

Review Article

Bird Flu, the Hanging Pandemic Threat for Human – It's Risk Assessment and Containment

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Bird flu, synonym of avian influenza (AI) caused by influenza A virus, become concern across the world for the possible incidence of the next human influenza pandemic. The latent danger of AI pandemic remains very real, though, the precise timing of occurrence and severity is uncertain. Each avian influenza type A (AIA) contains one of the 16 subtypes of haemagglutinin (HA) and 9 neuraminidases (NA) implicating theoretically 144 subtypes of AIA are possible in circulation, but only H1N1, H2N2 and H3N2 subtypes are documented for past pandemics in humans. In recent years H5N1, H7N3, H7N2, H7N7 and H9N2 are isolated from human samples, though H1N1 and H3N2 are still in circulation. Avian influenza viruses preferentially recognize receptor containing sialosugar chains terminating in sialic acid α -2,3-galactose in bird, whereas, human preferentially contain α -2,6-galactose subtype-receptor. To initiate a pandemic outbreak in human, the AIA viruses need alteration of receptor recognition specificity; and perfect match between HA and NA along with optimal cellular tropism. Cyclic nature of bird-flu emergence, and moreover, sporadic human incident reported around Asia and Europe in recent years anticipating a pandemic appearance of bird-flu in time to come. As we are on the edge of this alarming situation, AI prevention and containment can be considered under categories of surveillance, intervention, antiviral drugs, vaccination together with environment management issues.

Keywords: Pandemic, Avian influenza, Genetic reassortment, Host specificity, Environment management

Introduction

“Avian influenza” or “fowl plague” was first recognized in chickens in 1878¹. Influenza, an RNA virus can be designated as A, B or C² based on their antigenic differences. Influenza A viruses being the most dangerous one mutate and spread rapidly, and can infect different birds to mammals including human and subtype on the basis of surface protein named as haemagglutinin (HA) and neuraminidase (NA)²⁻⁷. There are 16 known HA and 9 known NA subtypes⁷. Many different combinations of HA and NA proteins are possible³⁻⁷. Theoretically, 144 subtypes are likely. So far about 100 subtypes have been notified². Generally, influenza viruses are highly species-specific^{3,6}. All known subtypes of influenza A viruses can be found in birds³. Intestinal cells of wild birds, especially shorebirds, ducks, and geese display receptors for AI viruses and regarded as the natural reservoir of all AI viruses⁸⁻⁹. Genetic mutation and re-assortment help viruses capable to jump over the species barrier making them capable to infect human^{7,10} (Figure 1 and Table 1).



Figure 1. Transmission of avian influenza viruses among hosts. Wild aquatic birds are the primary reservoir for influenza A viruses from which viruses can be transmitted to other hosts such as horses, pigs, poultry, whales, seals, and humans. Pigs and poultry can also infect humans. (Adapted from Trampuz et al.¹¹).

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Table 1. Instances of various subtypes of avian influenza A virus infections of human¹²⁻¹³

Subtype	Region	Year
H7N7 ^a	USA	1959
H7N7	UK	1995
H7N7	UK	1996
H5N1 ^a	Hong Kong	1997
H9N2	China and Hong Kong	1999
H7N2	USA (Virginia)	2002
H5N1 ^a	China and Hong Kong	2003
H7N7 ^a	Netherlands	2003
H9N2	Hong Kong	2003
H7N2	USA (New York)	2003
H7N3 ^a	Canada	2004
H5N1 ^a	Thailand and Vietnam	2004
H5N1 ^a	Thailand, Vietnam, Cambodia, China and Indonesia	2005
H5N1 ^a	Thailand, Vietnam, Cambodia, China, Indonesia, Azerbaijan, Djibouti, Egypt, Iraq and Turkey	2006
H5N1 ^a	Bangladesh, Vietnam, Cambodia, China, Indonesia, Laos, Nigeria and Egypt	2007
H7N2	UK	2007
H9N2	China and Bangladesh	2007
H5N1 ^b	Bangladesh	2008

^aHigh pathogenic; ^bPathogenicity is not clear, because the infected 16-month-old boy did not show any avian influenza (AI) symptom.

