Review

Clinical review: Update of avian influenza A infections in humans

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Abstract

Influenza A viruses have a wide host range for infection, from wild waterfowl to poultry to humans. Recently, the cross-species transmission of avian influenza A, particularly subtype H5N1, has highlighted the importance of the non-human subtypes and their incidence in the human population has increased over the past decade. During cross-species transmission, human disease can range from the asymptomatic to mild conjunctivitis to fulminant pneumonia and death. With these cases, however, the risk for genetic change and development of a novel virus increases, heightening the need for public health and hospital measures. This review discusses the epidemiology, host range, human disease, outcome, treatment, and prevention of cross-transmission of avian influenza A into humans.

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 virus subtypes (that is, H5, H7) 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 have heightened concerns globally, both in regards to mortality from HPAI H5N1 infection in humans and the potential of a new pandemic. This paper will review the current human infections with avian influenza and their public health and medical implications.

Influenza A viruses

Influenza A, B and C are the most important genera of the Orthomyxoviridae family, casusing both pandemic and seasonal disease in humans. Influenza A viruses are enveloped, single-stranded RNA viruses with a segmented genome (Table 1) [1]. They are classified into subtypes on the basis of the antigenic properties of the hemagglutinin (HA) and neuraminidase (NA) glycoproteins expressed on the surface of the virus [1,2]. Influenza A viruses are characterized by their pathogenicity, with highly pathogenic avian influenza (HPAI) causing severe disease or death in domestic poultry [3]. Molecular changes in the RNA genome occur through two main mechanisms: point mutation (antigenic drift) and RNA segment reassortment (antigenic shift) [4,5]. Point mutations cause minor changes in the antigenic character of viruses and are the primary reason a vaccination for influenza A is given yearly. Reassortment occurs when a host cell is infected with two or more influenza A viruses, leading to the creation of a novel subtype. The influenza subtypes of the 1957 (H2N2) and 1968 (H3N2) pandemics occurred through reassortment, while the origins of the 1918 (H1N1) pandemic are unclear.

The HA glycoprotein mediates attachment and entry of the virus by binding to sialic acid receptors on the cell surface. The binding affinity of the HA to the host sialic acid allows for the host specificity of influenza A [6,7]. Avian influenza subtypes prefer to bind to sialic acid linked to galactose by α-2,3 linkages, which are found in avian intestinal and respiratory epithelium (Table 2) [8]. Human virus subtypes bind to α-2,6 linkages found in human respiratory epithelium [8,9]. Swine contain both α-2,3 and α-2,6 linkages in their respiratory epithelium, allowing for easy co-infection with both human and avian subtypes (thus acting as a 'mixing vessel' for new strains) [10]. 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 by avian subtypes [9,11,12]. The HA glycoprotein is the main target for immunity by neutralizing antibodies.

The NA glycoprotein allows the spread of the virus by cleaving the glycosidic linkages to sialic acid on host cells and the surface of the virus. The virus is then spread in secretions or other bodily fluids. The NA glycoprotein is not the major target site for neutralization of the virus by antibodies.

ARDS = acute respiratory distress syndrome; CDC = Centers for Disease Control and Prevention; HA = hemagglutinin; HPAI = highly pathogenic avian influenza; NA = neuroaminidase; WHO = World Health Organization.

Table 1

Characteristics of influenza viruses

	Influenza A	Influenza B	Influenza C
Genetic structure	8 segments	8 segments	7 segments
Viral proteins	10 total	11 total	9 total
Unique viral protein	M2	NB	HEF
Antigenic determinants	Hemagglutinin and neuroaminidase	Hemagglutinin and neuroaminidase	Hemagglutinin and neuroaminidase
Genetic change	Antigenic shift and drift	Antigenic drift	Antigentic drift
Host range	Avians, humans swine, marine mammals, horses	Humans	Humans and swine
Human epidemiology	Pandemics and seasonal epidemics	Seasonal epidemics	No seasonality

Table 2

Characteristics and pathogenicity of influenza A viruses

	Viral features
Number of HA subtypes	16
Number of NA subtypes	9
Predominant human subtypes	H1, H2, H3
Avian subtypes	H1-H16
HPAI subtypes	H5 and H7
Conversion to HPAI	Basic amino acid insertion in HA
Avian sialic acid-galactose linkages	α-2,3 linkages
Human sialic acid-galactose linkages	α-2,6 linkages

HA, hemagglutinin; HPAI, highly pathogenic avian influenza; NA, neuroaminidase.

