AVIAN INFLUENZA A (H5N1) VIRUS: A BRIEF REVIEW

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ABSTRACT

Sudden emergence and re-emergence of new viral diseases in human beings has surprised the medical scientists from time to time. “Avian influenza” or “Bird flu” caused by H5N1 epidemics is one such surprise. Since their reemergence in 2003, highly pathogenic avian influenza A (H5N1) viruses have reached endemic levels among poultry in several Southeast Asian countries and have caused a still increasing number of more than 100 reported human infections with high mortality. These developments have ignited global fears of an imminent influenza pandemic. Scientific data to date showed some strains of avian influenza viruses including H5N1 are capable of going through mutations to develop into a novel, pandemic strain of influenza virus. This article reviews information about the morphology, genes and proteins, receptor specificity, epidemiology, transmission, clinical features, laboratory diagnosis, treatment and vaccination of human influenza H5N1 virus.
فإيروسات الإنفلونزا الطيرية A (H5N1): مراجعة مختصرة

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الخلاصة

إن الظهور المفاجئ وعودة الظهور للأمراض الفايروسية الجديدة في الكائنات البشرية قد فاجئ العلماء الطبيين من وقت إلى آخر. إن الإنفلونزا الطيرية الناتجة عن اوبئة H5N1 ظهرت في عام 2003، أن فايروسات الإنفلونزا الطيرية (H5N1) شديدة الإمارضة قد وصلت المستويات المستوطنة بين الدجاج في عدة بلدان جنوب شرق آسيا، مما يمثل إصابة بشرية مسجدة مع وفيات عالية. إن هذه التطورات قد أثارت مخاوف عالمية حول وباء الإنفلونزا وشيك، إن البيانات العلمية الحالية قد أظهرت بأن بعض سلالات فايروسات الإنفلونزا الطيرية ومنها H5N1 أظهرت على المضي بالتطور من أجل التطور إلى سلالة ووبائية جديدة من فايروس الإنفلونزا. إن هذه المقالة تستعرض معلومات حول الظهور، الجينات والبروتينات، نوعية المستقبلة، الوبائية، انتقال، المعالم السريرية، التشخيص المختبري، العلاج والتحقيقات الخاصة بفايروس الإنفلونزا H5N1.
INTRODUCTION

Avian influenza A (H5N1), a member of the Orthomyxoviridae family, is a common organism found in the digestive tracts of mainly aquatic wild birds worldwide. It has been described in chickens, ducks, turkeys, guinea fowl, geese, quails, pheasants, partridges, mynahs, passerine birds, psittacine birds, budgerigars, gulls, shorebirds, seabirds, and emus. It typically causes few, if any, symptoms in wild birds, as it preferentially replicates in their digestive tracts and is excreted in their feces (27). The presence of (H5N1) viruses in migratory birds suggested that migratory waterfowl maybe involved in spreading these viruses over a wide range (9, 40).

The increasing incidence of H5N1 in birds and the accompanying increase in opportunities for direct infection of humans pose the likelihood that humans concurrently infected with human and avian influenza strains could become “mixing vessels” for the emergence of a novel subtype with sufficient human genes to be transmitted easily from person to person. The pattern of disease appears to be changing in a manner consistent with this possibility. The changes in epidemiological patterns indicate that H5N1 viruses may be more infectious for humans, and sequencing analyses indicated that they were becoming more antigenically diverse and may be forming distinguishable groupings, based on phylogenetic analyses (38).

Morphology of avian influenza A (H5N1) viruses

Influenza virus particles are highly pleiomorphics, mostly spherical with 80-120 nm in diameter (72) figure(1). Present on its surface two distinct glycoproteins, the hemagglutinin (HA) and the neuraminidase (NA), enclosed in the phospholipidic envelope by sequences of hydrophobic amino acids localized close to ends COOH (HA), and NH2 (NA) (21,43). The HA and NA molecules range from 10 to 12 nm in length, the mean ratio of HA to NA is (5:1). The HA spikes are rod shaped whereas the NA spikes are mushroom shape with stalk (36). A third membrane protein, the M2 protein, is present in small quantities in influenza A viruses (19).
Figure (1): Particles of influenza virus, showing their pleomorphic shape. Bars represent 50 nm in length. a: two round particles, b: large particle stippled with end views of the surface spikes, c and d: disrupted particles (72).

