

**XIAN WEN JIN, MD, PhD**Department of General Internal Medicine,
The Cleveland Clinic Foundation**SHERIF B. MOSSAD, MD**Department of Infectious Diseases,
The Cleveland Clinic Foundation

Avian influenza: An emerging pandemic threat

ABSTRACT

While we are facing the threat of an emerging pandemic from the current avian flu outbreak in Asia, we have learned important traits of the virus responsible for the 1918 Spanish influenza pandemic that made it so deadly. By using stockpiled antiviral drugs effectively and developing an effective vaccine, we can be in a better position than ever to mitigate the global impact of an avian influenza pandemic.

KEY POINTS

The viruses responsible for the influenza pandemics of 1957 and 1968 were the product of genetic reassortment in hosts infected with both an avian and a human influenza virus. In contrast, the 1918 virus, which was more deadly, was a mutated form of a purely avian virus.

As seen in all three pandemics, a change in influenza viral antigenicity poses a significant pandemic threat to populations with no immunity to the new strain.

As of November 14, 2005, 125 cases of avian H5N1 influenza in humans have been reported, and 64 people have died. Although some cases of human-to-human transmission between close contacts may have occurred, as yet there has been no evidence that the virus can be transmitted efficiently by aerosol.

The neuraminidase inhibitors oseltamivir and zanamivir currently are the only drugs that could be used in the event of a pandemic outbreak of H5N1 in humans. But current supplies are inadequate. A vaccine is being developed.

AVIAN INFLUENZA is raising grave concern about a potential pandemic, as we are currently seeing an unprecedented outbreak in birds of a highly pathogenic strain of influenza type A H5N1 and lethal human infections from bird-to-human and probably human-to-human transmission in Asia. It also has become a major public health care challenge in today's highly mobile world.

This article briefly reviews the lessons learned from past pandemics, the threat of a current avian flu outbreak, and recommendations for prevention.

LESSONS LEARNED FROM 20TH CENTURY PANDEMICS

There were three influenza pandemics in the 20th century, in 1918, 1957, and 1968. Each was caused by a novel type-A virus of avian origin: the 1918 (Spanish) influenza pandemic by an H1N1 avian virus, the 1957 (Asian) pandemic by an H2N2 virus, and the 1968 (Hong Kong) pandemic by an H3N2 virus.¹

The 1957 virus was the product of gene reassortment: somewhere, a host was co-infected with both a human and an avian influenza virus, and the two were able to swap genes to form a hybrid virus that could be transmitted from person to person (FIGURE 1).² Thus, the resulting virus contained some genes from the avian virus—hemagglutinin type 2, neuraminidase type 2 (hence the designation H2N2), and an RNA polymerase gene segment, polymerase basic 1 (PB1)—and the rest from the human virus.^{3,4} The 1957 pandemic killed about 70,000 people in the United States.

Similarly, the 1968 virus was also the

product of gene reassortment, containing avian hemagglutinin type 3 and PB1 segments in the background of human influenza viral genes. The 1968 pandemic killed approximately 34,000 people in the United States.

Why was the 1918 pandemic different?

The 1918 pandemic was different. The worst pandemic by far, it killed at least 40 million people worldwide and 675,000 people in the United States. Most strikingly, it killed 50% of healthy adults age 15 to 34 years who contracted it. The overall case mortality rate averaged 2.5% in the United States.⁵ As a result, the average life expectancy in the United States was lowered by more than 10 years.⁶

Why was this virus different? Its biological properties remained unclear until recently, owing to the lack of viral isolates available for study. However, recent work has found that it was not the result of gene reassortment of avian and human influenza viral genes, but rather the result of avian viral gene mutation.⁷

In October 2005, scientists from the US Centers for Disease Control and Prevention (CDC), the Armed Forces Institute of Pathology, the US Department of Agriculture, and Mount Sinai School of Medicine resurrected the 1918 virus by reverse genetics, using viral gene sequences recovered from preserved tissue from 1918 flu victims.⁸ This elegant study revealed important properties associated with this virus's extraordinary virulence.

