

# Pandemic influenza is a zoonosis, as it requires introduction of avian-like gene segments in the human population

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## Abstract

Human influenza viruses manage to cause epidemics almost every year. The circulating viruses change their surface glycoproteins by accumulating mutations (antigenic drift) which results in variant viruses of the same subtype that are able to evade the immune pressure in the population. Every now and then, a completely new subtype of influenza A virus is introduced in the human population, which can result in an influenza pandemic. Pandemic human influenza viruses have been emerging for many centuries. Based on the genetic information of influenza viruses that have been isolated in this century, introduction of genes of the avian influenza virus reservoir obviously is required. Interspecies transmission, via another mammalian host and reassortment of avian and human influenza viruses are potential mechanisms for such an introduction.

A summary of the cases in which influenza viruses containing avian-like gene segments were introduced into the human population is presented. In three cases, such infections resulted in conjunctivitis. Influenza-like illness and even pneumonia was reported in some other infections. Finally, a mortality rate of 33% was observed in the avian influenza A (H5N1) viruses that infected 18 people in Hong Kong in 1997. Although some of these viruses fulfilled some criteria of pandemic influenza viruses, they lacked the ability to rapidly spread through the human population. © 2000 Elsevier Science B.V. All rights reserved.

*Keywords:* Influenza; Pandemic; Antigenic drift; Antigenic shift; Interspecies transmission; Zoonosis

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## 1. The impact of human influenza

Human influenza viruses annually cause minor or major epidemics with considerable morbidity and mortality. It was Hippocrates in ancient Greece who described the first case

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of influenza-like illness. Although it took about two millennia before reliable documentation on influenza epidemics was generated, it is likely that the virus has been circulating since ancient history-but even in the 20th century, influenza has been a serious disease. The notorious Spanish flu pandemic with more than 20 million victims is recorded as one of the most devastating epidemics that ever hit mankind. The search for the mechanism of virulence of this influenza A (H1N1) virus still continues (Taubenberger et al., 1997; Goto and Kawaoka, 1998; Reid et al., 1999). During the Spanish flu pandemic, the origin of the disease was unknown, and it took more than a decade before the first influenza virus was isolated from pigs (Shope, 1931). A few years later, Smith et al. (1933) isolated the first human influenza virus, influenza A/WS/33 (H1N1). Since that time, influenza viruses have been extensively studied, but we are still unable to control the disease. Although many of the victims of the Spanish flu died because of secondary bacterial infections and nowadays would be treated with antibiotics, influenza still causes considerable mortality in the nineties. Especially, the elderly with underlying diseases of the cardiovascular and respiratory system and diabetics are at risk during epidemics. A study carried out in The Netherlands revealed that about 2200 people have died due or related to influenza epidemics over the period from 1967–1989 (Sprenger et al., 1993). In 1993, the season with the highest influenza morbidity in The Netherlands of the last decade, approximately 6000 influenza related deaths were estimated according to the figures of the Dutch Central Bureau of Statistics (CBS). Figures from other countries in the Western Hemisphere are in the same order of magnitude, indicating that influenza still claims many lives.

## **2. Changes in human influenza viruses**

The success of influenza virus can be attributed to its ability to change. The high mutation rate of RNA viruses results in accumulation of mutations in the genome. If these affect the most immunogenic surface glycoprotein, the hemagglutinin, the antibodies present in the population will no longer protect against the mutants. This phenomenon, called antigenic drift, is the reason that influenza viruses manage to cause yearly epidemics. In the human population, currently, two subtypes of influenza A (H1N1 and H3N2) viruses and an influenza B virus are circulating and variants of all three (sub)types of virus are generated each year. As a result, the trivalent influenza vaccines have to be updated yearly because the protection that is induced by the strains in the vaccine is depending on their antigenic reactivity to the variant strains that actually cause the epidemic.

A more drastic way by which influenza viruses change their surface proteins is an antigenic shift. This introduces a new subtype in the human population and has so far only been found with influenza A viruses (Fig. 1). Nucleotide sequencing of all genome segments of influenza viruses revealed that the 1957 Asian pandemic (introduction of an influenza A/H2N2 virus) and the 1968 Hong Kong pandemic (introduction of an influenza A/H3N2 virus) were caused by influenza viruses that were the result of genetic reassortment (Scholtissek et al., 1978). As shown in Fig. 2, three and two gene segments, respectively, of these pandemic viruses were clearly distinguishable from the segments of

