

# Avian influenza — a pandemic waiting to happen?

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Cross-species transmission of a highly pathogenic subtype of influenza A virus directly from birds to humans has raised many concerns. The radical methods of immune evasion and the possibility of human-to-human transmission as a result of gene reassortment between the human and avian viral subtypes pose an imminent threat of a global pandemic. The growing reservoir of circulating influenza among the bird population and the perpetuating human demographic factors promote the emergence of a novel viral strain. This article discusses current methods of identifying and treating the illness in individuals, and outlines principles of public health measures for preventing and containing an influenza pandemic.

**Key words:** Disease outbreaks, influenza A virus H5N1 subtype, influenza in birds, reassortant viruses, swine diseases

## Introduction

With the terror of severe acute respiratory syndrome (SARS) still fresh in people's memory, the world is facing another potentially devastating communicable disease. Avian influenza, commonly known as "bird flu", is a viral infection that normally affects the fowl. The pathogen, influenza A virus, is a single-stranded RNA virus of the *Orthomyxoviridae* family [1]. Influenza viruses are classified by the differences in their nucleocapsid and matrix proteins (structures that facilitate viral attachment to host cells and help spread the viral offspring, respectively) into A, B and C groups. The A group is further sub-typed according to variations in its surface glycoproteins haemagglutinin (H) and neuraminidase (N). There are 16 H and 9 N subtypes which permute to form all the influenza A subtypes.

In the light of recent epidemiologic and scientific discoveries, this article will discuss the distinguishing features of avian influenza in contrast to "the common flu" and the causes for public health concerns.

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## Reasons for Concern

### Cross-species transmission

All influenza A subtypes can be found in waterfowl, but only the H1-3 and N1-2 subtypes are commonly known to infect humans. Mild forms of avian influenza only cause ruffled feathers or reduced egg production, but the highly pathogenic H5N1 subtype first discovered in Italy in 1978 is extremely infectious and rapidly fatal to birds. Most importantly, this viral subtype is now known to have jumped the species barrier to infect humans. The first documented leap occurred in Hong Kong in 1997, where 18 patients were uncharacteristically ill with severe flu, which eventually led to 6 deaths [2]. Genetic sequencing of a virus isolated from a child who died revealed an H5N1 strain indistinguishable (>99% sequence homology) from the virus that caused preceding outbreaks in the local poultry [3,4]. The exceptionally high case-fatality rate (around 51% overall) was again observed when at least 62 deaths resulted from 122 human cases of H5N1 influenza in Indonesia, Cambodia, Thailand and Vietnam since December 2003 [5].

### Increasing pathogenicity

Different viral subtypes and strains have variable ability to cause disease in its human hosts. Besides H5N1,

**Table 1.** Confirmed instances of avian influenza viruses infecting humans since 1997<sup>a</sup>

Subtype	Place	Year	Outcome
H5N1	Hong Kong, China	1997	Direct transmission from birds to human. Eighteen hospitalised, 6 of them died. 1.5 million chickens killed to remove source of virus
H9N2	Hong Kong, China	1999	Low pathogenic strain infected 2 children. Source unknown. Human-to-human infection not ruled out. Both recovered
H7N2	Virginia, USA	2002	One person with serological evidence of infection with H7N2, as for preceding outbreak among poultry
H5N1	Hong Kong, China	2003	Two cases of human infection among family members who travelled to China. One recovered. One died
H7N7	The Netherlands	2003	H7N7 outbreaks in several poultry farms. Eighty nine humans were later infected, mostly poultry workers with evidence of direct transmission. One died
H9N2	Hong Kong, China	2003	Low pathogenic infection in a child, who later recovered
H7N2	USA	2003	One patient developed respiratory symptoms and recovered. No evident contacts with birds
H7N3	Canada	2004	Infection in poultry workers following outbreak among birds
H5N1	Thailand and Vietnam	2004	Several outbreaks of this highly pathogenic strain reported by World Health Organisation

