

REVIEW

Is avian influenza virus A(H5N1) a real threat to human health?

A. AMENDOLA, A. RANGHIERO, A. ZANETTI, E. PARIANI
Public Health - Microbiology - Virology Department, University of Milan, Italy

Key words

Avian influenza • A(H5N1) • Pandemic

Summary

The A(H5N1) influenza remains a disease of birds with a significant species barrier: in the presence of some tens million cases of infection in poultry - with a wide geographical spread -, only a few hundreds cases have occurred in humans. To date, human cases have been reported in 15 countries - mainly in Asia - and all were related to the onset of outbreaks in poultry. A peak of H5N1 human cases was recorded in 2006, then decreasing in subsequent years. Despite this trend, the H5N1 virus still represents a possible threat to human health, considering that more than half of human cases of H5N1 have been fatal. Moreover, despite the drop in the number of cases, the risk of a novel pandemic cannot be excluded, since H5N1

continues to circulate in poultry in countries with elevated human population density and where monitoring systems are not fully appropriate. In addition, there is a major global concern about the potential occurrence of a reassortment between the 2009 pandemic H1N1 and the highly pathogenic H5N1 influenza viruses following a co-infection in a susceptible host. Therefore, the implementation of appropriate surveillance and containment measures is crucial in order to minimize such risk.

In conclusion, H5N1 avian influenza is still a rare disease in humans but its clinical severe outcome requires a careful monitoring of the virus's ability to evolve and to trigger a new pandemic.

Avian influenza viruses

Influenza viruses belong to the *Orthomyxoviridae* family, genus *Orthomyxovirus*, and are divided into three types – A, B, and C – based on the antigenic differences between their nucleoprotein and matrix proteins. Influenza A viruses are further subdivided into several subtypes. A most striking feature of influenza viruses is their ability to continually change their genetic structure – in particular, hemagglutinin (H) and neuraminidase (N) – so as to reach the antigenic heterogeneity that allows them to infect several animal species [1]. According to the current nomenclature, influenza A viruses are classified on the basis of the characteristics of their surface antigens hemagglutinin and neuraminidase [1]. Sixteen hemagglutinin subtypes (H1-H16) and nine neuraminidase subtypes (N1-N9) have been identified so far [2]. The hemagglutinin and neuraminidase subtypes seem to be able to assort into any combination, and most of the 144 possible combinations have been found in natural reservoir species [3].

The natural host reservoir species of influenza A viruses is the avifauna, particularly waterfowl that can harbour all subtypes of influenza A viruses identified so far. Besides birds, influenza A viruses can also infect a wide range of mammals such as pigs, horses, dolphins, seals, whales, and humans [1, 3]. Phylogenetic studies have revealed that the gene sequences of different species-specific lineages originated from those belonging to viruses isolated from aquatic birds. This indicates that birds are

the natural reservoir of all influenza A virus subtypes spread in the animal kingdom [4-6].

Influenza A viruses are usually not pathogenic for wild aquatic birds, revealing that they have obtained an optimal level of adaptation in these natural reservoirs. In these hosts, the annual peak of influenza infections coincides with the migratory period (late summer and early winter for the Northern hemisphere) [6, 7]. Avian influenza viruses (AIV) show a tropism for cells of the intestinal tract, therefore indicating that the main transmission route is via faecal-oral. The direct or indirect contact between migratory species and breeding species is often the cause of the occurrence of epizootic [8, 9]. Most AIVs cause asymptomatic or mildly symptomatic infections in birds, but symptoms can vary depending on both virus strains and type of poultry [8, 9].

AIVs are classified according to their potential pathogenicity in: a) highly pathogenic avian influenza (HPAI) viruses, which cause systemic infections in domestic birds with rapid progression and death rates as high as 100%, and b) low pathogenic avian influenza (LPAI) viruses, usually responsible for outbreaks of moderate severity in poultry [10, 11]. Phylogenetic studies have revealed the existence of two geographically distinct sub-lineages (Eurasian and American, respectively), probably related to different patterns of migratory birds [6, 12]. Both HPAI and LPAI viruses belong to these two sub-lineages, indicating that the viral pathogenicity is not associated with a particular geographical distribution. All HPAI strains identified to date belong to H5 and H7 subtypes, and are mainly responsible for

outbreaks with high case lethality among domestic avian species (chickens, turkeys and quails) [10, 11].

