

Predictions for Future Human Influenza Pandemics

Robert G. Webster

*Department of Virology/Molecular Biology, St. Jude Children's
Research Hospital, Memphis, Tennessee*

Will there be another human influenza pandemic? The certainty is that there will be, and the probability is that the virus will emerge from Eurasian aquatic bird reservoirs and involve reassortment between a human and avian strain, with accumulation of mutations or true recombinational events (or both) that will permit spread and pathogenicity among humans. This process will probably occur in pigs because they possess receptors for both avian and human influenza viruses, and emergence may occur in southern China. Prediction of the subtype is impossible, but there is a hypothesis based on seroarcheology that only H1, H2, and H3 subtypes can infect humans; however, it is arguable that H7 or H2 strains might be equally capable of infecting humans.

As the world prepares for the next pandemic of human influenza, the scientific community is looking for indicators of the imminence of this event. Certain predictions can be made on the basis of knowledge accumulated since 1933, when influenza A viruses were first isolated: the certainty that there will be another human pandemic, the probability that the precursor will be of Eurasian avian origin, and how and where this may occur. It is not possible to say when the pandemic will occur, just that likelihood increases proportionally with time. Herein, I will consider each of these predictions; however, time will be the arbiter.

The Certainty

The purpose of this report is to examine the available information about the source of and the mechanisms involved in human influenza pandemics and to utilize the antigenic and molecular information accumulated over the past 50 years to make predictions about future pandemics. Information from historical records, seroarcheology, and molecular epidemiology [1] indicates that there will be another influenza pandemic among humans. It has been ~28 years since the Hong Kong/68 (H3N2) pandemic appeared in humans and 19 years since the Russian influenza strain (H1N1) reemerged in 1977. As judged by the reappearance of H1 after an absence of 27 years, there are enough susceptible people to support a pandemic of the H2 subtype. Representatives of other influenza subtypes (H4–H15) that have not infected humans for at least 100 years might also cause a pandemic since humans have no immunity

to them. On the basis of the historical record of this century, it is probable that only the H1, H2, and H3 subtypes affect humans; however, it cannot be assumed with certainty that only these subtypes can cause human pandemics. I will begin with the assumption that the other subtypes (H4–H15) cannot be excluded and with the certainty that a human pandemic can be anticipated and that its occurrence becomes more imminent with time.

The Probabilities for the Origins of Pandemics

Eurasian origin. The primordial source of all influenza A viruses in mammals and domestic avian species is the aquatic bird reservoirs, in which each of the known subtypes are perpetuated [2]. These avian viruses can be divided into two different populations, one in Eurasia and one in the Americas. Evidence indicates that human influenza pandemics of this century originated from the Eurasian avian lineage. Epidemiologic evidence supports the proposition that the Asian/57 (H2N2), Hong Kong/68 (H3N2), and Russian/77 (H1N1) viruses all originated from China. Phylogenetic studies suggest that the catastrophic "Spanish" influenza of 1918 may also have been of Eurasian origin [3] and raise the question of whether the precursors of the outbreak were of American or Asian origin. Since the high mortality associated with the epidemic occurred in the second year of viral spread [4], it is possible that the virus originated in Asia and spread to North America before it became highly lethal in humans.

Both the Asian/57 (H2N2) and Hong Kong/68 (H3N2) pandemics originated by reassortment. In 1957, the Asian pandemic virus acquired three genes (for PB1, hemagglutinin [HA], and neuraminidase) from the avian influenza gene pool in wild ducks by genetic reassortment and retained five other genes from the circulating human strain [5]. After the Asian strain appeared, the H1N1 strains disappeared from humans. In 1968, the Hong Kong pandemic virus acquired two genes (PB1 and HA) from the duck reservoir by reassortment and kept six genes from the virus circulating in humans (figure 1).

A surprising discovery from phylogenetic analyses of amino acid changes was that avian influenza viruses, unlike mamma-

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Reprints or correspondence: Dr. Robert G. Webster, Dept. of Virology/Molecular Biology, St. Jude Children's Research Hospital, 332 N. Lauderdale, P.O. Box 318, Memphis, TN 38101.

