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CHEST Global Medicine

Avian Influenza Virus Infections in Humans*

Samson S. Y. Wong, MRCPath; and Kwok-yung Yuen, MD

Seroepidemiologic and virologic studies since 1889 suggested that human influenza pandemics were caused by H1, H2, and H3 subtypes of influenza A viruses. If not for the 1997 avian A/H5N1 outbreak in Hong Kong of China, subtype H2 is the likely candidate for the next pandemic. However, unlike previous poultry outbreaks of highly pathogenic avian influenza due to H5 that were controlled by depopulation with or without vaccination, the presently circulating A/H5N1 genotype Z virus has since been spreading from Southern China to other parts of the world. Migratory birds and, less likely, bird trafficking are believed to be globalizing the avian influenza A/H5N1 epidemic in poultry. More than 200 human cases of avian influenza virus infection due to A/H5, A/H7, and A/H9 subtypes mainly as a result of poultry-to-human transmission have been reported with a > 50% case fatality rate for A/H5N1 infections. A mutant or reassortant virus capable of efficient human-to-human transmission could trigger another influenza pandemic. The recent isolation of this virus in extrapulmonary sites of human diseases suggests that the high fatality of this infection may be more than just the result of a cytokine storm triggered by the pulmonary disease. The emergence of resistance to adamantanes (amantadine and rimantadine) and recently oseltamivir while H5N1 vaccines are still at the developmental stage of phase I clinical trial are causes for grave concern. Moreover, the to-be pandemic strain may have little cross immunogenicity to the presently tested vaccine strain. The relative importance and usefulness of airborne, droplet, or contact precautions in infection control are still uncertain. Laboratory-acquired avian influenza H7N7 has been reported, and the laboratory strains of human influenza H2N2 could also be the cause of another pandemic. The control of this impending disaster requires more research in addition to national and international preparedness at various levels. The epidemiology, virology, clinical features, laboratory diagnosis, management, and hospital infection control measures are reviewed from a clinical perspective.
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Key words: adamantane; avian influenza; H5N1; H7N7; influenza A virus; neuraminidase inhibitors

Abbreviations: IC_{50} = 50% inhibitory concentration; WHO = World Health Organization

Influenza Viruses

The high mutation rate, the ability of gene segments to reassort, and the huge pool of influenza viruses in birds and mammals explain their changing behavior and the difficulty in developing a permanent, long-lasting, and effective vaccine. The influenza viruses A, B, and C are the three most important genera of Orthomyxoviridae, a group of single-stranded minus-sense RNA viruses with a segmented genome. The eight RNA segments of the influenza A virus genome encode 11 viral proteins. These include the polymerase proteins (PB1, PB2, PA, PB1-F2), nucleocapsid protein, hemagglutinin, neuraminidase, matrix proteins (M1, M2), and nonstructural

*From the Department of Microbiology, Research Centre of Infection and Immunology, State Key Laboratory of Emerging Infectious Disease, Faculty of Medicine, The University of Hong Kong, Hong Kong.
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Correspondence to: Kwok-yung Yuen, MD, Department of Microbiology, The University of Hong Kong, University Pathology Building, Queen Mary Hospital, 102 Pokfulam Rd, Hong Kong; e-mail: hkumicro@hkucc.hku.hk

proteins (NS1, NS2). Hemagglutinin and neuraminidase are the major antigenic determinants of influenza A viruses and serve as the basis for their subtype classification. There are 16 hemagglutinin (H1 to H16) and 9 neuraminidase types (N1 to N9). Hemagglutinin mediates attachment to and entry of the virus into host cells by binding to sialic acid receptors at the cell surface. Hemagglutinin is also the main viral target of protective humoral immunity by neutralizing antibody. The binding affinity of hemagglutinin to the sialic acid residues partly accounts for the host specificity of the various influenza A virus subtypes. Human viruses preferentially bind to sialic acid linked to galactose by α-2,6 linkages that are the main type found on the epithelial cells of the human respiratory tract, while avian viruses tend to bind to α-2,3 linkages that are found on duck intestinal epithelium.[1,2] The specificity for different receptors has long been one of the explanations for the species barrier between avian and human influenza viruses. The presence of both α-2,3 and α-2,6 linkages in the pig tracheal epithelium is the reason why pigs may serve as the "mixing vessel" for the genesis of new viral types through co-infection.[2] Chickens may have a similar role, in that their lung and intestinal epithelia contain both types of linkages.[3] In the human respiratory epithelium, it has been shown that α-2,3 and α-2,6 linkages are found on ciliated and nonciliated cells, respectively, thereby allowing human infection by avian influenza viruses.[4,5] The change of one amino acid of the H5 protein is sufficient to change the receptor binding specificity of A/H5N1 viruses.[6] Thus, the barrier to interspecies infection can be overcome easily.