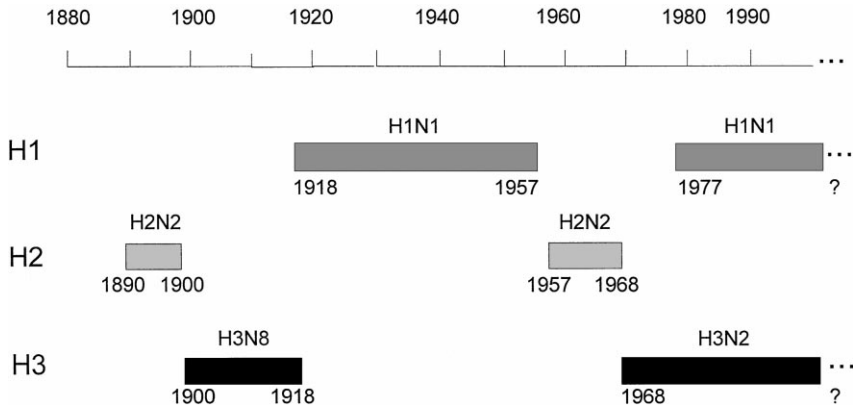


Fig. 1. Influenza A virus subtypes circulating in the human population in the last century. The data before 1993 are based on serological surveys. Yearly epidemics are caused by variant viruses of the same subtype. In 1957 and 1968, an antigenic shift occurred and a new subtype of influenza A virus was introduced in the human population. In 1977, the influenza A (H1N1) virus subtype was reintroduced by an unknown mechanism (Nakajima et al., 1978).

the viruses they replaced, while the others were closely related to those of the preceding human virus strains. In both cases, also the gene coding for the hemagglutinin was replaced and, therefore, this virus entered an unprotected or poorly protected population. Phylogenetic studies showed that the newly introduced gene segments were closely related to those of avian influenza viruses. Obviously, the 1957 and 1968 viruses were the result of reassortment of an avian and human influenza virus strain after co-infection of a susceptible host (Scholtissek et al., 1978). However, as avian influenza viruses do not

Antigenic shift in influenza A viruses

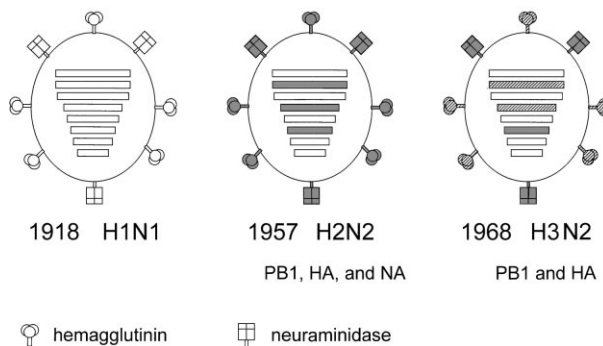


Fig. 2. A schematic representation of the reassortment event of the 1975 influenza A (H2N2) virus, which had only three new gene segments (PB1, HA and NA; in grey) as compared to the influenza A (H1N1) virus that circulated years before. The 1968 influenza A (H3N2) virus was a reassortant of this influenza A (H2N2) virus and an avian H3 subtype virus that provided a new PB1 and H3-HA gene (hatched).

efficiently replicate in humans (Kida et al., 1980; Beare and Webster, 1991), it was proposed by Scholtissek et al. (1985) that pigs could host this reassortment event. All subtypes of avian influenza viruses manage to replicate in pigs (Kida et al., 1994) and this species can also be infected by human influenza viruses (Kundin, 1970; Shortridge et al., 1977). This hypothesis was supported by the finding that avian-human reassortant influenza A (H3N2) viruses were actually identified in pigs (Castrucci et al., 1993) and that these viruses infected humans as well (Claas et al., 1994).

The 1918 influenza A (H1N1) virus causing the Spanish flu pandemic, however, apparently had entered the human population without a reassortment event (Gorman et al., 1991; Reid et al., 1999). Another avian-like influenza A (H1N1) virus had entered the European pig population in 1979 (Pensaert et al., 1981; Scholtissek et al., 1983). In 1986, it was shown that this virus was transmitted from pigs to humans, where it replicated and caused disease as well. Three human isolates of this virus were characterised, two from Switzerland and one from The Netherlands (de Jong et al., 1988; Claas et al., 1996). Thus avian influenza viruses can be transmitted to humans without a reassortment event. However, such a virus may have to adapt to a mammalian host first. Whether this has also been the case for the 1918 influenza A (H1N1) virus is unknown. This 1918 virus had unique properties which resulted in its extreme virulence.

