
Update on Avian Influenza for Critical Care Physicians

C. Sandrock

■ Introduction

Human influenza pandemics over the last 100 years have been caused by H1, H2, and H3 subtypes of influenza A viruses. More recently, avian influenza viruses have been found to directly infect humans from their avian hosts. The recent emergence, host expansion, and spread of a highly pathogenic avian influenza (HPAI) H5N1 subtype in Asia has heightened concerns globally, both in regards to mortality of HPAI H5N1 in humans and the potential of a new pandemic. In response, many agencies and organizations have been working collaboratively to develop early detection systems, preparedness plans, and objectives for further research. As a result, there has been a large influx of published information regarding potential risk, surveillance, prevention and control of highly pathogenic avian influenza, particularly in regards to animal to human and subsequent human to human transmission. This chapter will review the current human infections with avian influenza and its public health and medical implications.

■ Influenza A Viruses

Influenza A, B and C are the most important genera of the *Orthomyxoviridae* family. Influenza A is responsible for human pandemic outbreaks and seasonal epidemics and influenza B is responsible for increasing cases of seasonal disease. Influenza A viruses are enveloped, single-stranded RNA viruses with a segmented genome. The eight RNA segments of the genome encode for 11 viral proteins, including the polymerase proteins (PB1, PB2), matrix proteins (M1, M2) and the surface glycoproteins hemagglutinin (HA) and neuraminidase (NA). Influenza A viruses are classified into subtypes on the basis of the antigenic properties of the hemagglutinin and neuraminidase glycoproteins expressed on the surface of the virion [1]. To date, 16 hemagglutinin and 9 neuraminidase subtypes have been identified and are found in 144 different combinations (e.g., H1N1, H3N2, H5N1, etc.) [2].

The hemagglutinin glycoprotein mediates attachment and entry of the virus by binding to sialic acid receptors on the cell surface [3, 4]. The binding affinity of hemagglutinin to the host sialic acid allows for the host specificity of influenza A. More specifically, avian influenza subtypes prefer to bind to sialic acid linked to galactose by α -2,3 linkages, which are found on duck intestinal epithelium and poultry and duck respiratory epithelium [5]. Human virus subtypes, H1, H2, and H3, bind to α -2,6 linkages found in human respiratory epithelium. Swine contain both α -2,3 and α -2,6 linkages in their respiratory epithelium allowing for easy co-infec-

tion with both human and avian subtypes [6]. This reason has been cited as the likely genesis of novel strains, as in the 1968 H3N2 human pandemic, and has given pigs the designation of a 'mixing vessel' for new strains [7, 8]. To a lesser degree, humans have been found to contain both α -2,3 and α -2,6 linkages in their lower respiratory tract and conjunctivae which allows for human infections of avian strains [9]. The hemagglutinin glycoprotein is the main target for immunity by neutralizing antibody.

The neuraminidase glycoprotein allows the spread of the virus by cleaving the glycosidic linkages to sialic acid on host cells and the surface of the virus [10]. The virus is then spread in secretions or other bodily fluids. The neuraminidase glycoprotein is a lesser target for immunity by neutralizing antibodies, but is the target site for the antiviral neuraminidase inhibitors.

In addition to hemagglutinin and neuraminidase classification, influenza A viruses are characterized by their pathogenicity. Highly pathogenic avian influenza (HPAI) is defined by the World Organization for Animal Health (OIE) as any influenza that causes severe disease or death in domestic poultry. HPAI viruses, with very few exceptions, are of the H5 or H7 subtype, but not all H5 and H7 subtypes are HPAI viruses. The potential pathogenicity of H5 and H7 subtypes can be evaluated by sequencing the hemagglutinin gene, since pathogenicity is associated with the presence of multiple basic amino acids at the hemagglutinin cleavage site. A change from a low pathogenic H5 or H7 subtype to a highly pathogenic form may occur upon introduction into poultry and is thought to occur primarily as a result of insertion of basic amino acids in the hemagglutinin cleavage site. Molecular studies have shown that the 1918 human pandemic H1N1 subtype originated as a low pathogenic avian virus in contrast with current human cases of H5N1 worldwide, which are the result of a highly pathogenic avian influenza virus.