Neuraminidase facilitates the spread of the virions in the host by cleaving the glycosidic linkages to sialic acid on host cells and the surface of the viral particles and is the target of neuraminidase inhibitors. The report of a mutation at position 274 of neuraminidase from histidine to tyrosine in an A/H5N1 isolate from a Vietnamese girl was associated with clinical and laboratory evidence of resistance to oseltamivir but not zanamivir.[36]

M2 is an ion channel crucial for the pH-dependent dissociation of matrix proteins from the nucleocapsid during viral uncoating and pH changes across the trans-Golgi network during maturation of hemagglutinin molecules. M2 is the target of the adamantanes (amantadine and rimantadine). Mutation in the M2 from serine to asparagine at residue 31 invariably confers resistance to adamantanes, and this is seen in the latest A/H5N1 isolates circulating in Southeast Asia since late 2003.[7,8] PB1-F2 causes cellular apoptosis by acting on the host mitochondria.[9] The hemagglutinin and PB2 proteins appear to be important in determining host specificity and virulence.[10]

The other notable feature of influenza A viruses is their propensity to undergo antigenic variations through antigenic drift and antigenic shift. Antigenic drift represents relatively minor alterations in the antigenicity of hemagglutinin or neuraminidase by mutation. This occurs continuously as a result of selection pressure from the host immunity and explains the need for yearly changes in the human influenza vaccine composition. For example, the H3N2 component of the vaccine for the Northern Hemisphere 2005–2006 season is changed to A/California/7/2004(H3N2), as compared to A/Fujian/411/2002(H3N2) used in the 2004–2005 season. Antigenic shift by genetic reassortment of the eight gene segments can result in the appearance of a novel hemagglutinin/neuraminidase combination for which the human population has little or no immunity. The appearance of such novel antigenic types may lead to pandemics of influenza if such strains can be transmitted efficiently from human to human. Major pandemics have occurred in the last century, in 1918–1919 (H1N1), 1957 (H2N2), and 1968 (H3N2). However, it is also possible that a pandemic virus is generated by mere mutation of an avian influenza virus such as the A/H5N1, which adapts to the human host without genetic reassortment.

Changing Epidemiology, Changing Pathogenicity

Waterfowl are the natural reservoir hosts of all influenza A virus subtypes. The viruses once existed in an evolutionary equilibrium with these avian hosts, in that the birds remain asymptomatic despite infection and shedding of large numbers of virions. Human avian influenza infections largely occur as a result of direct transmission of viruses from infected birds to humans. Sporadic cases of human A/H7N7 infections have occurred as a result of direct animal-to-human transmission or laboratory accidents; most of these infections resulted in conjunctivitis (Table 1).[11–13,15–20] Between 1999 and 2003, 3.8% (7 of 185 poultry workers) in Italy had serologic evidence of infection by H7 avian influenza viruses during an H7N3 epizootic; only 1 worker had conjunctivitis, while the rest were asymptomatic.[14]

The first ominous sign that avian influenza viruses could directly infect humans from avian species in a large scale occurred in 1997 in Hong Kong, resulting in 18 documented cases and six fatalities.[15,21] The outbreak was controlled after depopulating 1.5 million chickens in Hong Kong farms and markets. A/H5N1 viruses reappeared again in 2001 and 2002 in poultry without human infections.[22] Human infections due to A/H5N1 resurfaced in Hong Kong in

Table 1—*Clinical Features of Reported Human Avian Influenza Virus Infections and Outbreaks

Features	United States, 1980[12]	United Kingdom, 1995[13]	Hong Kong, 1997[15,16]	Hong Kong, 1999[16]	Hong Kong, 2003[17]	The Netherlands, 2003[18]	Southeast Asia, 2,003 to present[19]†	Canada, 2004[20]
Animal source of human infection	Seal	Avian	Avian	Avian	Avian	Avian	Avian	Avian
Etiologic agent	A/H7N7	A/H7N7	A/H5N1	A/H9N2	A/H5N1	A/H7N7	H5N1	A/H7N3
Confirmed human cases, No.	3	1	18	2	2 (imported)	89	133	2
Fatalities, No. (case-fatality rate, %)	0 (0)	0 (0)	6 (33.3)	0 (0)	1 (50)	1 ( )	68 (51.1)	0 (0)
Presenting clinical syndromes	Conjunctivitis	Conjunctivitis	ILI, pneumonia	ILI	ILI, pneumonia	Conjunctivitis, ILI, pneumonia	ILI, pneumonia, diarrhea, encephalitis	Conjunctivitis, ILI

*ILI = influenza-like illness.
†Up to October 20, 2005.

February 2003 when a family of five returned from the Fujian Province of China. A 7-year-old girl died from pneumonia of unknown etiology in China. Her father had virologically confirmed A/H5N1 pneumonia and ARDS after returning to Hong Kong.[17]

In December 2003, an outbreak of A/H5N1 infection occurred among poultry in South Korea. Shortly afterwards, Vietnam, Japan, Thailand, Laos, Vietnam, Cambodia, China, Indonesia, and Malaysia successively experienced the largest-ever A/H5N1 poultry epidemic in history. Direct transmission to humans occurred in three waves involving 133 people, with 68 deaths in Vietnam, Thailand, Cambodia, Indonesia, and China.[19] At the same time, poultry outbreaks of less virulent avian influenza viruses also occurred in Taiwan (A/H5N2) and Pakistan (A/H7 and A/H9).