Unlike the contemporary strain of human influenza virus, the 1918 virus could replicate even in the absence of proteolytic cleavage of its surface glycoprotein hemagglutinin by most proteases. Most strains of influenza replicate best in tissues in which proteases are abundant, such as the lungs. But in the 1918 virus, another viral surface glycoprotein, neuraminidase, appears to have facilitated hemagglutinin proteolytic cleavage. This suggests that the 1918 virus could grow in any cell type, not just in protease-rich lung cells.

In addition, the 1918 virus also could replicate rapidly in human bronchial epithelial cells. The hemagglutinin and polymerase genes are essential for maximal viral replication in human bronchial epithelial cells. These properties are clearly associated with

the exceptional virulence of the 1918 virus. Furthermore, the 1918 virus also kills chicken embryos, a pathogenic feature of avian H1N1 virus.

Molecular characterization demonstrated that the polymerase genes in the 1918 virus differed from avian polymerase consensus sequences at only a small number of amino acids. This evidence strongly suggests that the 1918 virus was an entirely avian-like virus that adapted to humans.⁹

Apparently, as seen in all three pandemics, a change in influenza viral antigenicity resulting from either viral gene reassortment between human and avian viruses or pure avian viral gene mutation poses a significant pandemic threat to populations with no immunity to the new strain.

■ THE CURRENT THREAT

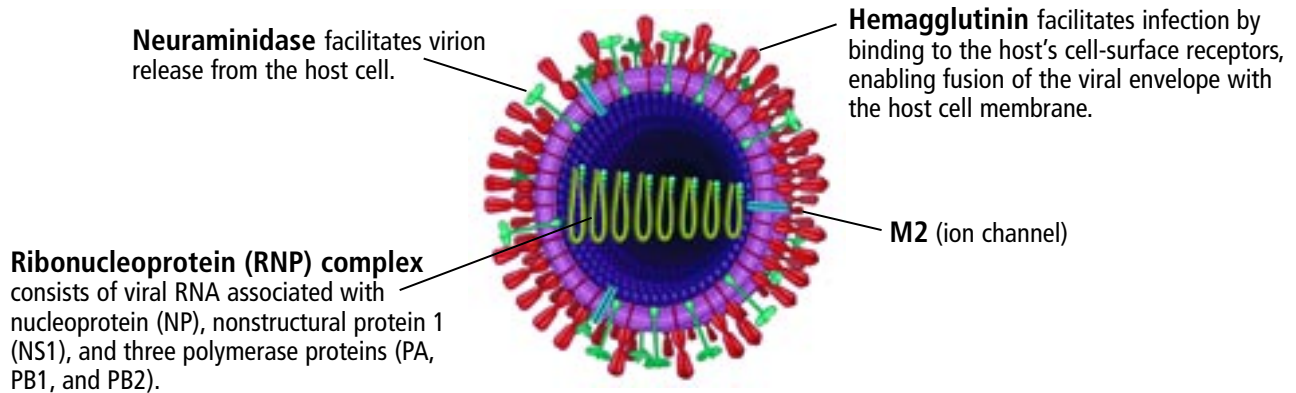
Since 2003, a highly pathogenic avian virus (influenza A H5N1) has broken out among poultry, sweeping across at least eight countries in Asia (Cambodia, China, Indonesia, Japan, Laos, South Korea, Thailand, and Vietnam)¹⁰ and killing more than 100 million birds. The geographic distribution of H5N1 virus infection continues to expand: outbreaks have been recently reported in Croatia, Kazakhstan, Mongolia, Romania, Russia, and Turkey.^{11,12}

Not only has this virus disseminated itself to an unprecedented degree among birds, but it also has crossed the species barrier to infect humans.¹³ Direct bird-to-human transmission was first reported in 1997. During the 1997 outbreak of H5N1 among poultry in Hong Kong, 6 of 18 people with confirmed infection died.¹⁴ And bird-to-human transmission has continued. As of November 14, 2005, there have been 125 human cases: 92 in Vietnam, 20 in Thailand, 4 in Cambodia, and 9 in Indonesia, resulting in 64 deaths.¹⁵ Most patients with H5N1 virus infection have had a history of direct contact with diseased poultry.

