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Note

The importance of animal influenza for human disease

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Abstract

Influenza is a zoonotic disease caused by a constantly varying RNA virus resulting in a need for continuous surveillance to update human vaccines. Our knowledge indicates that the intermittent pandemics of influenza originate from influenza viruses or gene segments from influenza viruses in lower animals and birds. These pandemics can be mild to catastrophic. While we have learned a great deal about the ecology and molecular properties of "animal" influenza viruses, we do not have a system for comprehensive international surveillance. The 1918 Spanish influenza pandemic that originated from lower animals and the recent H5NI bird flu incident in Hong Kong serves to remind us that influenza is an emerging disease. The challenge for the 21st century is to accumulate the necessary epidemiological data on animal influenza viruses so that an international surveillance system can be devised. This epidemiological data may provide clues on how to reduce interspecies transmission of influenza. The separation of aquatic birds from other "land based" domestic poultry in Hong Kong after the H5NI bird flu incident indicates that animal husbandry practices could influence the interspecies transmission of influenza viruses. © 2002 Published by Elsevier Science Ltd.

Keywords: Pandemic influenza; Zoonotic disease; Aquatic bird reservoir; H5N1 bird flu; Hong Kong poultry markets

1. Introduction

Influenza viruses continue to evolve, and new antigenic variants (drift strains) emerge constantly, giving rise to yearly epidemics. In addition, strains to which most humans have no immunity appear suddenly, and the resulting pandemics vary from serious to catastrophic. Influenza viruses are unique among respiratory tract viruses in that, they undergo continuous genetic variation. Both surface antigens of the influenza A viruses undergo two types of variation: drift and shift [1]. Antigenic drift involves minor changes in the haemagglutinin (HA) and neuraminidase (NA), whereas antigenic shift involves major changes in these molecules resulting from replacement of the gene segment.

2. Reservoirs of influenza A viruses

Aquatic birds are the reservoirs of all 15 subtypes of influenza A viruses. In wild ducks, influenza viruses replicate preferentially in the cells lining the intestinal tract, cause no disease signs, and are excreted in high concentration in the feces (up to $10^{8.7}$, 50% egg infectious doses/g) [2]. Avian influenza viruses have been isolated from freshly deposited fecal material and from unconcentrated lake water, which indicates that waterfowl have a very efficient way to transmit viruses, i.e. by fecal material in the water supply. Since a large number of susceptible young ducks are hatched each year throughout the world, many birds are infected by virus shed into water. This explains the high incidence of virus infection in Canadian ducks, particularly juveniles, when up to 30% can shed virus before fall migration. Transmission by feces also provides a way for wild ducks, as they migrate through an area, to spread their viruses to other domestic and wild birds [3].

The avirulent nature of avian influenza infection in ducks and wading birds may result from virus adaptation to this host over many centuries, which created a reservoir that ensures perpetuation of the virus; therefore, ducks and wading birds may occupy an important position in the natural history of influenza viruses. Influenza viruses of avian origin have been implicated in outbreaks of influenza in mammals, such as seals [4], whales [5], and pigs [6], as well as in domestic poultry [7]. While influenza A viruses transmit relatively frequently to other species, they rarely establish permanent lineages in these species.

3. Evolutionary pathways for influenza viruses

Studies on the ecology of influenza viruses have led to the hypothesis that all mammalian influenza viruses de-

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rive from the avian influenza reservoir. Support for this theory comes from phylogenetic analyses of nucleic acid sequences of influenza A viruses from a variety of hosts, geographic regions, and virus subtypes. Analyses of the nucleoprotein (*NP*) gene show that avian influenza viruses have evolved into five host-specific lineages: ancient equine, which has not been isolated in over 15 years; recent equine; gull; swine; and human [8,9]. The human and classic swine viruses have a genetic "sister group" relationship, which suggests a common origin. The ancestor of the human and classic swine virus appears to have been an intact avian virus that, like the influenza virus currently circulating in pigs in Europe, derived all its genes from avian sources. Sequence analysis of the *HA* gene from tissues of persons who died in 1918 does not support this concept [10]. This data indicates that the 1918 human strains are most closely related to classical swine influenza virus.

