Influenza virus A (H5N1): a pandemic risk?

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SUMMARY

Influenza A subtype H5N1 has represented a growing alarm since its recent identification in Asia. Previously thought to infect only wild birds and poultry, H5N1 has now infected humans, cats, pigs and other mammals in an ongoing outbreak, often with a fatal outcome. In order to evaluate the risk factors for human infection with influenza virus H5N1, here we summarize 53 case patients confirmed with H5N1 infection during 2006. The review also compares the mortality rate among human cases from late 2003 until 15 June 2006 in different countries. Neither how these viruses are transmitted to humans nor the most effective way to reduce the risk for infection is fully understood. The association between household contact with diseased poultry in human infection has been demonstrated. This association could possibly operate by 2 mechanisms. First, transmission may be by inhalation or conjunctival deposition of large infectious droplets which may travel only in short distances. Second, having infected poultry in the home and preparation of infected poultry for consumption may result in exposure to higher virus concentrations than other types of exposure. There is so far no significant evidence for repeated human to human transmission, yet some cases of human to human transmission among the family relatives in Indonesia, Azerbaijan, Iraq and Turkey have been described. Recent outbreaks of highly pathogenic avian influenza A virus (H5N1 subtype) infections in poultry and humans (through direct contact with infected birds) have raised concerns that a new influenza pandemic might occur in the near future.

KEY WORDS: Avian Influenza, Pandemic, Transmission, Pathogenicity

INTRODUCTION

The term influenza refers to illness caused by influenza virus. This is commonly called flu, but many different illnesses cause flu-like systemic and respiratory symptoms such as fever, chills, aches and pains, cough, and sore throat. In addition, influenza itself causes many different illness patterns, ranging from mild common cold symptoms to typical flu to life-threatening pneumonia and other complications, including secondary bacterial infections particularly in elderly persons with underlying chronic diseases. Influenza viruses constitute the genus Orthomyxovirus, which consists of three species A, B, and C (Stephenson and Democratis, 2006). The differences among these viruses are based upon the antigenic characteristics of the M protein of the virus envelope and the nucleoprotein within the viral particles. Influenza epidemics have been recorded throughout history. In temperate climates, the epidemics typically occur in the winter and cause considerable morbidity in all age groups. The likelihood of a human influenza pandemic has increased over the past few years with the emergence and outbreak of a highly virulent avian influenza virus, including influenza virus A(H5N1) that causes a contagious disease. Usually, influenza viruses attack the respiratory tract in humans, like throat...
and lungs. The presence of certain receptors in these target regions are very important in the flu virus infectivity and pathogenicity. In the past, the disease was termed fowl plague, described in 1878 as a serious disease of chickens in Italy (Kamps et al., 2006). All influenza viruses affecting domestic animals (equine, swine, avian) belong to Type A which is the most common type producing serious epidemics in humans. Other influenza types, B and C, do not affect domestic animals, Influenza C infects most people when they are young and rarely causes serious illness. Type B occasionally causes local outbreaks of influenza and is usually confined to youngsters. Despite the WHO efforts to control the avian influenza pandemic, sporadic cases of human influenza A H5N1 infection are still being reported from time to time in the endemic countries and the disease was high in severity in the year 2006. The failure to control avian influenza infections in the domestic birds and animals make the virus probably reassortant, then increase the ability of the virus to infect humans that improves human to human transmissibility. The WHO has advised all nations on vigilance for an influenza pandemic in view of the growing threat of H5N1.

**HISTORY OF AVIAN INFLUENZA VIRUS AND ITS GEOGRAPHIC DISTRIBUTION**

On the basis of their ability to cause disease, influenza A viruses infecting poultry can be divided into two distinct groups: highly pathogenic avian influenza (HPAI) and low pathogenic avian influenza (LPAI). Highly pathogenic avian influenza (HPAI) viruses have periodically occurred in recent years in Australia (H7), England (H7), Italy (H5), South Africa (H5), Scotland (H5), Ireland (H5), Mexico (H5), Pakistan (H7), and the United States (H5). Because laboratory facilities are not readily available in some parts of the world to correctly diagnose HPAI, the actual incidence of HPAI in the world’s poultry flocks is difficult to define. It can occur in any country, regardless of disease control measures, probably because of its prevalence in wild migratory waterfowl, sea birds and shore birds. Outbreaks of less virulent avian influenza (AI) have frequently been described in domestic ducks in many areas of the world. The AI viruses are often recovered from apparently healthy migratory waterfowl, shore birds, and sea birds worldwide. The epidemiologic significance of these isolations relative to outbreaks in domestic poultry has led to the generally accepted belief that waterfowl serve as the reservoir of influenza viruses.