This direct transmission of the H5N1 virus from birds to humans is certainly worrisome. However, the worst fear is that sustained human-to-human transmission will occur as the virus continues to evolve. Indeed, probable human-to-human transmission

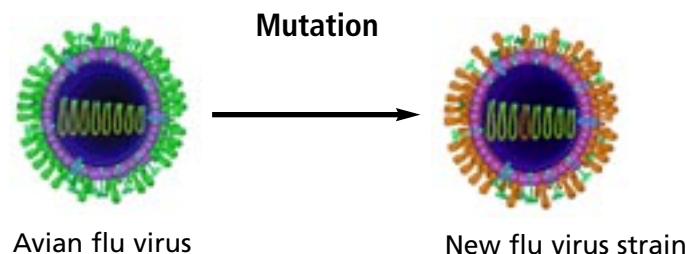
As of mid-November, 2005, the toll of avian flu is 125 human cases, 64 deaths

Anatomy of an influenza A virion

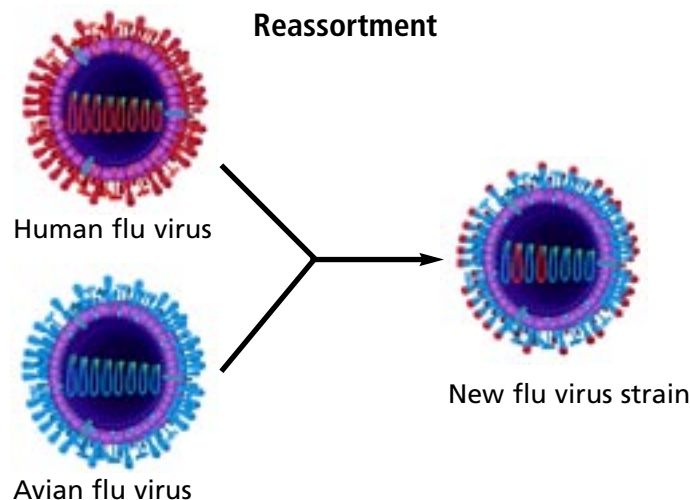


Each strain of influenza takes its name from the hemagglutinin (H) and neuraminidase (N) subtypes. There are 16 subtypes of hemagglutinin and 9 subtypes of neuraminidase for influenza A. Thus, strains are identified by those subtypes, such as H5N1.

How avian influenza could become a human pandemic strain



As the avian flu virus evolves, it may change its viral genes and antigenicity. The acquisition of new surface antigens may allow the new virus strain to circumvent the human immune response.



In reassortment, human flu virus and avian flu virus infect the same host simultaneously. The genes from the two strains swap, giving rise to a hybrid strain made from some of the genetic material from the two original strains. The new hybrid virus strain possesses altered surface antigens that the human immune system may not recognize.

CCF
Medical Illustrator: David R. Schumick ©2005

FIGURE 1

(although not sustained) has already been suggested in several household clusters in Vietnam and, recently, in close child-to-mother contact in Thailand.^{13,16} Of 10 patients reported in Vietnam, 8 died. Both the index patient (a child) and her mother in Thailand died. This mode of transmission with a high death rate is of great concern for a potential global pandemic.

How is the H5N1 virus different?