Genes and proteins of avian influenza A (H5N1) viruses

Influenza A viruses contain eight genome segments which encode for 11 proteins. The nomination of influenza A genes is based on their size and is as follows: RNA segment 1 encodes for PB2, segment 2 for PB1 and PB1-F2, segment 3 for PA, segment 4 for HA, segment 5 for NP, segment 6 for NA, segment 7 for M1 and M2, and segment 8 for NS1 and NS2 (36) figure(2).
Segment 1, 2 and 3: The polymerase proteins
The RNA polymerase of the influenza virus comprises of three subunits, namely PB1 (Polymerase basic protein 1), PB2 (Polymerase basic protein 2), and PA. This heterotrimeric complex is responsible for transcription of the viral genomic RNAs into mRNAs and replication of the genomic RNAs (11,2,54).

Segment 4: Hemagglutinin
Hemagglutinin (HA) is a transmembrane glycoprotein that binds to the receptor sialic acid, and is the major surface antigen of virus. It is the main target for host immune system and changing in antigenicity of HA can make the virus escape from host immunity (52). HA has two major functions; first is to mediate the recognition of the virus to host cell through sialic acid containing receptor, and second is to mediate the fusion between viral membrane and host endosomal membrane to deliver the RNP into the cytoplasm (18).

Segment 5: Nucleoprotein
Nucleoprotein (NP) is one of the type specific antigens used to classify influenza A, B and C virus. NP is also the major target of cross-reactive cytotoxic T lymphocytes generated against all influenza subtypes in mice and humans. The major function of NP protein is encapsidation of the viral RNA segment to form RNP complex (33).

Segment 6: Neuraminidase
Neuraminidase(NA) is a transmembrane mushroom-shaped tetrameric protein ,containing a head domain that is enzymatically active and a stalk region which is attach to the membrane, anchored to the viral membrane by a single hydrophobic sequence of some 29 amino acids near the N-terminus (14) figure (3).

Figure (3): Schematic diagram of the neuraminidase molecule (14).
NA is important during the final stages of influenza virus infection, where it removes sialic acid from infected cell surfaces and newly formed virions, thus facilitating progeny virus release and spread of the infection to neighboring cells (39).

**Segment 7: Matrix**

Viral RNA segment 7 encodes for two matrix (M) proteins, M1 and M2 proteins. M1 forms dimer underneath the viral envelope and interacts with both RNP and the cytoplasmic tails of the surface glycoproteins to maintain the structure of the virion (23).

M2 protein is encoded by spliced mRNA, of which 51 nucleotide sequences are from M1 mRNA, follows by 689 nucleotide intron and a 271 nucleotides of body region. In the viral envelope, two disulfide-linked M2 dimer associated to form a tetramer that function as an ion channel which allows acidification of the endosome helping virus uncoating and releasing the nucleocapsid into the cytoplasm, this function is blocked by the antiviral drugs amantadine and rimantadine (29).

**Segment 8: Nonstructural**

Viral RNA segment 8 encode for non structural NS1 and NS2 proteins. NS1 protein of the human influenza A virus is the non-structural protein that has multiple roles in enhancing virus survival. It has been shown to inhibit the antiviral response of the host by suppressing production of type I IFNs (35). Contradicting its unfavorable effects on cellular protein synthesis, the NS1 protein selectively enhances the rate of translation initiation rate of viral mRNAs (22, 34). Differently, NS2 protein can be found in virion (51). Its function is to mediate the nuclear export of viral RNA by acting as an adaptor molecule between viral RNP complex and the nuclear export machinery of the infected cell (46).