Studies on the *NP* and other gene lineages in avian species show separate sublineages of influenza in Eurasia and the Americas, indicating that migratory birds moving between these continents (latitudinal migration) have little or no role in the transmission of influenza, while birds that migrate longitudinally appear to play a key role in the continuing process of virus evolution.

Phylogenetic analyses of amino acid changes show that avian influenza viruses, unlike mammalian strains, have low evolutionary rates. In fact, influenza viruses, in their aquatic bird reservoirs, appear to be in evolutionary stasis, with no evidence of net evolution over the past 60 years. Nucleotide changes have continued at a similar rate in avian and mammalian influenza viruses; however, these changes no longer result in amino acid changes in the avian viruses, whereas all eight mammalian influenza gene segments continue to accumulate changes in amino acids. The high level of genetic conservation suggests that avian viruses in their natural reservoirs are approaching or have reached optimum, wherein nucleotide changes provide no selective advantage. It also means that the source of genes for pandemic influenza viruses exist phenotypically unchanged in aquatic bird reservoir.

4. Influenza A viruses in lower animals and birds are the source of genetic information for the emergence and re-emergence of new influenza A virus in humans

Over the past two and a half centuries, 10–20 human influenza pandemics have swept the globe. The most devastating pandemic, the so-called Spanish flu of 1918–1919, caused more than 20 million deaths and affected more than 200 million people. This pandemic probably obtained its gene segments from the avian reservoir and pigs may have been involved in interspecies transmission.

Since the first human influenza virus was isolated in 1933, new subtypes of human type A influenza viruses have occurred: H2N2 (Asian influenza) replaced H1N1 in 1957, H3N2 (Hong Kong) virus appeared in 1968, and H1N1 virus reappeared in 1977. Each of these new subtypes first appeared in China. In 1957, the Asian pandemic virus acquired three genes (*PB1*, *HA*, and *NA*) from the avian influenza gene pool in aquatic birds by genetic re-assortment and kept five other genes from the circulating human strains ([11], Fig. 1). 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 re-assortment and kept six genes from the virus