The recent epidemic of A/H5N1 virus infection is notable in two ways. Firstly, the geographic extent of the epidemic is unprecedented. Traditionally, southern China is considered to be the epicenter for both human and highly pathogenic avian influenza viruses. Circulation of highly pathogenic avian influenza viruses among avian species, domestic mammals, and farm workers have been documented in this region.[23] Since 2004, the A/H5N1 virus has been stretching its borders northwards and westwards, and avian infection has now been described in western China, Mongolia, Russia, Kazakhstan, Inner Mongolia, Turkey, and Romania. This puts an increasingly larger human population at risk of infection. Secondly, genetic changes of the contemporary viruses and a changing ecology with different animals are evident. Since 2002, waterfowl in Hong Kong started to succumb to A/H5N1 infections. This is also seen in the current epidemic in Eurasia. Studies[24,25] of A/H5N1 viruses collected from 2003–2004 in Southeast Asia showed that these viruses infect not only the intestinal tract but also the respiratory tract of ducks, with a higher viral load at the trachea than the cloaca. Viruses may be shed for up to 17 days after infection of the ducks (in contrast to 2 to 5 days in older studies). More importantly, they demonstrated increased virulence to mammals as shown in animal studies[10,26–29] and natural infection of caged felines in endemic areas. This increase in virulence has been associated with specific amino acid changes in the sequence of the PB2 protein (such as aspartic acid to asparagine at position 701 and glutamic acid to lysine at position 627).[10,29–31] Disseminated extrapulmonary infection, including the brain, was demonstrated in mice and ferrets.[29] Since 1997, the Z genotype of A/H5N1 viruses has emerged as the predominant virus in southern China and many Z genotype strains also carry M2 gene mutations, thereby conferring resistance to the adamantanes.[7]

Other avian influenza viruses have also been associated with symptomatic human infections in recent years (Table 1). Human infections due to the A/H9N2 virus manifesting as influenza-like illness was documented in two patients in Hong Kong in 1999.[16] H7N3 infection occurred in two poultry workers in Canada in 2004.[20] The largest outbreak of non-H5N1 avian influenza infection in humans occurred in 2003 in the Netherlands, with 89 virologically confirmed cases and 1 fatal case.[18]

Humans acquire avian influenza viruses primarily through direct contact of the mucous membranes with infectious secretions and excreta from infected birds or contaminated poultry products. Personnel involved in culling operations have occasionally been infected.[31,32] The main portal of entry appears to be the upper respiratory tract and conjunctivae; the latter appears to be an important route for A/H7N7 and A/H7N3 infections. Direct introduction to the lower respiratory tract might occur following massive exposure (such as during a culling exercise), although the importance of this is uncertain. The role of infection through the GI tract remains to be established.

To date, human-to-human transmission of avian influenza viruses has occurred sporadically with very low efficiency. In the 1997 Hong Kong outbreak, one household contact of an A/H5N1-infected patient without any history of exposure to poultry and 3.7% of the health-care workers who had looked after the patients were subsequently found to be seropositive.[33,34] In the recent Southeast Asian A/H5N1 outbreak, the possibility of human-to-human transmission has been suggested, although two studies[35–38] of health-care workers failed to demonstrate such transmission. As for A/H7N7 infection, there is evidence that person-to-person transmission occurred in the 2003 outbreak in the Netherlands.[18] Documented A/H7N7 infections developed in three household contacts of the cases (two cases presented as conjunctivitis, and one case presented as influenza-like illness). In summary, the changing epidemiology and pathogenicity of avian influenza viruses has set the stage for a potential and particularly severe global epidemic.

Clinical Manifestations and Management

Clinical Features and Pathology

The main clinical manifestations of avian influenza infections depend on the viral subtype causing the disease. A/H7N7 infections mainly result in conjunctivitis and/or an influenza-like illness (Table 1).[11–13,31] In the 2003 outbreak in the Netherlands, 82 of the 89 cases (92.1%) manifested as conjunctivitis, and the remaining patients presented with influenza-like illness.[18] One veterinarian had an influenza-like illness 2 days after visiting a farm, which progressed to pneumonia 7 days later. The pneumonia persisted despite treatment, and the patient died of ARDS 15 days after exposure.

In the 1997 Hong Kong A/H5N1 outbreak, an influenza-like illness typically appeared early in the course of the disease, and conjunctivitis was seen in some patients.[15,21] The ages of patients ranged from 1 to 60 years (mean, 17.2 years; median, 9.5 years); 11 of the 18 patients were aged $\leq$ 14 years. Some patients had prominent GI symptoms with abdominal pain, diarrhea, and vomiting. Seven of the 18 patients recovered following the influenza-like illness; 11 patients progressed to pneumonia, and 6 of them died from ARDS or multiorgan failure. Reye syndrome and pulmonary hemorrhage were other complications. Advanced age, a longer symptomatic period before admission, pneumonia, leukopenia, and lymphopenia were risk factors associated with severe disease. Amantadine was administered to eight patients, although clinical benefits from its use could not be confirmed.

In the 2004 series of 10 patients from Vietnam with A/H5N1 infection, the disease again predominantly affected the younger population (mean age, 13.7 years; range, 5 to 24 years).[39] Among the 12 confirmed cases in Thailand, the mean age of the patients was 12 years (range, 2 to 58 years).[40] A history of contact with poultry could be found in 58% to 90% of the cases. Onset of disease occurred at a median of 3 to 4 days after exposure (range, 2 to 8 days).[41] The main presenting syndrome was community-acquired pneumonia, and fever was universally present. The time from disease onset to hospitalization ranged from 1 to 8 days (median, 3 to 8 days).[41] An important symptom was diarrhea, which was present in 42 to 70% of the patients. Severe diarrhea was also the presenting symptom of a 4-year-old boy in Vietnam whose sister died 2 weeks earlier as a result of A/H5N1 infection; both died of disseminated infection and encephalitis.[42]

Lymphopenia and thrombocytopenia were common findings in all series; these were prognostic indicators for ARDS and death.[39,40] All patients had abnormal chest radiographic findings with features of interstitial infiltration, lobar infiltration, collapse/consolidation, and air bronchograms. Pneumothorax occurred in patients who received mechanical ventilation. The median time to the appearance of ARDS was 6 days after disease onset in the Thai series (range, 4 to 13 days).[40] The case fatality rate of the two series in Vietnam and Thailand ranged from 67

to 80%. The time from onset to death ranged from 4 to 30 days (median, 8 to 23 days).[41] The incidence of asymptomatic or mild infections vs pneumonia is unknown.

