird trade routes could also explain some

FIGURE 1.3

Bar-headed Goose (*Anser indicus*) carcass found during an H5N1 AI mortality event in Mongolia in August 2005

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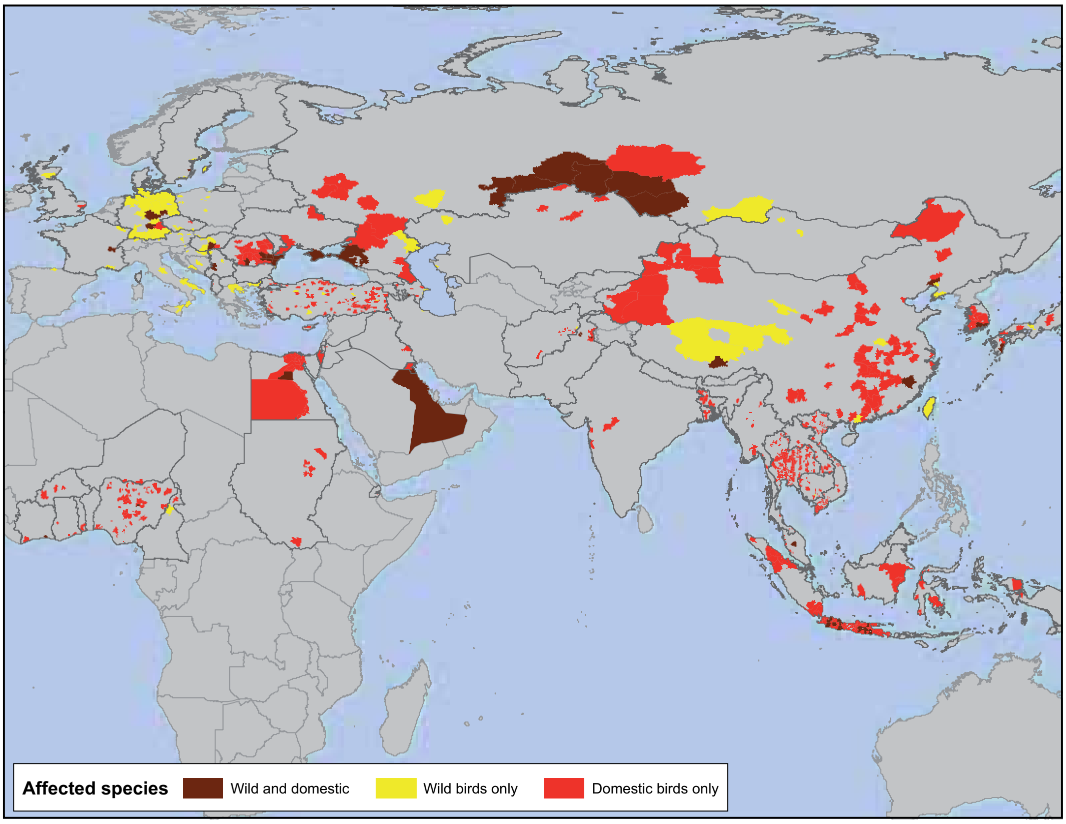
FIGURE 1.4

Countries in which the H5N1 HPAI virus has been detected in poultry and/or wild birds (updated 5 September 2007)



of the outbreaks (Gauthier-Clerc *et al.* 2007). Outbreaks in some of the domestic flocks in Siberia and Kazakhstan occurred at the same time as reports of mortalities in wild migratory waterbirds in the vicinity of infected poultry farms, but the original source of infections could not be determined. Confirmed H5N1 AI-related mortalities in Mongolia were limited to a Bar-headed Goose and four Whooper Swans (*Cygnus cygnus*) in 2005.

The H5N1 HPAI virus continued its westward expansion during the northern autumn of 2005 and by October it was detected in poultry in Turkey, and subsequently in Croatia and Romania, the first occurrences in Europe. The arrival of the H5N1 HPAI virus in Turkey and Eastern Europe heralded the swift spread of the disease throughout Europe and into the Persian Gulf region by December 2005, and the Middle East and Africa by February/ March 2006.

