



Highly pathogenic H5N1 avian influenza virus: Cause of the next pandemic?

Marguerite Pappaioanou *

*Division of Epidemiology and Community Health, School of Public Health, University of Minnesota,
1300 S. Second Street, Suite 300, Minneapolis, MN 55454, USA*

Abstract

Since 1997, when human infections with a highly pathogenic (HP) avian influenza A virus (AIV) subtype H5N1 – previously infecting only birds – were identified in a Hong Kong outbreak, global attention has focused on the potential for this virus to cause the next pandemic. From December 2003, an unprecedented H5N1 epizootic in poultry and migrating wild birds has spread across Asia and into Europe, the Middle East, and Africa. Humans in close contact with sick poultry and on rare occasion with other infected humans, have become infected. As of early March 2007, 12 countries have reported 167 deaths among 277 laboratory-confirmed human infections to WHO. WHO has declared the world to be in Phase 3 of a Pandemic Alert Period. This paper reviews the evolution of HP AIV H5N1, molecular changes that enable AIVs to infect and replicate in human cells and spread efficiently from person-to-person, and strategies to prevent the emergence of a pandemic virus.

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Pandemic influenza in humans has occurred every 10–50 years over several centuries, causing major morbidity and mortality, and severe economic consequences [1,2]. Given the last pandemic occurred 37 years ago in 1968, the next pandemic is anticipated at any time. Since 1997, when humans in a Hong Kong influenza outbreak were found to be infected with a highly pathogenic (HP) avian influenza A virus (AIV) of subtype H5N1 – a virus previously known to infect only birds [3] – global attention has focused on the potential for

* Tel.: +1 612 624 7554.

E-mail address: pappa046@umn.edu.

this virus to evolve into a pandemic strain [4]. With the severity of illness and high case fatality rate of approximately 60% that has been observed among humans infected with this subtype [5–7], there is great concern that a pandemic caused by this virus would result in extremely high morbidity and mortality, and have major economic consequences [2].

Beginning in December 2003, an unprecedented epizootic of HP AIV H5N1 has occurred in poultry and migrating wild bird populations, spreading across Asia, into Europe, the Middle East, and Africa, with 38 countries reporting a combined total of 4598 poultry and/or wild bird outbreaks to the Office International Epizooties (World Organization for Animal Health) as of 2 March 2007 [8]. Concurrent with this epizootic, as of 1 March 2007, 12 countries have reported a total of 277 laboratory-confirmed human HP AIV H5N1 infections and 167 deaths to WHO [5]. Although the majority of infections have resulted from close human contact with infected and ill birds [11], limited human-to-human transmission is suspected to have occurred in Thailand [9], and more recently in Indonesia [10]. Based on these events, WHO has determined the world to be in Phase 3 of a Pandemic Alert Period [12].

Pandemic influenza viruses are novel influenza A viruses that infect humans lacking any natural immunity, that spread efficiently from person-to-person, and that cause severe illness [13]. This paper reviews the basic mechanisms by which AIVs adapt to infect and replicate in human host cells and achieve efficient person-to-person spread, factors that affect pathogenicity, and strategies that can be employed to prevent the emergence of pandemic viruses. Particular attention will be given to HP AIV H5N1 and whether it is evolving on a pathway to becoming the next pandemic virus.

Influenza A viruses are enveloped and contain a single, segmented, negative-strand ribonucleic acid (RNA) genome. The genome comprises eight segments of negative-sense RNA, which are named hemagglutinin (HA), neuraminidase (NA), matrix (M), nucleoprotein (NP), and nonstructural (NS) genes, and three polymerase segments named PB1, PB2, and PA [14]. Multiple segments of the genome are believed to play important roles in infection and replication [15–19], and pathogenicity [19–21].

Influenza A viruses are categorized into subtypes based on its two glycoprotein surface antigens—HA and NA. To date, 16 HA and 9 NA antigens have been identified [14,22]. Segmented influenza A viruses are continually changing. Small changes occur year-to-year by point mutations, a process referred to as genetic drift. Major changes occur less frequently by reassortment (i.e., mixing) of genes of two different viruses in co-infected cells, a process referred to as genetic shift. Genetic reassortment can result in the emergence of novel subtypes with a completely new HA antigen, not previously experienced by potential host species [14]. In addition, whole AIVs can jump into new, previously unsusceptible species, and adapt to successfully infect new hosts [16,23–25].

1. The emergence of pandemic viruses

During the 20th century, influenza pandemics occurred in 1918, 1957, and 1968, and were caused by novel H1N1, H2N2, and H3N2 viruses, respectively. The 1918 Spanish influenza pandemic virus originated from direct infection and adaptation of a whole H1N1 AIV into humans [16]. The 1957 Asian influenza and 1968 Hong Kong influenza pandemic

viruses are believed to have originated from reassortment between currently circulating human and AIVs. In 1957, the novel H2N2 pandemic virus contained three genes of an H2 AIV, the result of an avian H2 virus reassorting with the circulating human H1N1 strain. In 1968, the new H3N2 pandemic virus contained three genes of an H3 AIV, which was again the result of an avian H3 virus reassorting with the circulating H2N2 strain [15].

Influenza A viruses infect a broad range of mammalian species including humans, wild aquatic and shore birds, domestic poultry and swine, horses, seals, whales, dogs, cats, and other species. All influenza A subtypes have been isolated from wild aquatic and shore birds, which are considered the reservoir of infection [14]. Interspecies transmission of AIVs, such as human AIV H5, H7, H9, H10, and H11 infections [21,25–29], swine AIV H1, H4, and H9 infections [30,31], feline AIV HP H5 infections [25,32,33], canine AIV HP H5 infections [34], and AIV infections in other mammals [35], have been described. Swine influenza viruses have infected humans [36,37], and equine influenza viruses have infected dogs [24]. Although recent studies have shed light on molecular changes in influenza A viruses that are involved with their adaptation to new species [38], in general, significant gaps in knowledge remain as to how interspecies transmission occurs—an important step in the emergence of pandemic viruses.