<sup>a</sup>Data extracted from Centers for Disease Control and Prevention [6].

other avian influenza subtypes (e.g., H9N2 and H7N7) are known to have infected humans in recent times (Table 1) [6], but the reported cases are sporadic and often resulted in mild illnesses [7,8]. However, it has been shown that certain influenza strains initially of lower pathogenicity can rapidly mutate (within 6 to 9 months) into a highly pathogenic strain if allowed to circulate in the poultry population. In particular, research groups from China and the USA have found, by injecting mice with confirmed H5N1 viral strains isolated from ducks, that between 1999 and 2002 there is a clear temporal pattern of progressive virulence [9]. The H5N1 influenza virus possesses certain amino acid residues near the cleavage sites of haemagglutinin. This allows subtilisin-like proteases, which can be found in all host cell types, to cleave the protein and expose its hydrophobic ends, and facilitates viral fusion to host cell membranes [10]. The H5N1 viral subtype can therefore affect different tissue types to cause systemic diseases and death.

### **Evasion of host immunity and progression to a pandemic**

Influenza is a common respiratory infection. Its annual epidemics in humans are mainly due to a phenomenon called ‘antigenic drift’ — mutations in the surface viral proteins as a result of errors made during RNA replication, since RNA polymerase lacks the proofreading mechanisms of DNA polymerase. These new mutations make the viral antigens less recognisable by the host’s acquired immunity. Every year the World

Health Organization (WHO) follows this antigenic drift and decides on the strains of virus to be used for the annual influenza vaccination in humans, because the antibody titre of the general population induced by older vaccines will not be sufficient to give adequate protection.

An even more radical way of immune evasion is that the segmented structure of influenza A genome allows swapping of major gene segments to occur between 2 different subtypes of influenza A virus that have infected the same cell [11]. The phenomenon known as antigenic shift helps create a novel virus that retains most of the human viral components, and may therefore be efficiently transmitted from human to human. Unlike the common flu, the human population will be immunologically naïve to its significantly altered antigens. Existing vaccines developed from previously known strains would also be ineffective against this genetically reassorted variant.

The above stages of cross-species transmission from animals to humans, followed by gene reassortment and ultimately viral infection between human beings are speculated to be the mechanisms behind the 1918 H1N1 “Spanish Flu” which claimed the lives of around 40-50 million victims globally (more than the official death toll of World War I during that period). Suspected antigenic shifts have occurred on 3 other occasions during the 20th century (H2N2 in 1957, H3N1 in 1968, H1N1 and H3N2 in 1977), each time resulting in a pandemic [12]. The current anticipation of a similar threat to the public’s health is therefore not unfounded.

### **Viral transmission and demographics**

The current cluster of avian influenza outbreaks in birds has occurred in areas where humans live in proximity to poultry. Here, live poultry market and backyard chicken farming are particularly prevalent, and this makes direct transmission from birds to humans much more likely. Out of the 18 confirmed human cases in Hong Kong, 1 patient had prior contacts with diseased birds on the farm while the other 14 had been in the traditional “wet market” [13].

Similar to its human counterpart, avian influenza is transmitted mainly by infected droplets adhering to the host conjunctiva and respiratory epithelium. Although H5N1 virus preferentially attaches itself via haemagglutinin protein to 2,3-sialic acid receptors on the respiratory and gut epithelium of birds, the same receptors can also be found in the human conjunctiva and ciliated portion of the respiratory columnar epithelium [14]. Since pigs have both the avian (2,3-sialic) and human (2,6-sialic) receptors in their respiratory tracts [15], they can be infected with both the human and avian viruses simultaneously and act as “mixing vessels” for gene swapping to occur. Pigs infected with avian influenza had been reported in Indonesia in 2005, and the affected areas were those where humans live close to their livestock. Thus, the human influenza virus can potentially be transmitted to the animal that in turn passes the reassorted strain back to the humans. Intermediate vectors such as the pig were initially thought necessary for cross-species transmission from birds to men. The hypothesis was, however, dismissed by strong evidence of direct transmissions during the 1997 Hong Kong H5N1 outbreak [4,16].

