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Influenza: interspecies transmission and emergence of new pandemics

R.G. Webster [a,*], K.F. Shortridge [b], Y. Kawaoka [a]

[a] *Department of Virology and Molecular Biology, St. Jude Children's Research Hospital, 332 N. Lauderdale, P.O. Box 318, Memphis, TN 38101-0318, USA*

[b] *Department of Microbiology, University of Hong Kong, Hong Kong*

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1. Introduction

Influenza is a well-known yearly threat, so why is it considered to be an emerging disease? It is because the virus evolves so rapidly that every year new viruses emerge, and every century three to four emergent strains are 'new' enough to cause pandemics.

Historically, pandemics have been both unpredictable and devastating. As we learn more about how and where pandemic strains evolve, strategies for blocking their emergence and for recognition of potentially dangerous strains will become possible [1].

2. Strain variability

The single most significant characteristic of the influenza virus that makes it such an important threat is its enormous variability. Several features of the virus contribute to its variability. The first is that the polymerase complex makes mistakes when replicating the viral genes, and, since there is no built-in proofreading mechanism for RNA viruses, a lot of variation accumulates. Many of these variant viruses will not survive, because the acquired changes will make them unable to grow, but many genetic mistakes are well tolerated. Any preparation of influenza virus is a mixture of these variants, one of which might emerge and become a dominant new strain.

The second, and probably more important, feature is the segmented nature of the genome. There are eight genetic segments, each specifying a unique viral function. When individual cells are infected by two different viruses, new strains can evolve that have some of their eight genetic segments derived from each of the infecting 'parents'. 'Genetic reassortment', as this is called, is a pivotal factor in emergence of pandemic strains. Adding to the potential for variability, occasionally there also can be true genetic recombination between segments.

The third feature is the large number of strains possible, because of the large number of potential permutations of the two viral surface glycoproteins, the principal determinants of humoral immunity. There are 15 different hemagglutinin subtypes (H1–H15) and nine neuraminidase subtypes (N1–N9), each of which can combine through genetic reassortment to yield a different strain (H1N1, H1N2, H1N3, etc.).

All 15 hemagglutinin subtypes of influenza virus are found in aquatic birds throughout the world. This raises an important question: how many of the 15 subtypes, which differ by only 30% in genetic homology, can transmit to humans? A dogma has

* Corresponding author. Tel.: +1 (901) 495-3400; fax: +1 (901) 523-2622; e-mail: robert.webster@stjude.org

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grown up that says that only three types, H1, H2 and H3, can, but we have only the experience of the past century on which to base this conclusion. The appearance and disappearance of subtypes in other mammals, such as the horse, over longer periods of time have been documented, raising the possibility that other subtypes have infected humans in the past and will infect humans in the future. Normally, we find two subtypes in pigs, two in horses, a number in seals and a few in whales. Occasionally, influenza virus has been isolated from other species including mink and, possibly, camels.

3. Emergence of pandemic strains

Since influenza was first grown in the laboratory in the early 1930s by Wilson Smith, we have had three pandemics. In 1957, we had Asian flu; in 1968, Hong Kong flu; and in 1977, we had the so-called Russian flu. Russian H1N1 is looked upon by many of us as an illegitimate strain or illegal strain, because this strain was genetically identical to one isolated in the mid-1930s and thus appears to have been reintroduced into the population as though it had been brought back from the freezer. It later reappeared, again in a totally conserved genetic state, in Northern China, suggesting the possibility that it, too, came out of the freezer.

The granddaddy of all influenza outbreaks was the one in 1918. About the only thing that could be done then was to wear face masks and bury the dead. That was a terrible pandemic. At least 20 million people in the world died, most of whom were young adults. It is estimated that one in four people in the world were affected. It is fairly well established that this virus was carried by American troops to Europe – probably the first unwitting example of biological warfare by American troops. It finished World War I. So many German and Allied troops died that neither side would acknowledge the fact that they were being decimated by this virus. As a consequence they blamed it on the Spanish, and that is how it became known as 'Spanish' flu. In fact, the pandemic killed more people in one year than all of the armed conflicts of the century. By comparison, the Asian/'57 and Hong Kong/'68 pandemics killed many fewer people, but cost an estimated 32 billion dollars.