The mechanisms by which novel influenza viruses infect human host cells and replicate efficiently are also important for the emergence and transmission of pandemic viruses. The process by which influenza A viruses infect host cells begins when the HA antigen binds to sialic acid (SA) receptors on host epithelial cell surfaces [14,17,39]. Thus, a major determinant of host range for an influenza A virus is the binding affinity between its HA antigen and host cell SA receptors [19]. Human adapted influenza A subtypes (i.e., circulating H1, H2, H3 viruses) preferentially bind to SA receptors having α 2,6 galactose (GAL) linkages, which are found in great numbers on human tracheal epithelial cells [40,41]. In contrast, influenza A viruses adapted to birds preferentially bind to epithelial cells having SA receptors having α 2,3 GAL linkages, which are located in the avian intestinal tract [19,42]. The difference between the composition and configuration of human and avian epithelial cell receptors, which determines host susceptibility to AIVs, has been as little as one to two amino acids [43–45]. For example, in 1967, a two amino acid change that occurred in an H3 duck virus during reassortment with the circulating H2N2 seasonal influenza virus, is believed to have led to the emergence of the 1968 Hong Kong influenza pandemic virus [39]. And although SA α 2,6 GAL receptors are dominant on human columnar epithelial cells in the upper and lower human respiratory tract, SA α 2,3 GAL receptors, which would allow infection by AIVs, have also been found but in much lower numbers [46,47].

In addition to receptor affinity, infection of a host cell also requires the HA antigen to cleave into two domains – HA1 and HA2 – at the terminal end of the HA1 domain, which is important for the virus to gain entry into the host cell where it can fuse with host endosomal membranes [48]. In general, host cell proteases are required for cleavage to occur, and basic amino acids near the cleavage site can block host protease access to the site, thereby interfering with cleavage and successful infection of the host cell [48,49].

Taking these processes into account, HP AIV N5N1 likely has been able to infect humans, at least in part, because of the presence of limited numbers of SA α 2,3 GAL receptors on human nasal epithelial cells—receptors to which the virus has an affinity

[46,47]. Replication of this virus in humans, however, has been found to be most efficient in alveolar epithelial cells of the lungs, where SA α 2,3 GAL receptors are present in greater numbers [47]. The pneumocyte has been postulated as the major site of H5N1 viral replication in the lung [42]. This scenario likely accounts for the observed inefficient person-to-person transmission of this virus. Close and intense human contact with infected birds or humans, when the virus can find its way to the lower respiratory tract, has been required for human infection to occur [44]. In addition, HP AIV H5N1 in humans has also been found to replicate in the intestine [42]. New research has documented HP AIV H5N1 infection of human *ex vivo* nasopharyngeal, adenoid and tonsillar tissues, which lack SA α 2,3 GAL receptors, leading investigators to conclude that other, currently unknown, binding sites on epithelial cells must be involved with virus entry [50].

Studies of 1957 and 1968 pandemic viruses found that the HA glycoprotein of the AIV's that reassorted with circulating human influenza viruses, adapting to SA α 2,6 GAL receptors in the epithelial cells of the upper respiratory tract of humans, gave the emergent virus the ability to more readily infect humans and spread efficiently from person-to-person [39,51]. Recent studies have suggested that the PB1 gene also may be critical for adaptation, since both it and the HA antigen were transferred during reassortment events from which the 1957 and 1968 pandemic viruses emerged [15,16]. In addition, PA genes are believed important for human adaptation, as four amino acids of the PA gene, one of PB1, and five of the PB2 gene have been identified in human influenza viruses, but not in AIVs [16].

2. Pathogenicity of HP AIV H5N1 in humans

The spectrum of clinical illness and outcomes experienced by patients infected with HP AIV H5N1 in Hong Kong [52], Vietnam [53], Thailand [54], Indonesia [10], and Turkey [55] have been described in detail. In contrast to human seasonal influenza A viruses, which affect very young children (under 2 years) and older adults (>65 years), patients infected with HP AIV H5N1 have been older children and young adults disproportionate in numbers to their respective populations [7]. Human infection with this virus has resulted in serious and often fatal illness, with sudden and severe pneumonia and multi-organ failure occurring prior to death [6,52,53]. A few mild cases and rare asymptomatic infections have been documented [7]. Recently, questions have arisen as to whether human genetic factors might be playing a role in predisposition to infection and severity of illness [10,56].

Factors that have been postulated as important in the pathogenesis of pandemic influenza viruses include (1) cleavability of the HA molecule [70–72] and host proteases involved with this process [48,57,58]; (2) efficient viral replication leading to high viral burdens [52,54,59,60]; (3) viral mechanisms for evading or suppressing host immune responses [19]; and (4) viral mechanisms for stimulating a hyper-immune response [60–62].

High viral burdens are achieved when newly synthesized viruses are released from infected cells and then infect new cells. The NA glycoprotein removes SA from the cell surface of infected epithelial cells, which is necessary for the release of newly synthesized viruses. Thus, efficient virus replication involves balanced actions of both HA and NA antigens [19].

The dysregulation of virus-induced cytokines also has been identified as important in affecting host immune response and therefore, severity of illness [60,61]. Research on the 1918 Spanish Influenza pandemic virus [63] and the 1997 Hong Kong HP AIV H5N1 virus [60] has suggested that more highly pathogenic influenza viruses have an NS gene that counteracts cellular interferon responses, and therefore, when compared to less pathogenic viruses, are more efficient in counteracting the host immune response. However, studies of the HA glycoprotein of the 1918 pandemic H1N1 virus [64,65] and the NS1 gene of the 1997 Hong Kong H5N1 virus [60,61] have shown that these genes played a role in stimulating an over production of proinflammatory macrophage-derived chemokines and cytokines in severely ill patients infected with those viruses. Viral isolates of HP H5N1 obtained from 1997 and 2004, when compared to the circulating H1N1 virus, were found to be more potent inducers of proinflammatory cytokines (e.g., tumor necrosis factor- α) and chemokines (e.g., IP-10) in primary human respiratory epithelial cells [61]. However, despite the growing understanding of molecular factors that are influencing the pathogenicity of influenza A viruses, substantial gaps in information remain and a robust research agenda remains.