The epidemic nature and the clinical features of this deadly influenza first recorded at the beginning of 19th century. Several epidemics recorded during the nineteenth century but the first pandemic outbreak of Bird flu not recorded precisely¹¹⁻¹⁴. In 1918-1919, a pandemic known as ‘Spanish flu’ recorded about 50 million deaths, principally the young adults¹⁵ suggesting unusual virulence nature of the strain. On the other hand, the large numbers of deaths may be due to the enfeebling conditions as an after effect of the First World War¹⁵⁻¹⁷. Pandemics continued to occur regularly after the Spanish influenza, in 1932-1933, 1947-1948, 1957 and 1968. The next pandemic is thought to be overdue. These latter pandemics resembled the pandemic of 1890, affecting millions of people with a mild upper respiratory tract infection (URTI) and a small number of deaths. The H1N1 (swine) viruses probably appeared in 1918 and continued in circulation until supplanted by the H2N2 (Asian) viruses in 1957¹⁸⁻²⁶. The H2N2 viruses predominantly circulated until H3N2 (Hong Kong) strains appeared in 1968. The H1N1 virus reappeared in 1977 and did not replace the H3N2 subtype and both subtypes continued to co-circulate^{6,19} (Figure 2).

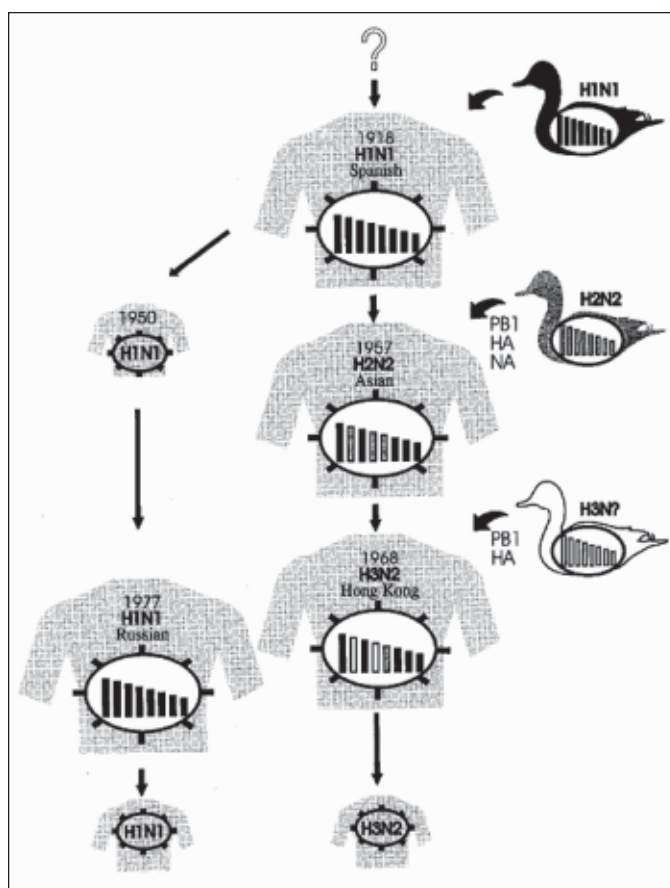


Figure 2. Possible origins of pandemic influenza viruses. Phylogenetic studies suggest that an avian influenza virus was transmitted to humans, caused the 1918 pandemic. A reassortment virus possessing its PB1, HA, and NA genes from a Eurasian avian virus, with the remainder coming from an H1N1 human virus, caused the Asian pandemic of 1957. In 1968, a reassortment possessing its PB1 and HA genes from a Eurasian avian virus and the remainder from an H2N2 human virus emerged, followed by the disappearance of the H2N2 virus. In 1977, a virus genetically almost identical to those circulating in humans in 1950 appeared and spread among children and young adults. The H1N1 and H3N2 viruses are now co-circulating in humans (Adapted from Taisuke et al.²⁷).

Depending on the severity all subtypes can be categorized into two kinds. One is high pathogenic avian influenza virus (HPAIV) and the other is low pathogenic avian influenza virus (LPAIV). When LPAIV strains are transmitted from avian reservoir hosts to highly susceptible poultry species such as chickens and turkeys, they undergo a series of mutations resulting adaptation to their new hosts²⁷⁻²⁸. Influenza A virus not only runs through a host adaptation phase in this way, HPAIVs may arise unpredictably *de novo* in poultry infected with LPAI progenitors²⁴. Over the last 30 years, highly virulent avian influenza viruses have caused outbreaks in poultry in Australia (1976 [H7]³⁰, 1985 [H7]³¹⁻³², 1992 [H7]³³, 1995 [H7] and 1997 [H7]), England (1979 [H7]³⁴ and 1991 [H5]³⁵), the United States (1983 to 1984 [H5]³⁶), Ireland (1983

to 1984 [H5]³⁷), Germany (1979 [H7]³⁸), Mexico (1994 to 1995 [H5]³⁹⁻⁴⁰), Pakistan (1995 [H7]⁴¹), Italy (1997 [H5]) and Hong Kong (1997 [H5]⁴²). Recently, H5 and H9 have been demonstrated to be involved with poultry flu in Bangladesh. Among the two, H5N1 (93%) is predominant according to the National Reference Laboratory, Bangladesh Livestock Research Institute (BLRI), Savar, Dhaka. The available information implicating with minor exception that all of the recent pathogenic avian influenza A viruses are of H5 or H7 containing subtypes (Table 1).