It has been 28 years since we had a human pandemic, and it is certain that there will be another. Where will the next pandemic stains come from? The accumulated evidence indicates that these viruses will come from aquatic birds. After the Hong Kong/'68 pandemic, the National Institutes of Health and World Health Organization decided it was time to find out where these viruses came from, and we and others have been involved in elucidating the world reservoirs of this virus.

Important clues were provided by studies done in the early 1970s in Canada, which found that, prior to their migration south, about 25% of young ducks tested from mid July to late August carried influenza virus. Duck infection is very different from human infection; there are no disease signs and the virus replicates in the intestinal tract more than in the respiratory tract. Shedding virus in their feces, these ducks make a wonderful system for transmission. As they migrate in the fall, they fly over turkey farms in the United States contaminating water as they go and probably infecting many turkeys on those farms. Similar avian reservoirs of influenza virus have been found all over the world, wherever studies have been done.

We do not know how influenza viruses are perpetuated, because the virus does not establish chronic infection in individual birds. In fact, it barely remains in the population, almost disappearing by the end of the season. But in the springtime other aquatic birds, migrating from South America, pick up these viruses and they, in turn, shed them in their feces onto the shore line. About 25% of ruddy turnstones and redknots, migrating from South America to the north slopes of Alaska, were found to be infected and capable of dropping viruses in their feces on the beaches [2]. Thus the biology suggests that the influenza viruses are transmitted to a limited range of other avian hosts.

Phylogenetic analyses of the nucleotide sequences of any of the eight genetic segments, suggest that there are five quite different host-specific lineages of influenza virus: two in horses (a very ancient one and a modern one), one in pigs that includes human strains, one in aquatic birds, and one in other avian species. Analysis of the pig lineage indicates that it has been mixed up with the avian lineage, showing that even though these lineages are host-

restricted, from time to time they transmit to other species.

Phylogenetic data also support the notions that avian species are the origin of all the mammalian lineages; there is geographical separation of the lineages; and the avian strains are in evolutionary stasis. In contrast to the mammalian viruses that are constantly changing their antigenic properties, some of the avian strains have not changed their antigenic epitopes in 50 years.

Which of these lineages of avian influenza virus have given rise to the pandemic strains? It is very clear that recent pandemic strains were Eurasian avian lineage viruses that came out of China. Spanish flu may have been an exception, but Dr. Shortridge maintains that this virus also may have come from Asia, spent a year or more in a benign form, and seeded the rest of the world before it accumulated the necessary mutations that made it the very lethal strain that it was. Genetic analysis of early strains provides some support for this idea.

Eurasian avian lineage viruses gave rise to pandemic strains by reassortment with the viruses circulating in humans. Three gene segments came from ducks in the case of the Asian/'57 strain (H2N2), and two gene segments were acquired in the Hong Kong/'68 strain (H3N2). A critical factor in their pandemic spread was the acquisition of new genes specifying outside viral components, hemagglutinin and/or the neuraminidase, to which the world population had no immunity. The frightening implication of these findings viewed together with the evolutionary stasis of the avian viruses is that viruses almost genetically identical to the ancestral strains that donated their gene segments to the pandemic strains earlier in the century are still circulating in wild birds.

4. Interspecies transmission

How do the pandemic strains get their avian genes? The available evidence indicates that the avian viruses do not transmit directly to humans, because humans do not have the appropriate cellular receptors. Avian influenza viruses prefer α2–3 sialic acid for attachment, and human viruses prefer α2–6. Recently, Dr. Kawaoka showed that the pig has both kinds of receptors and can be infected with both avian and human strains (personal communication). Moreover, as elegantly shown by Dr. Kida, the pig can be infected with all of the avian influenza viruses [3]. In China at least one-third of the pigs are still raised within the home on farms where ducks and other avian species are raised, making the pig a good 'mixing bowl' for reassortment of influenza viruses.

There is a lot of evidence of transmission of influenza viruses from pigs to humans and vice versa. In 1977, at the time of the famous 'swine flu' incident when so many people were vaccinated in the United States, we did not know how readily influenza transmits from pigs to humans, but we later learned that every year there are examples of people who contract swine influenza. In one instance, a pregnant woman walked through the pig barns at an agricultural fair in Wisconsin and 2 days later she was dead. Usually the virus does not become established, because it is not transmitted from one person to another.