3. The source and evolution of highly pathogenic avian influenza virus H5N1

Since the HP AIV H5N1 outbreak in humans in Hong Kong in 1997, the virus has been studied and monitored, and it continues to evolve. Significant concerns remain, that at any moment, the virus could acquire the capability to more easily infect people and spread person-to-person more efficiently, while retaining its high pathogenicity.

The human Hong Kong outbreak of 1997 was concurrent with poultry outbreaks in the region, and human viral isolates obtained at that time were related directly to chicken isolates obtained in Hong Kong live bird markets (LBMs) just before the human outbreak [66]. Additional studies have suggested that the virus causing the 1997 human outbreak in Hong Kong emerged from a reassortment of an influenza A/Goose/Guangdong/1/96-like virus that had caused outbreaks in geese on goose farms (with a morbidity rate of 40%), in Guangdong Province China in 1996 [67], and H9N2 or H6N1 viruses similar to strains circulating in Hong Kong LBMs in 1997 [68,69]. The HA gene of a 1997 H5N1 isolate was found to be 98.8% homologous with the 1996 Guangdong Province goose strain, and internal genes were 98–99% homologous with the circulating H9N2 virus in Hong Kong at that time [61,69]. Moreover, the NA of the 1997 Hong Kong H5N1 virus, containing a 19 amino acid deletion in its stalk region, was more than 98% homologous to an H6N1 avian virus (A/Teal/HK/W312/97) isolated during the same time period [68].

Despite rigorous control measures instituted in Hong Kong during the 1997 outbreak [70], sporadic HP AIV H5N1 outbreaks in poultry continued to occur and virologic surveillance was instituted [62,70]. The HA antigen of viral isolates that were obtained remained very close to those isolated in 1997 [62]. Since 1997, several genotypes have emerged, likely a consequence of genetic reassortment between the A/Goose/Guangdong/1/96-like 1997 Hong Kong viral precursor and viruses circulating during 2001 and 2002. Based on studies of internal genes isolated before or during 2001, six genotypes were identified—A, B, C, D, E, and Xo. Beginning in 2002, however, viruses with genotypes A,

C, D, and E could not be detected, and eight new genotypes – V, W, X1, X2, X3, Y, Z, and Z+ – were identified. By 2004, genotype Z, which contains both NA and NS1 deletions, was identified as the dominant H5N1 virus in southern China in [71].

Phylogenetically, the virus has evolved into genetic groups or “clades”. Viruses isolated from birds and humans in Hong Kong in 1997 and 2003 initially were classified as belonging to clades 1' and 3 respectively, differing by only one amino acid [68]. Based on continuing analyses of the HA gene, two genetically and antigenically distinct clades – Clades 1 and 2 – have since been identified and tracked. From late 2003 into 2005, Clade 1 viruses circulated in Cambodia, Thailand, and Vietnam, where they caused outbreaks in poultry and human infections, and in Laos and Malaysia, where they were isolated predominantly from birds (in February 2007, the first human case in Laos was identified, however, viral clade had not been identified at the time of manuscript submission). From 2004 into the first half of 2005, Clade 2 viruses circulated in China, Indonesia, Japan, and South Korea, where they caused poultry outbreaks but no human infections. In mid-2005, however, Clade 2 viruses and their epidemiology changed. Infected wild birds were found to be shedding virus from their respiratory tracts. In addition, the clade moved westward via both migrating wild birds and movement of poultry or poultry products. Poultry outbreaks and human infections from Clade 2 viruses have been documented in Turkey, Azerbaijan, Iraq, Egypt, Djibouti, Indonesia, and China; and since late 2005, Clade 2 viruses have been identified as the cause of a majority of human infections reported. Six viral subgroups have been identified as comprising Clade 2 viruses, with three of the six circulating in different geographic areas. One continues to circulate in Indonesia. A second (called the Qinghai Lake-like virus, which caused a large migrating wild bird die off at the Chinese lake in 2005) has caused outbreaks in Europe, the Middle East, and Africa, and the third is circulating in China and recently has moved into Vietnam [72–75]. The third sublineage, detected through surveillance of LBMs, has been found in southern China, Hong Kong, Laos, Malaysia, and Thailand, and has caused both poultry outbreaks and human infections. Since 2005, China has implemented a large compulsory poultry vaccination program for HP AIV H5N1. Surveillance of poultry in LBMs has shown low H5N1 seroprevalence rates, leading to a hypothesis that the emergence of this new sublineage possibly has resulted from selection pressure caused by the vaccination program [75].

Phylogenetic analysis of Clades 1 and 2 viruses isolated from poultry and humans in Asia during 2004–2005 has revealed that all eight gene segments have been avian in origin and have not acquired human influenza genes through reassortment. In addition, the majority of human H5N1 isolates obtained during 2004–2005 have been found to be antigenically homogeneous but distinct from avian viruses obtained prior to 2004. Molecular analysis of the HA antigen from isolates collected in 2005 has suggested that several amino acids located near the receptor-binding site are changing in ways that could affect antigenicity or transmissibility. For example, all H5N1 viruses isolated from 2004 to 2005 outbreaks were found to have a multiple basic amino acid sequence at the cleavage site, which is indicative of a HP AIV [62].

Characteristics of HP AIV H5N1 infection in different species have varied over time; and ducks, traditionally serving as a reservoir of AIVs, appear to be playing an important role in the evolution of the virus. During the earliest years of the epizootic, between 1997

and 2001, the virus was found to be non-pathogenic in, and inconsistently and inefficiently transmitted among ducks [76]. By 2002, however, when isolates of H5N1 viruses obtained from birds at the same time were experimentally inoculated into mallards, outcomes included high virus titers with systemic infection, pathogenic changes in multiple organs, and death [77]. In subsequent years, ducks naturally infected with the HP H5N1 virus did not develop clinical signs or symptoms, but were found to shed virus for extended periods of up to 17 days [76]. In addition, viruses isolated from healthy ducks caused increased pathogenicity in mice under experimental conditions [78]. Investigators in Thailand found a strong association between the pattern of H5N1 outbreaks in chickens and free-grazing ducks [79]. During late December 2006 and early January 2007, however, new HP AIV H5N1 outbreaks occurred in Thailand's poultry, including ducks showing clinical signs of infection [80]. New outbreaks in poultry have also been reported from Vietnam during the same period with infections detected in healthy ducks [81,82], greatly complicating efforts for disease control and substantially increasing risks for human exposure. That healthy ducks can be infected with HP AIV H5N1 viruses and shed virus for extended periods of time, placing poultry, swine, and humans at increased risk of exposure, is an environment conducive to the emergence of a pandemic strain either through reassortment, or through adaptation of the whole virus to the human host [76].