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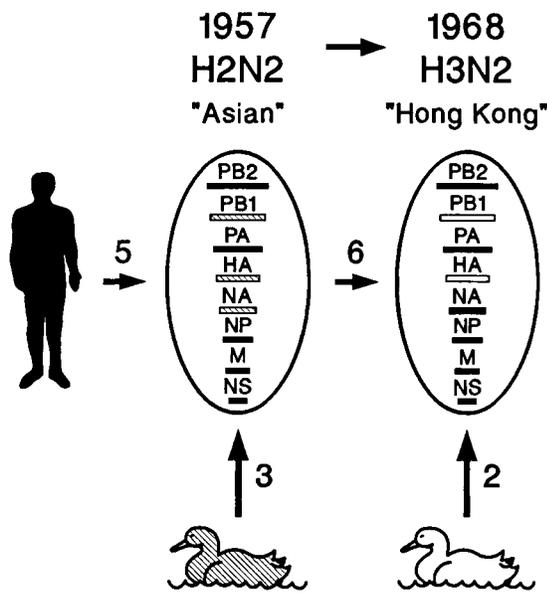


Figure 1. All human influenza pandemics since 1930 have originated in China. In 1957, Asian/57 (H2N2) acquired 3 genes (segments underlined with hatched bars) by reassortment from Eurasian avian viruses and kept 5 gene segments from circulating human strains (black bars). In 1968, Hong Kong/68 (H3N2) acquired 2 genes (segments underlined with white bars) by reassortment from Eurasian avian viruses and kept 6 gene segments from circulating human strains (black bars).

lian strains, show low evolutionary rates [6]. In fact, influenza viruses in aquatic birds appear to be in evolutionary stasis, with no evidence of net evolution over the past 60 years. Nucleotide changes have continued to occur at a similar rate in avian and mammalian influenza viruses; however, these changes do not result in amino acid changes in the avian viruses, but all eight mammalian influenza gene segments continue to accumulate changes in amino acids. The high level of genetic conservation suggests that avian viruses are approaching or have reached an adaptive optimum, wherein nucleotide changes provide no selective advantage. It also means that the source of genes for pandemic influenza viruses exists phenotypically unchanged in the aquatic bird reservoir.

Overall, the most important implication from phylogenetic studies is that the ancestral viruses that caused the Spanish influenza in 1918 and the viruses that provided gene segments for the Asian/57 and Hong Kong/68 pandemics are still circulating in wild birds, with few or no mutational changes.

How?—Mechanisms for generating pandemics. Influenza viruses, like other RNA viruses, are highly variable and undergo rapid genetic change. The reason for this variability is the very high mutation frequencies of their polymerases. The error frequencies for several viral RNA polymerases can average $\sim 10^{-4}$ – 10^{-5} base substitutions per single base site following a limited number of replications [7, 8]. This results in the generation of genetically heterogeneous populations known as

quasispecies [9], which consist of collections of virion RNAs with slightly divergent nucleotide sequences. This enormous genetic plasticity can result in genetic drift in all gene products, including antigenic drift in the surface glycoproteins; host range variation; and alterations in the severity of disease pathogenicity. The segmented nature of the influenza virus genome also permits genetic shift. As noted above, genetic shift was responsible for the generation of the Asian/57 (H2N2) and Hong Kong/68 (H3N2) pandemics (figure 1).

Genetic recombination of influenza virus is a much rarer event but has been demonstrated in RNA viruses, notably in coronaviruses and alphaviruses [10]. Nonhomologous recombination has also been demonstrated for influenza viruses [11]. Recombination resulting in increased cleavability and pathogenicity has also been demonstrated between chicken host cell 28S rRNA and the HA of A/turkey/Oregon/71 (H7N3) [12]. In addition, a variant of A/seal/Mass/1/80 (H7N7) influenza virus had a 60-nucleotide insertion in the HA gene that probably originated from the NP gene and increased the host range and pathogenicity of the virus [13].