Influenza A viruses are highly variable as a result of molecular changes in the RNA segments that occur through a number of mechanisms; the most important of which are point mutation (antigenic drift) and RNA segment reassortment (antigenic shift) [10]. Like other RNA viruses, the influenza A viruses lack proofreading ability, and are, therefore, subject to point mutations [10]. These individual mutations in the viral genome cause minor changes in the antigenic character of virus, with amino acid changes in hemagglutinin and neuraminidase of principal importance. Reassortment occurs when a host cell is infected with two or more influenza A viruses and leads to the creation of a novel subtype containing a new hemagglutinin or neuraminidase that is immunologically distinct from those of the previous circulating strains, as can be seen in pigs, which possess receptors for both the human and avian subtypes [8]. Three major pandemics have occurred in the last century (1918 H1N1, 1957 H2N2, and 1968 H3N2) through reassortment. However, point mutations leading to viral adaptation to a human host can occur with any avian influenza subtype.

■ Host Range of Influenza A Viruses

Influenza A viruses infect a wide range of hosts including many avian species, and various mammalian species such as swine, ferrets, felids, mink, whales, horses, seals, dogs, civets, and humans [11–13]. Wild birds (ducks, geese, swans, and shorebirds) are important natural reservoirs of these viruses, and all of the known 16 hemagglutinin and 9 neuraminidase subtypes have been found in these birds. In most cases,

these subtypes are found within the gastrointestinal tract of the birds, shed in their feces, and rarely cause disease. Since 2002, however, HPAI H5N1 viruses originating in Asia have been reported from approximately 960 wild bird species, causing disease in some instances and asymptomatic shedding in others [14]. The virus has now spread across Asia, Europe, the Middle East, and some African countries. Additional species, such as tigers, leopards, cats, stone martens, and humans have also become infected with HPAI H5N1. The wide host range of many of these bird species may be one potential mechanism of spread of HPAI H5N1 worldwide, thus complicating the potential contact, transmission, and mutability of HPAI H5N1 in animal and human populations.

■ Epidemiology and Pathogenicity of Avian Influenza Infections in Humans

The incidence of avian influenza infections in humans has increased over the last decade (Table 1) [11, 12, 15–20]. Initially, cases of avian influenza (H7N7) in humans occurred in association with poultry outbreaks, manifesting as self-limiting conjunctivitis [11]. Then, in 1997, a large scale HPAI H5N1 outbreak occurred among poultry in Hong Kong, with 18 documented human cases [17]. Two subsequent poultry outbreaks in Hong Kong in 1999 and 2003 with HPAI H5N1 occurred without human cases until 2003 when two members of a family in Hong Kong contracted HPAI H5N1. In December of 2003, HPAI H5N1 surfaced in poultry in Korea and China, and from 2003–2006 the outbreak stretched worldwide in the largest outbreak in poultry history. Human cases of HPAI H5N1 followed the poultry outbreak, with a total of 256 cases and 151 fatalities thus far. Other limited outbreaks have occurred, causing variable human disease (Table 1) [21]. However, HPAI H5N1 remains the largest and most significant poultry and human avian influenza outbreak.

