Ecology of Influenza Viruses in Nature, Birds, and Humans

Hiroshi KIDA$^{1,2,3}$

$^1$Department of Disease Control, Graduate School of Veterinary Medicine
$^2$Research Center for Zoonosis Control
$^3$OIE World Reference Laboratory for Highly Pathogenic Avian Influenza
Hokkaido University
Kita 18, Nishi 9, Kita-ku, Sapporo 060-0818, Japan
e-mail: kida@vetmed.hokudai.ac.jp

Abstract

Recent outbreaks of highly pathogenic avian influenza (HPAI) have spread from Southeast Asia to 62 Asian, Middle-Eastern, African and European countries. H5N1 viruses isolated from dead waterbirds in Mongolia on the way back to their nesting areas in Siberia in May 2005 and 2006 were genetically closely related to those isolated from birds in China, Iraq, Croatia, Nigeria, Korea and Japan, but different from those from poultry in Vietnam, Thailand and Indonesia. It is a serious concern that these HPAI viruses may perpetuate in lakes where migratory water birds nest in summer. In intensive surveillance studies on avian influenza fulfilled in the autumn of 2005, 2006 and 2007 in Mongolia and Japan, no HPAI viruses were isolated from migratory water birds that flew from Siberia, indicating that the virus has not yet persisted in their nesting lakes in Siberia. Careful surveillance of avian influenza in migratory water birds needs to continue.

These H5N1 HPAI virus strains have caused severe disease with high mortality in a few humans who appear particularly susceptible to infection with the virus strain in Asia, the Middle East and Africa since 2003. More than 240 fatalities have occurred out of 380 confirmed cases in fifteen countries in four years worldwide (as of June 2008). The second concern is that the H5N1 avian influenza virus alone is expected to cause the next pandemic influenza in humans. Since each of the known subtypes of influenza viruses perpetuates among migratory ducks and their nesting lake water in nature and avian viruses of any hemagglutinin (HA) subtype could contribute genes in the generation of reassortants in pigs, none of the 16 HA and 9 neuraminidase (NA) subtypes can be ruled out as potential candidates for future pandemic strains. Surveillance of swine influenza is, thus, extremely important as well as that of avian influenza to ensure preparedness for the emergence of pandemic strains in humans.

Avian influenza virus strains isolated from migratory ducks in our global surveillance studies have been stored for use in vaccines and diagnosis. Influenza virus isolates of 61 combinations of HA and NA subtypes have been isolated from fecal samples of ducks and viruses of 78 other combinations have been generated by genetic reassortment procedure in chicken embryos. Thus, 246 avian influenza virus strains of 139 combinations of HA and NA subtypes have been stocked in a library of a panel of influenza virus strains and their genes. Their pathogenicity, antigenicity, genetic information and yield in chicken embryos have been analyzed, registered in the database and opened for the member countries of the World Health Organization (WHO) and Office of International Epizootics (OIE) through the Internet.

Key words: antigenic and genetic variation of influenza viruses, host range, interspecies transmission, natural reservoir, perpetuation, vaccine

1. Introduction

Although many infectious diseases have been eradicated or controlled in the last century, influenza remains to be overcome, with influenza A viruses causing occasional catastrophic pandemics in poultry and mammals including humans. In addition, recent outbreaks of highly pathogenic avian influenza (HPAI) around the world cause us alarm to realize that there are no borders for infections and give rise to concern for human health as well as for the livestock industry. H5N1 avian influenza virus strains have jumped the species barrier and caused severe disease with high mortality in humans, although the number of cases remains very small, first in Hong Kong in 1997, then in Viet Nam, Thailand and Cambodia through 2004. It is obvious that live bird
markets have played important roles in the generation and supply of highly pathogenic avian influenza virus strains. Subsequently, the new HPAI virus strains have returned to feral water birds and spread to 62 countries. More than 240 victims have died out of 380 confirmed cases in fifteen countries worldwide in the four years since 2005. It is also obvious that the reason why the H5N1 HPAI virus strains have persisted for more than ten years in Asia is attributable to the failure of control measures, using inactivated vaccines, forgetting about basically essential stamping-out strategies in three countries. In addition, vaccination has accelerated the selection of multiple antigenic variants (Wu et al., 2008). Moreover, these three countries are ironically ranked as the top three for the number of human cases of H5N1 virus infection.