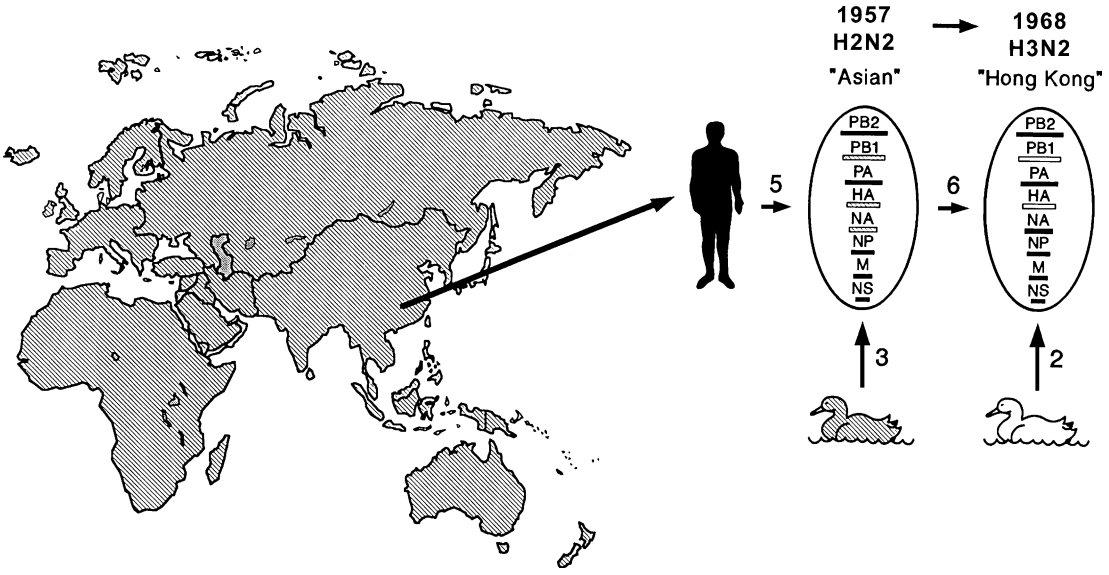

Fig. 1. Emergence of the Asian-57 and Hong Kong-68 influenza pandemics. The Asian-57 and Hong Kong-68 pandemics originated in Asia by re-assortment between the influenza virus circulating in humans and viruses of EurAsian avian origin. The Asian-57 virus acquired three gene segments (*PB1*, *HA*, and *NA*) from avian sources and the Hong Kong-68 obtained two gene segments (*PB1* and *HA*) from EurAsian avian sources [11].

circulating in humans (Fig. 1). After the appearance of the Hong Kong strain, the H2N2 Asian strains were no longer detectable in humans. In 1977, the Russian H1N1 influenza virus that had circulated in humans in 1950 reappeared and spread in children and young adults. This virus has probably escaped from a laboratory and continued to co-circulate with the H3N2 influenza viruses in the human population.

5. Is the pig the intermediate host?

Current human influenza viruses are believed to have arisen by genetic re-assortment between previous human influenza viruses and non-human viruses (Fig. 1). Where did the re-assortment between genes of humans and avian influenza viruses occur? Swine have been considered a logical intermediate for the re-assortment of influenza viruses, for they can serve as hosts for viruses from either birds or humans [12]. Additionally, pigs have receptors for both avian and human influenza viruses [13] and are susceptible to infection with all of the avian subtypes so far tested (H1–H13) [14]. It is now over 30 years since the Asian-57 H2N2 strain disappeared from humans. It is important that we do not reintroduce this strain into humans from the laboratory.

6. The H5N1 avian influenza incident

One of the best examples of the importance of influenza viruses in lower animals and birds to human disease was the bird flu incident in Hong Kong, Special Administrative Region of China in 1997. In late March and early May 1997, an H5N1 influenza virus caused high mortality on three chicken farms in the New Territories, Hong Kong, China. Approximately 75% mortality occurred on the three farms, with a loss of over 6500 chickens. Also in early May, a descendant of the H5N1 virus contracted by a young child caused fatal viral pneumonia with severe complications. This was the first reported avian influenza to cause clinical respiratory illness in humans [15,16,17]. Following a lag phase of about 6 months, a second wave of infection in humans in November and December 1997 caused 17 additional cases and five deaths. The ages of the 18 patients ranged from 1 to 60 years. The clinical features of the first 12 cases, described by Yuen et al. [18], included an onset typical of classical influenza, with fever and upper respiratory tract infection. However, a high percentage of patients (7 out of 12 cases) had severe complications with pneumonia; gastrointestinal manifestations, elevated liver enzymes, and renal failure were also usually prominent. The authors noted that except for the index case, all of the children infected fared better than the adults; the children under 13 years had uneventful recoveries, whereas all seven of the older patients had severe disease and four died.

By early December 1997, there was great concern that an incipient pandemic of influenza was emerging in Hong Kong. Human influenza cases were occurring with apparently random distribution, and the viruses isolated were avian H5N1 strains. Because it was urgent to determine the source of the viruses, an international task force of virologists was assembled for surveillance of the poultry markets. Influenza H5Nl was isolated from approximately 20% of fecal samples from chickens and from approximately 2% of fecal samples from ducks and geese. It became apparent that poultry markets were the source of H5N1 influenza viruses, setting the stage for the decision to depopulate Hong Kong's poultry.

Prior to this outbreak, H5 influenza viruses had been isolated only from avian species. Available evidence indicates that the highly pathogenic avian H5, as well as the H7 strains, evolve from non-pathogenic precursors [19]. Although previous studies found no evidence that A/chicken/Pennsylvania/l370/83 (H5N2) virus had been transmitted to poultry workers [20], more recent studies in southern China indicated low levels of antibodies to all avian influenza virus subtypes tested in the rural human population [21].