Postmortem findings in two patients in Hong Kong who died from A/H5N1 infection showed multiorgan damage, disseminated intravascular coagulation, lymphoid tissue necrosis and atrophy, and the expected pulmonary pathology of diffuse alveolar damage.[43] Hemophagocytic syndrome was a prominent feature. In another boy who succumbed to A/H5N1 infection in Thailand, viral RNA was detected by reverse transcription-polymerase chain reaction in the lungs, intestine, and spleen, but active viral replication was limited to the lungs and intestine.[44] Intestinal involvement by A/H5N1 viruses may explain the common occurrence of diarrhea. The reason for severe disease and high mortality in A/H5N1 infections that surpasses the previous pandemics of human influenza is not known. The presence of a new viral subtype to which the human host has no prior immunity cannot totally explain this phenomenon. The ability of the virus to cause disseminated infection—including viremia and encephalitis—could be an important factor. Pronounced activation of the proinflammatory cytokine cascade perpetuates the inflammatory response and could contribute to further tissue damage and persistence of the systemic inflammatory response syndrome.[45]

Clinical Approach and Laboratory Diagnosis

There are no pathognomonic signs and symptoms of A/H5N1 infections. The clinical, laboratory, and radiologic findings are not distinguishable from other causes of influenza-like illness, severe community acquired pneumonia, or ARDS. The only feature that raises the suspicion of avian influenza infection is the epidemiologic linkage to endemic areas and the history of contact with poultry. The frontline clinicians should therefore always try to elicit a detailed history of travel and exposure to animals in suspected patients. Those with a positive travel or contact history should receive appropriate radiologic and microbiological investigations, together with proper infection control precautions. Patients with mild influenza-like illness may be isolated and closely observed while waiting for results of laboratory investigations. A chest radiograph should be performed to exclude pulmonary involvement. The decision for hospitalization is based on a clinical assessment of disease severity, whether the patient can be readily followed up, and the likelihood of having avian influenza infection. Those with severe pneumonia and risk factors for avian influenza should initially be empirically treated with oseltamivir in addition to broad-spectrum antibiotics (*eg*, a β-lactam plus a macrolide).

The definitive diagnosis for avian influenza is a positive viral culture result from clinical specimens or demonstration of a fourfold rise in serum neutralizing antibody titer toward the presently circulating genotype of avian viruses in a biosafety level 3 laboratory. The convalescent serum should be taken at least 14 days after the onset of illness. The antibody neutralization test should preferably be confirmed by a baculovirus-expressed recombinant H5 Western blot test.[32] Neither of these tests are readily available, nor do they provide rapid results. Nevertheless, they should still be ordered for suspected cases because they may provide crucial information including the availability of viral strains for antiviral susceptibility testing and epidemiologic studies.

Rapid diagnosis by antigen detection or reverse transcription-polymerase chain reaction for influenza viruses can be performed on the throat swab or nasopharyngeal aspirate put in viral transport medium.[41] Aerosol-generating procedures for specimen collection should be performed with proper infection control precautions. The first specimen should be collected before commencement of antiviral therapy whenever possible. Viral antigens in the specimens may be detected by indirect immunofluorescence, enzyme immunoassays, or rapid immunochromatographic assays. Unfortunately, the commercially available test kits that detect nucleoprotein of influenza A viruses do not distinguish human from avian influenza viruses or their subtypes (A/H5, A/H7, and A/H9). The sensitivity of these kits for detecting A/H5N1 infection ranged from 33.3 to 85.7% based on the small number of patients being tested.[39,40] Specimens that test positive for influenza antigens must be confirmed by definitive virologic studies to confirm the subtype of the virus.

Reverse transcription-polymerase chain reaction appears to be the most promising test for rapid detection of avian influenza viruses. Various protocols have been described for reverse transcription-polymerase chain reaction to detect all influenza A viruses or specific H5 and N1 genes.[46–48] This test can be used on RNA extracted from plasma, cerebrospinal fluid, tissues, and feces in addition to the respiratory secretions. Again, there are no definitive recommendations on the ideal reverse transcription-polymerase chain reaction primer combinations or choice of laboratory investigations, and the results must be confirmed by viral culture.

Antiviral and Adjunctive Therapies

The adamantanes (amantadine and rimantadine) and neuraminidase inhibitors (oseltamivir and zanamivir) are most commonly used for influenza treatment and chemoprophylaxis. Adamantanes are active against influenza A viruses, while neuraminidase inhibitors are active against both influenza A and B viruses. Adamantanes are currently not considered to be the drug of choice for avian influenza virus infections because of two reasons. Firstly, adamantane resistance emerges rapidly following therapeutic use in human influenza, and the resistant viruses are fully transmissible and pathogenic. Up to 30% of patients with human influenza A being treated with amantadine may shed resistant viruses, sometimes as early as day 2 to 3 after treatment.[49] Such adamantane-resistant viruses can readily be transmitted to contacts. Secondly, recent isolates of A/H5N1 from the Indochina clade in Cambodia-Thailand-Vietnam often carry mutations in the M2 gene, thereby rendering this group of antiviral agents ineffective in the treatment and prevention of this emerging epidemic.[7] But it is important to note that the China-Indonesia clade are still quite susceptible to amantadine. Thus, adamantanes should still be considered for prophylaxis in the community contacts if the pandemic strain is still susceptible. The long shelf life of amantadine of >25 years and its low cost make it an attractive choice for stockpiling.