Molecular characterization of the H5N1 avian influenza virus isolated from patients in the 1997 outbreak revealed the following important virulence factors:

- Multiple basic amino acids at the proteolytic cleavage site of hemagglutinin, enhancing this molecule's cleavability and making the virus more pathogenic^{17,18}
- A specific amino acid substitution (Glu to Lys) at position 627 of the polymerase basic protein 2 (PB2), which enhances the efficacy of viral replication^{19,20}
- An amino acid substitution (Asp to Glu) at position 92 of nonstructural protein 1 (NS1), which enhances viral resistance to the antiviral effects of interferon and tumor necrosis factor alpha.^{21,22}

NS1 also increases transcription of cytokines, leading to an elaborate host inflammatory response and multiorgan failure.²³ Furthermore, H5N1 viral replication is prolonged in patients: viral loads in pharyngeal swabs 4 to 8 days after the onset of illness were at least 10 times higher than those in people infected with contemporary human influenza virus H1N1 or H3N2 strains.^{24,25}

Recently, there has been evidence of higher plasma levels of inflammatory mediators (interleukin-6, interleukin-8, interleukin-1, and monocyte chemoattractant protein-1) in patients who died from avian influenza H5N1 infection.^{24,25} Such an up-regulation of human inflammatory cytokines in H5N1 viral infection may have contributed to the sepsis syndrome, acute respiratory distress syndrome, and multiorgan failure seen clinically with the H5N1 avian influenza virus.

Potential for pandemic is real

Influenza pandemics happen when new viral strains emerge that can be readily transmitted

from human to human in a population with no immunity to the new strain of virus.

The H5N1 virus has already shown that it can replicate efficiently in human cells. Sustained human-to-human transmission has not been documented, although a probable transmission between a child and mother has been suggested. There is no evidence to date to suggest that H5N1 can be transmitted efficiently from human to human by small-particle aerosols.²⁶

Nevertheless, the threat of a flu pandemic is real. Either avian viral gene mutation or gene reassortment between avian and human influenza virus, as a result of the current continuing outbreak or viral evolution, clearly can lead to efficient human-to-human transmission by small-particle aerosols in an immunologically naive human population.

The danger of a potential avian influenza pandemic is further heightened because no vaccine against H5N1 is commercially available yet, and H5N1 has been documented to be resistant to several antiviral drugs (amantadine, rimantadine).²⁵ And, like the 1918 virus, H5N1 is associated with an unusually high death rate in humans. In fact, the human case death rate with the current H5N1 virus is 20 times higher than that of the 1918 virus (50% vs 2.5%).

RECOMMENDATIONS FOR PREVENTION

Facing the emerging avian influenza pandemic threat, the CDC and the World Health Organization have issued guidelines and recommendations for surveillance and prevention of avian influenza A H5N1 infection in humans^{27,28} (recently summarized by Beigel et al²⁵).

To reduce opportunities for human infection

To reduce opportunities for human infection, travelers should:

- Be immunized with human influenza vaccine at least 2 weeks before traveling to areas with avian influenza activity
- Avoid all direct contact with poultry and touching surfaces contaminated with poultry feces or secretions
- Avoid eating undercooked eggs or poultry
- Wash their hands carefully and frequently

Our worst fear is that H5N1 will be transmitted efficiently from human to human as it evolves



- Seek medical attention if they become ill with respiratory symptoms within 10 days of returning from an affected area.²⁵

To contain the spread of infection

To contain the spread of infection in hospitalized patients:

- Patients with avian influenza should be isolated in a negative-pressure room
- Health care workers should wear N-95 masks (non-oilproof respirators with at least 95% efficiency in filtering particles more than 3 μm in diameter), gloves, long-sleeved cuffed gowns, and eye protection when within 3 feet of patients
- Prophylaxis with oseltamivir is recommended for health care workers with fever (temperature $> 38^{\circ}\text{C}$ [100.4°F]) who had possible exposure to infectious aerosol secretions or were involved in an aerosol-generating procedure.²⁵

To prevent a pandemic

The most effective strategies for preventing a pandemic are probably to try to contain the outbreak with antiviral drugs and to develop a vaccine against H5N1.

Containment with antiviral drugs

The antiviral agents amantadine and rimantadine inhibit influenza virus uncoating inside the host cells by interfering with the M2 ion-channel protein.²⁹ Due to mutations in the M2 gene, the H5N1 virus is resistant to amantadine and rimantadine.¹² Therefore, these agents have no therapeutic role.