The frequency with which avian viruses transmit to other hosts, like the pig, is difficult to estimate. Dr. Shortridge has an enormous collection of viruses that he collected from pigs in Hong Kong and China between 1975 and 1980. None of more than 100 isolates examined by RNA-RNA hybridization and partial sequencing had gene segments characteristic of avian viruses or human viruses. On the other hand, his more recent studies and studies in 1979 in Europe by Dr. Scholtissek established that avian influenza virus is transmitted into the pig population. All eight avian virus genes transmitted and this virus became established in the pig. Not only did it transmit, it reassorted with human influenza viruses, and pigs in Italy now have reassortants possessing six avian virus genes and two human virus genes, the latter coding for the 'overcoat' of a human influenza virus. Not surprisingly, studies by Eric Claas in the Netherlands showed that these reassortants can infect children.

Why has this virus not taken off and caused a pandemic? We speculate that the illegal introduction of and subsequent pandemic by Russian/'77 may have provided immunity that is preventing these viruses from spreading and causing a pandemic. Dr. Shortridge has looked in Chinese pigs to see if there is evidence of avian influenza viruses in the pig pop-

ulation of Asia, and in 1993 he found influenza viruses in pigs every month of the year, with a peak in March. He also found evidence of avian influenza viruses in September in pigs. He also showed that, as in Europe, an avian influenza virus had transmitted all its genes to the pig population, but we do not have enough information to know whether this virus will become established in that population. The evidence so far indicates that the virus found in Asia is not the same as the one characterized in Europe, but that is not known with certainty at this time.

If we look in the households in China, is there evidence of avian influenza viruses in humans? There is no detectable antibody for the H2 determinant associated with the 1957 pandemic in young people less than 27 years of age. That could be considered good news, since H2N2 virus is one that we might guess would come back and it does not appear to be seeding into the young people in China. Since no protective antibody is detectable, however, this population is totally susceptible. On the other hand, we did find H7 antibodies indicating circulation of a 'new' virus in humans, and we did isolate duck viruses with H7 hemagglutinin in these homes. Evidence for H7 viruses in pigs has yet to be found.

5. Intraspecies variation and altered virulence

There are informative, but frightening, lessons to be learned from studying the behavior of influenza viruses in lower animals. In the early 1980s in Pennsylvania an influenza virus spread into a chicken house. One day the chickens were alive and apparently well; the next day they were all dead. The same thing happened in Mexico in 1993. Again an influenza virus transmitted from aquatic birds into the chicken population and for about a year the Mexicans did not know that a new virus was there. The virus spread throughout Mexico, and in the course of the next year, the virus became highly pathogenic and killed chickens in the high-density chicken farms near Mexico City. We know what the molecular changes were in that virus. The carboxyl end of the hemagglutinin, which has to be cleaved to make the hemagglutinin infectious, acquired a series of basic amino acids by mutation and probably by genetic recombination. As a result, this genetically altered virus, instead of infecting and growing just in the intestinal tract, now spreads throughout the body and the birds die of hemorrhage and central nervous system disorders.

6. Strategies for the future

What possibilities do we see for future human pandemics? We predict that the virus strains will be Eurasian in origin, probably from China, and will acquire new hemagglutinin (possibly H2) and neuraminidase determinants, as in the past, from avian viruses reassorted in the domestic pig.

A great deal of emphasis has been placed on human surveillance, and the US Centers for Disease Control and the World Health Organization do a fantastic job in monitoring the changes in humans so that the pandemic or epidemic strains can be recognized promptly and vaccines modified and utilized in time to avert disaster. Until now, however, little attention has been paid to monitoring animals. Pigs, especially in China, have not been looked at in sufficient detail, but, as we have shown, they are a likely intermediate host for the next pandemic strains.

Finally, it seems appropriate to comment on strategic planning for influenza pandemics. An alarming statistic is that in Japan there are more elderly people than in the United States, the United Kingdom and Germany combined. By 2020, 25% of the population will be over 65 years of age. Why is it of concern? It is because Japan is using less influenza vaccine than any other developed country. In fact, from 1988 until 1996 influenza vaccine usage has fallen consistently, and it is the only developed country where this has occurred. This is a great concern, because it suggests that the infrastructure is not there to respond quickly when the next pandemic occurs, possibly within the next 5 years. Besides giving consideration to having something equivalent to the US Centers for Disease Control, our Japanese colleagues are encouraged to give special attention to vaccines and to the infrastructure of producing them. They are encouraged to work diligently on a strategic plan, like the ones developed in the United States and many other countries, for dealing with a pandemic when it does come, as it is sure to do.

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