To elucidate the origin and evolution of pandemic influenza viruses, we have conducted global surveillance of influenza in birds, animals and humans. On the basis of antigenic and genetic analyses of influenza virus isolates from migratory ducks, domestic ducks, pigs and humans and experimental infection studies on birds and mammals with those viruses, we proposed that the hemagglutinin (HA) gene of A/Hong Kong/68 (H3N2) strain was introduced into the precedent human H2N2 Asian influenza virus by genetic reassortment, occurring in the epithelial cells lining the upper respiratory tract of pigs, through domestic ducks from an H3 influenza virus circulating in migratory ducks in southern China (Kida et al., 1987; Kida et al., 1988; Yasuda et al., 1991; Kida et al., 1994).

Ecological studies have revealed that a vast influenza virus gene pool for avian and mammalian influenza exists in migratory ducks. Each of the known subtypes of influenza A viruses perpetuates among migratory ducks and their nesting lake water in nature (Ito et al., 1995; Okazaki et al., 2000). Experimental infection studies have established that influenza viruses preferentially replicate in the columnar epithelial cells of the colon, causing no disease signs and are excreted in high concentrations in ducks (Kida et al., 1980). Influenza viruses have been isolated from freshly deposited fecal materials and from lake water, indicating that migratory ducks have an efficient way to transmit viruses, i.e., via fecal material in the water supply.

In comparison with influenza viruses of humans, influenza viruses of ducks are in evolutionary stasis (Kida et al., 1987). The remarkable lack of amino acid changes among influenza viruses isolated from migratory ducks suggests that the virus genes in this host are subject to stringent stabilizing selection. The genetic and antigenic conservation of influenza viruses in ducks suggests that the virus and host have reached a long established adaptive optimum. Such a stasis in migratory ducks is further evidence that duck populations are the natural reservoir of influenza viruses.

In experimental infection of pigs, most tested strains of each of the known HA subtypes of avian influenza viruses replicated in the upper respiratory tract of the animals. Co-infection of pigs with a swine virus and an avian virus unable to replicate in this animal generated reassortants, whose polymerase and HA genes were entirely of avian origin, that could be passed in pigs (Kida et al., 1994). The results indicate that avian viruses of any subtype can contribute genes in the generation of reassortants so that none of the 16 HA and 9 neuraminidase (NA) subtypes can be ruled out as potential candidates for future pandemics in humans and poultry. The direct transmission of H5N1 influenza viruses from domestic poultry to humans in fifteen countries in Asia, the Middle East and Africa in recent years have further emphasized the need to have information on all influenza A subtype viruses circulating in avian species worldwide.

To provide information on influenza viruses in their natural hosts, virological surveillance of avian influenza was carried out in Alaska and Siberia during their breeding season, summer. A number of influenza viruses of different subtypes were isolated from fecal samples of ducks and surprisingly also from fresh water samples of the lakes where they nest. Even in autumn when the ducks had left for migration to the south, viruses could still be isolated from the lake water, indicating that influenza viruses are maintained in duck populations by water-borne transmission in nature and revealing the mechanism of year-by-year perpetuation of the viruses in the frozen lake water while ducks are absent in winter. Phylogenetic analysis of the isolates from Alaska indicates that ducks carrying these viruses migrate to the south through the continent of North America and not to Asia (Ito et al., 1995). Influenza virus isolates from fecal samples of ducks in their nesting areas in Siberia phylogenetically belong to the Eurasian lineage and are closely related to those from birds, pigs and horses in Asia. It was noted that these isolates are closely correlated to the H5N1 influenza virus isolates from chickens and humans (Okazaki et al., 2000). Phylogenetic analysis of the HA of H5 influenza virus isolates from ducks in Japan reveals a close relationship with those of H5N1 influenza viruses from Hong Kong, southern China, Thailand, Viet Nam and Japan indicating that the H5HA of these viruses originated from influenza viruses maintained in migratory ducks nesting in Siberia. These results indicate that the precursor genes of pandemic influenza viruses are perpetuated in water in the lakes where ducks nest in the northern regions. Vaccine strains should be antigenically closely related to the pandemic strain, avirulent for humans and chicken embryos, of high yield in chicken embryos, and immediately prepared when the causative virus is characterized. We tested the immunogenicity to mice of inactivated avirulent H5N4 influenza viruses isolated from reservoir host ducks. The results showed that they were potent enough to protect animals from a challenge with a lethal dose of the highly pathogenic H5N1 virus (Takada et al., 1999).
2. Influenza is a Zoonosis.