Molecular Perspective of Pandemic Risk

For a pandemic an agent should have three properties⁴³.

1. A new type of flu virus has to be introduced into the human population.
2. The new type must have a serious impact on the health of humans.
3. The new type must have the capability to spread easily from one person to the next (human specific variant).

Currently circulating H5 and H7 subtypes attained the first two properties and the third one is yet to acquire³⁰. Host specification primarily depends on HA, the surface glycoprotein³³⁻³⁴. In addition, there is increasing evidence that NA can promote virus entry into host cells during the initial stage of infection⁴⁴. The pandemic risk lies within the molecular composition of these two structural proteins. Influenza infection requires binding of the HA protein to sialic acid-containing receptors on the host cell surface where the precise linkage of HA to host receptors determines species preference³⁸. For example, a switch in receptor specificity from receptors containing sialic acids connected to galactose in a alpha 2-3 linkage (avian receptors) to a alpha 2-6 linkage (human receptors) is required for influenza A virus to cross the species barrier to adapt in human host⁴⁵⁻⁴⁸. Antigenic properties also change with it⁴⁹. Pandemic strains of H1, H2, and H3 subtypes recognize alpha 2-6 linked sialic acid, the prevalent form found on cells of the human respiratory tract⁵⁰. But researchers found that human airway epithelium harbours alpha 2-3 linked sialic acids on ciliated cells. These findings suggest that although avian influenza viruses can infect human ciliated airway epithelium, their replication may be limited by a non-optimal cellular tropism⁵¹. Bangladesh health authorities confirmed on the 22nd May 2008 that a 16-month-old boy infected with H5N1 without showing any symptom of bird flu, had recovered and released from hospital. Thus, the present data and experimental evidence implicating that to be a HPAIV strain – the strain must have α -2,6 receptor specificity and capable to evade cellular control that limit its replication or pathogenesis.

Genetic alteration is likely high for avian influenza A virus⁵². For the lack of proofreading activity of RNA polymerases, there is much more inherent variation at the nucleotide sequence level in a replicating population of an RNA virus than for an otherwise similar DNA viruses⁵³⁻⁵⁴. The effects of natural selection can

produce evolutionary change in viruses over a shorter time scale. For influenza, according to natural selection, superior evader variants of body's immune response have better replicative success in human populations⁵⁵.

Influenza virus genome is spliced into 8 pieces⁷. And various strains of influenza A virus can productively infect multiple mammalian and avian species. This co-hosting phenomenon is a good reason for genetic reassortment to evolve viruses with new-fangled characteristics. Mixed infections occur frequently in nature and thus lead to genetic reassortment⁵⁶⁻⁵⁸. Furthermore, major antibody-interacting domains of HA lack specific conserved structure and distinct from the main functional domains⁵⁹. Per year a given strain of influenza virus may change by a percent at the nucleotide sequence level⁵³. HA contains about 566 amino acid residues (1.75 kb)⁶⁰. Over a decade, a given "strain" of influenza virus can change quite significantly resulting flu from the same strain, even if, the host is vaccinated. One important phenomenon for the potency of pandemic is the crosstalk and match between two surface proteins residing on the viral particle. High pathogenic HA is accompanied better with low-functional NA⁶¹.

Classification of Pandemic Phases and Recent Pandemic Layout

According to WHO experts' investigation, AI virus did not spread within the community. No healthcare worker was infected either. It showed that infection is only possible with very close contact with H5N1 infected patients⁶⁴. Therefore, the world remains at phase 3 of the WHO alert scale (Table 2). One key point to focus, besides H5N1 (the recently isolated pathogenic form of AIA from the human patient of different countries) other potential pandemic viruses H7N7, H9N2, and H2N2 are also the threat and candidate for pandemic, because they are still in circulation and showed high virulence potential in laboratory samples^{12-13,65}.