Epidemiologic investigations of human cases of avian influenza shows that the virus was acquired by direct contact from infected birds. Influenza A is transmitted

Table 1. Avian influenza A outbreaks reported in humans

	United Kingdom 1995	Hong Kong 1997	Hong Kong 1999	The Netherlands 2003	Canada 2004	Worldwide (southeast Asia, Africa, Middle East) 2003-present
Influenza A subtype	H7N7	H5N1	H9N2	H7N7	H7N3	H5N1
Source of infection	Poultry	Poultry and waterfowl	Poultry	Poultry	Poultry	Poultry and waterfowl
Clinical presentation	Conjunctivitis	Conjunctivitis and ILI	ILI	Conjunctivitis pneumonia, ILI	Conjunctivitis, ILI	Conjunctivitis, ILI, pneumonia, multi-organ failure
Number of human cases	1	18	2	89	2	256
Number of fatalities (%)	0 (0)	6 (33)	0 (0)	1 (1)	0(0)	151 (59)

H: hemagglutinin; N: neuraminidase; ILI: Influenza-like illness

Table 2. Person-to-person transmission of avian influenza

	Hong Kong 1997	Hong Kong 1997	Netherlands 2003	Thailand 2004	Vietnam 2004	Indonesia 2006
Influenza subtype	H5N1	H5N1	H7N7	H5N1	H5N1	H5N1
Location	Household	Hospital	Household	Hospital	Hospital	Household
Transmission to	Family member	Health care worker	Family member	Family member	Health Care worker	Family member
No of cases	1	8	3	2	0	7
Clinical presentation	seropositive	seropositive	Conjunctivitis and ILI	Pneumonia, death	N/A	Pneumonia, death

ILI = influenza-like illness

through the fecal-oral and respiratory routes among wild birds and poultry [13, 14]. Human interaction with these infected secretions and birds was the major mode of transmission, with contact including consumption of undercooked or raw poultry products, handling of sick or dead birds without protection, or food processing at bird cleaning sites [11, 12, 15–17, 19, 22–24]. All birds were domesticated poultry or waterfowl, and no transmission from wild birds or contaminated water has been reported. In a few cases, limited human to human transmission was reported among health care workers and family members (Table 2) [24–28]. In each of these cases, no personal protective equipment was utilized and was the major factor in transmission between humans.

■ Clinical Manifestations of Avian Influenza in Humans

The clinical manifestations of avian influenza in humans have ranged from mild conjunctivitis to severe pneumonia with multiple organ failure (MOF) [11, 12, 15–20, 22, 23, 29]. While the ages of the patients have varied, the majority of cases in both the 1997 and 2003 HPAI outbreaks were young. In 1997, the median age of the cases was 17.2 years, while the cases from 2003–2004 in Southeast Asia had a median age of 16 (range 2 months to 90 years). Nearly all cases were linked with sick and infected poultry, and the incubation period ranged from 2 to 8 days from contact to symptoms. The symptoms in each outbreak have varied with the avian influenza A subtype. In 2003 during the Netherlands outbreak with subtype H7N7, 92% (82 of 89) presented with conjunctivitis [11, 29]. The other cases in Canada and the UK also presented with conjunctivitis [22]. However, with HPAI in Hong Kong in 1997, 18 of the cases had an influenza-like illness [17, 19]. In 11 cases, pneumonia developed with 6 of these progressing to MOF, acute respiratory distress syndrome (ARDS), and death [17]. Reye syndrome, pulmonary hemorrhage, and predominant nausea, vomiting, and diarrhea complicated cases.

Cases from the worldwide outbreak originating in Southeast Asia had similar presentations to the 1997 HPAI H5N1 cases [30–33]. The main presenting symptom was pneumonia with fever and an influenza-like illness. Diarrhea was present in up to 70% of the cases. Many cases had both thrombocytopenia and lymphopenia. Chest radiographic findings included interstitial infiltrates, lobar consolidation, and

air bronchograms. Sixty-eight percent of patients developed ARDS and MOF within 6 days of disease onset. The case fatality rate has ranged from 67–80%, depending on the case series [34]. Once the cases reached the critical care unit, however, the mortality was 90%. The average time of death from disease onset was 9–10 days.