In January 2006, the first human H5N1 AI infections reported outside East Asia occurred in Turkey. Within a few months human infections were also reported in Iraq, Azerbaijan, Egypt and Djibouti, raising to 10 the number of countries reporting H5N1 virus infections in humans (258 cases, 154 fatal as of 29 November 2006). As in Asia, human cases in most of these countries were associated with handling of infected domestic poultry. However, the first fatality in Azerbaijan in March 2006 was linked to plucking a dead infected swan. This marked the first, and only, known case of H5N1 virus transmission from a wild bird to a human.

TABLE 1.2

Countries affected by the H5N1 avian influenza virus in domestic poultry, free-ranging wild birds, captive wild birds and humans since 1996 (as of 7 September 2007)

Country	Year*	Poultry	Wild birds	Captive birds	Humans
ASIA					
Afghanistan	2006	•	×		
Bangladesh	2007	•			
Cambodia	2004	•		•	×
China**	1996	•	×	•	×
India	2006	•			
Indonesia	2004	•			×
Iran	2006		×		
Iraq	2006	•			×
Israel	2006	•			
Japan	2004	•	×		
Jordan	2006	•			
Kazakhstan	2005	•	×		
Kuwait	2005	•		•	
Korea, Rep of	2003	•	×		
Lao PDR	2004	•			
Malaysia	2004	•	×		
Mongolia	2005		×		
Myanmar	2006	•			
Pakistan	2006	•	×	•	
Saudi Arabia	2007	•		•	
Thailand	2003	•	×		×
Viet Nam	2004	•			×
West Bank and Gaza Strip	2006	•			
AFRICA					
Burkina Faso	2006	•			
Cameroon	2006	•	×		
Côte d'Ivoire	2006	•	×		
Djibouti	2006	•			×
Egypt		•			×
Ghana	2006	•			×
Niger	2006	•			
Nigeria	2006	•	×		
Sudan	2006	•			
Togo	2007	•			

(Continued)

TABLE 1.2 *(Continued)*

Countries affected by the H5N1 avian influenza virus in domestic poultry, free-ranging wild birds, captive wild birds and humans since 1996 (as of 7 September 2007)

Country	Year*	Poultry	Wild birds	Captive birds	Humans
EUROPE					
Albania	2006	•			
Austria	2006		×	•	
Azerbaijan	2006	•	×		×
Bosnia-Herzegovina	2006		×		
Bulgaria	2006		×		
Croatia	2005		×		
Czech Republic	2006	•	×		
Denmark	2006	•	×		
France	2006	•	×		
Georgia	2006		×		
Germany	2006	•	×	•	
Greece	2006		×		
Hungary	2006	•	×		
Italy	2006		×		
Poland	2006		×		
Romania	2005	•	×		
Russian Federation	2005	•	×		
Serbia	2006	•	×		
Slovakia	2006		×		
Slovenia	2006		×		
Spain	2006		×		
Sweden	2006		×	•	
Switzerland	2006		×		
Turkey	2005	•	×		×
Ukraine	2005	•	×		
United Kingdom	2006	•	×		

* The year indicates when the virus was first confirmed - data compiled from various sources, including OIE, WHO and FAO.

** Including Hong Kong and Tibet.

Over a two-month period during the northern summer of 2007, H5N1 was detected in over 200 dead wild birds from three countries (Czech Republic, France and Germany) with two of them (Czech Republic and Germany) experiencing a concurrent outbreak in domestic birds. These mortalities in wild birds involved primarily non-migratory species, and took place at a time of year (June-July) when birds may have been flightless due to moult, and were not migrating into or away from Europe.

As of September 2007, the H5N1 HPAI virus had been confirmed in poultry or wild birds in 59 different countries on three continents (Figure 1.4 and Table 1.2). In Europe, the virus has been detected in both wild birds and poultry in 12 countries (Azerbaijan, Denmark, France, Germany, Hungary, Romania, Russia, Serbia, Sweden, Turkey, Ukraine and the United Kingdom), only in wild birds in 12 countries (Austria, Bosnia-Herzegovina, Bulgaria, Croatia, Czech Republic, Greece, Italy, Poland, Slovakia, Slovenia, Spain and Switzerland), and only in poultry in one country (Albania).