Controlled clinical trials on the efficacy of neuraminidase inhibitors for treatment and prophylaxis of human avian influenza infections have not been performed. Given the severity of the disease, such clinical trials will unlikely be performed in the near future. The use of neuraminidase inhibitors in this clinical setting is therefore based on *in vitro* data and animal experiments.

Both neuraminidase inhibitors have been found to be effective in animal models in preventing death and improving survival following infection by A/H5N1 viruses.[50–52] As in human influenza, the time of commencement of antiviral therapy is directly related to the survival of animals.[50,51] Highest levels of protection were seen when neuraminidase inhibitors were administered within 48 h of infection. Protective efficacy diminished substantially when neuraminidase inhibitors were administered >60 h after infection. However, the length of this window of opportunity for human avian influenza infection is currently unknown. In the Thai series in 2004, patients who had survived after oseltamivir treatment appeared to have received the agent earlier than those who subsequently died (4.5 days vs 9 days after disease onset).[40] The benefits of early antiviral intervention may be nullified by the fact that patients with influenza-like illness early in the course of disease often do not seek medical attention, and patients in remote or rural areas of developing countries often have delayed access to medical services.

Until recently, there was little evidence of naturally occurring resistance to neuraminidase inhibitors.[53,54] Both A/N1 and A/N2 isolates are highly susceptible to neuraminidase inhibitors with a mean 50% inhibitory concentration (IC_{50}) usually <5 nmol/L.[53–56] The susceptibility of avian influenza viruses to neuraminidase inhibitors is variable and appears to be related to the specific neuraminidase type (Table 2). A/H5N1 viruses isolated in 1997 and in the recent outbreaks are readily inhibited by clinically achievable levels of neuraminidase inhibitors. Nevertheless, an oseltamivir-resistant A/H5N1 isolate was recently described in Vietnam.[36] In this report,[36] an oseltamivir-resistant A/H5N1 virus was isolated from a symptomatic girl who had received 4 days of postexposure oseltamivir prophylaxis (75 mg qd). The virus had a histidine-to-tyrosine substitution at position 274 of the neuraminidase protein, and the IC_{50} for oseltamivir was found to be 90 nmol/L, still within clinically achievable peak plasma level of oseltamivir. The girl subsequently received a therapeutic dose of oseltamivir (75 mg bid) for 7 days, and no viruses were then isolated. This level of resistance apparently did not lead to treatment failure in this case, although it is impossible to draw definite conclusions based on a single case report. One of the viral clones of the girl's isolate, however, has an IC_{50} for oseltamivir of >763 nmol/L. Extensive use of oseltamivir for the treatment of human influenza in children has been shown to be associated with a substantial risk of resistance developing to the agent.[57] Therefore, it will hardly be surprising to see more oseltamivir-resistant A/H5N1 viruses when the number of human cases increases and the use of this drug for prophylactic or therapeutic purposes becomes more common. Other strategies for antiviral therapy and prophylaxis are urgently required.

Combination therapy of oseltamivir with rimantadine was found to be synergistic in preventing mortality from A/H9N2 infections in animal studies, although *in vitro* or animal studies on other avian influenza viruses have not been performed.[50,51] Furthermore, the higher virulence of recent isolates of A/H5N1 viruses appears to reduce the efficacy of neuraminidase inhibitors in animal models.[74] A higher daily dose (10 mg/kg/d vs 1 mg/kg/d in mice) and a longer duration of treatment (8 days vs 5 days) of oseltamivir significantly improves survival of animals. The findings of this study is definitely worth exploring in humans in order to determine the