The neuraminidase inhibitors oseltamivir and zanamivir interfere with the release of the influenza virion from infected host cells and prevent the spread of infection, and avian H5N1 viruses remain sensitive to oseltamivir.³⁰ Thus, neuraminidase inhibitors are currently

the only drugs available for chemotherapy and prophylaxis against human H5N1 virus infection. Current supplies of neuraminidase inhibitors are inadequate for pandemic control, however.³¹

To contain an outbreak, we would need a stockpile of the drugs, controlled by government and health officials for rapid distribution to the site of the initial outbreak. Hoarding of the drugs should be strongly discouraged, as it could seriously undermine any coordinated pandemic control effort. Furthermore, misuse of the drugs can contribute to the emergence of resistant viral strains. A recent report of the isolation of oseltamivir-resistant H5N1 virus from a patient treated in Vietnam highlights the importance of monitoring drug resistance.³²

Work continues on a vaccine

Currently, there are no commercially available vaccines for human H5N1 infection. However, on August 6, 2005, government scientists at the National Institute of Allergy and Infectious Disease announced results from an initial clinical trial of an H5N1 avian influenza vaccine being developed. Preliminary data indicate that the experimental vaccine evoked an immune response in a small group of healthy adults.³³

Despite the promising data from the experimental vaccine clinical trial, serious concerns remain. Since the avian influenza strain responsible for the next pandemic may have different antigens resulting from mutation, the current experimental H5N1 vaccine may not be completely effective. Furthermore, production of enough effective vaccines for the entire US population in a short period of time by conventional methods poses a significant challenge. Efforts to stockpile oseltamivir and expedite the vaccine production are currently under way.

**Hoarding
of flu drugs
could
undermine
public health
efforts**

REFERENCES

1. Horimoto T, Kawaoka Y. Influenza: lessons from past pandemics, warnings from current incidents. *Nat Rev: Microbiol* 2005; 3:591–600.
2. Monto AS. The threat of an avian influenza pandemic. *N Engl J Med* 2005; 352:323–325.
3. Scholtissek C, Rohde W, Von Hoyningen V, Rott R. On the origin of the human influenza virus subtypes H2N2 and H3N2. *Virology* 1978; 87:13–20.
4. 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; 63:4603–4608.
5. Simonsen L, Clarke MJ, Schonberger LB, Arden NH, Cox NJ, Fukuda K. Pandemic versus epidemic influenza mortality: a pattern of changing age distribution. *J Infect Dis* 1978; 178:53–60.
6. Glezen WP. Emerging infections: pandemic influenza. *Epidemiol Rev* 1996; 18:64–76.
7. Reid AH, Taubenberger JK, Fanning TG. Evidence of an absence: the genetic origins of the 1918 pandemic influenza virus. *Nat Rev Microbiol* 2004; 2:909–914.
8. Tumpey TM, Basler CF, Aguilar PV, et al. Characterization of the reconstructed 1918 Spanish influenza pandemic virus. *Science* 2005; 310:77–80.