During characterization of a new subtype of influenza A (H15) from aquatic birds in Australia, it was found that the HA gene had a 30-nucleotide insertion in the HA at residue 253, which is located in the globular head of the molecule [14] (figure 2). In that study, we were unable to trace the 30-nucleotide insert of our Australian H15 viruses to any viral or cellular gene, but nonhomologous recombination between viral and cellular RNA may have led to the insertion. Since the H15 containing the insertion is translated into a stable surface glycoprotein, this example emphasizes the possible importance of RNA recombination in creating genetic diversity among influenza viruses. Although recombination in influenza viruses appears to be a rare event, it is still a potentially important mechanism in the evolution of human pandemic viruses.

Where?—Do influenza pandemics originate in China? Historical records and the appearance of the Asian, Hong Kong, and Russian pandemic strains of influenza virus in China suggest that most pandemics of human influenza since about 1850 have originated in China. The exception seems to be Spanish influenza, which may have originated in military camps in Kansas and subsequently spread to Europe by US troops in 1918 [4]; however, as considered above, it is also possible that this virus originated in Eurasia.

The possibility has been raised that southern China is an influenza epicenter [15]. The farming practices [16], religious customs (unlike those of Muslim and Jewish religions, which do not promote pigs as a source of protein [2]), and climate [2] of the region combine to make southern China a place where influenza viruses could originate, and the large population of people, pigs, and ducks provides the opportunity for interspecies transmission and genetic exchange among influenza viruses [2]. However, agricultural practices are changing in China, and the percentage of farm families raising pigs in close proximity to humans is steadily declining.

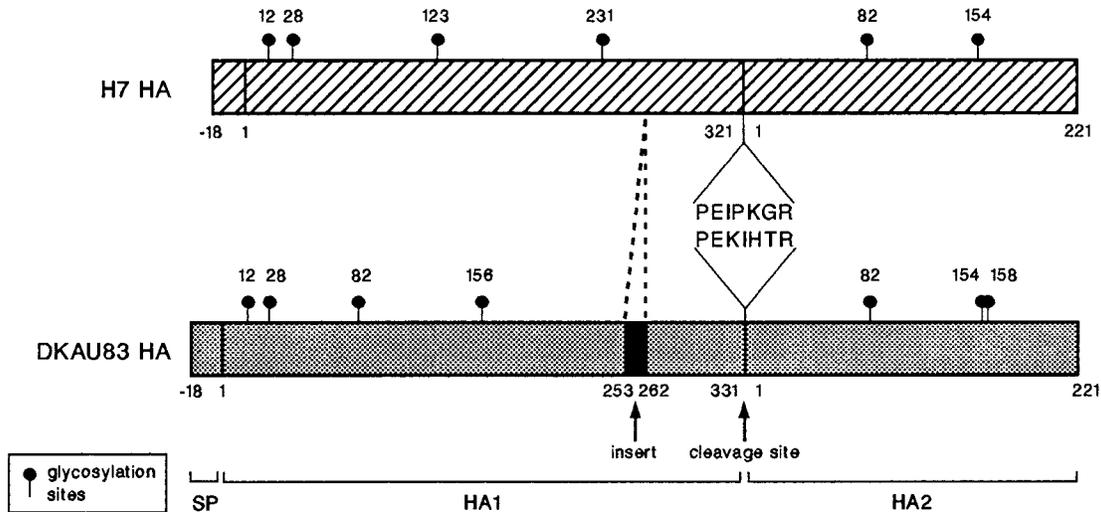


Figure 2. Recombination in hemagglutinin (HA) of H15? H15 HA has 10–amino acid insert at residue 253 that is in globular head of molecule [14] and may have originated by nonhomologous recombination that has been demonstrated for influenza viruses in laboratories [12, 13].

A recent study showed that transmission of avian influenza virus genes to swine occurred in Europe in 1979 [17]. Surveillance studies in Europe from 1977–1989 indicated that H1, H2, and H4 subtypes predominate in wild aquatic birds in this region [18], while H3, H4, and H6 predominate in domestic poultry in southern China [19]. It is important to note that the distribution of influenza virus subtypes has never been established in wild aquatic birds in China or Southeast Asia, but studies over 25 years in the former USSR established that all known subtypes, including H1, H2, and H3, were commonly isolated from water birds, including ducks, gulls, terns, and sandpipers [20]. Studies on the frequency of transfer of avian influenza viruses or gene segments to humans in China have been limited, and preliminary serologic studies suggest that interspecies transmission probably occurs [21]; however, there is no evidence for transfer of avian influenza virus gene segments in interpandemic periods [22].