Since 2,3-sialic acid receptors are present also in the human gut, humans can theoretically be infected with avian influenza by ingesting under-cooked poultry (the virus is easily killed by heat at 60°C for 30 min). Nevertheless, such cases are rare and unconfirmed, probably because most victims have had contacts with live birds as well. The methods of food preparation a week before the onset of illnesses were not significantly different between the affected subjects and controls [17].

### **Natural viral reservoir**

Evidence of a correlation between the sizes of epidemics in birds and the number of humans affected is rising. For example, in Thailand and Vietnam where most of the human cases have occurred, there had been widespread outbreaks in birds [17]. Current scientific

models suggest that as more people become infected with avian influenza, the emergence of a new virus also becomes more likely. Limiting the infection amongst animals is therefore crucial in preventing a human pandemic.

The spread of H5N1 in birds, however, is rapid and particularly difficult to control. Bird excretion containing large amount of viruses contaminates dusts and soil. Mechanical vectors such as vehicles and bird feed then carry the virus from farm to farm. The recent appearance of avian influenza in Europe suggests that long-distance spread of the virus is also possible [8], as a result of international trade in live poultry. Migratory birds may serve as ideal H5N1 virus carriers, since they are naturally more resilient to developing illnesses from the infection, and can fly freely across continents spreading the disease to the local bird population [18].

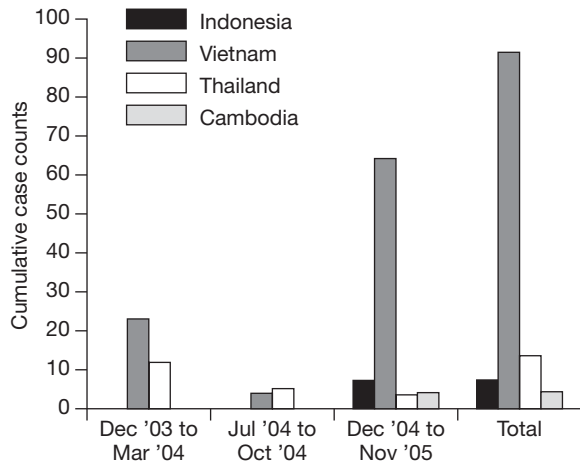
### **Vaccine failures and drug resistance**

Previous experiences with H7N7 influenza infection in humans suggest that 2 classes of drugs licensed to treat the common flu, the M2 inhibitors and neuraminidase inhibitors, may be effective against avian influenza as well [19,20]. Unfortunately, the H5N1 viruses obtained postmortem from the 2004 Vietnamese outbreak hardly responded to the M2 inhibitors amantadine and rimantadine [21]. Scientists attribute this to the widespread yet unpublicised practice of giving antiviral drugs prophylactically to poultry stocks, which helped the virus develop resistance.

Although a vaccine prototype had been created from a seed strain of H5N1 influenza isolated in 2003, further development was halted because the circulating viral strains have since mutated extensively [22]. The alternative of creating a vaccine de novo at the beginning of an epidemic is also impractical. Such a vaccine takes at least 4 months to produce, while millions of people would have already become ill with the infection. Rapid viral mutations would also be occurring in the mean time.

### **Disease Prevention and Containment**

Given that currently very few people are known to have been infected with H5N1 influenza A virus, and that the majority of them have had histories of contact with birds, there is hitherto no firm evidence of efficient spread between humans. Nevertheless, in September 2002, the Thai Ministry of Health announced a possible case of human-to-human transmission from a sick child



**Fig. 1.** Cumulative number of human cases of H5N1 influenza A infection reported to the World Health Organization [5].

to her mother [23]. The proximity of people to birds in Southeast Asia and the rising incidence of human infections (Fig. 1) perpetuate the risk of creating a human-transmissible virus, which could dramatically change the present state of public health.