When influenza virus is introduced into a new host, the proportion of nucleotide changes that result in amino acid changes is usually highest in the *HA* gene. In the H5N1 influenza viruses isolated from Hong Kong poultry markets, this was not the case; the highest percentage of coding changes that altered amino acids was approximately six times higher in the *PA* gene than in the *HA* gene. The emergence of highly pathogenic, H5 influenza viruses in chickens in Mexico represents the most comparable case for which information is available. Those isolates showed a large proportion of amino acid changes in the HA1 protein (57.3%) and a lower proportion in the products of the internal genes. This reversal of the pattern of coding changes suggests that the *HA* is better adapted to chickens than are the other genes. The Hong Kong H5N1 viruses may be re-assortants that acquired the *HA* gene from an H5 virus that is well adapted to domestic poultry, while the other seven genes may have come from another source, such as wild aquatic birds. The *HA* could possibly have come from A/goose/Guangdong/l/96 (H5N1), a virus that was highly lethal in geese ([22], Fig. 2). Guangdong Province lies adjacent to Hong Kong and provides much of Hong Kong's domestic poultry. The other seven genes are of EurAsian avian origin, but their source is unknown. Additional sequence information from EurAsian influenza viruses should elucidate the origin of these gene segments.

7. Live bird markets and the epidemiology of influenza

The chicken/Pennsylvania (H5N2) influenza outbreak in 1983 to 1984 demonstrated that live bird markets play an important part in the spread of influenza viruses in avian species. In 1997, Senne et al., [23] described live bird