Post-mortem studies have illustrated findings consistent with MOF and overwhelming systemic inflammatory response syndrome (SIRS), including diffuse alveolar damage, acute tubular necrosis and atrophy, disseminated intravascular coagulation (DIC), and multi-organ damage [35]. Interestingly, the virus has been isolated from the lungs, intestine, spleen, and brain, suggesting viremia. However, active replication of the virus was limited to the lungs. This overwhelming inflammatory response, with acute lung injury (ALI) and ARDS as the predominant feature, coincides with the findings of a preferential binding of the avian influenza A viruses to α -2,3 linkages in type II pneumocytes of the lower respiratory tract of humans [36]. Subsequent viral replication, cytokine release, overwhelming host immune response, and the subsequent systemic manifestations then occur.

■ Diagnosis

The clinical diagnosis of avian influenza infection in humans is difficult and relies on the epidemiological link to endemic areas, contact with sick or dead poultry, or contact with a confirmed case of avian influenza. Since many infectious diseases present with these findings, the only feature significant to the clinician may be contact in an endemic area, through travel or infected poultry, and the clinician should always elicit this detailed history.

The definitive diagnosis is made from isolation of the virus in culture from clinical specimens. This method not only provides the definitive diagnosis, but the viral isolate is now available for further testing, including pathogenicity, antiviral resistance, and DNA sequencing and analysis. Alternatively, antibody testing can be performed, with a standard four-fold titer increase to the specific subtype of avian influenza virus. Neutralizing antibody titer assays for H5, H7 and H9 are performed by a microneutralization technique. Western blot analysis with recombinant H5 is the confirmatory test for any positive microneutralization assay. More recently, rapid diagnosis can be performed with reverse transcription-polymerase chain reaction on clinical samples with primers specific for the viral subtype [37]. This test should only be performed on patients meeting the case definition and with an indirect immunofluorescence or enzyme immunoassay test confirming influenza A.

Any suspected case of avian influenza in a human should be investigated by the public health officials in the province or country of origin [38]. Additionally, governmental laboratories are often equipped with the appropriate biolevel safety 3 laboratories, primer libraries, and associated expertise to confirm the diagnosis quickly and efficiently. Any clinical specimens should be submitted with the assistance of the public health experts

■ Treatment

Treatment of avian influenza infections in humans includes antiviral therapy, supportive care, and adjunctive therapies [39–43]. Controlled clinical trials on the efficacy of antivirals (neuraminidase inhibitors), supportive therapy, or adjuvant care

have never been performed, so current recommendations stem from the experiences of past avian influenza outbreaks and animal models.

The adamantanes (rimantadine and amantadine) and neuraminidase inhibitors (oseltamivir and zanamivir) are the antivirals used for treatment and prophylaxis of influenza infections in humans [42]. Adamantanes bind to the M2 protein on the viral capsule, inhibiting dissociation of the matrix proteins from the nucleocapsid during viral uncoating. In avian influenza virus infections, adamantanes have no role due to widespread resistance. HPAI H5N1 isolated from Southeast Asia carried the mutation in M2 that conferred resistance to this group of antivirals. In fact, over 90% of isolates of H1 and H3 human subtypes during seasonal influenza have had resistance to the adamantanes, thereby limiting their use in seasonal epidemics with human subtypes. Their role has been limited now to prophylaxis in the community when the circulating strain is known to be susceptible to the adamantanes.

Neuraminidase inhibitors (oseltamivir and zanamivir) have been extensively studied for both treatment and prophylaxis in the human influenza A subtypes, H1, H2, and H3, as well as influenza B [40, 42]. In avian influenza, the efficacy has been well documented in animal models where improved survival has been seen after infection with HPAI H5N1. The timing of treatment is paramount, as earlier therapy is directly related to improved survival. The greatest level of protection was seen if the neuraminidase inhibitors were started within 48 hours of infection, and protection rapidly dropped after 60 hours. In HPAI H5N1 cases from Southeast Asia, survival appeared to be improved in patients who received oseltamivir earlier at 4.5 days compared to 9 days. Both of these times are much longer than documented in the animal models, so the window of optimal therapy is largely unknown. Additionally, therapy with oseltamivir has been shown to decrease the viral level in nasal secretion in patients infected with HPAI H5N1. For oseltamivir, therapy has been at 75 mg twice daily, with 75 mg once daily reserved for prophylaxis. The drug has a 90% oral bioavailability and reaches significant plasma and bronchoalveolar lining fluid levels. Zanamivir is available in a dry powder inhalation at 10 mg twice daily for treatment and 10 mg daily for prophylaxis. Zanamivir has not been used in human avian influenza cases, and some concern exists over treatment with an inhalation powder as plasma levels are significantly lower than with oseltamivir.

