FRONTIERS

Avian influenza – a new challenge

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Abstract

Objective: **To present a wide-ranging review of the literature on avian influenza A (H5N1).**
Background: **The recent epidemics caused by the avian influenza A virus in Asia, have demonstrated the capacity of this agent to cause serious illness in humans.**
Methods: **Most articles were obtained from the Medline database using the keywords "Influenza A virus", "avian flu", "epidemiology", "disease vectors" and "H5N1 infection" for the period between 1996 and 2006.**
Results: **We selected 25 original articles addressing the recent outbreaks of infection with the H5N1 subtype of avian influenza A in domesticated birds in Asia, which have resulted in significant economic losses and repercussions for public health, as well as some cases of human infection presenting high lethality. In most cases, infection has been associated with direct exposure to infected birds or contact with surfaces infected with bird excrement. However, cases of human-to-human transmission have been confirmed.**
Conclusion: **Controlling outbreaks in domestic fowl and limiting contact between humans and infected birds must be the priorities in the management of this disease at the public health level. In addition, techniques and knowledge regarding the disease should be widely disseminated *(Ref. 28)*.**
Key words: influenza A virus, H5N1 infection; influenza; disease outbreaks; public health; clinical trials.

Recent outbreaks of infection with subtype H5N1 of the avian influenza A virus on poultry farms in Asia have resulted in significant economics losses and have serious public health implications. In January of 2004, as had previously occurred in 1997 and 2003, cases of human infection with this virus, presenting high lethality, were reported in various parts of Asia, leading public health officials to declare a state of alert regarding the possibility of a new influenza pandemic (1–2).

The present study aims to review the literature with the objective of presenting the history of influenza pandemics in the twentieth century, as well as addressing the epidemiology, pathogenesis, transmissibility, clinical profile, diagnosis and treatment of avian influenza A, based on articles published within the last ten years.

History of influenza pandemics

The twentieth century witnessed three influenza pandemics: the Spanish flu (viral subtype H1N1) in 1918; the Asian flu (H2N2) in 1957; and the Hong Kong flu (H3N2) in 1968. These epidemics caused serious illness with high mortality rates. The Spanish flu, in particular, killed at least 20 million people worldwide in 1918. The gene sequence of the H1N1 influenza virus, which was responsible for this epidemic, suggests that it originated in an avian reservoir. The viral subtypes responsible for the Asian flu and Hong Kong flu pandemics had two important characteristics in common: they both first appeared in southeast Asia, and they were both antigenically distinct from the viral subtypes that had previously circulated among humans. Southeast China is considered the epicenter of the influenza virus, based on the two previous epidemics that occurred there. The close contact among pigs, people and ducks in this region creates an ideal environment for the generation of recombinant viruses or, in other words, a genetic recombination of human and avian vi-

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ruses. Genetic studies and biochemical analyses indicate that the Asian flu and Hong Kong flu pandemics were generated by a recombinant virus. It is important to note that the H5N1 isolated in humans is not a recombinant virus like those of 1957 and 1968, and that all of its genes originate from avian viruses (1–3).

In 1996, the H7N7 subtype was isolated in England in the eye of a patient with conjunctivitis. That patient had been in frequent contact with ducks. This subtype was identical to the virus infecting birds, presenting 98 % homology among the nucleotides (1–2).

In view of these aspects, one can perceive that avian viruses play a key role in the emergence of pandemic strains. Despite the fact that humans and other mammals can be experimentally infected with avian viruses, these viruses were not, until recently, transmitted directly to humans. The reservoir of influenza A viruses, in populations of aquatic birds, is the origin of the influenza A viruses that infect humans, other mammals and domestic fowl (3).

The theory that the avian influenza virus does not replicate efficiently in humans led to the formulation of the hypothesis that an intermediary would be needed in order to promote the infection in humans. However, the recent H5N1 epidemics in Asia led researchers to re-examine this concept (3–4).