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 [13-31]. Wild birds (ducks, geese, swans, and shorebirds) are important natural reservoirs of these viruses, and all of the known 16 HA and 9 NA subtypes have been found in these birds [32-35]. In most cases, these subtypes are found within the gastrointestinal tract of the birds, are shed in their feces, and rarely cause disease [32]. 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 [36-48]. 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 [49]. This spread of H5N1 into a wide range of animal and avian species may enhance the spread of the virus into the human population as it interacts with animals in a number of ways (increased land use, markets, consumption) [44]. Thus, the potential contact, transmission, and mutability of HPAI H5N1 worldwide will increase as the number of species and their interactions increase, complicating prevention, surveillance and treatment possibilities.

Epidemiology and pathogenicity of avian influenza infections in humans

The incidence of avian influenza infections in humans has increased over the past decade (Table 3). Initially, cases of avian influenza (H7N7) in humans occurred in association with poultry outbreaks, manifesting as self-limiting conjunctivitis [30,50-53]. Then, in 1997, a large scale HPAI H5N1 outbreak occurred among poultry in Hong Kong, with 18 documented human cases [29,31,54,55]. 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 [56]. In December of 2003, HPAI H5N1 surfaced in poultry in Korea and China, and from 2003 to 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 [57]. Other limited outbreaks have occurred, causing variable human disease (Table 3) [52,58]. However, HPAI H5N1 remains the largest and most significant poultry and human avian influenza outbreak.

Epidemiological investigations of human cases of avian influenza show that the virus was acquired by direct contact with infected birds [29-31,50-56]. Influenza A is transmitted through the fecal-oral and respiratory routes among wild birds and poultry [32]. 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. All birds were domesticated (chicken, duck, goose) and no transmission from birds in the wild (migrating) or

Table 3

Avian influenza A outbreaks reported in humans

	United Kingdom 1995 [51]	Hong Kong 1997 [54]	Hong Kong 1999 [52]	The Netherlands 2003 [53]	Canada 2004 [58]	Worldwide (Southeast Asia, Africa, Middle East) 2003 to present [57,65,66,68]
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 (percent)	0 (0)	6 (33)	0 (0)	1 (1)	0 (0)	151 (59)

H, hemagglutinin; ILI, influenza like illness; N, neuroaminidase.

Table 4

Person to person transmission of avian influenza

	Hong Kong 1997 [59]	Hong Kong 1997 [60]	Netherlands 2003 [53]	Thailand 2004 [61]	Vietnam 2004 [62]	Indonesia 2006 [64]
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
Number 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.

contaminated waterways has been documented. In a few cases, limited human to human transmission has been reported among health care workers and family members (Table 4) [59-63]. In each of these cases, no personal protective equipment was used, which is the major factor in transmission between humans [60].

Clinical manifestations of avian influenza in humans

The clinical manifestations of avian influenza in humans has ranged from mild conjunctivitis to severe pneumonia with multi-organ system failure (Table 5) [50,51]. The median age of patients was 17.2 years in the 1997 HPAI H5N1 outbreak and 16 years in the 2003 to 2006 Southeast Asian cases (range 2 months to 90 years) [17,55,65-68]. The incubation period ranged from two to eight days from contact with sick or dead birds to symptom onset. The predominant clinical findings appear to vary with each influenza A subtype; for example, in 2003 during the Netherlands outbreak (H7N7) 92% (82 of 89) of patients presented with conjunctivitis and a minority with respiratory symptoms [53]. However, with HPAI in Hong Kong in 1997 and in Southeast Asia currently, pneumonia progressing to multiorgan failure, acute respiratory distress syndrome (ARDS), and death are the predominant findings [17,55,65-68]. Rye syndrome, pulmonary hemorrhage, and predominant nausea, vomiting, and diarrhea complicate these cases [68]. Laboratory findings include both thrombocytopenia and lymphopenia [65,66]. Chest radiographic findings include interstitial infiltrates, lobar consolidation, and air bronchograms. The clinical course of patients with HPAI H5N1 is rapid, with 68% percent of patients developing ARDS and multiorgan failure within 6 days of disease onset [69]. The case fatality rate ranges form 67% to 80%, depending on the case series [17,55,65,66]. Once the patients reached the critical care unit, however, the mortality rate was 90% [69]. The average time of death from disease onset was nine to ten days.