The main difference in the pathogenicity between HPAI and LPAI viruses is determined by the cleavage site in the precursor of the hemagglutinin molecule. The hemagglutinin is a glycoprotein that mediates the binding to the cellular receptor and promotes the release of the ribonucleoprotein-complex (viral RNA, polymerases and nucleoprotein) in the host cell. This glycoprotein is the primary mediator of pathogenicity, where the proteolytic cleavage site sequence determines whether infection will be systemic (highly pathogenic) or restricted to the respiratory and enteric tracts (low pathogenic). This difference is based on which type of proteases recognizes the sequence that is present in the hemagglutinin molecule [10]. Indeed, the hemagglutinin of LPAI viruses has – at its cleavage site – a single amino acid arginine, which is usually recognized by proteases located in a limited number of host cells. For this reason, the LPAI viruses can cause only mild or moderate infections. Otherwise, the hemagglutinin cleavage site of HPAI viruses is characterized by a sequence of basic amino acids; this multibasic sequence is recognized by proteases present in a wide range of host cells, which may favour systemic infections at high lethality in populations of domestic birds [10].

H5N1 virus

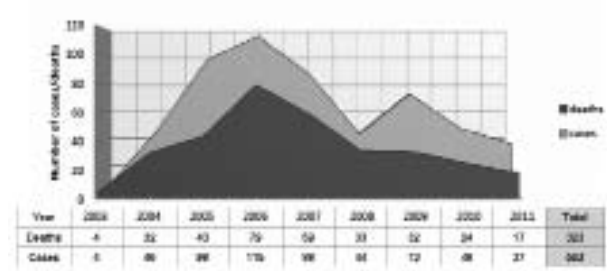
The ancestor of the HPAI H5N1 virus was isolated from infected geese in 1996 [13] in Guangdong, Southern China, the same region where SARS virus emerged in 2003. The following year, the H5N1 virus was identified as the cause of disease in poultry in Hong Kong, and was promptly eliminated by culling infected animals [14]. From 1999 to 2002, the virus continued to circulate both in waterfowl and in poultry markets in Hong Kong, causing several outbreaks in poultry flocks [15]. In 2004, H5N1 was isolated from crows in Japan [16] and, in 2005, from migratory waterfowls in Mongolia [17]. Finally, – that year – the discovery of more than 6,000 dead migratory birds in Qinghai Lake was renowned [18, 19]. A major element in the determination of this new scenario was the expansion of the range of geographical spread of H5N1 virus across borders in Southeast Asia [20]. Between July and August 2005, outbreaks of avian influenza were reported in poultry flocks in Russia and Kazakhstan. In both these countries, outbreaks of H5N1 were ascribed to the contact between migratory and domestic birds through the sharing of water sources [21]. In early August 2005, an outbreak in poultry was identified in Tibet and a plague of migratory birds was reported in Mongolia [17, 21]. Between February and May 2006, H5N1 virus reached 13 countries: Iraq, Nigeria, Azerbaijan, Bulgaria, Greece, Italy, Slovenia, Iran, Austria, Germany, Egypt, India and France [22]. The mute swans played an important role in the spread of the virus in the European countries such as to be considered as sentinel animals [6, 23]. Within the poultry flocks, the virus is transmitted very efficiently

and infection can easily spread from farm to farm by the movement of infected live birds, people (especially when shoes and other clothing are contaminated) and contaminated vehicles, equipment, feed, and cages. All these aspects have to be taken into due consideration so as to implement suitable control measures within poultry farming. However, it is still difficult to control and prevent the spread of this infection among poultry and the onset of recurrent outbreaks increases the risk for human exposure [24].

H5N1 infection in humans

In May 1997, the H5N1 virus was isolated in a throat swab collected from a 3-year-old child, who died from pulmonary complications due to an acute respiratory infection, in Hong Kong [25]. In the same year, the H5N1 virus caused 18 additional cases of severe respiratory disease, six of which were fatal [7, 26]. Human infections coincided with the onset of a widespread epidemic in poultry, supported by the same virus strain. Epidemiological studies suggested that the virus was transmitted directly from birds; in addition, the H5N1 viruses isolated from human biological samples did not show the characteristics of reassortant strains, but were similar to A/goose/Guangdong/1/96 strain (an AIV belonging to the Eurasian lineage previously isolated from a goose died in the Guangdong province in China) [13]. In 2002, an antigenic drifted H5N1 strain – different from those previously circulating – emerged in Hong Kong, and came out to be highly pathogenic to several species of breeding birds. In early 2003, the H5N1 virus passed to humans again, infecting two members of a family (father and son) in Hong Kong [27]. Overall, from December 2003 to May 2011, a total of 553 cases and 323 deaths (58.4%) were reported in 15 countries in three continents (Africa, Asia, and Europe) (Fig. 1) [28]. Since the beginning of 2011, the surveillance of human cases of H5N1 allowed to record 37 cases and 17 deaths in Cambodia, Indonesia, Bangladesh, and Egypt. Up to now, human cases have occurred in rural or semi-urban areas and have always coincided with the onset of outbreaks in poultry. Human infection is rare and requires the direct transmission of H5N1 virus to people in close contact with sick or dead poultry. Indeed, it has been demonstrated that humans have alpha-2-3-sialic-recep-