Influenza A viruses are widely distributed in birds and mammals including humans. Among those, viruses of each of the known hemagglutinin (HA) and neuraminidase (NA) subtypes (H1-H15 and N1-N9, respectively) other than H16 (the H16N3 virus has only been isolated once, from a seagull in 2005, Olsen et al., 2006) have been isolated from migratory ducks. Ecological studies have revealed that a vast influenza virus gene pool for avian and mammalian influenza exists in migratory ducks and their nesting lake water and that influenza is a typical zoonosis (Figs. 1 and 2). Influenza viruses in ducks are in evolutionary stasis, suggesting that the virus and host have reached a long established adaptive optimum.

3. Perpetuation of Influenza Viruses in Nature

Ducks are orally infected with influenza viruses by waterborne transmission at their nesting lakes in Siberia, Alaska and Canada around the Arctic Circle during their breeding season, summer. These viruses then replicate in the columnar epithelial cells forming crypts in the large intestine, and are excreted together with feces. The viruses in the lake water become frozen after the ducks migrate to the south and are preserved there over the winter. In other words, northern nesting lakes for migratory ducks serve as influenza virus gene pools in nature.

Fig. 1 Electron micrograph of an influenza virus particle of A/Hong Kong/68 (H3N2) and its schematic diagram.

The influenza A virus contains eight segmented negative-strand RNA genomes within its bi-layered lipid membrane and envelope. Two spike glycoproteins, the hemagglutinin (HA) and the neuraminidase (NA) are embedded in the envelope. On the basis of antigenic specificity of the HA and NA, the influenza A virus is divided into 16 HA and 9 NA subtypes.

Fig. 2 Host animal species and HA and NA subtypes of the influenza A virus.

Influenza A viruses infect humans, pigs, horses, various species of birds, mink, seals, and whales. Of these, from waterfowl such as ducks, each of the known subtypes of the HA and NA, except H16 that has only been isolated from a seagull, has been isolated.
4. Mechanism of Emergence of New Pandemic Influenza Viruses in Humans

The phrase “a new virus” describes an influenza A virus of a subtype of the HA or NA other than H1N1, H2N2 and H3N2, which have spread among humans in the last century, that is transmitted to humans and causes a pandemic (Fig. 3). We have revealed that the A/Hong Kong/68 (H3N2) pandemic influenza virus emerged in 1968 via genetic reassortment in pigs concurrently infected with an H3Nx influenza virus circulating in migratory ducks through domestic ducks and the H2N2 virus, which was circulating among humans at the time, in southern China (Figs. 4 and 5). Furthermore, experimental infection studies have revealed that pigs are highly susceptible to avian influenza viruses of all HA subtypes, and that since epithelial cells lining the upper respiratory tract of pigs have receptors for both human and avian influenza viruses, pigs can act as reservoirs for influenza viruses. In addition, pigs have been shown to be capable of efficiently transmitting influenza viruses to humans, which has been observed in epidemic outbreaks of highly pathogenic avian influenza (H5N1) in South East Asia and India. The A/Hong Kong/68 (H3N2) pandemic influenza virus is now believed to have been introduced into the human population from an influenza virus circulating in migratory ducks through domestic ducks and pigs in southern China. It is now believed that the Asian influenza virus (H2N2) strain must have emerged similarly. It is also assumed that the 1918 Spanish influenza pandemic strain originated from a swine influenza virus strain in early 1918.