Although the above considerations about the regions of the world where influenza pandemics originate are interesting, they are still largely speculative. Only circumstantial evidence exists for the appearance of pandemic influenza viruses in southern China. Further studies on influenza in Southeast Asia are necessary to determine whether an epicenter does exist in this region, whether the next human pandemic strains can be isolated from lower animals (e.g., pigs), and whether the next pandemic will occur first in this region of the world.

What?—Which subtype of influenza A will cause the next human pandemic? Serologic and virologic evidence suggest that since 1889 there have been six introductions of a virus bearing an HA subtype that had been absent from the human population for some time: Three human subtypes have appeared and reappeared cyclically, beginning with the sequential emergence of the virus subtypes H2 in 1889, H3 in 1900, and H1

in 1918 and the reemergence of H2 in 1957, H3 in 1968, and H1 in 1977 [2].

If recycling occurs, then H2 will probably be the subtype that causes the next human pandemic. It has been ~28 years since the H2 subtype last infected humans, and as determined on the basis of the reappearance of H1 after an absence of 27 years, there are enough susceptible people to support a pandemic. Influenza viruses of the H2 subtype have continued to circulate in the aquatic avian reservoir [23], the HA of which is essentially unchanged from that of the viruses that affected humans in 1957. During 1994–1995, H2N2 virus was isolated 49 times from live-bird markets and poultry in the United States (Senne D., personal communication). Do these viruses have the capacity to infect humans, including immunosuppressed individuals, or swine? There is no convincing evidence for the detection of H2 influenza viruses in pigs. Since the Eurasian lineage of avian influenza viruses has been the source for gene segments that have reassorted with the circulating human viruses to produce the Asian/57 and Hong Kong/68 pandemic strains, we need to know if H2 viruses are currently prevalent in avian species in Eurasia.

If the next pandemic in humans is not caused by an H2 virus subtype, then which of the remaining subtypes (H4–H15) are possibilities? H7 might cause a pandemic, for it is known to infect mammals. H7N7 was 1 of 2 influenza A strains that caused epidemics in horses, and it has also infected seals and caused conjunctivitis in humans [1]. Preliminary studies have found antibodies to subtype H7 in humans in China [21]. Other subtypes, including H4, have been isolated from seals [24], and H10 has been isolated from minks; however, these strains have not become established in these species. Other subtypes seem less likely to cause a pandemic among humans, despite their high prevalence in aquatic birds throughout the world

(H4, H3, and H6 predominate in aquatic birds in China [19] and North America, and H1, H2, and H4 predominate in Europe [18]).

The Emergence of “New” Influenza Viruses in Lower Animals and Birds

Periodically, influenza viruses in the aquatic bird reservoir spread to swine, horses, domestic poultry, and sea mammals [2], causing major outbreaks of infections ranging in severity from inapparent to severe. In lower animals, the entire virus is often transmitted without reassortment to the new host, as typified by a recent example of the emergence in chickens of a highly pathogenic influenza virus from the aquatic bird reservoir [25]. In October of 1993, there was decreased egg production and increased mortality among Mexican chickens in association with serologic evidence of an H5N2 influenza virus (figure 3). First isolated from chickens in May of 1994, after spreading widely in the country, the virus caused only a mild respiratory syndrome in specific pathogen-free chickens. Eradication of the virus would have required the destruction of infected birds, a step that was not taken because it would have devastated the poultry industry in Mexico. Therefore, a “field