4. Preventing the emergence of pandemic viruses

Continued human exposure to and infection with HP AIV H5N1 increases the likelihood that this virus will mutate or reassort in a way that will result in efficient human-to-human transmission [44,71]. In addition, because pigs apparently can be infected with HP AIV H5N1 [83] and are also susceptible to prevailing circulating seasonal human influenza A strains, the viruses could reassort in co-infected swine, and a new virus could emerge capable of efficient human-to-human transmission.

Consequently, a basic strategy to minimize the potential for a pandemic strain to emerge is to control infections in poultry and limit the opportunity for humans and pigs to be exposed and become infected with AIVs [84,85]. Important measures to control avian influenza in poultry include the institution of strong biosecurity measures and animal husbandry practices on farms (i.e., keeping species separate and preventing poultry contact with wild birds), proper and effective use of sanitation and disinfectants, vaccination with an accompanying strategy to differentiate infected from vaccinated birds (i.e. DIVA), and safe marketing practices in LBMs that prevent the mixing of species or movement of infected birds from markets to backyard farms [86–89].

A second important strategy to prevent the emergence of a pandemic virus is to prevent or minimize infections in humans at high risk of exposure. Almost all cases of human HP AIV H5N1 infection have occurred from close and direct contact with infected and ill birds [90–92]. Backyard poultry and swine farmers; owners and or managers of fighting cocks; LBM managers, vendors, and customers; poultry and pork industry employees having contact with poultry and swine; slaughterhouse/abattoir workers; veterinary and animal health professionals responsible for culling or vaccinating birds and pigs as part of disease control efforts; wild bird rehabilitation workers; population groups with dietary practices

that include drinking raw duck blood, and any other group that is in close contact with birds and or swine are considered at increased risk for infection with HP AIV H5N1 [93]. Considerable guidance has been issued on the use of personal protective equipment to prevent human infection in these populations, including the use of respirators, proper hand hygiene, and the use of antivirals for prophylaxis in high risk settings [94–99]. Recent attention has focused on the importance of vaccinating poultry and swine farmers and food industry workers with seasonal influenza vaccine for overall disease prevention, but importantly as a key strategy to prevent reassortment of influenza A viruses which could lead to the emergence of a pandemic virus [100].

5. Vaccines and antiviral drugs to control an influenza pandemic and reduce morbidity and mortality

Mass vaccination of the human population with an effective vaccine is considered the most effective approach to prevent the spread of potentially pandemic viruses, and to reduce illness and death. Influenza vaccines are known to be most protective when they are antigenically similar to circulating strains [80,81]. Early HP H5N1 vaccines were developed using two Clade 1 viruses – a 2003 H5N1 isolate from Hong Kong and 2004 isolates from Vietnam – and have been in clinical trials. These inactivated (2003) and subunit (2004) vaccines were poorly immunogenic, requiring two doses to produce neutralizing antibody titers [80]. Preliminary data have indicated a lack of cross-protecting neutralizing antibody from candidate Clade 1 virus vaccines to more recently emerged Clade 2 H5N1 viruses. WHO, in response to the rapidly evolving H5N1 virus into multiple sublineages, has made isolates of three Clade 2 subclades available for the development of new human vaccines [101]. Currently, a number of vaccine seed viruses are being generated, and plasmid-based reverse genetics used to generate influenza vaccine candidates [80]. In late February 2007, a US Food and Drug Administration (FDA) advisory panel supported approval for an H5N1 vaccine that would be used in the event of a pandemic (i.e., not sold commercially), although supporting evidence indicated limited effectiveness [102].

The use of antiviral drugs is also important for reducing the severe morbidity and mortality of an influenza pandemic [80,103]. With HP AIV H5N1 having infected humans for the first time in 1997, humans populations in general lack any natural immunity, and are not protected by antibodies that might have been developed against previous or current seasonal circulating strains or vaccines. Given the importance of high viral loads to the pathogenesis of human AIV H5N1 infections, suppressing viral replication by the use of effective antiviral drugs is a potentially important intervention to reduce mortality associated with infection [59,104]. Although limited clinical efficacy of antiviral treatment in patients ill with H5N1 influenza has been observed [7], most patients diagnosed to date have been started on antivirals well into their illness; and antivirals are unable to interrupt the cascading host reaction leading to hypercytokinemia and associated complications in humans infected with AIV H5N1, once the process has begun [59]. Isolates from 2004 to 2005 have been shown sensitive to two neuraminidase inhibitors—oseltamivir and zanamivir. WHO has recommended oseltamivir for prophylactic or therapeutic

intervention with H5N1 infections [72,105]. Neuraminidase inhibitors are thought less likely to select for resistant influenza viruses than other antiviral drugs such as the adamantanes, however, resistance recently was detected in some HP AIV H5N1 isolates [106]. WHO has underscored the importance of monitoring the susceptibility of potentially pandemic viruses to antiviral drugs for evidence-based decisions on the most effective control measures to recommend [72].

Recently, WHO and others in the public health community have explored the effectiveness of social distancing measures that could be instituted during a pandemic to reduce person-to-person transmission until stockpiled vaccine and drugs can be made available [107,108].

6. The importance of surveillance in preventing and preparing for future pandemics

HP AIV H5N1 has become endemic in Asia [105]. Molecular methods, unavailable at the time of previous pandemics, have permitted the close monitoring of molecular and genetic changes of virus subtypes, and have contributed to a better understanding of how AIVs evolve, how they adapt to and infect humans, and of factors important for efficient person-to-person transmission, and pathogenicity. These methods have also permitted identifying and tracking sublineages as they spread across populations and country borders. At this time, there is growing recognition that legal and illegal movement of infected poultry and healthy ducks, and migrating wild birds are playing important roles in the spread of the virus [109,110], and that the virus acquires different characteristics as it transitions between poultry and wild birds [62]. Continued virologic and disease surveillance of AIVs in humans, poultry, and swine is essential to detect outbreaks or unusual occurrences of disease [30] and to monitor pandemic risk [104], so that timely and effective interventions can be applied to control, and potentially to stop transmission. Surveillance on the sensitivity of circulating viral subtypes to available antiviral drugs and the effectiveness of candidate vaccines is also needed to provide a rational basis for guiding their use.