Neuraminidase inhibitor resistance has been documented in HPAI H5N1 subtype in a Vietnamese girl treated with 75 mg daily for 4 days for post-exposure prophylaxis [44]. The NA glycoprotein had a histidine to tyrosine substitution at position 274, conveying markedly higher IC₅₀ for oseltamivir. Zanamivir resistance was not found with this change [39, 41]. Neuraminidase resistance has not been documented in other HPAI H5N1 cases to date.

Combination therapy has not been studied in influenza A viruses. Ribavirin by inhalation has been evaluated *in vitro* with some avian influenza A subtypes and has been found to reduce mortality from influenza B in a mouse model. Further animal model studies are indicated to determine if there is a role for ribavirin or combination therapy with avian influenza A viruses [42].

Supportive care with i.v. rehydration, mechanical ventilation, vasopressor therapy, and renal replacement therapy are required if MOF and ARDS are a feature of disease [43]. Due to the progression of pneumonia to ARDS, non-invasive ventilation is not recommended, and early intubation may be beneficial before overt respiratory failure ensues. Corticosteroids have been used in some patients with HPAI H5N1, but no definitive role for steroids has been determined. Other immunomodulatory therapy has not been reported.

■ Vaccination

Human vaccination for avian influenza viruses has not been widely used, although multiple vaccination trials are underway. Prior avian vaccines in humans have been poorly immunogenic and thus have limited use [45–48]. An inactivated H5N3 has been tested and was tolerated but with limited immunogenicity [45]. Other H5 vaccines have developed neutralizing antibodies, but to a limited degree. Recently, a large randomized trial looked at an H5N1 attenuated vaccine from the Vietnam strain. Only a modest immunologic response was seen, with microneutralization antibodies being developed at 12 times the dose for seasonal influenza. The side effects were minimal. A number of other industry trials with adjuvant vaccines are currently ongoing. Sandofi-Adventis has recently reported success with a H5N1 attenuated vaccine at low doses, but the results have not been released thus far. Although promising, human vaccination against avian influenza viruses is still under development. Underscoring this development is the uncertainty of a pandemic strain, which may have vastly different antigenic properties of any developed H5 vaccine.

■ Infection Control and Preventative Measures

Health care infection control is a crucial component in the management of avian influenza infection or a new pandemic strain. Experience with the severe acute respiratory syndrome (SARS) outbreak in 2002 has illustrated that appropriate infection control measures are paramount to reducing spread to health care workers and possibly the community [49]. Therefore, the World Health Organization (WHO) and Centers for Disease Control and Prevention (CDC) recommend contact and airborne precautions for any initial suspected case of avian influenza in a human. In late October 2006, the CDC released an updated interim guidance on the use of masks and respirators in the health care setting (Table 3). In certain high risk procedures, additional protection with an N-95 particulate respirator may be considered given the likelihood of generating aerosol particles that may enhance transmission (Table 4). Respiratory protection should be worn along with an impermeable gown, face shield, and gloves. Initial cases should be placed in a negative pressure isolation room with 6–12 air changes per hour. Hand hygiene with antibacterial soap or alcohol based washless gel should be standard, with appropriate basins at each patient room. Seasonal vaccination of all health care workers should be preformed and emphasized to reduce spread. Visitors and family members should be strictly monitored and limited to reduce the likelihood of spread. Finally, antiviral chemoprophylaxis should be available to any health care workers with exposure to an infected