Avian influenza A infections in humans differ from seasonal influenza in several ways. The presence of conjunctivitis is

Table 5

Clinical, laboratory, and radiographic findings of avian influenza in humans

Clinical presentation
Conjunctivitis
Influenza-like illness
Nausea
Emesis
Diarrhea
Shortness of breath
Pneumonia
Laboratory findings
Lymphopenia
Thrombocytopenia
Elevated creatinine
Abnormal transaminases
Chest radiographic findings
Interstitial infiltrates
Lobar infiltration
Consolidation
Pneumothorax (on mechanical ventilation)

Table 6

Case definition of avian influenza

Suspected H5N1 case
Lower respiratory tract infection
Fever (>38°C)
Cough
Shortness of breath
AND one of the following in the past 7 days
Close contact with a known or suspected human case of H5N1 disease
Contact with sick or dead birds (handling) or the environment where H5N1 is present
Consumption of a sick or dead bird in an H5N1 endemic area
Contact or exposure to laboratory specimens of H5N1

more common with avian influenza A infections than with seasonal influenza. Gastrointestinal symptoms, as seen with HPAI H5N1, and reports of primary influenza pneumonia and development of ARDS are also more common with avian influenza A infections [65,67,69]. Finally, the rapid progression to multi-organ failure and eventually death occurs at a much higher rate with avian influenza A infections [69].

Post-mortem studies have illustrated findings consistent with an overwhelming systemic inflammatory response syndrome, including diffuse alveolar damage, acute tubular necrosis and atrophy, disseminated intravascular coagulation, and multi-organ damage [70,71]. Interestingly, the virus has been isolated from the lungs, intestine, spleen, and brain, suggesting viremia, but active replication of the virus has been limited to the lungs [71]. This overwhelming inflammatory response, with acute lung injury and ARDS as the predominant features, coincides with the findings of preferential binding of the avian influenza A viruses to α-2,3 linkages in type II pneumocytes of the lower respiratory tract of humans and a vigorous cytokine response, including increased interleukin-6, interleukin-10, and interferon beta release [11,12,70,71].

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 (Table 6). Since many infectious diseases present with similar symptoms, 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 a detailed patient 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 the micorneutralization technique [72]. Western blot analysis with recombinant H5 is the confirmatory test for any positive microneutralization assay [59,60,72]. More recently, rapid diagnosis can be performed with reverse transcription-PCR on clinical samples with primers specific for the viral subtype [73-75]. This test should be performed only on patients meeting the case definition of possible avian influenza A infection.

Any suspected case of avian influenza in a human should be investigated by the public health officials in the province or country of origin [39,76]. Additionally, governmental labs 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 and supportive care. Controlled clinical trials on the efficacy of antivirals (NA 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.

Table 7

Neuroaminidase inhibitors

	Oseltamivir	Zanamivir
Spectrum of activity	Influenza A and B	Influenza A and B
Administration	Oral	Inhalation
Prophylaxis	≥13 years; 75 mg daily	>13 years; 10 mg daily
Treatment	≥1 year; 75 BID × 5 to 10 days	≥7 years; 10 mg BID × 5 to 10 days
Select adverse effects	GI symptoms: N/V, abdominal pain	Bronchospasm, cough
Resistance potential	Drug-resistant strain of H5N1 reported	None yet
Efficacy	Estimated efficacy: 30 to 70 percent	Not well studied
Generic available	No	No

Data are from [80-82]. BID, twice a day; GI, gastrointestinal; N/V, nausea and vomiting.

The adamantanes (rimantadine and amantadine) and NA inhibitors (oseltamivir and zanamivir) are the antivirals used for treatment and prophylaxis of influenza infections in humans. In avian influenza virus infections, adamantanes have no role due to widespread resistance through a M2 protein alteration. In addition, over 90% of isolates of H1 and H3 human subtypes during seasonal influenza have had resistance to the adamantanes [77]. Their role has now been limited to prophylaxis in the community when the circulation strain is know to be susceptible to the adamantanes [78-80].