7. H5N1 avian influenza virus—cause of the next pandemic?

Currently HP AIV H5N1 is not a pandemic influenza virus, but it continues to change and evolve. It is impossible to predict whether or when the H5N1 virus will change in ways that will result in more efficient human infection and human-to-human spread, and whether the virus will retain its high pathogenicity should those changes occur. The virus continues to spread globally and to cause unprecedented poultry outbreaks and human infections. Each day, new twists and turns on its host range and impact on affected species are reported. Considerable variability exists among affected countries in their ability to mount effective surveillance and disease control programs, and in the formulation of feasible and effective pandemic preparedness plans. Effective influenza virologic and epidemiologic surveillance requires quality laboratory support services, which are often lacking in many

areas affected by this virus. WHO has identified the following five priority actions for optimal pandemic prevention and preparedness: (1) reduce human exposure to the H5N1 virus; (2) strengthen early warning systems; (3) intensify rapid containment operations; (4) build coping capacity; and (5) coordinate global research [111]. Whether it is HP AIV H5N1 or another viral subtype altogether that ultimately emerges as the next influenza pandemic virus, the research on and attention to the evolving HP AIV H5N1 virus are advancing scientific understanding of how pandemic influenza viruses emerge, and are motivating countries and the world at large to become prepared to address the challenges of the next influenza pandemic, whenever it occurs.

References

- [1] Kilbourne ED. Influenza pandemics of the 20th century. *Emerg Infect Dis* 2006;12:9–14.
- [2] Osterholm M. Preparing for the next pandemic. *Foreign Aff* July/August 2005.
- [3] CDC. Update: isolation of avian influenza A (H5N1) viruses from humans—Hong Kong, 1997–1998. *MMWR* 1998;46:1245–7.
- [4] Osterholm MT. Preparing for the next pandemic. *N Engl J Med* 2005;352:1839–42.
- [5] WHO. Cumulative number of confirmed human cases of avian influenza A/(H5N1) reported to WHO; 2006. Accessed online March 1, 2007: http://www.who.int/csr/disease/avian_influenza/country/cases_table_2007_03_01/en/index.html.
- [6] The Writing Committee of the World Health Organization. Avian Influenza A (H5N1) infection in humans. *N Engl J Med* 2005;353:1374–85.
- [7] WHO. Epidemiology of WHO-confirmed human cases of avian influenza A (H5N1) infection. *WER* 2006;81:249–57.
- [8] OIE. Update on avian influenza in animals (Type H5), as of June 20, 2007. Accessed on line March 4, 2007 at: http://www.oie.int/downld/AVIAN%20INFLUENZA/Graph%20HPAI/graphs%20HPAI%2002_03_2007.pdf.
- [9] CDC. Avian influenza in humans. Accessed online January 20, 2007 at: <http://www.cdc.gov/flu/avian/gen-info/avian-flu-humans.htm>.
- [10] Ungchusak K, Auewarakul P, Dowell SF, et al. Probable person-to-person transmission of avian influenza A (H5N1). *N Engl J Med* 2005;352:333–40.
- [11] Kandun IN, Wibisono H, Sedyaningsih ER, et al. Three Indonesian clusters of H5N1 virus infection in 2005. *N Engl J Med* 2006;355(21):2186–94.
- [12] WHO. Current WHO phase of pandemic alert. Accessed online January 20, 2007 at: http://www.who.int/csr/disease/avian_influenza/phase/en/index.html.
- [13] National vaccine program office. Influenza pandemics: how they start, how they spread, and their potential impact. Accessed online January 20, 2007 at: <http://www.hhs.gov/nvpo/pandemics/flu2.htm#3>.
- [14] Webster RG, Bean WJ, Gorman OT, Chambers TM, et al. Evolution and ecology of influenza A viruses. *Microbiol Rev* 1992;56:152–79.
- [15] Belshe RB. The origins of pandemic influenza—lessons from the 1918 virus. *N Engl J Med* 2005;353:2209–11.
- [16] Taubenberger JK, Reid AH, Lourens RM, et al. Characterization of the 1918 influenza virus polymerase genes. *Nature* 2005;437:889–93.
- [17] Yamada S, Suzuki Y, Suzuki T, et al. Haemagglutinin mutations responsible for the binding of H5N1 influenza A viruses. *Nature* 2006;444:378–82.
- [18] Kawaoka Y, Krauss S, Webster RG. Avian-to-human transmission of the Pb1 gene of influenza A viruses in the 1957 and 1968 pandemics. *J Virol* 1989;63:4603–8.
- [19] Neumann G, Kawaoka Y. Host range restriction and pathogenicity in the context of influenza pandemic. *Emerg Infect Dis* 2006;12(6):881–6.