The WHO has given warning about a possible influenza pandemic within the next few years (Table 2). In the face of this grave global challenge, the organisation developed a “Global Influenza Preparedness Plan” in 2001 (last updated in April 2005) [24].

### Prevention

Preventing reassortment of influenza viruses is the first priority in avoiding a human pandemic, and can be achieved by:

1. Eliminating the natural viral reservoir: reducing the pool of exposed poultry stops the virus circulating in the animal population. Systematic

and controlled elimination of livestock in Holland has successfully eradicated outbreaks in the past [25]. The culling operation must be performed safely with protective measures for workers and proper disposal of carcasses.

2. Reducing human exposure to the infected animal: avoiding rearing poultry in proximity to humans and pigs, and curbing live poultry trading. Rapid detection of outbreaks in birds helps warn the local community about their risk of infection. Continuous global surveillance tracks unusual outbreaks and informs the WHO of the need to take urgent action.
3. Vaccinating humans against the common influenza virus: although there is currently no effective vaccine against H5N1 in humans, immunising high-risk personnel (i.e., healthcare workers and those in close contact with birds) against human influenza strains helps prevent gene reassortment once these people do become co-infected with avian influenza.

### Containment

1. Developing contingency plans: as with all serious communicable diseases, emergency measures involving good communication and coordination between departments and nations would facilitate the detection, isolation, and treatment of affected people. Lessons from SARS have demonstrated that keeping outbreaks secret is no longer an option. On the contrary, the policy of openness during Netherlands’ poultry infections helped warn the surrounding countries via the Office International des Epizooties, and was also key to its domestic infection controls.
2. Stockpiling antivirals and chemoprophylaxis: epidemiologic experiences with influenza suggest

**Table 2.** Phases of the World Health Organisation (WHO) Global Influenza Preparedness Plan<sup>a</sup>

Interpandemic period	
Phase 1	No new influenza virus subtypes detected in humans. Low risks of human infection or disease
Phase 2	No new influenza virus subtypes detected in humans, but a circulating animal influenza virus subtype poses a substantial risk of human disease
Pandemic alert period	
Phase 3	Human infections with a new subtype, but no human-to-human spread, or at most rare instances of spread to a close contact
Phase 4	Small clusters with limited human-to-human transmission but spread is highly localised, suggesting the virus is not well adapted to humans
Phase 5	Larger clusters but human-to-human spread still localised, suggesting virus is becoming increasingly better adapted to humans but may not yet be fully transmissible
Phase 6	Increased and sustained transmission in general population

<sup>a</sup>Data extracted from World Health Organisation [24].

that the highly infectious H5N1 virus is unlikely to be controllable by SARS-like quarantine measures [26]. Although resistance may be rampant, the neuraminidase inhibitors may still be able to alleviate the severity and slow the spread of infection.

3. New vaccine development: although hindered by the slow speed of production, newer technologies including reverse genetics (e.g., plasmid-based cloning of viral segments) and the use of adjuvants may improve the efficacy of immunisation [27]. A subtype-specific vaccine as an alternative could reduce the severity of illnesses until a strain-matching vaccine emerges. The WHO is urgently working together with laboratories and manufacturers in the WHO Global Influenza Surveillance Network to develop better H5N1 vaccines.

### Clinical Presentation and Diagnosis

There is a lack of clinical information about avian influenza and how it may present differently from the “common flu”. Nevertheless, experiences from the 1997 case series have identified some key clinical features [28]. Nonspecific symptoms of high or persistent fever and a general deterioration in health were most frequently noted. Initial symptoms such as conjunctivitis, rhinitis and pharyngitis are common, and cannot be reliably differentiated from other acute lower and upper respiratory tract infections (Fig. 2). However, many cases showed a rapid progression towards acute respiratory distress syndrome (ARDS) and an extensive systemic involvement (e.g., lymphopenia, impaired clotting) not normally seen in common chest infections. Most patients required high-dose oxygen therapy and intensive care, and many developed