experiment” was conducted to determine the fate of an avirulent virus after repeated cycles of replication in millions of chickens. By the end of 1994, the virus had mutated to contain a highly cleavable HA but remained only mildly pathogenic in chickens. Within months, however, it had become lethal in poultry. Nucleotide sequence analysis of the HA cleavage site of the original avirulent strain revealed a sequence (RETR) typical of avirulent viruses and unlike the sequence (KKKR) that characterized viruses responsible for the 1983 outbreak in US poultry. The highly pathogenic isolates contained insertions and substitutions in the HA connecting peptide, RKRKTR, which made the HA highly cleavable in trypsin-free chicken embryo fibroblasts. Phylogenetic analysis of the HA of H5 avian influenza viruses, including the Mexican isolates, indicated that the epidemic virus originated from the introduction of a single virus of the North American lineage into Mexican chickens. This sequence of events demonstrates the step-wise acquisition of virulence by an avian influenza virus in nature.

Until a decision is made to eradicate H5N2 virus from Mexican poultry, the field experiment continues, with three possible outcomes. First, it is possible that both nonpathogenic and pathogenic viruses will continue to spread in Mexico and internationally. Second, an effective vaccination and biosecurity program might eradicate currently circulating nonpathogenic

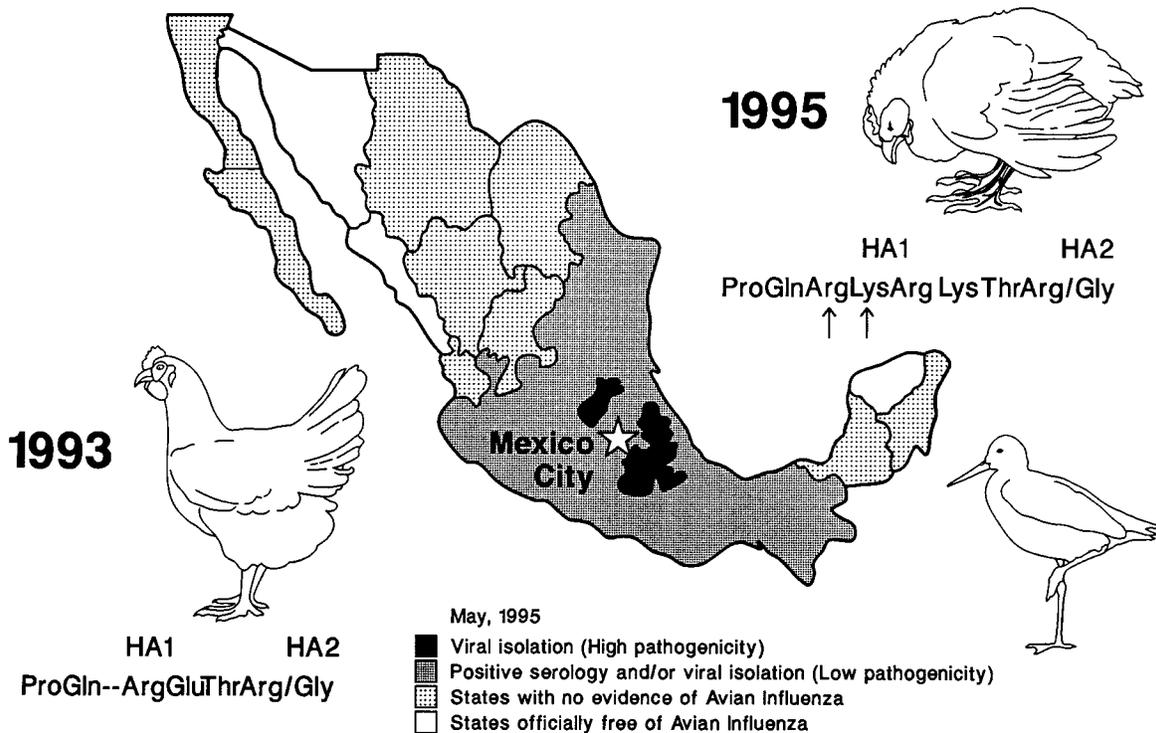


Figure 3. Emergence of highly pathogenic H5N2 influenza virus in chickens in Mexico. In 1994, nonpathogenic H5N2 influenza virus was detected in Mexican chickens; virus was related to H5N2 virus isolated in 1991 from Ruddy turnstones (*Arenaria interpres*) in Delaware Bay (arm of Atlantic Ocean, between the southwest coast of New Jersey and the east coast of Delaware). Over course of next year, virus became highly pathogenic, and hemagglutinin (HA) had acquired insert of 2 basic amino acids (Arg and Lys) and mutation of Glu to Lys at position -3 from cleavage site of HA1/HA2.