**Main influenza pandemics**

Influenza pandemics can occur when a novel strain of virus causes an epidemic that spreads over a wide geographic area and affects an exceptionally high proportion of the population. The novel strain will be produced when there is a certain genetic change, antigenic shift, in the circulating strain of influenza (Figure 1). At present influenza A virus causes the most severe disease in humans, and has good chance of triggering a pandemic. Three main pandemics occurred in the 20th century. The pandemic influenza of 1918-1919, termed Spanish flu, was the biggest disaster in flu history, with more than 20 million deaths (Mills et al., 2004); most of them were children and young adults (see below). As expected, many of the deaths in 1918 were from pneumonia caused by secondary bacterial infections. The Spanish flu also caused a form of primary viral pneumonia, with extensive hemorrhaging of the lungs. The second pandemic in 1957, Asian flu, was on the whole a milder illness than that of 1918, yet the global death toll was estimated to be about 1 million. At that time, the causative agent was quickly identified, due to advances in scientific technology and the vaccine was available in limited supply by August 1957. An antigenic shift occurred between an avian virus (H2N2) and the causative agent of the 1918 pandemic (H1N1) (Belshe, 2005). After eleven years, another pandemic appeared, known as Hong Kong flu (H3N2), by which around 1 million persons died. Earlier infections by the Asian flu virus might have provided some immunity against the Hong Kong flu virus that may have helped to reduce the severity of illness. Instead of peaking in September or October, as had in the previous two pandemics (Spanish and Asian flu), the Hong Kong flu pandemic did not occur until near the school holidays in December. Since children were at home and did not infect one another at school, the rate of influenza illness among schoolchildren and their families was blocked. Also,
improved medical care and antibiotics that are more effective for secondary bacterial infections, were available for those who became ill. There is no evidence that the 1957 or 1968 pandemic viruses originated in pigs (Neumann and Kawaoka, 2006) even though the flu viruses can become reassortant in this intermediate host. There are three notable flu outbreaks, but they are not categorized as true pandemics, which include a pseudopandemic in 1947 with low severity and death rates, an epidemic in 1977 that was a pandemic in children, and a potential epidemic of swine influenza in 1976 that was termed abortive pandemic (Kilbourne, 2006). There is no exact information which type of influenza will be the next pandemic strain, but influenza virus A(H5N1) is the first candidate (Figure 5B).

Recent scares of flu pandemic in humans
1) 1997: In Hong Kong, avian influenza A (H5N1) infected both chickens and humans. This was the first time an avian influenza virus had ever been found to transmit directly from birds to humans. During that outbreak, 18 people were hospitalized and 6 of them died (Zhou et al., 1999).
2) 1999: In Hong Kong, cases of avian influenza A (H9N2) were confirmed in children, but this infection resulted in only mild, self-limiting illnesses. The evidence suggested that poultry was the source of infection and the main mode of transmission was from bird to human (Hien et al., 2004).
3) 2003: Two cases of avian influenza A (H5N1) infection occurred among members of a Hong Kong family that had traveled to China (Enders et al., 2005), from whom one person recovered and the other died. How or where these 2 family members were infected was not determined. Also in the same year another family member died of a respiratory illness in China, but no testing was done. No additional cases were reported.
4) 2003: Avian influenza A (H7N7) infections among poultry workers and their families were confirmed in the Netherlands during an outbreak of avian flu among poultry. More cases of H7N7 illness were reported: the symptoms were mostly confined to eye infections, yet some respiratory symptoms occurred: 1 patient died (Fouchier et al., 2004). There was evidence of some human to human transmission. In the same year another 2 mild clinical cases in Hong Kong were reported with H9N2 subtype virus (Chotpitayasunondh et al., 2005).
5) 2004: In an outbreak in Vietnam and Thailand many birds and humans were infected with H5N1; among humans 32 fatal cases have been documented (Ungchusak et al., 2005).