The first case of human infection with H5N1 occurred in May of 1997. No other cases were reported for six months, although an epidemic occurred in November and December of the same year, during which 17 additional cases were reported (3).

From December of 2003 until February of 2004, a total of 23 cases of human infection with this same virus, confirmed through laboratory testing, were reported in Myanmar, China, Indonesia, Japan, Laos, South Korea, Thailand and Vietnam (4).

Epidemiology

In May of 1997, the H5N1 subtype was isolated in the tracheal aspirate of a 3-year-old child who presented odynophagia, fever and cough. The patient was medicated with acetylsalicylic acid and antibiotics. However, the symptoms persisted and the child was hospitalized. Despite the use of mechanical ventilation and broad-spectrum antibiotics, the patient died sixteen days after the onset of symptoms, with extensive influenza-related pneumonia complicated by Reye's syndrome. This was o first documented case of H5N1 infection in humans. In December of 1997, the same virus was isolated in 18 patients, 7 of whom had a history of contact with domestic fowl. Of those 7, 6 did not survive (1–2, 4).

Epidemiological and molecular evidence suggests that domestic fowl are the source of the H5N1 virus. Research has revealed that fatal epidemics of infection with the avian influenza occurred on chicken farms in northeast Hong Kong in March and April of 1997, just prior to the first reported case of human infection (4). Subsequently, similar epidemics were reported in October, November and December of the same year, virtually in parallel with the epidemic in humans. Epidemiological surveillance in Hong Kong identified H5N1 in approximately 20 % of the fecal samples obtained from chicken and in 2 % of those from ducks and geese sold in public markets. All of the strains isolated in these samples were lethal to chicken. These findings implicate the contaminated birds bought in these markets as the source of the H5N1 infection in Hong Kong (4).

The comparison between the eight RNA segments of the virus isolated in humans and birds showed homology between the sequences of greater than 99 %. The sequence of events and the temporal relationship between epidemics in birds and those in humans strongly suggest direct transmission between the two species, without the involvement of an intermediary host. Human influenza viruses are generally transmitted by inhalation of aerosolized droplets. However, although birds excrete the viruses in their feces, it is unknown whether H5N1 infection is transmitted by inhalation of the virus in aerosol form, direct contact with the feces of domestic fowl or by some other vector. Notably, epidemiological studies in the literature suggest that human-to-human transmission can occur, albeit with low efficiency (3–7).

A timely article presented ten cases of human infection diagnosed in Vietnam between December of 2003 and January of 2004. The mean age of the patients was 13.7 years. Nine of those ten patients reported having direct contact with domestic fowl (through breeding, slaughter or preparation for cooking). The symptoms appeared, on average, three days after contact. None of the patients presented pre-existing pathologies and resided in both urban and rural areas. None of the patients had been in contact with each other prior to hospitalization. The estimated interval between the exposure to infected birds and the onset of symptoms suggests that the period of incubation is from two to four days. There have been no reports of similar illness among the professionals who cared for these patients, despite the lack of measures to control respiratory infections from the beginning of the outbreak. Another 34 cases were confirmed later. Of the 44 cases diagnosed between the beginning of the epidemic and December of 2004, 32 patients died. There are approximately 100 suspected cases currently under investigation by national health authorities in Thailand and Vietnam. The Centers for Diseases Control, the World Health Organization and national health authorities in Asian countries are working to monitor and control the situation, providing laboratory testing and epidemiological support (8).

As previously mentioned, despite the fact that all of the genes are of avian origin, the H5N1 subtype, author of the epidemic of 2004, is antigenically different than that isolated in humans in Hong Kong in 1997 and 2003, suggesting that the pandemic strain evolved through adaptation in humans by mutation genetic or by recombination with human influenza virus. Despite the fact that this appears not to have happened yet, recent studies have demonstrated the ongoing evolution of the virus since the H5N1 epidemic in Hong Kong in 1997. The precursor strain of the avian virus that caused the influenza epidemic evolved to a dominant pathogenic genotype, now endemic among the domestic fowl population of Asia, with a spectrum of hosts that includes domestic as well as wild birds. Other studies have demonstrated that the H5N1 strain isolated between 1999 and 2002 seems to

have acquired the ability to replicate itself in mammals, possibly as a result of transmission between ducks and pigs, which has been underscored by recent reports from Thailand of infection in cats and tigers that died after ingesting contaminated chicken (8–9).