NA inhibitors (oseltamivir and zanamivir) have been studied for both treatment and prophylaxis with the human influenza A subtypes H1, H2, and H3 as well as influenza B (Table 7) [80-82]. In animal models with HPAI H5N1, their efficacy has been well documented, with improved survival rates seen after infection [83-85]. Oseltamivir has been used in avian influenza outbreaks involving H7N7 and HPAI H5N1, and therapy with oseltamivir has been shown to decrease the viral load in nasal secretions in patients infected with HPAI H5N1 [11,86,87]. Resistance to oseltamivir has been documented in a HPAI H5N1 subtype in a Vietnamese girl treated with 75 mg daily for 4 days as post-exposure prophylaxis [68]. The NA glycoprotein had a histidine to tyrosine substitution at position 274, conveying a markedly higher IC50 for oseltamivir [68,88]. In one study, the viral count of HPAI H5N1 in nasal secretions did not decrease with the administration of oseltamivir when the H5N1 isolate carried this resistance mutation [68]. However, resistance produced by this change may be overcome with higher doses of oseltamivir *in vitro*, and this change has not been documented to confer resistance to zanamivir [88].

The timing of treatment with NA inhibitors is paramount, as early therapy is directly related to improved survival [66,83-85]. The greatest level of protection was seen if the NA inhibitors were started within 48 hours of infection, and protection rapidly dropped after 60 hours [78,79]. These initial studies, however, were performed with seasonal human influenza A and B, where the period of viral shedding is approximately 48 to 72 hours. In HPAI H5N1 cases from Southeast Asia, survival appeared to be improved in patients who received oseltamavir earlier (4.5 days versus 9 days after onset of symptoms) [66]. Both of these time periods are much longer than documented in animal models, so the window of optimal therapy is still unknown, particularly if viral shedding exceeds the average 48 to 72 hour period seen in seasonal influenza A and B infections.

Combination therapy with influenza A viruses has not been studied [84]. Ribaviron 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 [89]. Further animal model studies are indicated to determine if there is a role for ribaviron or combination therapy with avian influenza A viruses.

Supportive care with intravenous rehydration, mechanical ventilation, vasopressor therapy, and renal replacement therapy are required if multiorgan failure and ARDS are a feature of disease [69,90]. 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 [91].

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. An inactivated H5N3 has been tested and was tolerated but with limited immunogenicity [91,92]. Other H5 vaccines have resulted in the development of neutralizing antibodies, but to a limited

Table 8

Masks and respirators for health care workers

	Surgical mask	N-95 respirator	N-95 cartridge mask	Powered air purifying respirator
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

Table 9

High risk aerosol procedures in avian influenza

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

degree [93,94]. Recently, a large randomized trial looked at an H5N1 attenuated vaccine from the Vietnam strain [95]. Only a modest immune response was seen, with micro-neutralization antibodies being developed at 12 times the dose used in the seasonal influenza vaccine. The side effects were minimal. A number of other industry trials with adjuvant vaccines are currently ongoing. 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 from any developed H5 vaccine.

Infection control

Health care infection control is a crucial component in the management of avian influenza infection or a new pandemic strain. Experience from the severe ARDS outbreak in 2002 has illustrated that appropriate infection control measures are paramount to reduce spread to health care workers and, possibly, the community [96-98]. 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 [99]. In late October 2006, the CDC released updated interim guidance on the use of masks and respirators in the health care setting (Table 8) [99]. In certain high risk procedures, additional protection may be considered given the likelihood of generating aerosol particles that may enhance transmission (Table 9) [99]. 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 to 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 further emphasized in order to reduce the likelihood of co-infection with two stains of influenza. Visitors and family members should be strictly monitored and their access to the patient limited to reduce the likelihood of spread. Finally, antiviral chemoprophylaxis should be available to any health care workers exposed to an infected individual. Any symptomatic worker should be taken off duty and workplace surveillance should occur. With these aggressive measures, risk to health care workers, patients, and family members will be reduced.

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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 through direct transmission from infected poultry or waterfowl, with only a few limited cases of human to human transmission. Treatment has been successful with the NA 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.

Competing interests

The authors declare that they have no competing interests.

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