6) In the year 2005, 42 fatal cases were confirmed in Cambodia, China, Indonesia, Thailand and Vietnam. Also in the year 2006, from January to 15th June, 53 fatal cases were confirmed in Azerbaijan, Cambodia, China, Egypt, Indonesia, Iraq and Turkey.

GENETIC VARIATION AND PANDEMIC ALERT

The Spanish flu pandemic virus that caused the highest number of known flu deaths did not originate through a reassortment event. The researchers indeed identified that the 1918-1919 pandemic evolved by mutational adaptation of the avian strain (Belshe, 2005). Also, genetic studies showed that all eight segments of the virus are more closely related to avian influenza viruses than any other influenza virus species (Taubenberger et al., 2005). Genetic variation makes the virus more virulent. For example, one of the five amino acid changes in the polymerase gene (PB2) of the 1918 virus made the virus infect humans (Belshe, 2005). This type of mutation was seen in several human isolates of the 1997 Hong Kong H5N1 virus and 2004 Vietnam H5N1 virus. So, if H5 viruses do persist they will likely continue to evolve and then be more easily transmitted among people (Chotpitayasunondh et al., 2005). Influenza viruses have a high level of recombination between genes because of the segmented nature of their genomes. As a consequence of this, gene reassortment through dual infection with human and avian strains of the Asian influenza and Hong Kong influenza may occur (Belshe, 2005). In recent years, there is no large genetic variation like the past pandemics. However, antigenic analysis of the new avian isolates shows reactivity pattern differences from the H5N1 viruses isolated in 1997 and 2001 (Sturm-Ramirez et al., 2004). On the other hand, the genomic sequence for the virus (A/Beijing/01/2003) which was isolated from a 24-year-old Chinese man suggests that the virus may be a mixed virus because its gene segments are closely related to some other species (Qin et al., 2006). In the 2 human deaths in Turkey (Figure 5B), two different mutations were observed. The first was at position 223 (see table 1) of the haemagglutinin receptor protein which increases the ability of the virus to bind the human receptors more than avian receptors (Butler, 2006). The same mutation was observed twice in a father and son in Hong Kong in 2003, and in one fatal case in Vietnam in 2005. The second mutation observed in the Turkish deaths (Glu627Lys) is in the polymerase protein. This mutation was seen in other flu sequences from Eurasian poultry throughout 2005 in fatal cases who died during an outbreak of H7N7 in the Netherlands in 2003 and in a few people in Vietnam and Thailand. Another new finding for genetic variation in the genome of the influenza virus A (H5N1) is that of those 32 mutations that were found in the isolates of the family clusters in Indonesia, the 21 mutations that occurred in the isolate of a 32-year-old man (Butler, 2006).

EPIEIDOIOLOGY AND TRANSMISSION OF THE VIRUS

Waterfowl is the natural reservoir of all influenza A viruses, usually carrying the infection with no sign of disease (Sturm-Ramirez et al., 2005).
Also migratory birds are considered to have an epidemiological role during flu outbreaks (Hlinak et al., 2006). The infection of humans with an H5 avian influenza virus in Hong Kong in 1997 resulted in a reconsideration of the role of the avian species in the epidemiology of human influenza (Buxton Bridges et al., 2002). The infected birds excrete virus in high concentrations in their faeces, nasal and ocular discharges. The disease generally spreads rapidly in a flock by direct contact, but on occasions spread is erratic. Airborne transmission may occur if birds are in close proximity and with appropriate air movement. Once introduced into a flock, the virus is spread from flock to flock by the usual methods involving the movement of infected birds, contaminated equipment, egg flats, feed trucks, and service crews. Usually, infection by H5N1 virus occurs in humans when there is a direct contact with infected birds. An epidemiological study in Vietnam showed that 9 of 10 patients had a clear history of direct contact with poultry (Hien et al., 2005). Normally, typical influenza is primarily transmitted from person to person via droplets from the nose and throat. Thus, close contacts are in general required for transmission. The transmission may also occur through indirect contact with respiratory secretions (for example touching contaminated surfaces then touching the eyes, nose or mouth). The in vivo studies illustrated that influenza viruses replicate to higher levels in the trachea than in the cloacae (Sturm-Ramirez et al., 2005), meaning that the chance of influenza virus transmission is greater via respiration than faeces. The conformation of the sialic acid linkage has been established to control tropism of the influenza viruses (Victor et al., 2004). Avian influenza viruses bind to cell-surface glycoproteins containing sialyl-galactosyl residues linked by a 2-3-linkage. By contrast, human influenza viruses bind to receptors that contain terminal 2-6-linked sialyl-galactosyl moieties, since alpha 2-3 sialic acid receptors are found mainly in the lower respiratory regions. Thus, avian flu viruses can replicate efficiently only in cells in the lower region of the respiratory tract where specific receptor is more prevalent (Shinya et al., 2006). Also, the findings imply that specimens from the lower respiratory tract, such as sputum or bronchoalveolar lavage, have a higher sensitivity for viral detection than an upper respiratory specimen like nasopharyngeal aspirates or throat swab specimens. Moreover, the absence of viral antigen in the trachea indicated that the upper airway is probably not an active site of avian viral replication (Uiprasertkul et al., 2005). This restriction may be the cause of limited human-to-human transmission of