Table 3. masks and respirators for health care workers

	Surgical Mask	N-95 Respirator	N-95 Cart-ridge mask	Powered air purifying respirator (PAPR)
Protection	Droplet	Aerosol	Aerosol	Aerosol
Disposable	Yes	Yes	Filter only	No
Fit testing	No	Yearly	Yearly	Yearly
Power source	No	No	No	Battery
Stockpiling	Yes	Yes	No	No
Cost	Very low	Low	High	Very high

High Risk Procedures

Nebulization of medication
 Endotracheal intubation
 Non-invasive mechanical ventilation
 Bronchoscopy
 Humidified oxygen delivery
 Non-rebreather mask without expiratory filter

Table 4. High risk aerosol procedures in avian influenza

individual. Any symptomatic worker should be taken off duty and workplace surveillance should occur. With these aggressive measures, risk to the health care worker, patients, and family members will be reduced.

Conclusion

Avian influenza viruses have occurred with increased incidence within the human population, reflecting the delicate and tangled interaction between wildlife, domesticated animals, and humans. Disease in humans can be limited to conjunctivitis or an influenza-like illness, but HPAI H5N1 causes mainly severe pneumonia, respiratory failure, and death. Most cases have occurred with direct transmission from infected poultry or waterfowl, with only a few limited cases of human to human transmission. Treatment has been successful with the neuraminidase inhibitors if started early, and vaccine development is underway with a more immunogenic attenuated H5N1 virus preparation. Infection control measures are the mainstay for prevention and disease reduction. Avian influenza viruses may constitute part of the next pandemic, so appropriate knowledge, prevention, and treatment will reduce the likelihood of this occurrence.

References

1. World Health Organization Expert Committee (1980) A revision of the system of nomenclature for influenza viruses: a WHO Memorandum. *Bull WHO*. 58:585-591
2. Fouchier RAM, Munster V, Wallensten A, Bestebroer TM, Herfst S (2005) Characterization of a novel influenza A virus hemagglutinin subtype (H16) obtained from black-headed gulls. *J Virol* 79:2814–2822
3. Kawaoka Y, Webster RG (1988) Molecular mechanism of acquisition of virulence in influenza virus in nature. *Microb Pathog* 5:311–318
4. Kendal AP (1987) Epidemiologic implications of changes in the influenza virus genome. *Am J Med* 82:4–14
5. Couceiro JN, Paulson JC, Baum, LG (1993) Influenza virus strains selectively recognize sialyloligosaccharides on human respiratory epithelium: the role of the host cell in selection of hemagglutinin receptor specificity. *Virus Res* 29:155–165
6. Ito T, Couceiro JN, Kelm S, et al (1998) Molecular basis for the generation in pigs of influenza A viruses with pandemic potential. *J Virol* 72:7367–7373
7. Matrosovich MN, Matrosovich TY, Gray T, et al (2004) Human and avian influenza (AI) viruses target different cell types in cultures of human airway epithelium. *Proc Natl Acad Sci USA* 101:4620–4624
8. Pensaert M, Ottis K, Vandeputte J, Kaplan MM, Bachman PA (1981) Evidence for the natural transmission of influenza A virus from wild ducks to swine and its potential importance for man. *Bull World Health Organ* 59:75–78
9. Scholtissek C, Burger H, Bachmann PA, Hannoun C (1983) Genetic relatedness of hemagglutinins of the H1 subtype of influenza A viruses isolated from swine and birds. *Virology* 129:521–523