Table 2. WHO classification of pandemic phases⁶²⁻⁶³

Warning status	Phase	Human risk situation
Inter-pandemic	Phase 1	Low risk of human cases
	Phase 2	High risk of human cases
	Phase 3	No or very limited human-to-human transmission
Pandemic alert	Phase 4	Evidence of increased human-to-human transmission
	Phase 5	Evidence of significant human-to-human transmission
Pandemic	Phase 6	Efficient and sustained human-to-human transmission

Major Strategies to Fight Bird Flu Pandemic

WHO had included surveillance for pandemic preparedness, public health interventions, the use and availability of antiviral, and access to vaccines produce against the infectious AIA as four major topics for discussion in "WHO consultation on priority public health interventions before and during an influenza pandemic" held in March 2004⁶⁶.

Strategy I – Surveillance and early detection

The important function of surveillance is to detect unusual cluster of cases at an early stage or to discover abnormal clinical manifestations in cases and then to understand virus characteristics through analysis. This will help us block the virus in time, once its transmission ability enhances and will facilitate the execution of epidemic control measures to prevent the epidemic situation from worsening.

Strategy II – Interruption of transmission

Besides medical interventions of bird flu using antiviral agents and vaccines, non-medical public health interventions such as personal hygiene practices, isolation, an reduction of social contact have been demonstrated extremely effective in preventing and minimizing the spread of the pathogen. WHO Global Influenza Preparedness Plan⁶⁷ categorized non-pharmaceutical public health interventions into 4 types:

1. Measures that limit the international transmission of virus, such as screening of fever at border and travel restriction,
2. Measures that reduce virus transmission, such as isolated treatment of patient, health self-management of contact, quarantine, cancellation of rallies and class suspension, etc.,
3. Decrease personal risks, such as frequent practice of hand-washing and
4. Communication of risks to the public.

A recent research found that border restrictions and/or internal travel restrictions are unlikely to delay spread by more than 2-3 weeks, school closure during the peak of a pandemic can reduce peak attack rates by up to 40%, and treatment of clinical cases can reduce transmission, but only if antiviral is given within a day of symptoms starting⁶⁸. Given enough drugs for 50% of the population, household-based prophylaxis coupled with reactive school closure could reduce clinical attack rates by 40-50%⁶⁸⁻⁶⁹. More widespread prophylaxis would be even more logistically challenging and might reduce attack rates by over 75%⁷⁰.

Strategy III – Antiviral drugs

At present, the cure and preventive function of neuraminidase inhibitor anti-viral drugs have been confirmed in seasonal influenza⁷¹. As a consequence, it is expected to be effective in treatment and after exposure prophylaxis for avian influenza and pandemic influenza.

There are 2 classes of anti-viral drugs specific for influenza: M2 inhibitors and neuraminidase inhibitors. M2 inhibitors launched earlier and are cheaper. A major concern is that H5N1 virus has been found to be resistant to M2 inhibitors⁷². Neuraminidase inhibitors, such as Oseltamivir and Zanamivir, are newly developed. Neuraminidase inhibitors are effective in the treatment of avian influenza especially when given early, in the first 48 hours of infection⁷³ but evidence shows higher incidence of resistance (AI H5N1 strains resistant to Oseltamivir have been collected in 0.4-4.0% of patients)⁷⁴. Factors, which might contribute to this

apparently limited efficacy, include suboptimal dosing or routes of administration, suboptimal timing of treatment, the inability of antiviral drugs to interfere with immunopathology and the development of drug resistance⁷⁵. The promising news is that last year, Neugene, a new antiviral drug still in the testing phase, is showing promise as an effective treatment for avian flu. The new drug is manufactured by BioPharma, Inc⁷⁶.

Strategy IV – Vaccination

Present vaccines have had mixed results. In the field of influenza vaccination, neither commercially available nor experimentally tested vaccines have been shown promising enough to fight this diseases perfectly⁷⁷. A variety of vaccine types are employed, including inactivated (whole virion, split virus and surface antigen), live attenuated and virosome. The majority of projects target specific strains of influenza virus (H2N2, wild type H5N1, H5N1, H5N3, H7N1, H7N7, H9N2)⁷⁸ (Table 3).

Table 3. Current development in avian influenza vaccine (2006 to 2007)

Vaccine type and formulation	Dose and year
^a Split H5N1, no adjuvant	2 x 90 µg (2006) ⁷⁷
Split H5N1, with alum	2 x 30-45 µg (2006) ⁷⁸
^b Whole virus H5N1 (egg grown), with alum	2 x 10-15 µg (2006) ⁷⁹⁻⁸⁰ 1 x 6 µg (2007) ⁸¹
Subunit H5N3, with MF59	2 x 7.5 µg* (2001) ⁸²
^b Subunit H5N1, with MF59	2 x 7.5 µg (2007) ⁸³
Vero cell whole virus H5N1 (wild type), no adjuvant	2 x 7.5 µg* (2007) ⁸⁴
Split H5N1 vaccine, with novel adjuvant	2 x 1.9*- 3.75 µg (2007) ⁸⁵

^aLicensed in the USA; ^bLicensed in the Europe; *Not evaluated.