FIGURE 2 - Transmission of influenza A viruses to wild birds, domestic birds, and intermediate hosts like pigs. The figure shows how the strains circulate among their hosts.
avian flu including H5N1 viruses. To be easily transmitted among humans, avian viruses must have the ability to bind cells that display the 2-6 receptors, so that it can enter the cells and replicate in them. Transmission of influenza viruses is illustrated in (Figure 2).

PATHOGENICITY AND INFLUENZA VIRUS VIRULENCE

HPAI are virulent viruses that cause fowl plague. Mortality rate may be as high as 100%. These viruses have been restricted to subtypes H5 and H7, but not all viruses of these subtypes are highly pathogenic. Haemagglutinin subtypes can be differentiated by developed multiplex reverse transcriptase polymerase chain reaction (Xie et al., 2006). This differentiation is important during any flu outbreak. Also, a discrimination among H5 subtypes must be made, because they are not always from HPAI strains (Payungporn et al., 2006). The hemagglutinin glycoprotein for influenza viruses is produced as a precursor, HA0, that cleaves to HA1 and HA2 by host proteases. These precursor derived subunits are a prerequisite for fusion of the viral and endosomal membranes and, therefore, for viral infectivity (Neumann and Kawaoka, 2006). Pathogenic avian influenza viruses present a single Arginine residue at the cleavage site recognized by extracellular trypsin-like proteases which are thought to be secreted only by cells of the respiratory and intestinal tract and consequently limit infections to these organs. In contrast, highly pathogenic avian viruses possess multiple basic amino acids at the cleavage site that are recognized by any intracellular subtilisin-like proteases that thus trigger systemic infection. The HA cleavability is affected by the absence or presence of a carbohydrate side chain near the cleavage site that may interfere with the facility of host proteases to the cleavage site. When the human airway epithelial cells are exposed to the avian influenza A (H5N1), virus haemagglutinin (HA) can recognize its receptors on the surface of the airway epithelial cells (Yuen and Won, 2005). After the target recognition, the viruses enter the cells, replicate and rapidly (hours) spread throughout the human respiratory tract, damaging vital organs and tissues. Genetic variation in the H5N1 genome may increase the pathogenic properties of the virus in humans and animals. For example, certain mutations in PB2 (E627K) affect the virulence of the virus in mice (Fouchier et al., 2004). Also, studies of the isolates of avian influenza virus A (H5N1) from patients in 1997 revealed that the virulence of the virus increases with the highly cleavable hemagglutinin, specific substitution in the polymerase basic protein PB2 (Glu627Lys), and by substitution in non-structural protein NS1 (Asp92Glu) (Writing Committee of the World Health Organization WHO Consultation on Human Influenza A/H5. Avian Influenza A(H5N1) Infection in Humans, 2005). For more information on the effect of genetic variation on influenza virus virulence see Table 1.