Within this context, it has been stated that the majority of the recent cases of H5N1 infection are apparently related to direct exposure to infected birds or contact with surfaces contaminated with the excrement of infected birds. However, in February of 2004, it was hypothesized that human-to-human transmission had occurred. This was based on the identification of a case of avian influenza in a female patient who had not been directly exposed to infected birds but had had direct contact with a patient who died, probably due to the illness (9). The data from the epidemic of 1997 had been suggestive of human-to-human transmission. However, this type of transmission appears to result in a milder, self-limiting form of the illness (10).

In a complementary fashion, a case-control study evaluated fifteen patients hospitalized for H5N1 infection regarding activities related to the preparation or ingestion of chicken, contact with wild birds, travel or exposure to persons with influenza-like illnesses, and concluded that these do not constitute significant risk factors. Despite the fact that the results of the study identified exposure to live domestic fowl as a major risk factor for infection with H5N1, the exact mode of transmission remains unclear (11).

In order to estimate the risk of human-to-human transmission, a retrospective cohort study comparing the prevalence of the H5N1 antibody among health professionals exposed to patients infected with this virus to that found among professionals not so exposed, was conducted in the year 2000. Data were collected from infected patients regarding exposure to domestic fowl and blood samples were drawn in order to test for the H5N1 antibody. Of the 217 exposed health professionals, 8 (3.7 %) were seropositive for H5N1, as were 2 (0.7 %) of the 309 not so exposed ($p=0.01$). The difference remained significant after the stratification by exposure to domestic fowl ($p=0.01$). This study presented the first epidemiological evidence of human-to-human transmission of H5N1 (12).

Transmissibility and etiology

The etiologic agent of the avian influenza is an RNA virus belonging to the Orthomyxoviridae family, genus influenza A, which is found in various bird species, as well as in humans, pigs, horses and occasionally in other mammals. The type A viruses are divided into subtypes according to the antigenic nature of their hemagglutinin (HA) and neuraminidase. There are at least fifteen different subtypes of the HA antigen and nine neuraminidase antigen subtypes. All of these subtypes, in every possible combination, have been isolated in birds. However, in mammals, only a few subtypes of the virus are found (13). The new combinations, resulting from the genetic re-arrangement of the virus, facilitate dissemination of the illness in populations having had no previous contact with that subtype of the virus (12–15). Simultaneous infection of humans or pigs with avian and human influenza viruses can, theoretically, generate new viruses with pandemic potential as a result of genetic recombination between these viral subtypes. Such hybrid viruses may be capable of expressing surface antigens of avian viruses to which the human population has no immunity (16).

The HA antigen is the most important antigen because it is responsible for the hemagglutinating activity of the virus and for its adherence to susceptible cells. In addition, the antigens present in the HA and in the neuraminidase vary constantly, making immunological control of the illness. It should be borne in mind that the influenza virus is capable of genetic permutation, thereby altering its pathogenic characteristics. Therefore, samples presenting low to medium pathogenicity can become highly pathogenic and cause illness in humans (12, 16).

All H5N1 virus types possess a set of basic amino acids at the HA antigen cleavage site and are highly lethal to chicken, producing systemic infection. The HA antigen of the influenza virus is synthesized as a polypeptide and later cleaves to the HA1 and HA2 antigens using host proteins. The cleavage of HA is essential for the infectivity of the virus because this event mediates the fusion between the viral envelope and the endosomal membrane. However, the relevance of this mechanism in H5N1 infection is unclear (4).