and pathogenic forms of the virus; however, this seems unlikely since the avirulent virus that is adapted to chickens spreads very effectively and causes minimal primary evidence of infection. Third, it is possible that the pathogenic variants will be self-limiting and will not be maintained; however, if the avirulent form is allowed to continue circulating, it is inevitable that there will be periodic outbreaks of highly pathogenic avian influenza.

Thus, influenza A viruses continue to emerge from the aquatic bird reservoir to cause disease of various degrees of severity. These outbreaks in lower animals and birds serve to remind us that it is only a matter of time before another influenza pandemic occurs among humans.

Direct or Indirect Spread

There is an increasing amount of information suggesting that pigs may be the intermediate host or “mixing vessel” for the spread of avian influenza virus to humans [26, 27]. This subject is extensively reviewed elsewhere in this supplement. There is convincing evidence for interspecies spread of swine, avian, and human influenza viruses and for genetic reassortment of the virus in pigs; thus, there is convincing evidence that the pig probably provides one avenue for the transfer of H1N1 and H3N2 influenza virus genes to humans.

There is also evidence that turkeys in the United States can be infected with classical H1N1 swine influenza viruses and that these viruses can reassort with avian strains [28]. There is no evidence yet that these viruses spread directly to humans; however, it could be envisioned that they might spread through pigs to humans.

At this time, there is no evidence for or against the direct spread of avian influenza viruses to humans. The sialic acid receptor specificity of human cells (α 2-6Gal linkage) is not compatible with that found on avian influenza viruses (α 2-3Gal linkage) (reviewed elsewhere in this supplement), but it is not known whether this is sufficient to preclude direct spread. There is indirect evidence that some avian strains infect humans, including H7N7 seal influenza virus [2], and there are reports of antibodies to avian strains (especially to H7) in humans [21].

Thus, the most compelling evidence supports the notion that there is indirect spread of avian influenza virus to humans, but the much rarer possibility of direct spread cannot be ruled out.

The Uncertainty of When the Next Human Pandemic Will Occur and Its Severity

According to Kennedy Shortridge [29], it is not possible to predict when the next human influenza pandemic will occur: “Put simply, each year brings us closer to the next pandemic.” It is also not possible to predict the severity of a pandemic. Although we know some features that determine pathogenicity in avian influenza viruses (the insertion of basic amino acids near the cleavage site of the HA), we know very little about the molecular basis of

pathogenicity of human influenza viruses and why one strain is more pathogenic than another.

Conclusions

Given the certainty that there will be another pandemic of human influenza, we must devote as much effort and as many resources as necessary to draw up a defense strategy and a battle plan for dealing with what could be a potential catastrophe. Since an H2 Eurasian avian influenza virus may be the precursor of a future human pandemic originating in China, it is recommended that the frequency of H2 isolates in aquatic birds and any evidence of H2 or other avian influenza viruses in pigs and humans be determined in China.

If we assume that people, pigs, and aquatic birds are the principal variables associated with the interspecies transfer of influenza virus and the emergence of new human pandemic strains, it may be possible to influence the occurrence of human pandemics of influenza. Live-bird markets that house a wide variety of avian species (chickens, ducks, turkeys, pheasants, and guinea fowl) and occasionally pigs together for sale directly to the public provide outstanding conditions for genetic mixing and spreading of influenza viruses. Monitoring of the birds entering these markets for influenza could provide information on prevalent subtypes. If pigs are the mixing vessel for influenza viruses, then monitoring them for evidence of avian influenza viruses, particularly of H2 subtypes, might provide an early warning of what might occur in humans. Finally, it is recommended that serologic surveillance of humans in contact with animals should be done regularly, particularly in Southeast Asia.

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