CASE CONFIRMATION AND LABORATORY PROCEDURE

Avian influenza virus is most commonly isolated by inoculation of swab material or tissue homogenates into embryonated chicken eggs by the allantoic sac route (Mahy, 1985). The embryos may or may not die, but in any case the presence of the virus can be detected by haemagglutination tests on harvested allantoic. Then its identity is confirmed by agar gel diffusion or haemagglutination inhibition tests using specific antiserum. Also, rapid diagnosis can be made by the detection of viral antigen in tissue impression smears using immunofluorescence, or by antigen detection enzyme-linked immunosorbent assay (ELISA) on tissue homogenates. Cytological tests (including those done at autopsy) can be performed for virus detection. Lung may be the candidate organ because it was demonstrated that the pneumocytes are the major site for H5N1 virus replication (Upprasertkul et al., 2005). Searching for H5N1 in plasma may be necessary, since a clear-cut viremia can be found (Chutinimitkul et al., 2006). Furthermore, the virus can be serotyped to determine its haemagglutinin and neuraminidase subtypes. In vitro tests use plaque assay for viral quantification (Mastrosovich et al., 2006). However, this assay depends on the ability of the virus to produce plaques in cell cultures. Also, polymerase chain reaction and gene sequencing
procedures can be used for rapid determination of the pathogenic potential of an AI virus. The real-time RT-PCR assay is a rapid, specific, and a relatively sensitive method for directly detecting influenza A subtype H5 virus and may be useful in routine diagnostic testing (Enders et al., 2005).

All cases described in this paper have been obtained from the WHO and the centre for infectious disease research and policy (CIDRAP). At first, cases were confirmed by the ministries of health of the countries in which the outbreak occurred. The ministry of agriculture had also given the epidemiological informations to the investigators. Then the samples were sent to the WHO centres for reconfirmation that are the United States Naval Medical Research Unit 3 (NAMRU-3) in Cairo, WHO H5 reference laboratories in Hong Kong and the USA, WHO collaborating laboratory in the United Kingdom, Pasteur Institute in Cambodia, and Collaborating Centre for Influenza at the National Institute for

<table>
<thead>
<tr>
<th>Type of the mutations</th>
<th>Type of the mutant genes</th>
<th>Effect of the mutations</th>
<th>Reference</th>
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</thead>
<tbody>
<tr>
<td>R152K</td>
<td>NA gene</td>
<td>Associated with oseltamivir-resistant</td>
<td>Kamps B.S., Hoffmann C., Preiser W., 2006</td>
</tr>
<tr>
<td>Dual mutation motif Leu26Ile-Ser31Asn</td>
<td>M2 gene</td>
<td>Associated with Amantadine resistance</td>
<td>Cheung C-L. et al., 2006</td>
</tr>
<tr>
<td>V27A, A30S and S31N</td>
<td>M2 gene</td>
<td>Associated with Amantadine resistance</td>
<td>Ilyushina N.A. et al., 2005</td>
</tr>
<tr>
<td>D126N and K152N</td>
<td>HA gene</td>
<td>Reducing virulence and lethality in mice</td>
<td>Kaverin N.V. et al., 2002</td>
</tr>
<tr>
<td>Lys88Arg, Asp701Asn, Phe103Leu</td>
<td>NS2 gene, PB2 gene, NS1 gene respectively</td>
<td>It is suggested that they may operate in a certain virus to mediate its virulence</td>
<td>Brown E.G. et al., 2001</td>
</tr>
<tr>
<td>Glu627Lys and Asp92Glu</td>
<td>PB2 gene</td>
<td>Both mutations that have been associated with increased virulence of influenza viruses</td>
<td>Smith G.J.D. et al., 2006</td>
</tr>
<tr>
<td>S129L</td>
<td>HA gene</td>
<td>Effect atomic contact with cellular sialosides receptors</td>
<td>The World Health Organization Global Influenza Program Surveillance Network, October 2005</td>
</tr>
<tr>
<td>A156T</td>
<td>HA gene</td>
<td>Reduce viral affinity for sialosides</td>
<td></td>
</tr>
<tr>
<td>S223N</td>
<td>HA gene</td>
<td>Facilitate binding of sialosides</td>
<td></td>
</tr>
<tr>
<td>E119A, E119G</td>
<td>NA gene</td>
<td>Associated with zanamivir-resistant</td>
<td>Mishin V.P. et al., 2005</td>
</tr>
<tr>
<td>E119D</td>
<td>NA gene</td>
<td>Associated with zanamivir and peramivir resistance</td>
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Some of these mutations make the virus resistant to anti-influenza drugs and others increase its virulence.
Medical Research (NIMR). In these centres, the RT-PCR assay, haemagglutination inhibition test, virus isolation in embryonated eggs and MDCK cells, and gene sequencing have been performed to detect avian influenza virus A (H5N1).