Note: Food and Drug Administration of China issued license for a vaccine against H5N1, April, 2008.

Researchers believe that universal influenza vaccine is possible, using an M-2 peptide conjugate protein⁸⁶. Recently A Novel Intranasal Virus-Like Particle (VLP) Vaccine have prepared bearing the surface glycoproteins HA and NA of the 1918 influenza A virus by infecting Sf9 cells with a quadruple recombinant baculovirus that expresses the four influenza proteins (HA, NA, M1 and M2)⁸⁷. Vaccines are primarily geared towards ducks and chickens to save economic lose, though, it may cause host immunopressure resulting generation of antigenic diversity or more pathogenic variants⁸⁸. Experimental result shows that when HA protein comes under selective immunopressure, it mutates to evade the host's immune system⁸⁹.

Vaccination vary broadly in regard to several local factors (*e.g.*, type of production, local pattern of disease, costs and potential losses) but vaccination should also be applied in the framework of poultry disease eradication program at national or regional

levels under the official supervision of public veterinary services⁹⁰. Recent data of N1 sequences in the NCBI database shows that N1 from H5N1 is distantly related to the H1N1 from 1918 and its descendants. N2 of different strains also feature the same type of relatedness¹³. So, new vaccine targeting both the N1 and N2 (combination vaccine) is however speculative to provide with partial protection because of cross-immunity. This might be enough to prevent death with a rapid pace.

Factory Farming and Environmental Degradation Boosting Pandemic Threat

Wild birds are the main reservoir of avian influenza virus¹¹. They have been blamed for the spread of present influenza threat. In Canada, Quebec outlawed the outdoor raising of poultry (12th November 2005)⁹¹. If wild birds had been spreading the disease across continents there would have been trails of dead birds following migration routes, which is not the case. Certain countries on flight paths of birds from Asia remain flu-free, whilst their neighbours suffer repeated infections⁹². Another interesting thing, in a low-density and dispersed population such as, flocks of wild birds a virus can only survive as a low pathogenic agent because if a virus mutates into a highly pathogenic form in these circumstances, it quickly dies out as it kills all available hosts⁹³.

The necessity for efficiency to produce the animal protein the agribusiness has been especially moved to poultry sector, where it is now a reality, as many as 10 million birds are raised within a few square kilometers⁹⁴⁻⁹⁵. In a factory farm situation, perfect conditions exist for a virus to mutate from a low pathogenic to a high pathogenic form. Thousands of hosts (chickens) with near identical genetic makeup, all the same age and size, crowded in close conditions allow a virus to kill its host and move onto the next victim with great speed and ease⁹⁶. The poultry industry should change and humanity must move toward raising poultry in smaller scale, under less stressful, less crowded and more hygienic conditions with outdoor access. Some useful practice can be introduced rearing animals (*e.g.*, straw bedding is linked to decrease risk of infection with the influenza virus⁹⁷).

Nutrition, sanitation and medication have improved in the last century but we have disrupted planetary ecological processes. Human activities like deforestation destroy birds natural habitat thus they are getting contract with our domesticated animals and giving the virus to widen its host range. Some of our strange farming strategies like fish-chicken integrated farm (where economic benefit is questionable⁹⁸) prompts pandemic risk. Direct contamination of chicken excreta in fish rearing water bodies⁹⁹ introduces a huge dose of pathogenic agent to a new environment (an infected chicken can contain enough virus to infect 1 million of individuals¹⁰⁰). Pathogens that enter the food chain of farm animal have good potency to flourish largely because of stress-related factors (overcrowding, competition, same age etc.¹⁰¹). This sort of desperate farming trend should be stopped under proper law and legislations.

Conclusion

Rapid spread rate in a dense population occurs in breeding ground of infectious diseases. The more the infectious agent circulates in human host, the more they get adapted with that. Most of the people in developing countries suffer from malnutrition. Respiratory diseases have good link with malnutrition. The condition is worse in sub-Saharan countries particularly in militaries. These people are veritably vulnerable to avian influenza. On the other hand, poultry is the fastest growing segment of global agriculture. Being in this dilemma, the Governments of over populated developing countries mainly in Asia and Africa should put in place an effective strategy for the prevention and control of bird flu in collaboration of FAO, WHO and other International Agencies to prevent the looming catastrophic potential of bird flu with great agency and implementation surety.

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