Fig. 1. Cumulative number of confirmed human cases and deaths of avian influenza A(H5N1) reported to World Health Organization (WHO) as to May 13, 2011 [28].



tors specific for AIVs, and that these receptors are located exclusively in the lower respiratory tract, particularly in the lungs [10, 29]. Thus, the AIVs can infect human cells only when present at high concentrations, as a result of a close contact with infected animals.

Wild waterfowls are the reservoir hosts of AIVs and are the population in which the best adaptation has occurred. The high degree of adaptation of AIVs in this natural reservoir is revealed by the absence of pathogenicity, that it is advantageous to the virus ecology [20]. From an epidemiological point of view, wild waterfowls are crucial since they allow the perpetuation of infection. The domestic birds act as “spill-over” hosts: they are susceptible to infection when exposed, eliminate the virus and transmit the infection to other hosts, but they are not able to maintain the infection for long periods. The “spill-over” hosts usually acquire a more severe disease than the reservoir hosts do. Humans are the “aberrant” hosts: they rarely become infected, but usually develop serious illness [7, 11].

The major route of transmission of H5N1 to human is represented by close contact with infected poultry. Sporadic cases have been described so far [30]. There is no evidence of human-to-human transmission to date. In poultry workers, despite the high potential for exposure, development of the disease has occurred on rare occasions. Nosocomial transmission in health care workers is possible but the probability of infection is very low compared to seasonal influenza, since H5N1 infection is not transmitted efficiently from human-to-human. Family clusters have been reported [30]; the explanation may be the result of a genetic susceptibility, common behavioural habits or the presence of specific environmental factors.

Since the beginning of the epidemic, the average lethality rate was around 60%, ranging between 33% (Egypt) and 83% (Indonesia) [28]. This variability depends on several factors including early availability of medical care, clinical management of infected patients, biological features of the infecting virus, surveillance bias, and characteristics of the population.

Is H5N1 a threat to human health?

Given the highly pathogenic impact for humans, the H5N1 virus poses a threat to public health worldwide. In addition, migratory birds can carry the virus to distant regions – as it happened in May 2005 –, and the virus can become capable of infecting other animal species, including cats, tigers and pigs, that could potentially act as mixing vessels [8, 9]. The greater the ability of H5N1 to spread in different geographical contexts and in different animal hosts is, the greater the likelihood that the virus will experience antigenic variation mechanisms – enabling it to become pandemic – will be. At the time of writing, the H5N1 virus meets two of the three criteria to become a virus capable of causing a pandemic in humans. These include the ability to infect human hosts and the susceptibility of human population to the infection. The ability to be transmitted efficiently from

an infected person to a susceptible one (the third criterion) has been excluded so far. However, the H5N1 virus might acquire – through genetic adaptive mechanisms – the ability to efficiently trigger the chain of inter-human contagion. At present, this hypothesis is considered possible, though not predictable.

While H5N1 virus has not yet acquired a pandemic status, a novel swine-origin influenza A virus of H1N1 subtype emerged and caused a pandemic in 2009. This H1N1 virus contains a unique combination of gene segments from swine, avian, and human viruses, and was first identified in humans in April 2009 [31]. Spontaneous reassortment of H5N1 virus with human influenza viruses has not been reported; however, the emergence and the establishment of this novel H1N1 virus in the human population may represent a new opportunity for such reassortment to happen with the creation of new potentially pandemic viruses [32].

The acquisition of transmissibility of H5N1 in human populations can involve two main mechanisms: a) genetic reassortment, and b) adaptive mutations. The mechanism of reassortment could lead to the emergence of a fully transmissible pandemic virus, announced by a sudden and unexpected increase in the number of infected cases as well as an explosive spread of the virus. The second mechanism can involve a gradual process of acquisition of mutations in the viral genome that could enable the virus to efficiently bind specific receptors present on human cells. In such occurrence, small clusters of human cases with evidence of human-to-human transmission would announce the pandemic. In this scenario, there will be more options for action with appropriate public health countermeasures.