10. Hinshaw VS, Webster RG, Easterday BC, Bean WJ Jr (1981) Replication of avian influenza A viruses in mammals. *Infect Immun* 34:354–361
11. Fouchier RA, Schneeberger PM, Rozendaal FW, et al (2004) Avian influenza A virus (H7N7) associated with human conjunctivitis and a fatal case of acute respiratory distress syndrome. *Proc Natl Acad Sci USA* 101:1356–1361
12. Subbarao K, Klimov A, Katz J, et al (1998) Characterization of an avian influenza A (H5N1) virus isolated from a child with a fatal respiratory illness. *Science* 279:393–396
13. Ellis TM, Bousfield RB, Bissett LA, et al (2004) Investigation of outbreaks of highly pathogenic H5N1 avian influenza in waterfowl and wild birds in Hong Kong in late 2002. *Avian Pathol* 33:492–505
14. Liu J, Xiao H, Lei F, et al (2005) Highly pathogenic H5N1 influenza virus infection in migratory birds. *Science* 309:1206
15. Webster RG, Geraci J, Petrusson G, et al (1981) Conjunctivitis in human beings caused by influenza A virus of seals. *N Engl J Med* 304:911
16. Kurtz J, Manvell RJ, Banks (1996) J Avian influenza virus isolated from a woman with conjunctivitis. *Lancet* 348:901–902
17. Yuen KY, Chan PKS, Peiris M, et al (1998) Clinical features and rapid viral diagnosis of human disease associated with avian influenza A H5N1 virus. *Lancet* 351:467–471
18. Peiris, M, Yuen, KY, Leung, CW, et al (1999) Human infection with influenza H9N2. *Lancet* 354:916–917
19. Peiris JS, Yu WC, Leung CW, et al (2004) Re-emergence of fatal human influenza A subtype H5N1 disease. *Lancet* 363:617–619
20. Koopmans, M, Wilbrink, B, Conyn, M, et al (2004) Transmission of H7N7 avian influenza A virus to human beings during a large outbreak in commercial poultry farms in the Netherlands. *Lancet* 363:587–593
21. World Health Organization (2006) Cumulative number of confirmed human cases of avian influenza A/(H5N1) reported to WHO, 16 October, 2006. Available at: www.who.int/csr/disease/avian_influenza/country/cases_table_2006_10_16/en/index.html. Accessed October 17, 2006
22. Tweed, SA, Skowronski, DM, David, ST, et al (2004) Human illness from avian influenza H7N3, British Columbia. *Emerg Infect Dis* 10:2196–2199
23. Chan PKS (2002) Outbreak of avian influenza A (H5N1) virus infection in Hong Kong in 1997. *Clin Infect Dis* 34 (Suppl 2):S58-S64
24. Bridges CB, Lim W, Primmer JH, et al (2002) Risk of influenza A (H5N1) infection among poultry workers, Hong Kong, 1997–1998. *J Infect Dis* 185:1005–1010
25. Bridges CB, Katz JM, Seto WH, et al (2000) Risk of influenza A (H5N1) infection among health care workers exposed to patients with influenza A (H5N1), Hong Kong. *J Infect Dis* 181:344–348
26. Ungchusak, K, Auewarakul, P, Dowell, SE, et al (2005) Probable person-to-person transmission of avian influenza A (H5N1). *N Engl J Med* 352:333–340
27. Liem NT, Lim W (2005) World Health Organization International Avian Influenza Investigation Team, Vietnam. Lack of H5N1 avian influenza transmission to hospital employees, Hanoi, 2004. *Emerg Infect Dis* 11:210–215
28. Schultz, C, Dong, VC, Chau, NVV, et al (2005) Avian influenza H5N1 and healthcare workers. *Emerg Infect Dis* 11:1158–1159
29. Koopmans M, Wilbrink B, Conyn M, et al (2004) Transmission of H7N7 avian influenza A virus to human beings during a large outbreak in commercial poultry farms in the Netherlands. *Lancet* 363:587–593
30. Tran TH, Nguyen TL, Nguyen TD, et al (2004) Avian influenza A (H5N1) in 10 patients in Vietnam. *N Engl J Med* 350:1179–1188
31. Chotpitayasunondh T, Ungchusak K, Hanshaoworakul W, et al (2005) Human disease from influenza A (H5N1), Thailand, 2004. *Emerg Infect Dis* 11:201–209
32. The Writing Committee of the World Health Organization (WHO) (2005) Consultation on Human Influenza A/H5. Avian influenza A (H5N1) infection in humans. *N Engl J Med* 353:1374–1385
33. de Jong MD, Bach VC, Phan TQ, et al (2005) Fatal avian influenza A (H5N1) in a child presenting with diarrhea followed by coma. *N Engl J Med* 352:686–691