Table 2—*Key Characteristics of Currently Available Anti-influenza Agents

Characteristics	Amantadine[58]	Rimantadine[58]	Oseltamivir†	Zanamivir†	Ribavirin[58,59]
Molecular weight	187.7	215.8	312.4 (base)	332.3	244.2
Usual adult dosage and route of administration	Therapeutic and prophylactic: 100 mg po bid; 100 mg po qd for elderly (> 65 yr)	Therapeutic: 100 mg po bid; 100 mg po qd for elderly (> 65 yr); prophylactic: 50 to 200 mg/d po	Therapeutic: 75 mg po bid; prophylactic: 75 mg po qd	Therapeutic: 10 mg po inhalation (dry powder) bid; prophylaxis: 10 mg po inhalation (dry powder) qd (not FDA-approved at the time of writing)	6 g/d by aerosolization at 18 h/d; 600 to 2,400 mg/d po in three to four divided doses; 1.5 mg/kg/h continuous infusion for 2 to 6 d
Half-life, h	12–18	24–36	6–10 (carboxylate, which is the active form of the drug)	4.14–5.05 (dry powder inhalation); 2 (IV)	24–36 (oral)
Oral bioavailability, %	86–94	> 90	90	1–5, median, 2 (oral); 10–20 systemic absorption after po inhalation	45
Protein binding, %	59–67	40	3 (carboxylate), 42 (phosphate)	< 10	Not bound
Peak plasma level (dosage used)	300–723 μg/L (100 mg po bid); 633–1,405 μg/L (300 mg/d po)[60]	140–442 μg/L (100 mg po bid); 301–913 μg/L (300 mg/d po)[60]	147–230 μg/L (50 mg po bid); 308–575 μg/L (100 mg po bid); 579–897 μg/L (150 mg po bid); 688–1,293 μg/L (200 mg po bid); 1,363–2,458 μg/L (500 mg po bid)[62]	39–54 μg/L (10 mg dry powder inhalation); 340–352.8 μg/L (600 mg IV q12h)[61]; 17–142 μg/L (10 mg dry powder inhalation)[63]	1.3 μg/mL (600 mg po); 12.5 μg/mL (1,200 mg po); 3.2 μg/mL (2,400 mg po); 17 μg/mL (500 mg IV); 24 μg/mL (1,000 mg IV); 0.5–2.2 μg/mL (after 8 h of aerosol); 0.8–3.3 μg/mL (after 20 h of aerosol)
Trough plasma level (dosage used)	350 μg/L (200 mg po single dose)[64]	280 μg/L (200 mg po single dose)[64]	115–137 μg/L (50 mg po bid); 219–233 μg/L (100 mg po bid); 468–587 μg/L (200 mg po bid); 1,101–1,128 μg/L (500 mg po bid)[62]	441.1–471.1 μg/L (600 mg IV q12h); mean concentration after oral inhalation over dosing interval, 40 μg/L[61]	1.25 μg/mL (200 mg po q8h); 3.22 μg/mL (400 mg po q8h); 4.49 μg/mL (800 mg po q8h)[59]
Maximum drug level in respiratory tract secretions	Nasal mucus: 0.45 μg/g (0.95 times of plasma level)[64]	Nasal mucus: 0.42 μg/g (1.75 times higher than plasma level)[64]	73.6% plasma level in bronchoalveolar lining fluid of rats[65]	Nasal washes: peak, 54.7 μg/L (50 mg IV), 485 μg/L (600 mg IV); trough 116–184 μg/L[61]; median concentration after 10 mg po inhalation: 1,336–47 μg/L (6–24 h) in sputum; 137 μg/L to undetectable (6–24 h) in nasal wash[66]; approximately 15% of the inhaled dose deposited in tracheobronchial tree and lung	1,000 μg/mL by aerosol[58]
Dosage adjustment in renal impairment	Yes	Yes, if creatinine clearance is < 10 mL/min	Yes	No, if administered by po inhalation	Yes, if creatinine clearance is < 50 mL/min
Dosage adjustment in liver impairment	No	Yes, for severe hepatic dysfunction	No recommendations	No recommendations	No

Table 2—*Continued*

Characteristics	Amantadine[58]	Rimantadine[58]	Oseltamivir†	Zanamivir†	Ribavirin[58,59]
Metabolism	Mainly excreted unchanged in urine	Extensive metabolized in liver	Extensive metabolized in liver (from phosphate to the active form carboxylate); > 99% of carboxylate form excreted in urine	Not significantly metabolized	Partially metabolized in liver
Major route of excretion	Renal	Liver (< 1% parent compound excreted unchanged in urine)	Renal (63% carboxylate excreted in urine); < 20% in feces	Renal (90% excreted unchanged in urine after IV; 16% excreted in urine after po inhalation)	Renal (40%)
Major adverse reactions	Neuropsychiatric	Similar to amantadine but much less common	Few major side effects; well tolerated at up to 1,000 mg single dose or 500 mg bid doses	Few major side effects; may cause bronchospasm in patients with underlying respiratory disease such as asthma and COPD, although not absolutely contraindicated; well tolerated at up to 600 mg bid IV[61,67]	Anemia, hyperbilirubinemia, teratogenicity
Important drug interactions	Caution when using other drugs with neurologic toxicity and nephrotoxicity	No clinically significant drug interactions	No clinically significant drug interactions	No clinically significant drug interactions	Antiretroviral agents
Mean IC_{50} of susceptible human influenza A viruses	100–400 μg/L	10–100 μg/L	0.16–0.31 μg/L; 0.19–8122 μg/L‡[70]; A/N1: 0.09–0.31 μg/L[70]; A/N2: 0.06–0.25 μg/L[69]	0.10–1.53 μg/L, 6.65–19938 μg/L‡[69]; A/N1: 0.17–0.83 μg/L[69]; A/N2: 0.30–1.86 μg/L[69]	2.6–6.8 μg/mL[68]
Mean IC_{50} of avian influenza A viruses (year of viral isolates)	A/H5N1 (2003–2004): > 8,000 μg/L[71]		A/H5N1 (1997): 2.19 μg/L, 2343 μg/L‡[50]	A/H5N1 (1997): 1.67 μg/L, 3323 μg/L‡[52]	A/H5N1 (1981): 2.3 μg/mL, 1.6 μg/mL‡[73]
	A/H5N1 (2003): > 18,770 μg/L[72]		A/H5N1 (2003–2004): 0.78–3.09 μg/L (median)[8]	A/H5N1 (2004): 0.27 μg/L, 299.07 μg/L‡[74]	A/H5N1 (1983): 4.3 μg/mL, 1.2 μg/mL‡[73]
	A/H5N3 (2003): 18.77 μg/L[72]		A/H5N1 (2004): 0.12 μg/L, 31.24 μg/L‡[58]	A/H5N1 (2005): 0.17–1.03 μg/L[36]	
	A/H7N2 (2002): 18.77 μg/L[86]		A/H5N1 (2005): 28.11 μg/L, one of the clones > 238.56 μg/L[36]	A/H6N1 (1997) 2.49 μg/L, 2.82 μg/L‡[52]	
	A/H7N2 (2003): 6231.64–8277.57 μg/L[72]		A/H7N7 (2003): 0.40 μg/L[18]	A/H7N7 (2003): 1.31 μg/L[18]	
	A/H9N2 (2000): > 18,770 μg/L[72]		A/H9N2 (1997–1999): 3.12–4.69 μg/L, 3.12–3.75 μg/L‡[50]	A/H9N2 (1997): 2.33–3.32 μg/L, 3.32–4.65 μg/L‡[52]	
	A/H9N2 (2001): 91.97 μg/L[72]		A/N1 to N9 (1949–1997): 0.59–21.62 μg/L, 312.40–13120.80 μg/L‡[75]	A/N1 to N9 (1949–1997): 0.73–10.00 μg/L,[1]1329.20–19373.09 μg/L‡[9]	

*FDA = US Food and Drug Administration.