SPREAD OF AVIAN INFLUENZA VIRUS IN HUMANS

We retrieved the epidemiological data for this review from the daily news of the WHO, CDC, and CIDRAP. According to the report of WHO, from 2003 to 15th June 2006, 230 persons resulted infected with H5N1 virus; 57% died (Figure 4). In 2003, there were 4 confirmed human cases: 3 in Vietnam and 1 in China. All of them were fatal (Figure 3). In 2004, 17 cases in Thailand and 29 in Vietnam were reported; about 70% of them died (Figure 3). In 2005, avian flu was present in Cambodia, China, Indonesia, Thailand and Vietnam. The confirmed human cases in these countries are 4, 8, 19, 5 and 61, respectively, of whom 43% died (Figure 3). Avian influenza cases caused by H5N1 were detected in numerous countries in 2006 compared with the years 2003, 2004, 2005. In the year 2006 there were 10 countries with virus outbreak (Figure 3): Iraq, Egypt, Azerbaijan, Djibouti and Turkey are the new ones. From January to 15th June 2006, 82 cases were confirmed in all countries (except Thailand and Vietnam); 65% were fatal (Figure 3). From the 53 confirmed deaths in 2006, 12 patients died in January, 8 in February, 12 in March, 6 in April, 14 in May and 1 in June (Figure 5A). The largest number of deaths was reported in Indonesia (27 cases). In 2006, the disease predominantly affected children and young adults.

The age distribution is shown in Figure 6: 81% of the deaths are between (5-34) years, 7.54% between (34-55) and 1.88% between (55-80); 9.4% are less than 5 years old. The total median age is 20.36 years. The causes of this prevalence in young people are not fully defined, but most probably nearly all people are immunologically naive for the virus (Bartlett, 2006). This age distribution of deaths in 2006 is compatible with 1918 pandemics, in which the ages most affected by the virus were between 15 and 35 years. Another study in Thailand shows that the fatality rate is very high in children younger than 15 years (Areechokchai et al., 2006). Not surprisingly, a recent epidemiological study in the area of Rome, Italy, showed that influenza viruses cir-

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FIGURE 4 - The total human cases and deaths with influenza virus A (H5N1) in late 2003 until 15th June 2006; 10 countries (Azerbaijan, Cambodia, China, Egypt, Indonesia, Iraq, Thailand, Turkey, Djibouti and Vietnam) are indicated with virus outbreak.

FIGURE 5 - a) comparison of the human death rate from January until 15th June 2006. The highest number of the deaths was documented in May. b) the relative human deaths by H5N1 in the year 2006 (until 15th June) in the four designated countries (Azerbaijan, Iraq, Indonesia and Turkey). The ages and dates of the relative deaths are shown. Each symbol represents a cluster.
FIGURE 6 - a) age distribution of all 53 deaths in the year 2006 (from January until 15th June). b) median age of all 53 deaths in the year 2006 (from January until 15th June) in each country. The figure shows that children and young adults are at high risk during influenza virus outbreaks.

FIGURE 7 - a) the percentage of both males and females among all 53 deaths in the year 2006 (from January until 15th June). b) the days between onset of symptoms to hospitalization (left columns) and the days between hospitalization and death (right columns) of 17 patients.
culates far more in younger patients. There was no documented case in persons over 65 years (Rezza et al., 2006).

The rates of male/female deaths due to the avian flu change over the world. As shown in Figure 7a, the death rates among females in Azerbaijan, Egypt, China and Turkey are more prevalent when compared to other countries. This difference depends on the traditions, customs and economy of these countries. In Azerbaijan 80% of the deaths were female because in this community defeathering of the birds is a task usually undertaken by adolescent and young women (Gilsdorf et al., 2006).

Avian flu causes death in humans a few days after the onset of symptoms. The period between onset of symptoms, hospitalization and death is shown in Figure 7b for 17 deaths in the year 2006 (for the others no information was available). All evidence indicates that no sustained human to human transmission has occurred, and direct or indirect contact with infected or dead birds remains the principal source of infection.