- [20] Chen J, Lee KH, Steinhauer DA, Stevens DJ, et al. Structure of the hemagglutinin precursor cleavage site, a determinant of influenza pathogenicity and the origin of the labile conformation. *Cell* 1998;95:409–17.
- [21] Subbarao K, Katz J. Avian influenza viruses infecting humans. *Cell Mol Life Sci* 2000;57:1770–84.
- [22] Fouchier RAM, Munster V, Wallensten A, et al. Characterization of a novel influenza A virus hemagglutinin subtype (H16) obtained from black-headed gulls. *J Virol* 2005;79:2814–22.
- [23] Subbarao K, Klimov A, Katz J, et al. Characterization of an avian influenza A (H5N1) virus isolated from a child with a fatal respiratory illness. *Science* 1998;279:393–6.
- [24] Crawford PC, Dubovi EJ, Castleman WL, et al. Transmission of equine influenza virus to dogs. *Science* 2005;310:482–5.
- [25] Keawcharoen J, Oraveerakul K, Kuiken T, et al. Avian influenza H5N1 in tigers and leopards. *Emerg Infect Dis* 2004;10:2189–91.
- [26] PAHO. Avian influenza virus A (H10N7) circulating among humans in Egypt. EID Weekly updates. Accessed online January 21, 2007 at: <http://www.paho.org/English/AD/DPC/CD/eid-eer-07-may-2004.htm>.
- [27] Lin YP, Shaw M, Gregory V, et al. Avian-to-human transmission of H9N2 subtype influenza A viruses: relationship between H9N2 and H5N1 human isolates. *Proc Natl Acad Sci USA* 2000;97:9654–8.
- [28] Koopmans M, Wilbrink B, Conyn M, et al. Transmission of H7N7 avian influenza A virus to human beings during a large outbreak in commercial poultry farms in the Netherlands. *Lancet* 2004;363(9409):587–93.
- [29] Gill JS, Webby R, Gilchrist MJR, Gray GC. Avian influenza among waterfowl hunters and wildlife professionals. *Emerg Infect Dis* 2006;12:1284–6.
- [30] Zhou NN, Senne DA, Landgraf JS, et al. Genetic reassortment of avian, swine, and human influenza A viruses in American pigs. *J Virol* 1999;73:8851–6.
- [31] Peiris JSM, Guan Y, Markwell D, et al. Cocirculation of avian H9N2 and contemporary “human” H3N2 influenza A viruses in pigs in southeastern China: potential for genetic reassortment. *J Virol* 2001;75:9679–86.
- [32] Songserm T, Amonsin A, Jam-on R, et al. Avian influenza H5N1 in naturally infected domestic cat. *Emerg Infect Dis* 2006;12:681–3.
- [33] Amonsin A, Payungporn S, Theamboonlers A, et al. Genetic characterization of H5N1 influenza A viruses isolated from zoo tigers in Thailand. *Virology* 2006;344:480–91.
- [34] Songserm T, Amonsin A, Jam-on R, et al. Fatal avian influenza A H5N1 in a dog. *Emerg Infect Dis* 2006;12:1744–7.
- [35] WHO. Avian influenza—H5N1 infection found in a stone marten in Germany. Accessed online January 21, 2007 at: http://www.who.int/csr/don/2006_03_09a/en/index.html.
- [36] Wells DL, Hopfensperger DJ, Arden NH, et al. Swine influenza virus infections: transmission from ill pigs to humans at a Wisconsin agricultural fair and subsequent probable person-to-person transmission. *J Am Med Assoc* 1991;265:478–81.
- [37] Rimmelzwaan GF, de Jong JC, Bestebroer TM, et al. Antigenic and genetic characterization of swine influenza A (H1N1) viruses isolated from pneumonia patients in the Netherlands. *Virology* 2001;282:301–6.
- [38] Webby R, Hoffman E, Webster R. Molecular constraints to interspecies transmission of viral pathogens. *Nat Med Suppl* 2004;10:S77–81.
- [39] Harvey R, Martin ACR, Zambon M, Barclay WS. Restrictions to the adaptation of influenza A virus H5 hemagglutinin to the human host. *J Virol* 2004;78:502–7.
- [40] Couceiro JN, Paulson JC, Baum LG. Influenza virus strains selectively recognize sialyloligosaccharides on human respiratory epithelium; the role of the host cell in selection of hemagglutinin receptor specificity. *Virus Res* 1993;29:155–65.
- [41] Connor RJ, Kawaoka Y, Webster RG, Paulson JC. Receptor specificity in human, avian, and equine H2 and H3 influenza virus isolates. *Virology* 2005;17–23.
- [42] Uiprasertkul M, Puthavathana P, Sangsiriwut K, et al. Influenza A H5N1 replication sites in humans. *Emerg Infect Dis* 2006;11:1036–41.
- [43] Rogers GN, Paulson JC, Daniels RS, et al. Single amino acid substitutions in influenza haemagglutinin change receptor binding specificity. *Nature* 1983;304:76–8.
- [44] Stevens J, Blixt O, Tumpey TM, Taubenberger JK, et al. Structure and receptor specificity of the hemagglutinin from an H5N1 influenza virus. *Science* 2006;312:404–10.