†The antiviral activity of neuraminidase inhibitors as denoted by IC_{50} values is determined either by inhibition of neuraminidase enzyme activity or plaque inhibition assay in cell cultures (also expressed as EC_{50}). The latter test method tends to give more variable results and higher IC_{50} levels. *In vivo* susceptibility of influenza viruses to this group of compounds is more closely related to the results NA enzyme inhibition assays.[76] Unless otherwise stated, values for IC_{50} are determined by the neuroaminidase enzyme inhibition. EC_{50} is determined by cell-culture–based virus reduction or microcentralization assays.

optimal dosing of oseltamivir for avian influenza, which most likely requires higher doses than currently recommended dosages for human influenza. Increased dose is especially indicated in patients with a high viral load due to delayed presentation, severe diseases with shock, or poor oral drug absorption as a result of severe diarrhea. Although oseltamivir has been administered in high doses to healthy volunteers at supratherapeutic doses without significant adverse reactions,[77] any potential toxicity associated with high-dose regimens must be closely monitored in therapeutic trials.

If the use of an increased dosage of neuraminidase inhibitors for avian influenza infection is indeed beneficial, this will have a major impact on the supply of these agents in a pandemic situation. One option is to use a combination of oseltamivir and probenecid to double the systemic exposure following a conventional dosage of oseltamivir.[78] Another option is to use combination therapy with two neuraminidase inhibitors, one neuraminidase inhibitor plus one adamantane if the circulating genotype is susceptible to adamantanes, or one neuraminidase inhibitor plus ribavirin (or viramidine when it becomes available). The synergistic effects of these combinations, if any, need to be studied urgently by *in vitro* and animal studies. Ribavirin has been used in the treatment of human influenza A virus infections, usually administered orally or by aerosolization, and occasionally by the IV route for severe infections or in immunocompromised hosts. A consistent benefit has not been observed in clinical studies, and currently ribavirin is not considered to be a drug of choice for influenza A infection. There are only limited *in vitro* data on the activity of ribavirin on avian influenza viruses.[73] But ribavirin was shown to be highly effective in reducing mortality in a mannan-enhanced mice model infected by influenza B even when treatment was delayed for 3 days after the infection, when oseltamivir treatment is no longer effective. Combination of oseltamivir with ribavirin treatment started at such delayed timing does not increase the efficacy in this mice model. Viramidine is a carboxamidine analog of ribavirin that also demonstrates broad-spectrum antiviral activities like ribavirin. Its anti-influenza activity has been confirmed in limited *in vitro* and animal studies.[73] Viramidine is currently not licensed for clinical use.

Stockpiling of neuraminidase inhibitors is an essential component in the global plan against pandemic influenza.[79] Despite comparable efficacies of the neuraminidase inhibitors in the therapy of human influenza, the present emphasis of most national pandemic plans and the World Health Organization (WHO) is to preferentially recommend oseltamivir, probably because of the relatively lower serum level of zanmavir and the lack of its treatment data for H5N1 infections in humans.[80,81] Oseltamivir is administered orally, while zanamivir is delivered by oral inhalation of a dry powder. This mode of delivery for zanamivir could be problematic in certain populations such as the very young and old patients, the intubated patients, and those who are severely ill who are not able to inhale the powder properly. Oseltamivir is easier to administer and theoretically more effective when there is systemic infection or severe pneumonia (with collapse and/or consolidation of the lungs that could hinder penetration of inhaled zanamivir). However, both neuraminidase inhibitors need to be available because cross-resistance between the two neuraminidase inhibitors is incomplete. For example, the oseltamivir-resistant A/H5N1 isolate from Vietnam described above remains fully susceptible to zanamavir. A healthy reserve of zanamivir not only relieves the tension over oseltamivir supply but may also prove necessary when oseltamivir-resistant viruses become prevalent in the future. Zanamavir should be considered for prophylaxis in health-care workers taking care of patients receiving treatment with oseltamivir for avian influenza. Zanamivir may be used for prophylaxis or treatment of less severely ill patients because higher blood levels of the drug may not be needed (the blood level of zanamivir is almost 5 times lower than that of oseltamivir). However, oseltamivir may be reserved for patients with systemic infection or severe pneumonia who require higher blood levels. It is unfortunate that other formulations of zanamivir and the newer agent peramivir are not commercially available. In preclinical trials, IV zanamivir, even when administered at doses up to 1,200 mg/d, was very well tolerated and achieves very high blood and respiratory secretion levels; protective efficacy was also demonstrated in experimental human infections.[61,67] The role of combinations of neuraminidase inhibitors are uncertain, as is the case of combinations with other groups of antivirals. However, a low serum level of inhaled zanamivir in patients with consolidated lungs may theoretically promote the emergence of zanamivir resistant virus that is cross-resistant to oseltamivir.