The influenza virus is highly contagious and can be transmitted among birds in various ways: directly, through the exchange of respiratory or digestive system secretions between a sick animal and a healthy animal; and indirectly, from equipment, clothing, shoes, insects, birds, wild animals, food or water (17). The virus multiplies in the nasal epithelium or pharynx and then spreads to the mucous membranes of the respiratory system. It can disseminate throughout the organism and cause the systemic form of the illness. The clinical signs of avian influenza can vary depending on the species affected, age, gender, virulence of the virus, environment, accompanying infections and disease management. However, the most important determining factor of the symptom profile and pathogenicity of the virus (3, 17).

In an attempt to understand the molecular basis of viral adaptation in humans, sequences of the virus isolated in patients in Hong Kong were compared with those of the viruses isolated in chicken, ducks and geese obtained from the public market. Like the human viruses, the avian viruses contain multiple basic amino acids at the HA antigen cleavage site, but there is no differing amino acid that might suggest a viral basis for adaptation in humans (3, 13).

The reactive hemophagocytic syndrome is the most characteristic pathological finding and may be the principal factor contributing to the lymphopenia and hepatic dysfunction observed in patients with severe illness. It is known that this syndrome is mediated by cytokines. In two patients who later died from the illness, serum samples, collected in the first ten days of illness, were analyzed, and increases in the numbers of receptors for IL-2, IL-6 and INF-gamma were found (17).

The clinical and pathological findings lead to the postulation that, in patients with sever forms of the illness, the initial

viral replication in the respiratory tract can provoke a state of hypercytokinemia, leading to the reactive hemophagocytic syndrome, which is at least partially responsible for lymphopenia, as well as for varying degrees of pancytopenia, hepatic dysfunction and occasionally multiple organ failure (9, 17–18).

In dry environments with a temperature of 25 °C, the H5N1 is inactivated within one day. However, in humid feces, it is stable and has been found to present infectivity for up to four days. This suggests that infection requires contact with fresh contaminated feces. Even in chicken, the virus is only transmitted via the fecal-oral route and no aerosol transmission has been detected (19). The birds that survive the infection excrete the virus, orally and in their feces, for a minimum of ten days, thereby facilitating the dissemination of the pathogen in domestic fowl sold in markets, as well as in wild birds (13).

Clinical manifestations

The clinical profile associated with H5N1 infection range from asymptomatic infection and minimal involvement of the upper respiratory tract to severe pneumonia and multiple organ failure. Some cases of H5N1 infection are characterized by rapid clinical progression, with signs of involvement of the lower respiratory tract, to hospital admission, after which the disease rapidly evolved to the stage in which mechanical ventilation becomes necessary. Patients with severe H5N1 infection develop primary viral pneumonia, early-onset lymphopenia and renal failure within one to two weeks after the onset of symptoms. Elevated transaminase levels have been detected prior to respiratory deterioration in the majority of patients presenting the severe forms (7).

Fever was the presentation complaint of all of the patients. Initial symptoms also included headache, fatigue, myalgia, odynophagia, cough and rhinorrhea. Dyspnea was reported by the majority of patients, appearing from one to five days after the onset of symptoms. In the early stage of the illness, it is difficult to predict which patients will develop the severe form. Abdominal pain, vomiting, diarrhea, hepatic dysfunction, Reye's syndrome, pancytopenia, renal failure, pulmonary hemorrhage, acute respiratory distress syndrome and septic shock have been reported with varying frequency (7–8).

The dichotomy of age-dependent differences in presentation is the finding that merits attention. Except for one patient who had Reye's syndrome, patients below five years of age presented milder forms than did hospitalized adults. Explanations for this finding include differences in occupation or behavior, with the adults being exposed to higher viral loads. In addition, when the patients were children, medical treatment/hospitalization was typically sought sooner. Furthermore, the immunopathology was mediated by an incomplete, or nonprotective, cross-reaction, by direct immunomediated impairment or by antibodies, as has been proposed in hemorrhagic dengue fever (7–9).