34. Gruber PC, Gomersall CD, Joynt GM (2006) Avian Influenza (H5N1): implications for intensive care. *Intensive Care Med* 32:823–829
35. To KF, Chan PKS, Chan KF, et al (2001) Pathology of fatal human infection associated with avian influenza A H5N1 virus. *J Med Virol* 63:242–246
36. Yu IT, Li Y, Wong TW, et al (2004) Evidence of airborne transmission of the severe acute respiratory syndrome virus. *N Engl J Med* 350:1731–1739
37. Payungporn S, Phakdeewirot P, Chutinimitkul S, et al (2004) Single-step multiplex reverse transcription-polymerase chain reaction (RT-PCR) for influenza A virus subtype H5N1 detection. *Viral Immunol* 17:588–593
38. Li KS, Guan Y, Wang J, et al (2004) Genesis of a highly pathogenic and potentially pandemic H5N1 influenza virus in eastern Asia. *Nature* 430:209–213
39. Le QM, Kiso M, Someya K, et al (2005) Avian flu: isolation of drug-resistant H5N1 virus. *Nature* 437:1108
40. Leneva IA, Roberts N, Govorkova EA, et al (2000) The neuraminidase inhibitor GS4104 (oseltamivir phosphate) is efficacious against A/Hong Kong/156/97 (H5N1) and A/Hong Kong/1074/99 (H9N2) influenza viruses. *Antiviral Res* 48:101–115
41. Kiso M, Mitamura K, Sakai-Tagawa Y, et al (2004) Resistant influenza A viruses in children treated with oseltamivir: descriptive study. *Lancet* 364:759–765
42. Inouye RT, Panther LA, Hay CM, et al (2002) Antiviral agents. In: Richman DD, Whitley RJ, Hayden, FG eds. *Clinical virology* 2nd ed. 2002,171–242 ASM Press. Washington DC:
43. Cheng VC, Tang BS, Wu AK, et al (2004) Medical treatment of viral pneumonia including SARS in immunocompetent adult. *J Infect* 49:262–273
44. De Jong MD, Tranh TT, Khanh TH, et al (2005) Oseltamivir resistance during treatment of influenza A (H5N1) infection. *N Engl J Med* 353:2667
45. Nicholson KG, Colegate AE, Podda A et al (2001) Safety and antigenicity of non-adjuvanted and F59-adjuvanted influenza A/Duck/Singapore/97 (h5N3) vaccine: a randomized trial of two potential vaccines against H5N1 influenza *Lancet* 357:1937
46. Nicholson KG, Colegate AE, Podda A, et al (2001) Safety and antigenicity of nonadjuvanted and MF59-adjuvanted influenza A/duck/Singapore/97 (H5N3) vaccine: a randomised trial of two potential vaccines against H5N1 influenza. *Lancet* 2001;357,1937–1943
47. Stephenson I, Bugarini R, Nicholson KG, et al (2005) Cross-reactivity to highly pathogenic avian influenza H5N1 viruses after vaccination with nonadjuvanted and MF59-adjuvanted influenza A/Duck/Singapore/97 (H5N3) vaccine: a potential priming strategy. *J Infect Dis* 191:1210–1215
48. Lipatov AS, Webby RJ, Govorkova EA, et al (2005) Efficacy of H5 influenza vaccines produced by reverse genetics in a lethal mouse model. *J Infect Dis* 191:1216–1220
49. Van Riel D, Munster VJ, de Wit E, et al (2006) H5N1 Virus attachment to lower respiratory tract. *Science* 312:399