DISCUSSION

Human cases of avian influenza virus A (H5N1) infection have remained rare and sporadic, but the disease is very severe and the case fatality is high. Genetic variation, including reassortment, is the main factor producing a flu pandemic (Figure 1). However, the development of the 1918 pandemic virus cannot be related to gene reassortment, since simple adaptive mutations (drift) were sufficient to generate a strain able to kill millions of people (Taubenberger et al., 2005). The recent genetic sequencing for the isolates of the 2 cases in Turkey (Figure 5B) demonstrates that the strains contain two mutations which probably make the virus easier to adapt to humans (Butler, 2006).

The first mutation is in the pol gene at position 627 (compatible to the mutation that occurred in the 1918 pandemic). The other mutation occurred in HA at position 223 (detected in Hong Kong in 2003 and in one fatal case in Vietnam last year). Both mutations found in Turkey cases show the importance of keeping the level of alert high, since a few mutations may confer pandemic potential on avian viruses that can efficiently replicate in humans and cause human to human transmission (Shinya et al., 2006). Indeed, in 2004 a possible human to human transmission was reported in a family cluster of the disease in Thailand (Ungchusak et al., 2005). A 10 year old boy was infected by a slightly mutated virus. He passed the mutated virus to this father, closely involved in caring for his son; this contact is considered a possible source of infection (http://www.cidrap.umn.edu/cidrap/content/influenza/avianflu/news/jun2306cluster.html). Thus, further studies are needed to shed more light on the epidemiology of the disease.

For 17 of the 53 deaths in 2006 the time intervals between onset of symptoms and hospitalization was about 5 days; between hospitalization and death it was about 3 days (Figure 7b). These data illustrate that the virus is very severe when it infects humans. For this reason the ministry of Public Health in each country must inform people about viral severity. Also infected persons should go directly to the health centers after onset of flu symptoms. Avian influenza is more severe in children, who should avoid handling dead and sick poultry during flu outbreak.

CONCLUSIONS

The conclusions that arise from these viro-epidemiological data are:

1) Infection of humans with the virus (H5N1) is very severe: only a few days are needed from onset of symptoms to death.

2) The disease is more severe in children and young adults. Also, female deaths are predominant when compared with males.

3) The mortality rate in the years 2004 and 2006 were nearly equal (69.5% and 64.6% respectively). However, there is a difference in the number of countries with flu outbreak; this may mean that the affinity and ability of the virus to infect human may increase on a day-by-day basis.

4) In the next possible pandemic, influenza virus A (H5N1) may be the first candidate, since the mutations in the virus genome make the virus more adapted to infect humans. Today there is no evidence of repeated events of human to human transmission. So, long-term pub-
lic health surveillance and control measures are needed to monitor for person-to-person transmission and the emergence of a potentially pandemic H5N1 influenza virus.

5) Handling of dead or sick poultry in an H5N1 infected area is the risk factor for humans. Close contact with them is the primary source for transmission of the virus to humans.

6) Influenza virus A (H5N1) can replicate efficiently only in cells in the lower regions of the respiratory tract where the avian virus receptor is prevalent. This is the main cause of inefficient transmission of the viruses among humans (Shinya et al., 2006), yet, if the virus continues to circulate widely among poultry, it has a greater potential to infect humans and other animals. Genetic reassortment may thus take place and create a new pandemic strain. In a recent report of H5N1 virus infection in a domestic cat infected by eating a pigeon carcass, the virus isolated from the pigeon and the cat shows the same cluster as the viruses obtained during the outbreak in Thailand (Kuiken et al., 2004). This affinity of the virus to infect other animals (as well as humans and birds) may cause genetic variation, that in turn may anticipate the next avian influenza outbreak.

7) Vaccines against the next flu pandemics are not available, because there is not enough instruction on which type of influenza virus will be the cause. Available vaccines for birds and humans may be useful for reducing the risk of pandemics, by decreasing the circulation and replication of common flu viruses (avian and humans), that in turn may lower the risk of viral reassortment and mutations. For instance, no significant flu outbreak was detected in Thailand and Vietnam in the year 2006 (from January until 15th June), because of the good vaccination process and surveillance performed in the previous year.

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