Immunomodulators such as corticosteroids and IV Ig have been used for the treatment of viral pneumonias.[82] Corticosteroids had been used for small numbers of patients with A/H5N1 pneumonia in Hong Kong, Vietnam, and Thailand, which is often complicated by hyperglycemia. The numbers of patients who received corticosteroids in the three outbreaks were three (two deaths), seven (six deaths), and eight (six deaths), respectively.[39,40,83] Although a cytokine storm has been incriminated as

a possible pathogenic mechanism of A/H5N1 pneumonia, current evidence does not support a beneficial role of corticosteroids or other immunomodulators in the management of severe A/H5N1 infections.[41]

Infection Control Within Health-care Facilities

The outbreak of severe acute respiratory syndrome from late 2002 to mid-2003 exposed the vulnerability of health-care workers to nosocomial transmission of infectious diseases.[83] Moreover, these hospital epicenters may serve as the source for subsequent large-scale community outbreaks.[84] Hence, hospital infection control is a crucial component in the management of emerging infectious disease. For patients suspected or confirmed of having avian influenza, the WHO recommended contact and droplet precautions as key measures.[85] Airborne precautions are also recommended because of the high mortality and the ability of human influenza viruses to be transmitted by droplet nuclei. The relative importance of these routes of transmission are uncertain at present. The findings of GI involvement by A/H5N1 viruses and the prominent diarrhea with a high fecal viral load in some patients should be not be overlooked. A similar combination of clinicopathologic findings during the SARS outbreak in Hong Kong led to an unprecedented outbreak in a housing estate via airborne transmission of a virus-laden aerosol plume generated from a faulty sewage system.[84] Besides hospital-acquired infections, laboratory-acquired infections during the postmortem examination of A/H7N2-infected seals have been reported. The 1977 A/H1N1 strain was believed to have been originated from a laboratory in Russia. In 2005, an isolate of A/H2N2 virus was erroneously sent to laboratories in 18 countries in a proficiency testing program. Therefore, faulty laboratory precautions can also be a source for pandemics. Avian influenza viruses are readily inactivated by common disinfectants, and the WHO presently recommends the use of 1% sodium hypochlorite or 70% alcohol for disinfection.[85–87]

Preventive Measures

Controlling highly pathogenic avian influenza in animals is a mammoth task that has been recently addressed.[88] Strict regulation and control of bird and poultry trafficking—be it legal import or smuggling—are crucial for the control of intercontinental spread of avian influenza. Proper poultry-handling practice and personal hygiene (such as handwashing) and minimizing contact with birds are basic precautions that must be enforced. In some instances, use of an H5 vaccine in poultry has been shown to be able to interrupt virus transmission in the field.[89] There are no H5 influenza vaccines licensed for human use at present, but developmental work is ongoing. An inactivated H5N3 vaccine was previously tested in human subjects and was found to be well tolerated.[90] An MF59-adjuvanted vaccine was immunogenic in volunteers with development of anti-A/H5N1 neutralizing antibodies.[91] Another recombinant baculovirus-expressed H5 hemagglutinin vaccine has also been tested in human subjects and was immunogenic.[92] Other candidate vaccines that have appeared to be protective in animal models include an A/H5N1 vaccine generated by reverse genetics.[93] Even though vaccine development is taking place, it is important to remember that the pandemic strain may have significant antigenic differences from the H5N1 vaccines being developed. Should this happen, a new vaccine would have to be developed with a significant time lag of 6 months before an effective vaccine is commercially available for mass vaccination.

In the absence of an effective human vaccine, antiviral chemoprophylaxis remains the only viable means for specific protection against avian influenza. Oseltamivir prophylaxis was used in the Netherlands during the A/H7N7 outbreak for high-risk individuals, such as personnel involved in culling operations on infected farms, and hospital and family contacts of a patient suffering from A/H7N7 pneumonia. Persons who had received oseltamivir prophylaxis appeared to have a lower risk of infection by avian influenza (2.6% vs 9.6% infection rate for those who had and had not received prophylaxis, respectively), although the difference was not statistically significant.[18]

Perspective

Avian influenza may seem to be the most likely candidate for the next influenza pandemic. Vaccines are probably the most effective means of specific protection, but the ability to produce effective vaccines on time will limit their role in the early phase of the pandemic. Indiscriminate use of neuraminidase inhibitors for human influenza virus infection should be discouraged because this will inevitably promote the emergence of neuraminidase inhibitor-resistant influenza viruses.[57] Another potential concern is the reassortment between neuraminidase inhibitor-resistant A/H1N1 or A/H3N2 virus with A/H5N1 virus, resulting in neuraminidase inhibitor-resistant A/H5N1 reassortants with enhanced human-to-human transmissibility. Studies need to be done urgently to include the role of combination

antiviral therapy in treating severe infections and its ability to prevent the emergence of resistant strains of viruses. The efficacy of other agents such as ribavirin (by aerosolization or systemic administration), either alone or in combinations, should be explored. Antiviral susceptibility testing must be performed on new epidemic strains, as not all A/H5N1 viruses are resistant to adamantanes (our unpublished data, 2001). While oseltamivir is toxic to newborn rats at a dose 250 times higher than the recommended pediatric dosage, the use of oseltamivir in infants < 1 year old has not been shown to be neurotoxic. This should be confirmed by larger clinical trials.[94,95] Efforts to avert or minimize the impact of the impending pandemic should not be based solely on therapeutic measures. Simple community infection control and personal hygienic measures are important for respiratory infections, as shown in the severe acute respiratory syndrome epidemic in 2003.[96] Human avian infection is currently limited to close contacts with infected animals. For travelers to endemic areas of avian influenza, avoiding close contacts with poultry or wild birds could significantly reduce the risk of infection.

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