- [45] Glaser L, Stevens J, Zamarin D, et al. A single amino acid substitution in the 1918 influenza virus hemagglutinin changes the receptor binding specificity. *J Virol* 2005;79:11533–6.
- [46] Matrosovich MN, Matrosovich TY, Gray T, et al. Human and avian influenza viruses target different cell types in cultures of human airway epithelium. *Proc Natl Acad Sci USA* 2004;101:4620–4.
- [47] Shinya K, Ebina M, Yamada S, et al. Influenza virus receptors in the human airway. *Nature* 2006;440:435–6.
- [48] Garten W, Klenk HD. Understanding influenza virus pathogenicity. *Trends Microbiol* 1999;7:99–100.
- [49] Kawaoka Y, Naeve CW, Webster RG. Is virulence of H5N2 influenza viruses in chickens associated with loss of carbohydrate from the hemagglutinin? *Virology* 1984;139:303–16.
- [50] Nicholls JM, Chan MCW, Chan WY, et al. Tropism of avian influenza A (H5N1) in the upper and lower respiratory tract. *Nat Med*, Published online January 7, 2007.
- [51] Matrosovich M, Tuzikov A, Bovin N, et al. Early alterations of the receptor-binding properties of H1, H2, and H3 avian influenza virus hemagglutinins after their introduction into mammals. *J Virol* 2000;74:8503–12.
- [52] Tran TH, Liem NT, Dung NT, et al. Avian influenza A (H5N1) in 10 patients in Vietnam. *N Engl J Med* 2004;350:1179–88.
- [53] Yuen KY, Chan PK, Peiris M, et al. Clinical features and rapid viral diagnosis of human disease associated with avian influenza A H5N1 virus. *Lancet* 1998;351(February 14 (9101)):467–71.
- [54] Chotpitayasunondh T, Ungchusak K, Hanshaoworakul W, et al. Human disease from influenza A (H5N1), Thailand, 2004. *Emerg Infect Dis* 2005;11:201–9.
- [55] Oner AF, Bay A, Arslan S, et al. Avian influenza A (H5N1) infection in eastern Turkey in 2006. *N Engl J Med* 2006;355(21):2179–85.
- [56] WHO. Recent findings countering genetics angle.
- [57] Bosch FX, Garten W, Klenk HD, Rott R. Proteolytic cleavage of influenza virus hemagglutinins: primary structure of the connecting peptide between HA1 and HA2 determines proteolytic cleavability and pathogenicity of avian influenza viruses. *Virology* 1981;113:725–35.
- [58] Tumpey TM, Basler CF, Aguilar PV, et al. Characterization of the reconstructed 1918 Spanish influenza pandemic virus. *Science* 2005;310:77–80.
- [59] De Jong MD, Simmons CP, Thanh TT, et al. Fatal outcome of human influenza A (H5N1) is associated with high viral load and hypercytokinemia. *Nat Med* 2006;12:1203–7.
- [60] Cheung CY, Poon LL, Lau AS, et al. Induction of proinflammatory cytokines in human macrophages by influenza A (H5N1) viruses: a mechanism for the unusual severity of human disease? *Lancet* 2002;360:1831–7.
- [61] Chan MCW, et al. Proinflammatory cytokine responses induced by influenza A (H5N1) viruses in primary human alveolar and bronchial epithelial cells. *Respir Res* 2005;6:135 (Available online from <http://respiratory-research.com/content/6/1/135>).
- [62] Guan Y, Poon LLM, Cheung CY, et al. H5N1 influenza: A protean pandemic threat. *Proc Natl Acad Sci USA* 2004;101:8156–61.
- [63] Geiss GK, Salvatore M, Tumpey TM, et al. Cellular transcriptional profiling in influenza A virus-infected lung epithelial cells: the role of the nonstructural NS1 protein in the evasion of the host innate defense and its potential contribution to pandemic influenza. *Proc Natl Acad Sci USA* 2002;99:10736–41.
- [64] Kobasa D, Takada A, Shinya K, et al. Enhanced virulence of influenza A viruses with the haemagglutinin of the 1918 pandemic virus. *Nature* 2004;431:703–7.
- [65] Kash JC, Basler CF, Garcia-Sastre A, et al. Global host immune response: pathogenesis and transcriptional profiling of type A influenza viruses expressing the hemagglutinin and neuraminidase genes from the 1918 pandemic virus. *J Virol* 2004;78:9499–511.
- [66] Bender C, Hall H, Huang J, et al. Characterization of the surface proteins of influenza A (H5N1) viruses isolated from humans in 1997–1998. *Virology* 1999;254:115–23.
- [67] Xu YY, Subbarao K, Cox NJ, Guo YJ. Genetic characteristics of the pathogenic influenza A/Goose/Guangdong/1/96 (H5N1) virus: similarity of its hemagglutinin gene to those of H5N1 viruses from the 1997 outbreaks in Hong Kong. *Virology* 1999;261:15–9.
- [68] Guan Y, Shortridge KF, Krauss S, Webster RG. Molecular characterization of H9N2 influenza viruses: were they the donors of the “internal” genes of H5N1 viruses in Hong Kong? *Proc Natl Acad Sci USA* 1999;96:9363–7.

- [69] Subbarao K, Shaw MW. Molecular aspects of avian influenza (H5N1) viruses isolated from humans. *Rev Med Virol* 2000;10:337–48.
- [70] Sims LD, Ellis TM, Liu KK, et al. Avian influenza in Hong Kong 1997–2002. *Avian Dis* 2003;47(3 Suppl):832–8.
- [71] Li KS, Guan Y, Wang J, et al. Genesis of a highly pathogenic and potentially pandemic H5N1 influenza virus in eastern Asia. *Nature* 2003;430:209–13.
- [72] WHO. WHO Global Influenza Program Surveillance Network. Evolution of HN1 avian influenza viruses in Asia. *Emerg Inf Dis* 2005;11:1515–21.
- [73] WHO. Influenza research at the human and animal interface. Report of a WHO working group. Geneva, Switzerland, September 21–22, 2006. WHO/CDS/EPR/GIP/2006.3. Accessed on line January 23, 2007 at: http://www.who.int/csr/resources/publications/influenza/WHO_CDS_EPR_GIP_2006_3C.pdf.
- [74] Webser RG, Govorkova EA. H5N1 influenza—evolving evolution and spread. *N Engl J Med* 2006;355:2174–7.
- [75] Smith GJD, Fan XH, Wang J, et al. Emergence and predominance of an H5N1 influenza variant in China. *Proc Natl Acad Sci USA* 2006;103:16936–41.
- [76] Hulse-Post DJ, Sturm-Ramirez KM, Seiler HP, et al. Role of domestic ducks in the propagation and biological evolution of highly pathogenic H5N1 influenza viruses in Asia. *Proc Natl Acad Sci USA* 2005;102:10682–7.
- [77] Sturm-Ramirez KM, Ellis T, Bousfield B, Bissett L, et al. Reemerging H5N1 influenza viruses in Hong Kong in 2002 are highly pathogenic to ducks. *J Virol* 2004;78:4892–901.
- [78] Chen H, Deng G, Li Z, et al. The evolution of H5N1 influenza viruses in ducks in southern China. *Proc Natl Acad Sci USA* 2004;101:10452–7.
- [79] Gilbert M, Chaitaweessub P, Parakamawongsa T, et al. Free-grazing ducks and highly pathogenic avian influenza, Thailand. *Emerg Infect Dis* 2006;12:227–34.
- [80] Luke CJ, Subbarao K. Vaccines for pandemic influenza. *Emerg Infect Dis* 2006;12:66–72.
- [81] Subbarao K, Murphy BR, Fauci AS. Development of effective vaccines against pandemic influenza. *Immunity* 2006;24:5–9.
- [82] Promed. Avian Influenza (14): Viet Nam, Thailand, South Korea. Ducks in Vietnamese province infected with bird flu. Posted January 22, 2007. Archive Number 20070122.0295; Accessed on line January 29, 2007 at: http://www.promedmail.org/pls/promed/f?p=2400:1001:7219590730279528404::NO:F2400_P1001_BACK_PAGE,F2400_P1001_PUB_MAIL_ID:1010,36018.
- [83] Choi YK, Nguyen TD, Ozaki H, et al. Studies of H5N1 influenza virus infection of pigs by using viruses isolated in Vietnam and Thailand in 2004. *J Virol* 2005;79:10821–5.
- [84] Webser R, Hulse D. Controlling avian flu at the source. Commentary. *Nature* 2005;435:415–6.
- [85] OIE. Recommendations. OIE/FAO international scientific conference on avian influenza, OIE, Paris, France, April 7–8, 2005. Accessed on line January 22, 2007 at: http://www.oie.int/eng/avian_influenza/OIE_FAO_Recom_05.pdf.
- [86] Perdue ML, Swayne DE. Public health risk from avian influenza viruses. *Avian Dis* 2005;49:317–27.
- [87] Capua I, Marangon S. Control of avian influenza in poultry. *Emerg Infect Dis* 2006;12:1319–24.
- [88] FAO/OIE/WHO Technical consultation on the control of avian influenza. February 3–4, 2004, conclusions and recommendations. Accessed on line January 22, 2007 at: http://www.oie.int/download/AVIAN%20INFLUENZA/avian_rome_feb04_report.pdf.
- [89] USDA. USDA Fact sheet (Release No. 0458.05). Questions and answers; March 2006. Accessed online January 22, 2007 at: http://www.usda.gov/wps/portal/tut/pl/s_7_0_A/7_01RD?printable=true&contentidonly=true&contentid=2005/10/0458.xml.
- [90] Dinh PN, Long HT, Tien NT, et al. Risk factors for human infection with avian influenza A H5N1, Vietnam, 2004. *Emerg Infect Dis* 2006;12:1841–7.
- [91] Sturm-Ramirez KM, Hulse-Post DJ, Govorkova EA, et al. Are ducks contributing to the endemicity of highly pathogenic H5N1 influenza virus in Asia? *J Virol* 2005;79:11269–7.
- [92] WHO. Avian influenza (“bird flu”)—Fact sheet; January 2006. Accessed on line January 22, 2007 at: http://www.who.int/csr/disease/avian_influenza/avianinfluenza_factsheetJan2006/en/print.html.
- [93] Schünemann HJ, Hill SR, Kakad M, et al. WHO rapid advice guidelines for pharmaceutical management of sporadic human infection with avian influenza A (H5N1) virus. *Lancet Infect Dis* 2007;7:21–31.