Introduction of genes originating from the avian influenza virus gene pool into humans is required for generating a new pandemic influenza virus. Two possible mechanisms for such an event have been described. First, these avian genes can be mixed with the genome of human influenza viruses by reassortment. Secondly, avian viruses may be transmitted without reassortment, but then may have to adapt to a mammalian host in other animal species.

### **3. Human influenza virus isolates containing avian-like genes**

Apart from the pandemic influenza viruses introduced in 1957 and 1968, not many human isolates contain avian-like genes. Serological studies in rural China have shown the presence of antibodies to many avian influenza virus subtypes in humans, providing evidence for some level of replication (Shortridge, 1992). Actual virus isolates are rare, possibly because of the inefficiency of the replication of these viruses resulting in a subclinical outcome of the infection that does not require medical attention.

In three cases, avian-like influenza virus subtypes have caused conjunctivitis in humans. The first case was reported in 1977, when accidental exposure in the laboratory to influenza A/FPV/Dutch/27 (H7N7) caused keratoconjunctivitis. The infection was confirmed by virus-isolation. (Taylor and Turner, 1977). Another case was diagnosed in relation to the harbour seal mortality of 1979–1980 in the Northeast of the USA. This mortality has been associated with influenza A/seal/Massachusetts/80 (H7N7) viruses, which were isolated from the lungs and brains of some of the animals (Webster et al., 1981a). Four people involved in the autopsies contracted purulent conjunctivitis, but unfortunately, no virus isolation was attempted. However, an investigator in subsequent experimental infections of harbour seals was sneezed in the face by an infected animal, developed severe conjunctivitis. Influenza A (H7N7) virus was

isolated from a swab of the conjunctival membrane (Webster et al., 1981b). The last case was in 1996, when again influenza A (H7N7) virus was isolated from a woman with conjunctivitis. Obviously a piece of straw had entered her eye while cleaning a duck house (Kurtz et al., 1996; Banks et al., 1998). In all three cases, the patients recovered uneventfully.

Actual respiratory disease was caused by the previously mentioned avian-like swine influenza A (H1N1) viruses in Switzerland and The Netherlands in 1986, and the avian-human reassortant influenza A (H3N2) viruses in The Netherlands in 1993 (de Jong et al., 1988; Claas et al., 1994). Although person-to-person spread must have occurred in some of the cases, in general these viruses obviously did not manage to spread efficiently through the human population.

The most serious outbreak of an avian-like influenza virus subtype in the human population was the notorious bird-flu in Hong Kong. In 1997, 18 people were infected by an influenza A (H5N1) virus and six of them died. The isolates were shown to be highly virulent avian influenza H5 subtype viruses, that were transmitted directly from infected poultry in live-bird markets. Although these viruses replicated efficiently in humans, they did not spread efficiently through the human population (de Jong et al., 1997; Subbarao et al., 1998; Claas et al., 1998a). When the authorities in the Chinese special administrative region of Hong Kong decided to kill all poultry, no more new infections were reported. By doing so, they may well have prevented a new influenza pandemic. If the avian influenza A (H5N1) viruses still would have been present during human influenza activity, someone could have been infected with both the bird-flu strain and a human influenza virus. Man himself could have hosted the reassortment event required to provide an H5 influenza virus with the ability to rapidly spread through the population and thus starting a new influenza pandemic (Claas et al., 1998b).

The last cases of avian influenza viruses that managed to infect humans and cause respiratory disease were isolated in February 1999 in Hong Kong. Influenza A (H9N2) viruses, closely related to a virus isolated from a quail, caused typical influenza-like illness in two young girls. Later, it was reported that the same subtype of virus had been infecting five children in mainland China in August 1998. These influenza A (H9N2) viruses were completely of avian origin and did not spread efficiently through the population (Anonymous, 1999). One possibility is that a reassortment event is required for generating a successful new pandemic subtype of human influenza virus. Another possibility is that efficient spread of influenza viruses other than those of the H1, H2 and H3 subtype are restricted by other host range determining factors.

Detection of the influenza A (H9N2) cases has been the result of the increased influenza surveillance in Hong Kong since the bird-flu crisis in 1997. Future will teach us whether more influenza virus subtypes occur in humans now that we have started to pay more attention to this possibility. In order to keep track of these events, it is necessary that all National Influenza Centres world-wide are able to identify all known subtypes of influenza viruses. Despite the impact of pandemic influenza we should keep in mind that cumulative mortality from yearly influenza epidemics caused by antigenic drift variants may even be higher. Identifying antigenic drift variants, therefore, also remains of great importance in our attempts to reduce the impact of influenza.

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