9. Taubenberger JK, Reid AH, Lourens RM, Wang R, Jin G, Fanning TG. Characterization of the 1918 influenza virus polymerase genes. *Nature* 2005; 437:889–893.
10. Centers for Disease Control and Prevention. Recent avian influenza outbreaks in Asia and Europe. www.cdc.gov/flu/avian/outbreaks/asia.htm#recent. Accessed November 7, 2005.
11. Liu J, Xiao H, Lei F, et al. Highly pathogenic H5N1 influenza virus infection in migratory birds. *Science* 2005; 309:1206.
12. World Organization for Animal Health. www.oie.int/download/AVIAN%20INFLUENZA/A_AI-Asia.htm. Accessed November 14, 2005.
13. Tran TH, Nguyen TL, Nguyen TD, et al. Avian influenza A (H5N1) in 10 patients in Vietnam. *N Engl J Med* 2004; 350:1179–1188.
14. 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; 351:467–471.
15. World Health Organization. Cumulative number of confirmed human cases of avian influenza A (H5N1) reported to WHO, November 9, 2005. (Accessed November 14, 2005, at http://www.who.int/csr/disease/avian_influenza/country/cases_table_2005_11_09/en/index.html.)
16. Ungchusak K, Auewarakul P, Dowell SF, et al. Probable person-to-person transmission of avian influenza A (H5N1). *N Engl J Med* 2005; 352:333–340.
17. 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; 279:393–396.
18. Claas EC, Osterhaus AD, van Beek R, et al. Human influenza A H5N1 virus related to a highly pathogenic avian influenza virus. *Lancet* 1998; 351:472–477.
19. Hatta M, Gao P, Halfmann P, Kawaoka Y. Molecular basis for high virulence of Hong Kong H5N1 influenza A viruses. *Science* 2001; 293:1840–1842.
20. Shinya K, Hamm S, Hatta M, Ito H, Ito T, Kawaoka Y. PB2 amino acid at position 627 affects replicative efficiency, but not cell tropism, of Hong Kong H5N1 influenza A viruses in mice. *Virology* 2004; 320:258–266.
21. Seo SH, Hoffmann E, Webster RG. Lethal H5N1 influenza viruses escape host anti-viral cytokine responses. *Nat Med* 2002; 8:950–954.
22. Seo SH, Hoffmann E, Webster RG. The NS1 gene of H5N1 influenza viruses circumvents the host anti-viral cytokine responses. *Virus Res* 2004; 103:107–113.
23. 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; 360:1831–1837.
24. Peiris JS, Yu WC, Leung CW, et al. Re-emergence of fatal human influenza A subtype H5N1 disease. *Lancet* 2004; 363:617–619.
25. Beigel JH, Farrar J, Han AM, et al. Avian influenza A (H5N1) infection in humans. *N Engl J Med* 2005; 353:1374–1385.
26. Centers for Disease Control and Prevention. Outbreak notice update: human infection with avian influenza a (H5N1) virus in Asia. www.cdc.gov/travel/other/avian_influenza_se_asia2005.htm. Accessed November 7, 2005.
27. Centers for Disease Control and Prevention. Outbreak notice update: guidelines and recommendations. Interim guidance about avian influenza (H5N1) for U.S. Citizens Living Abroad. October 26, 2005. www.cdc.gov/travel/other/avian_flu_ig_americans_abroad_032405.htm. Accessed November 14, 2005.
28. World Health Organization. WHO recommendations relating to travelers coming from and going to countries experiencing outbreaks of highly pathogenic H5N1 avian influenza. www.who.int/csr/disease/avian_influenza/travel/2005_11_3/en/. Accessed November 14, 2005.
29. Wang C, Takeuchi K, Pinto LH, Lamb RA. Ion channel activity of influenza A virus M2 protein: characterization of the amantadine block. *J Virol* 1993; 67:5585–5594.
30. Moscona A. Neuraminidase inhibitors for influenza. *N Engl J Med* 2005; 353:1363–1373.
31. Hayden FG. Pandemic influenza: is an antiviral response realistic? *Pediatr Infect Dis J* 2004; 23(suppl):S262–S269.
32. Mai Le Q, Kiso M, Someya K, et al. Avian flu. Isolation of drug-resistant H5N1 virus (brief communication). *Nature* 2005; 437:1108.
33. World Health Organization. Scientists at the National Institute of Allergy and Infectious Disease announce results of clinical trials of H5N1 avian influenza vaccine. www.who.int/csr/disease/avian_influenza/statement_2005_08_12/en/index.html. Accessed October 24, 2005.

ADDRESS: Xian Wen Jin, MD, PhD, Department of General Internal Medicine, S70, The Cleveland Clinic Foundation, 9500 Euclid Avenue, Cleveland, OH 44195; e-mail jinx@ccf.org.