**Fig. 3** History of the occurrences of pandemic influenza in humans and highly pathogenic avian influenza.

There are three instances of naturally occurring emergence of new pandemic strains in humans in the last century. A new pandemic strain is defined as a virus possessing new HA and/or NA subtypes that humans have not experienced for the last several decades. On the other hand, highly pathogenic avian influenza viruses with H5 or H7 HA must have emerged everywhere in the world since feral water birds were domesticated.

**Fig. 4** Genetic reassortment.

If two different influenza A viruses concomitantly infect one host cell, viruses of different constellations of the gene segments derived from either parental viruses, are produced when they mature and are released from the cell. Thus, the mathematical number of different combinations of genome segments is 256. This is the mechanism of genetic reassortment, and some of these reassortant viruses bearing the hemagglutinin gene derived from a parental virus of non-human origin, which can infect and be transmitted to humans, are potential candidates for new pandemic strains. The A/Hong Kong/68 (H3N2) pandemic strain bears PB1 and HA genes from an H3Nx virus of duck origin and the others from a human H2N2 Asian influenza virus strain prevailing at that time.

**Fig. 5** Route of transmission of the genes of pandemic influenza virus strains.

Ecological studies of animal influenza viruses and antigenic and genetic analyses of H3 influenza viruses isolated from migratory ducks, domestic ducks and pigs, together with experimental infection studies indicate that the hemagglutinin gene of A/Hong Kong/68 (H3N2) influenza virus was introduced into the precedent human H2N2 virus from an influenza virus circulating in migratory ducks through domestic ducks and pigs in southern China. It is now believed that the Asian influenza virus (H2N2) strain must have emerged similarly. It is also assumed that the 1918 Spanish influenza pandemic strain originated from a swine influenza virus strain in early 1918.
and avian viruses, genetic reassortment takes place and new viruses are thus produced in pigs. These findings indicate that genetic reassortants of any HA subtype will be generated in pigs, and new viruses will emerge as a result.

5. Highly Pathogenic Avian Influenza Viruses

Since late 2003, the highly pathogenic avian influenza virus of the H5N1 subtype has seriously affected poultry in Asia. Well over 400 million birds have died from infection or have been killed for control purposes. A highly pathogenic avian influenza virus is generated when a nonpathogenic virus brought in by migratory birds from nesting lakes in the north is transmitted to chickens via domestic ducks, geese, quails, turkeys, etc. and acquires pathogenicity for chickens with repeated multiple infections in the chicken population. This pathogenicity does not apply to other kinds of birds or mammals, nor does it to humans (Fig. 6).

6. Do the Highly Pathogenic Avian Influenza Viruses that were Conversely Transmitted from Poultry to Feral Water Birds Perpetuate in the Lakes where Migratory Birds Nest?

During over-wintering, some migratory birds were conversely infected with the highly pathogenic H5N1 virus from poultry and have been found dead at lakes in northern China, Mongolia, Russia, Europe and Africa in May on their way back to their nesting lakes in northern regions (Fig. 6). It was found that all viruses isolated from these birds were the same viral strains isolated from poultry in China. Since it is of concern that this H5N1 virus may perpetuate in lakes in Siberia where migratory ducks nest in summer, intensive surveillance of avian influenza has been carried out in autumn when migratory birds fly from Siberia to the south in Mongolia, China and Japan. In the four-year investigation, no highly pathogenic avian influenza virus has been isolated, indicating that the highly pathogenic H5N1 avian influenza virus strain has not yet perpetuated in the lakes where the migratory birds nest in summer.