Hence, which are the viral features of public health interest that must be constantly monitored? Above all, the receptor-binding sites in the hemagglutinin molecule. If the virus mutates in these sites – thus acquiring the ability to bind alpha-2-6-sialic receptors –, it will become efficiently transmissible to humans [10]. Another important aspect is related to the viral polymerase complex. For example, H5N1 strains presenting the amino acid mutation E627K in their PB2 gene have been shown to be associated with a higher efficiency of replication in mammals [33, 34]. Other viral factors that should be taken into account are: the hemagglutinin-cleavage sites, determining the susceptibility to ubiquitous proteases and, thus, bringing about systemic infections, and the NS1, PB2 and PB1 genes, all involved in the induction of cytokine cascade – triggering the so called “cytokine storm” [35-38].

Concluding remarks

At the time of writing, the H5N1 influenza remains a disease of birds with a significant species barrier: in the presence of some tens million cases of infection in poultry – with a wide geographical spread –, only just over hundreds cases have occurred in humans. To date, human cases have been reported in 15 countries, mainly in Asia. Indonesia, Egypt and Vietnam have been the most

hit countries. So far, all the recorded human cases were related to the onset of outbreaks in poultry. The cumulative number of H5N1 cases in humans since the beginning of 2003 to May 2011 is 553, with a total of 323 (58.4%) deaths [28]. A peak was recorded in 2006 and a decreasing trend was then observed in subsequent years (Fig. 1). Despite this trend, the H5N1 virus still represents a possible threat to human health, considering that more than half of human cases of H5N1 have been fatal. Moreover, despite the drop in the number of cases, the risk of a novel pandemic cannot be excluded, since H5N1 continues to circulate in poultry in coun-

tries with elevated human population density and where monitoring systems are not fully appropriate. There is a major global concern about the potential occurrence of a reassortment between the 2009 pandemic H1N1 and the highly pathogenic H5N1 influenza viruses following a co-infection in a susceptible host. Therefore, the implementation of appropriate surveillance and containment measures is crucial in order to minimize such risk. In conclusion, H5N1 avian influenza is still a rare disease in humans but its clinical severe outcome requires a careful monitoring of the virus's ability to evolve and to trigger a new pandemic.