By contrast, outbreaks in 10 African countries (Burkina Faso, Cameroon, Côte d'Ivoire, Djibouti, Egypt, Ghana, Niger, Nigeria, Sudan and Togo) have been limited almost entirely to poultry. Only three H5N1 AI cases have been recorded in wild birds: a Sparrow Hawk (*Accipter nisus*)[2] in Côte d'Ivoire and unspecified duck and vulture species in Cameroon and Nigeria respectively.

As the H5N1 virus spread over Eurasia and Africa in 2006, recurrent outbreaks in Southeast Asia suggested that the virus had become endemic in many regions and was still expanding. Wildlife mortality events in China were fewer in number, with some 1,800 wild birds, but over a broader geographic range compared to 2005. Four new countries (Afghanistan, India, Myanmar, and Pakistan) reported presence of the H5N1 HPAI virus in early 2006, raising to 19 the number of Asian countries with confirmed outbreaks in poultry or wild birds. Although Japan had effectively controlled H5N1 HPAI virus outbreaks in poultry and declared itself disease free in the northern summer of 2004, outbreaks continued in most other countries, including Malaysia and the Republic of Korea, which had been able to eliminate the disease earlier but probably experienced reintroductions. In early 2007, a commercial turkey farm in the United Kingdom reported the first domestic turkey outbreak in the country that was possibly linked to importation of frozen turkey meat from Hungary. The H5N1 HPAI virus spread in poultry in Ghana and Togo in Africa and Bangladesh in Asia.

SURVEILLANCE STRATEGIES FOR AVIAN INFLUENZA

The scientific community has acknowledged that the H5N1 HPAI virus is primarily responsible for a poultry disease and that more emphasis on surveillance, prevention and control measures should be addressed at the animal (agricultural) production level to improve husbandry and marketing biosecurity practices in order to halt the risk of human infections and curb further spread in poultry. However, concern remains about the role that wild birds may play in harbouring and transmitting the disease. Most of the information regarding the relationship between wild birds and the H5N1 virus has relied on samples collected from sick or dead birds during mortality events. While this "opportunistic" surveillance has provided important data (e.g. host range and susceptibility), it is a biased collection technique and does not offer insight into identification of the *reservoir role* that wild birds might play in the propagation and spread of the H5N1 virus or other infectious diseases.

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[2] Other sources refer to a Yellow-billed Kite (*Milvus migrans parasiticus*) which highlights the problem of wild bird identification in the official reporting channels for avian influenza. Limited involvement by qualified wildlife biologists often results in failure to identify or misidentification of wild birds both in the vicinity of outbreaks and in the wider countryside.

Recently, several surveillance programmes specifically designed to collect samples from healthy free-ranging wild birds have been undertaken by a number of international or national agencies, and non-governmental organisations. However, active surveillance in wild birds presents practical, logistic and financial obstacles that make it a challenge. Given the expected low prevalence of H5N1 AI viruses in healthy wild birds and the often limited resources available for what are costly efforts, it is important to approach active surveillance sampling in a strategic manner with clearly defined goals, sound epidemiological justification and sufficient technical skills and capabilities to perform both field and laboratory activities. The primary goals of effective and active wildlife surveillance programmes for the H5N1 virus should be: 1) to determine which species can host the virus; 2) to determine temporal and spatial variation in disease prevalence; 3) to determine the role of wildlife in the ecology of the disease; and 4) to develop protocols that will reduce the potential for human and poultry exposure to the virus from wildlife sources and vice versa.

Active surveillance programmes for free-ranging healthy wild birds should be targeted at species with the following characteristics: 1) species known to have been infected with the H5N1 AI virus; 2) species known to be epidemiological reservoirs for LPAI viruses; 3) social species that are known to aggregate seasonally at breeding, roosting, migration stopover and non-breeding (wintering) sites; 4) species that potentially share habitats with poultry farms, integrated livestock-aquaculture systems, backyard poultry flocks and croplands such as rice fields; and 5) species whose seasonal movements or migratory patterns may explain disease dispersal and/or emergence. Selection of sampling sites will primarily be dictated by the habitat preferences of the species to be sampled and occurrence of outbreaks in poultry, although other factors such as bird and researcher safety, and project logistics should also be considered (see *Chapter 3*).

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