7. Transmission of Highly Pathogenic Avian Influenza Viruses to Humans

It has been confirmed that more than 380 people have been infected with the H5N1 virus, 60% of whom have died in Asia, the Middle East and Africa since 2003. Against this backdrop, it is assumed that the H5N1 virus will acquire the ability to be transmitted among humans and wreak havoc as a new virus. The WHO has warned for the past decade that this avian influenza virus is in the final stages of acquiring the ability to infect humans. In response, industrialized nations have formulated emergency plans, including measures such as stocking an inactivated H5N1 vaccine for humans.

The world is now confused as to the extent that the avian influenza can pass as a human disease. Both avian influenza and human influenza are infections with the influenza A virus. Although the potential for a virus with the H5HA gene to cause an influenza pandemic in humans cannot be denied, we should not focus on the H5N1 virus alone. Rather, we should keep in mind the possibility of influenza viruses of other subtypes emerging as new pandemic influenza virus strains.

Research to date has revealed that all the genes of influenza viruses for birds and mammals including humans are derived from non-pathogenic viruses maintained in migratory ducks. The basics of practical avian influenza control measures involve minimizing damage and protecting human health and food safety by eradication policies to contain avian influenza within poultry (Fig. 7).

Efforts should also be made to conquer human influenza through prediction and prevention. It is necessary

![Fig. 6](image1.png)

**Fig. 6** Return of the H5N1 highly pathogenic avian influenza virus strain from domestic poultry to migrating water birds.

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![Fig. 7](image2.png)

**Fig. 7** The origin and routes of transmission of the genes of highly pathogenic avian influenza virus and human pandemic influenza virus strains.
to establish and implement a preemptive strategy to control highly pathogenic avian influenza and human pandemic viruses by investigating mechanisms of the emergence of human pandemic influenza virus strains and the acquisition of pathogenicity of avian influenza viruses. To this end, we have completed a library of all HA- and NA-subtype virus strains and genes that exist in nature, created a database and provided it over the Internet (Fig. 8). It has already been used for vaccine production and diagnosis by 26 research institutions in ten countries.

8. Zoonosis Control Measures

The strategy fostered in influenza research has been applied to combat other zoonotic infections. First of all, it is necessary to identify the natural host of the causal agent and clarify the root of transmission. That is to say, the ecological elucidation of wildlife and agents in nature leads to fundamental control of infections. The Research Center for Zoonosis Control, inaugurated at Hokkaido University in 2005, conducts global surveillance studies and engages in research and education toward the establishment of methods of diagnosis, prevention and control. Its aim is to predict and prevent the outbreaks of influenza, West Nile fever, Ebola-Marburg hemorrhagic fever, Hantavirus infections, rabies and other unknown infections.

References


Hiroshi KIDA

Hiroshi Kida, DVM, PhD, is a Professor at the School of Veterinary Medicine and Director of the Research Center for Zoonosis Control, Hokkaido University, Sapporo, Japan. He has devoted himself to clarifying the origin, evolution, perpetuation mechanisms and ecology of influenza A viruses in nature and among birds, and mammals including humans through extensive global surveillance, molecular epidemiology and experimental infection studies. He has demonstrated that aquatic birds and their nesting lake water are the natural reservoir for influenza A viruses and that past pandemic influenza viruses arose via genetic reassortment between human and avian viruses in pigs. In 2005, he was awarded the Japan Academy Prize for a series of accomplishments and selected as a member of the Japan Academy in 2007. He has been designated Director of the OIE Reference Laboratory for Highly Pathogenic Avian Influenza and is also a member of the OIE Scientific Committee, the WHO Human/Animal Interface in Influenza Surveillance Network and the Scientific Committee of the OIE and FAO Influenza (OFFLU) Network. He has established an influenza virus and gene library consisting of all the known HA and NA subtype combinations, opened it to other researchers as a database and started to provide these strains and genes as vaccine seed candidates and for diagnostic use. He has also demonstrated that test vaccines prepared from H5N1 and H7N7 influenza virus strains selected from the library induced protective immunity against a challenge with highly pathogenic avian influenza viruses in a monkey model.

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