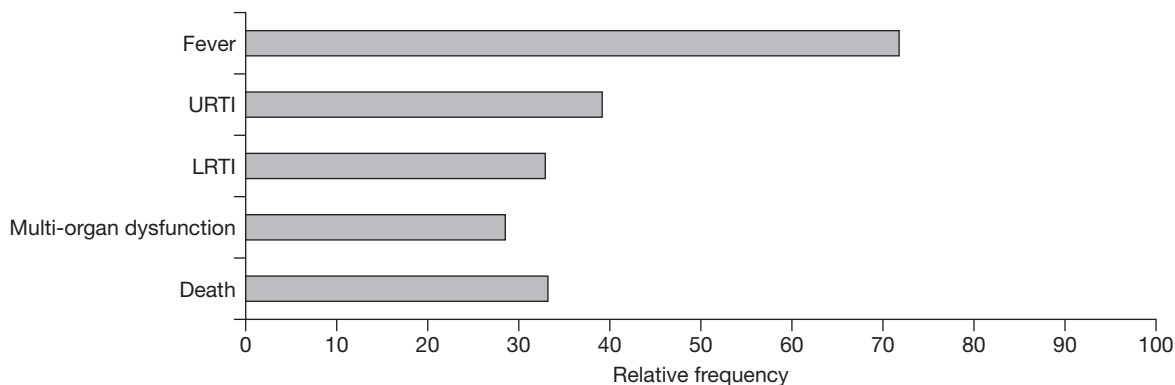
renal dysfunction days later. Secondary bacterial respiratory infection is rare, unlike the common viral infections.

The immediate causes of all 18 deaths were multi-organ dysfunction and ARDS, and were independent of patients’ comorbidities. The major radiological findings were extensive bilateral infiltrations, lobar collapses, focal consolidations and air bronchograms [29]. Interstitial infiltrations were observed less commonly. Eliciting risk factors such as recent travels to endemic areas, occupation and contact with poultry is important in determining the necessity for further laboratory investigations and starting empirical treatment.

A definite laboratory diagnosis requires a positive culture for H5N1 virus in Madin-Darby canine kidney cell line or chick embryo allantoic sac inoculated with the collected specimen [4]. For a more rapid diagnosis, immunoassays (e.g., H5-specific monoclonal antibody-based immunofluorescence) and reverse transcriptase-polymerase chain reaction (RT-PCR) that detect viral antigens and genes, respectively, have been used [4].

### Treatment

Resistance to amantadine has prompted the use of neuraminidase inhibitors (e.g., oseltamivir, zanamivir) as the recommended antiviral agents against avian influenza. However emerging cases have revealed new resistances to oseltamivir, which many European countries have stockpiled in preparation for a human pandemic [30]. Antiviral therapy should be given early and preferably within 48 h, before its efficacy declines. The human immune system being naïve to H5N1 virus would respond slowly to the infection, and therefore a prolonged antiviral treatment is probably necessary.



**Fig. 2.** Characteristics of 18 patients who acquired H5N1 during the 1997 epidemic in Hong Kong [27]. URTI = upper respiratory tract infection, LRTI = lower respiratory tract infection.

The mainstay and most effective approach to treating the disease involves good supportive management and intensive care during the critical stages of organ failures. The use of immunomodulation is yet to be widely supported, as they have resulted in more adverse outcomes than expected, although this may be because the only agents used have been steroids without adequate antiviral therapies [28].

## Conclusion

A lack of experience with avian influenza means no one knows if a human pandemic can be avoided. Although there is no confirmed effective transmission between humans, the existing risks pose an imminent threat to public health. International collaborations in antiviral and vaccine development are currently underway. Clear understanding about the disease and an instructive public health policy would help prevent its occurrence and contain its effect. However, an effective solution would not be feasible without giving support to countries that do not have the resources to implement the recommended measures.

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