Gastrointestinal symptoms such vomiting, diarrhea and abdominal pain were not typical in children infected with the H5N1 virus. However, their presence, with complications, in adult patients infected with this subtype is surprising. Hepatic dysfunction, in relation to previous epidemics, was rare: 0.1 % of hospitalized cases (19–20).

Autopsies of the victims of the illness revealed edema, hemorrhage and fibrin exudate in the lungs, as well as numerous interalveolar macrophages and CD3+ T cells in the pulmonary interstice, hyperplasia from type 2 pneumocystis, and marked expression of TNF-alfa in these cells, although no viral antigen was detected. In the hilar and peribronchial lymph nodes, reactive parafollicular histiocytosis, with hemophagocytosis, was observed. In the bone marrow, hypercellularity and reactive hemophagocytosis were observed. No significant clinical alterations were observed in other organs (17).

There is no known explanation for the fact that so few individuals among all of those apparently exposed to the virus developed infection. Neither is it known why the mortality rate among the infected individuals is so high. Genetic predisposition must be considered, as well as the possibility that a strain that is more virulent for humans might be found in a subtype from the population of domestic fowl.

Diagnosis

Records of documented cases indicate that a chest X-ray is essential in the initial examination. The chest X-ray may reveal bilateral interstitial infiltrate, lobar collapse, focal consolidation and air bronchogram with no pleural effusion. However, none of these alterations, either isolated or in conjunction, is specific to infection with influenza A (H5N1). The lymphocyte count is the most valuable parameter for the identification of patients with worse prognoses (9–10).

Radioimmunoassay, with reverse transcriptase-polymerase chain reaction specific for the H5 gene has been quite useful for rapid detection of the virus in samples collected from the respiratory tract (nasopharyngeal swab, endotracheal aspirate or bronchoalveolar lavage). Direct immunofluorescence with the monoclonal H5 antibody has been used to rule out infection with the H5 subtype. The neutralizing antibody is generally detected fourteen days or more after the onset of symptoms. Titers >640 of the neutralizing antibody have been observed both in adults and in children at twenty days or more after the onset of symptoms. The H5-specific immunoglobulins IgG and IgM have been detected in the majority of patients. The rapid diagnosis has proven to be cost-effective and to reduce the duration of hospital stays by allowing the early initiation of antiviral therapy and implementation of isolation measures, as well as the investigation of the history of contact (18, 21).

Influenza A (H5N1) can be easily cultured in cell lines routinely used for detection of the influenza virus. In contrast with the typical human strains (H1 – H3), the majority of H5N1 strains have a cytopathic effect that is detectable after four to five days of incubation (9).

Hospitalized patients should be tested for H5N1 infection when presenting the following: radiologically-confirmed pneumonia, acute respiratory distress syndrome (or other serious res-

piratory disease for which a diagnosis has yet to be established); and a history of travel to a country in which H5N1 infection has been documented in humans or birds within ten days prior to the onset of symptoms. Special cases should be monitored for infection if presenting the following: documented temperature > 38 °C; cough, odynophagia or dyspnea; and history of contact with domestic fowl or patients known or suspected to be infected with H5N1 within ten days of the onset of symptoms (8).

Treatment

Corticosteroid treatment (methylprednisolone, 5 mg/kg/d) and antiviral therapy (oseltamivir, 75 mg twice a day or ribavirin, 400 to 800 mg three times a day) in some patients infected in the outbreak of 1997, seemed inefficacious. Six of the seven patients receiving corticosteroids died, as did 80 % of the patients receiving oseltamivir. Some patients required mechanical ventilation, especially in the first two days of the illness. Maintenance treatment appears to be the only acceptable treatment. Controlled clinical trials are needed in order to evaluate the role of antivirals and corticosteroids in the management of the infection (8).

Broad-spectrum antibiotics should be used empirically (for example, for Streptococcus pneumoniae), including the possibility of superinfection with Staphylococcus aureus. The efficacy of antivirals and the period after which their use produces little or no benefit remain unknown (20–21).