- [94] CDC. Interim guidance for protection of persons involved in U.S. avian influenza outbreak disease control and eradication activities. Accessed online January 29, 2007 at: <http://www.cdc.gov/flu/avian/professional/protect-guid.htm>.
- [95] CDC. Avian influenza: protecting workers from exposure. Accessed online January 29, 2007 at: <http://www.cdc.gov/niosh/topics/avianflu/>.
- [96] CDC. Prevention and control of influenza: recommendations of the advisory committee on immunization practices (ACIP). *MMWR Recomm Rep* 2006;55:1–42.
- [97] FDA. Personal protective equipment and influenza outbreaks, including bird flu (avian influenza). Accessed online January 29, 2007 at: <http://www.fda.gov/cdrh/ppe/fluoutbreaks.html>.
- [98] US Department of Labor, Occupational Safety & Health Administration. Guidelines for protecting workers against avian influenza. Accessed online January 29, 2007 at: <http://www.osha.gov/dsg/guidance/avianflu.html>.
- [99] Department of Interior. Employee health and safety guidance for avian influenza surveillance and control activities in wild bird populations. Memorandum, January 2007. Accessed online January 29, 2007 at: <http://www.doi.gov/issues/appendixOHSguidanceforAvian%20Influenza12-18.pdf>.
- [100] Saenz RA, Hetgicite HW, Gray G. Confined animal feeding operations as amplifiers of influenza. *Vector Borne Zoonot* 2006;4:338–46.
- [101] WHO. Antigenic and genetic characteristics of H5N1 viruses and candidate H5N1 vaccine viruses developed for potential use as pre-pandemic vaccines. Accessed on line January 23, 2007 at: http://www.who.int/csr/disease/avian_influenza/guidelines/recommendationvaccine.pdf.
- [102] Center for Infectious Disease and Research. FDA panel supports H5N1 vaccine approval. February 27, 2007. Accessed on line March 4, 2007 at: <http://www.cidrap.umn.edu/cidrap/content/influenza/avianflu/news/feb2707vaccine.html>.
- [103] Monto AS. Vaccine and antiviral drugs in pandemic preparedness. *Emerg Infect Dis* 2006;12:55–60.
- [104] de Jong MD, Tran TT, Khanh TH, et al. Oseltamivir resistance during treatment of influenza A (H5N1) infection. *N Engl J Med* 2005;353:2667–72.
- [105] WHO. Advice on use of oseltamivir; March 17, 2006. Accessed on line January 29, 2007 at: http://www.who.int/csr/disease/avian_influenza/guidelines/useofoseltamivir2006_03_17A.pdf.
- [106] Moscona A. Oseltamivir resistance—disabling our influenza defenses. *N Engl J Med* 2005;353:2633–6.
- [107] WHO Writing Group. Nonpharmaceutical interventions for pandemic influenza, international measures. *Emerg Inf Dis* 2006;12:81–7.
- [108] Glass RJ, Glass LM, Beyeler W, Min HJ. Targeted social distancing design for pandemic influenza. *Emerg Inf Dis* 2006;12:1671–81.
- [109] Chen H, Smith GJ, Li KS, et al. Establishment of multiple sublineages of H5N1 influenza virus in Asia: implications for pandemic control. *Proc Natl Acad Sci USA* 2006;103:2845–50.
- [110] Kilpatrick AM, Chmura AA, Gibbons DW, et al. Predicting the global spread of H5N1 avian influenza. *Proc Natl Acad Sci USA* 2006;103:19368–73.
- [111] WHO. Avian influenza and human pandemic influenza. Summary report. Meeting held in Geneva, Switzerland, November 7–9, 2005. Accessed on line January 29, 2007 at: http://www.who.int/mediacentre/events/2005/avian_influenza/summary_report_Nov_2005_meeting.pdf.