References

- [1] Nicholson KG, Wood JM, Zambon M. *Influenza*. Lancet 2003;362:1733-45.
- [2] Fouchier RAM, Munster V, Wallensten A, et al. *Characterization of a novel influenza A virus hemagglutinin subtype (H16) obtained from black-headed gulls*. J Virol 2005;79:2814-22.
- [3] Spackman E. *A brief introduction to the avian influenza virus*. Methods Mol Biol 2008;436:1-6.
- [4] Reid AH, Taubenberger JK, Fanning TG. *Evidence of an absence: the genetic origins of the 1918 pandemic influenza virus*. Nat Rev Microbiol 2004;2:909-14.
- [5] Reid AH, Fanning TG, Janczewski TA, et al. *Novel origin of the 1918 pandemic influenza virus nucleoprotein gene*. J Virol 2004;78:12462-70.
- [6] Olsen B, Munster VJ, Wallensten A, et al. *Global patterns of influenza A virus in wild birds*. Science 2006;312:384-8.
- [7] Peiris JSM, de Jong M, Guan Y. *Avian influenza (H5N1): a threat to human health*. Clin Microbiol Rev 2007;20:243-67.
- [8] Vandegrift KJ, Sokolow SH, Daszak P, et al. *Ecology of avian influenza viruses in a changing world*. Ann N Y Acad Sci 2010;1195:113-28.
- [9] Krauss S, Webster RG. *Avian influenza virus surveillance and wild birds: past and present*. Avian Dis 2010;54:394-8.
- [10] Horimoto T, Kawaoka Y. *Influenza: lessons from past pandemics, warnings from current incidents*. Nat Rev Microbiol 2005;3:591-600.
- [11] De Jong MD, Hien TT. *Avian influenza A (H5N1)*. J Clin Virol 2006;35:2-13.
- [12] Si Y, Skidmore AK, Wang T, et al. *Spatio-temporal dynamics of global H5N1 outbreaks match bird migration patterns*. Geospat Health 2009;4:65-78.
- [13] Tang X, Tian G, Zhao C, et al. *Isolation and characterization of prevalent strains of avian influenza viruses in China*. Chinese J Anim Poultry Infect Dis 1998;20:1-5.
- [14] Xu X, Subbarao E, Cox NJ, et al. *Genetic characterization of the pathogenic influenza A/goose/Guangdong/1/96 (H5N1) virus: similarity of its hemagglutinin gene to those of H5N1 viruses from the 1997 outbreaks in Hong Kong*. Virology 1999;261:15-9.
- [15] Sims LD, Ellis TM, Liu KK, et al. *Avian influenza in Hong Kong 1997-2002*. Avian Dis 2003;47:832-8.
- [16] Tanimura N, Tsukamoto K, Okamatsu M, et al. *Pathology of fatal highly pathogenic H5N1 avian influenza virus infection in large-billed crows (Corvus macrorhynchos) during the 2004 outbreak in Japan*. Vet Pathol 2006;43:500-9.
- [17] Chen GJ, Smith SY, Zhang K, et al. *Avian flu: H5N1 virus outbreak in migratory waterfowl*. Nature 2005;436:191-2.
- [18] Li KS, Guan Y, Wang J, et al. *Genesis of a highly pathogenic and potentially pandemic H5N1 influenza virus in eastern Asia*. Nature 2004;430:209-13.
- [19] Chen H, Li Y, Li Z, et al. *Properties and dissemination of H5N1 viruses isolated during an influenza outbreak in migratory waterfowl in Western China*. J Virol 2006;80:5976-83.
- [20] Weir E. *The changing ecology of avian flu*. CMAJ 2005;173:869-70.
- [21] Gilbert M, Xiao X, Domenech J, et al. *Anatidae migration in the western Palearctic and spread of highly pathogenic avian influenza H5N1 virus*. Emerg Infect Dis 2006;12:1650-6.
- [22] World Organization for Animal Health (OIE). <http://www.oie.int/>
- [23] Terregino C, Milani A, Capua I, et al. *Highly pathogenic avian influenza H5N1 subtype in mute swans in Italy*. Vet Rec 2006;158:491.
- [24] Ferguson NM, Fraser C, Donnelly CA, et al. *Public health risk from the avian H5N1 influenza epidemic*. Science 2004;304:968-9.
- [25] Subbarao K, Klimov A, Katz J, et al. *Characterization of an avian influenza A (H5N1) virus isolated from a child with a fatal respiratory illness*. Science 1998;279:393-6.
- [26] Yuen KY, Chan PK, Peiris M, et al. *Clinical features and rapid viral diagnosis of human disease associated with avian influenza A H5N1 virus*. Lancet 1998;351:467-71.
- [27] Peiris JS, Yu WC, Leung CW, et al. *Re-emergence of fatal human influenza A subtype H5N1 disease*. Lancet 2004;363:617-9.
- [28] World Health Organization (WHO). <http://www.who.int>
- [29] Matrosovich MN, Matrosovich TY, Gray T, et al. *Human and avian influenza viruses target different cell types in cultures of human airway epithelium*. Proc Natl Acad Sci USA 2004;101:4620-4.
- [30] Ungchusak K, Auewarakul P, Dowell SF, et al. *Probable person-to-person transmission of avian influenza A (H5N1)*. N Engl J Med 2005;352:333-40.
- [31] Dawood FS, Jain S, Finelli L, et al. *Emergence of a novel swine-origin influenza A (H1N1) virus in humans*. N Engl J Med 2009;360:2605-15.
- [32] Octaviani CP, Ozawa M, Yamada S, et al. *High level of genetic compatibility between swine-origin H1N1 and highly pathogenic avian H5N1 influenza viruses*. J Virol 2010;84:10918-22.
- [33] Subbarao EK, London W, Murphy BR. *A single amino acid in the PB2 gene of influenza A virus is a determinant of host range*. J Virol 1993;67:1761-4.
- [34] Taubenberger JK, Reid AH, Lourens RM, et al. *Characterization of the 1918 influenza virus polymerase genes*. Nature 2005;437:889-93.
- [35] Taubenberger JK, Morens DM. *The pathology of influenza virus infections*. Annu Rev Pathol 2008;3:499-522.
- [36] Maines TR, Szretter KJ, Perrone L, et al. *Pathogenesis of emerging avian influenza viruses in mammals and the host innate immune response*. Immunol Rev 2008;225:68-84.
- [37] Doherty PC, Turner SJ, Webby RG, et al. *Influenza and the challenge for immunology*. Nat Immunol 2006;7:449-55.
- [38] de Jong MD, Simmons CP, Thanh TT, et al. *Fatal outcome of human influenza A (H5N1) is associated with high viral load and hypercytokinemia*. Nat Med 2006;12:1203-7.

■ Accepted on July 18, 2011.

■ Correspondence: Antonella Amendola, Public Health - Microbiology - Virology Department, University of Milan, via Pascal 36, 20133 Milan, Italy - E-mail: antonella.amendola@unimi.it