Genetic sequencing of the H5N1 subtype, isolated in five patients in 2004, indicates that the virus has genetic characteristics associated with resistance to antiviral agents such as amantadine and rimantadine. Tests of H5N1 susceptibility to the neuraminidase inhibitor oseltamivir have demonstrated that the virus is sensitive to that drug (8).

Prognosis

Prognostic risk factors for developing the severe form of the illness include advanced age, delayed hospitalization, involvement of the lower respiratory tract, low total leukocyte counts and lymphopenia upon admission (21).

Epidemiological surveillance

In 2003, the World Health Organization developed the Global Agenda on Influenza Surveillance and Control, that defines and prioritizes activities designed to reduce morbidity and mortality secondary to the annual influenza epidemics. The agenda provides a basis for national and global intervention plans, facilitating the involvement of all countries in the prevention and control of these epidemics (22).

The principal aims of this standard are to provide impartial orientation regarding priorities in research, as well as in national and global interventions for the control of influenza and to promote the implementation of the interventions identified as priorities through technical legal and financial support. These interventions are intended to achieve several goals: rigorous and constant epidemiological surveillance; increased knowledge regarding the impact of the illness; the development of vaccines; and preparation to deal with the next epidemic (22).

One of the most significant characteristics of the various findings regarding the influenza virus is that its structure is constantly changing (a process known as “drift”), which results in the appearance of different circulating strains each year, thereby requiring that the influenza vaccine is modified annually. In certain years, the structure of these viruses changes drastically (a process known as “shift”), which results in the advent of a virus to which few individuals are immune. This new virus is easily transmitted from person to person and is capable of rapidly crossing geographic borders, characteristic of a pandemic (23).

When an epidemic occurs, epidemiological surveillance and the timely development of a vaccine, as well as the ability to produce the vaccine and administer that vaccine to a great number of people within a short period of time, is fundamental. The warning period preceding an epidemic is short, and, due to the fact that at least six months are needed to produce a vaccine, it is imperative that surveillance systems remain on alert for early detection of a possible pandemic (23).

One obstacle to the development of a vaccine is the high virulence of the agent, requiring that it be handled under stringent biosafety conditions. In a double-blind, randomized study, a recombinant vaccine was tested, thereby avoiding the need to handle the infectious agent. The vaccine was well tolerated, but the immune response provoked was suboptimal (24–25).

More recently, viruses obtained from two fatal cases of confirmed H5N1 infection were isolated in Vietnam and were used for analysis at the molecular level. Such studies will determine the antigenic and genetic characteristics of the virus, which is a necessary step in the production of a vaccine. The initial results allowed a vaccine to be produced in less than four weeks after the isolation of the virus. The removal of polybasic amino acids, which have been associated with the high pathogenicity of the virus, produces an attenuated agent, guaranteeing simplicity in preparation of manipulation (26–27). In the eventuality that H5N1 infection becomes pandemic in humans, vaccination, for the time being, will not be an option. Plasmid-based reverse genetics could be used, clinical trials are still needed in order to produce a vaccine by this method (28).

Conclusions

The recent epidemics caused by the avian influenza A virus in Asia, in particular those caused by the H5N1 subtype, have demonstrated the capacity of this agent to cause serious illness in humans, without any recombination between human and avian viruses or any intermediate mammalian host, such as the pig. This alerts us to the fact that any influenza A subtype can cross the interspecies barrier and become a latent pandemic strain. Human beings themselves can function as intermediate hosts, in which avian viruses recombine with human viruses. This can result in a virus with a new surface glycoprotein and a constellation of genes that facilitate the rapid transmission of the virus to susceptible populations.

We cannot rule out the possibility of mild or asymptomatic infection in persons exposed to infected birds or humans. There is no way to evaluate the importance of this "carrier" factor in the risk of transmission and appearance of new pandemics.

Within this context, efforts to control outbreaks in domestic fowl and contact between humans and such birds should be a priority in the management of the illness at the public health level.

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