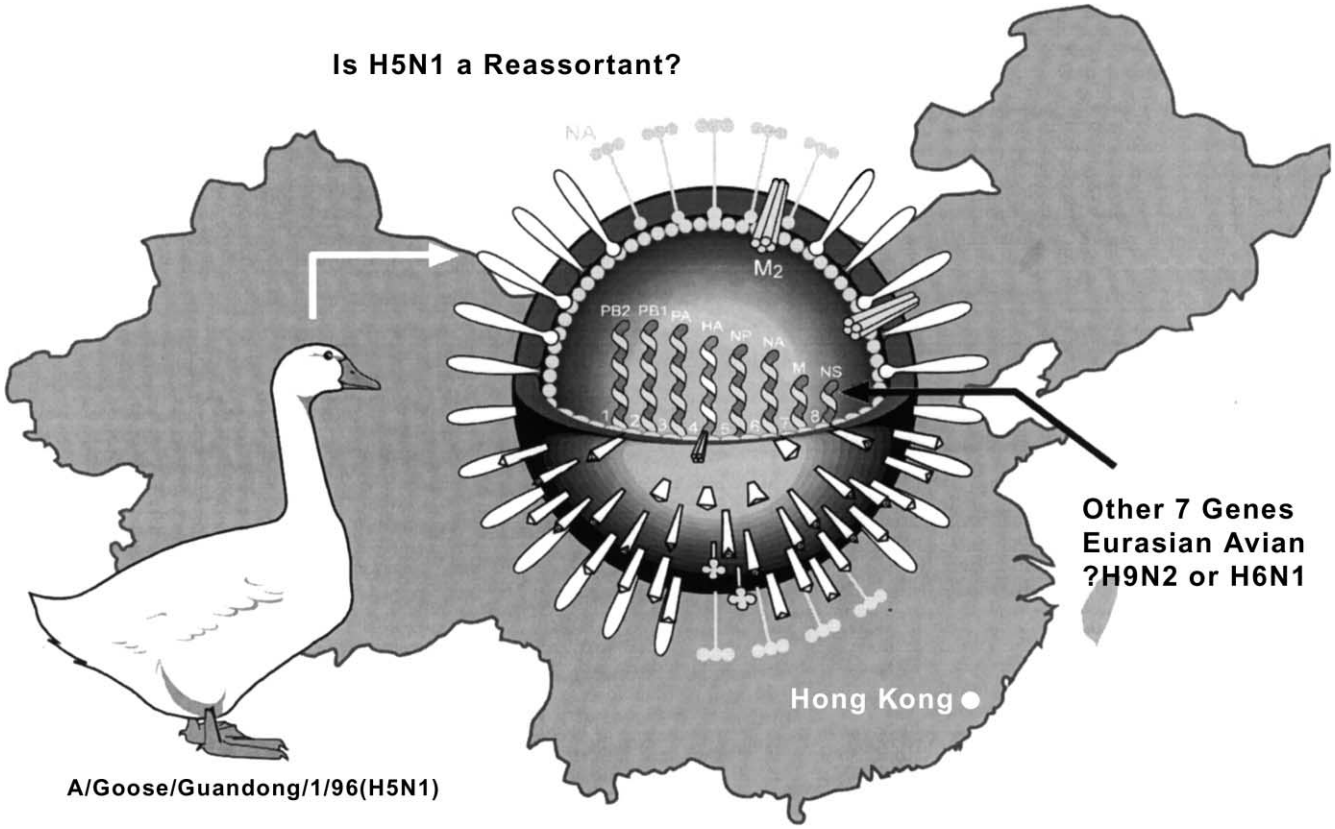

Fig. 2. Origin of the H5N1 bird flu. It is proposed that the H5N1 influenza virus that infected humans in Hong Kong in 1997 is a re-assortant. The *HA* may have come from A/goose/Guandong/l/96 (H5N1) influenza virus and the "internal genes" from a EurAsian avian influenza virus.

markets as the "missing link in the epidemiology of avian influenza", for H5N2 viruses related to chicken/Pennsylvania/83 had been isolated from live bird markets until 1986, several years after they had been eradicated from poultry in Pennsylvania. These H5N2 viruses caused subclinical infection in chickens in the markets, as did H5Nl viruses in live bird markets in Hong Kong in 1997. Moreover, ducks in the markets in the United States were infected with many different subtypes of influenza A viruses, including H2N2 viruses related antigenically to the Asian-57 (H2N2) viruses that have disappeared from humans.

The live bird markets in the United States continue to harbor many influenza viruses. The ancestor of the H5N2 influenza virus that caused the epidemic in Mexico in 1993 to 1995 was isolated from market birds. H7N2 viruses are currently found in live bird markets. These viruses are potentially pathogenic for chickens and are of great concern to chicken farmers in the north-eastern United States.

The depopulation of live bird markets and farms in the New Territories of Hong Kong (29 December 1997) stopped the spread of H5N1 influenza viruses. An important lesson can be learned from this action in Hong Kong. Live bird markets are potential breeding grounds for both avian and mammalian influenza viruses. One action taken in response to the bird flu incident in 1997 was the separation of avian host species. Thus, chickens are now marketed separately from aquatic birds. Aquatic birds are limited to ducks and geese, which are killed at a separate wholesale market. This practice of separating avian species is strongly recommended to all countries that market poultry through live bird markets.

8. Challenge for the 21st century

The World Health Organization can be justly proud of the international surveillance system for influenza in humans that has been improved and strengthened over the past 50 years. A further challenge is to utilize the information about the emergence of human pandemic influenza viruses from lower animals and birds to design a surveillance system for influenza in lower animals and birds that is workable and serves both agricultural and human needs. The absence of non-pathogenic H5N1 viruses that could serve as surrogate vaccine strains in humans indicates that surveillance of aquatic bird reservoirs for influenza should be done systematically worldwide. Sampling of wild aquatic birds in both the EurAsian and American clads is necessary. How many sites are necessary? Surveillance in poultry markets, pigs, horses, and of outbreaks of influenza in sea mammals, mink and other species must continue to be evaluated. At

this time, there is insufficient background knowledge to develop a comprehensive surveillance plan. The immediate needs are to collect the necessary epidemiological data and then develop a surveillance system in lower animals.

It is critical that surveillance for influenza viruses in lower animals and birds is done at different sites from human surveillance. Any attempts to combine human and animal surveillance will result in cross contamination and disaster. Regardless, the possible risks of cross contamination must not be used as an excuse for not conducting animal surveillance for there is no doubt that influenza is a zoonotic infection and it would serve the World Health Organization to be pro-active in establishing a workable system.

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References

[1] Murphy BR, Webster RG. Orthomyxoviruses. In: Fields BN, et al., editors. Fields Virology. New York: Raven, 1998. p. 1397–1445.
[2] Webster RG, et al. Intestinal influenza: replication and characterization of influenza viruses in ducks. Virology 1978;84:268–78.
[3] Halvorson D, et al. Epizootiology of avian influenza—simultaneous monitoring of sentinel ducks and turkeys in Minnesota. Avian Dis 1983;27:77–85.
[4] Geraci JR, et al. Mass morality of harbor seals: pneumonia associated with influenza A virus. Science 1982;215:1129–31.
[5] Hinshaw V, et al. Characterization of two influenza A viruses from a pilot whale. J Virol 1986;58:655–6.
[6] Scholtissek C, et al. Genetic relatedness of haemagglutinins of the H1 subtype of influenza A viruses isolated from swine and birds. Virology 1983;129:521–3.
[7] Horimoto T, et al. Origin and molecular changes associated with emergence of a highly pathogenic H5N2 influenza virus in Mexico. Virology 1995;213:223–30.
[8] Gorman OT, et al. Evolution of the nucleoprotein gene of influenza A virus. J Virol 1990;64:1487–97.
[9] Gammelin M, et al. Phylogenetic analysis of nucleoproteins suggests that human influenza A viruses emerged from a 19th-century avian ancestor. Mol Biol Evol 1990;7:194–200.
[10] Reid AH, et al. Origin and evolution of the 1918 Spanish influenza virus haemagglutinin gene. Proc Natl Acad Sci 1999;96:1651–6.
[11] Kawaoka Y, Krauss S, Webster RG. Avian-to-human transmission of the *PB1* gene of influenza A virus in the 1957 and 1968 pandemics. J Virol 1989;63:4603–8.
[12] Scholtissek C, et al. The role of swine in the origin of pandemic influenza. In: Hannoun C, editor. Options for the Control of Influenza-II. New York: Elsevier, 1993. p. 193–201.
[13] Ito T. Molecular basis for the generation in pigs of influenza A viruses with pandemic potential. J Virol 1998;72:7367–73.
[14] Kida H. Potential for transmission of avian influenza viruses to pigs. J Gen Virol 1994;75:2183–8.
[15] de Jong JC. A pandemic warning. Nature 1997;389:554.
[16] Claas EC, et al. Human influenza A(H5N1) virus related to highly pathogenic avian influenza virus. Lancet 1998;351:472–7.
[17] Subbarao K, et al. Characterization of an avian influenza virus isolated from a child with a fatal respiratory illness. Science 1998;279:393–6.
[18] Yuen KY, et al. Clinical features and rapid viral diagnosis of human disease associated with avian influenza A H5N1 virus. Lancet 1998;351:467–71.
[19] Röhm C. Different haemagglutinin cleavage site variants of H7N7 in an influenza outbreak in chickens in Leipzig, Germany. Virology 1996;218:253–7.
[20] Bean WJ. Characterization of virulent and avirulent A/chicken/Pennsylvania/83 influenza A viruses: potential role of defective interfering RNAs in nature. J Virol 1985;54:151–60.
[21] Shortridge KF. Pandemic influenza—a zoonosis. Semin Respir Infect 1992;7:l–25.
[22] Xu X, Cox N, Guo Y. Emergence of influenza A (H5N1) viruses in geese in China. In: Proceedings of the 17th Annual Meeting of the American Society of Virology, vol. W29-5. University of British Columbia, Vancouver, BC, 11–15 July 1998. p. 110.
[23] Senne DA, Pearson JE, Panigraphy B. Live poultry markets: a missing link in the epidemiology of avian influenza. In: Proceedings of the 3rd International Symposium on Avian Influenza. The University of Wisconsin, Madison, WI, 1997. p. 50–8.