**Binding of avian influenza A (H5N1) viruses to the host cells receptors**

The receptor specificity of avian and human influenza viruses differ; avian influenza A viruses bind preferentially to the N-acetylneuraminic acid-α 2,3-galactose linkages, which are found in avian intestinal and respiratory epithelium (42). Human influenza A viruses bind the N-acetylneuraminic acid-α 2, 6-galactose linkage on sialyoligosaccharides, that are found in human respiratory epithelium (66,30).

Sialic acid receptors with α 2, 3 receptors are found in human conjunctiva and nasal ciliated columnar epithelium, which explains why many human infections with avian influenza virus in the past have tended to manifest as conjunctivitis or upper respiratory infections (74). The current H5N1 avian influenza virus retains this 2, 3 linkage receptor preference but it has been theorized that only 1 or 2 mutations are needed to change that to a preference for a α 2, 6 linkage (68,56).
Respiratory epithelial cells in the pig contain both $\alpha_2, 3$ and $\alpha_2, 6$ linkages, which explains why this animal is susceptible to both human and avian influenza viruses (28). Because of this trait, pigs are widely regarded as a potential source of new pandemic strains, since they could serve as a non-selective host in which mixed infection of avian and human strains efficiently occurs, potentially resulting in new reassortant viruses, or in which purely avian strains can adapt to human receptor recognition (69).

**Epidemiology of avian influenza A (H5N1) viruses in humans**

The world’s first cases of human infection with the H5N1 strain were documented in May 1997, following outbreaks of influenza H5N1 among poultry on three farms in the New Territories of Hong Kong, when the H5N1 strain caused severe respiratory disease in 18 people, of whom six died (58). In November and December of the same year, concomitant with outbreaks of influenza H5N1 among chickens in poultry markets and on farms in Hong Kong, 17 additional cases of human (H5N1) infections were identified, 5 of which were fatal. The outbreak was contained after the slaughtering of all 1.5 million chickens in Hong Kong. Since that date, sporadic cases of avian influenza caused by various strains have been reported (8). Interspecies transmission occurred again in Hong Kong SAR in February 2003. Two human cases, one of which was fatal, were reported in a family that recently had traveled to southern China. Another child in the family had died during the visit (48).

Between December 30, 2003, and March 17, 2004, 12 confirmed human cases of (H5N1) were reported in Thailand and 23 in Vietnam, with a total of 23 deaths. Sporadic human cases were reported during August to October 2004 in Vietnam and Thailand. A total of nine cases, of which eight were fatal, were reported between August 1st and October 9th in Thailand (5) and Vietnam (4) (13).

On February 2, 2005, WHO reported the first case of Cambodian human infection with H5N1 in a 25 year old woman from Kampot Province in Cambodia. After developing respiratory symptoms in the latter part of January, she sought medical care in neighboring Vietnam, where she died in the Kien Giang Provincial Hospital on January 30 (38). By May 4, three other persons had died of confirmed H5N1 virus (10).

By June 28, 2005 a total of 108 cases (87 in Vietnam, 17 in Thailand, and 4 in Cambodia) and 54 deaths (38 in Vietnam, 12 in Thailand, and 4 in Cambodia) had been reported (38). As of 31 October 2006, ten countries, Azerbaijan, Cambodia, China, Djibouti, Egypt, Indonesia, Iraq, Thailand, Turkey and Vietnam had reported 256 cases of H5N1 virus in humans to the World Health Organization, One hundred fifty-two of these cases have died. Most human cases, if not all, were linked to direct exposure to dead or sick poultry (17).
Transmission of avian influenza A (H5N1) viruses to human

Transmission of (H5N1) viruses from animal to human
Influenza A is transmitted through the fecal-oral and respiratory routes among wild birds and poultry (60). 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 (5, 55).

Transmission of (H5N1) viruses from environment to human
Given the survival of influenza A (H5N1) in the environment, several other modes of transmission are theoretically possible (70). Plausible transmission routes include contact with virus-contaminated fomites or with fertilizer containing poultry feces, followed by self inoculation of the respiratory tract or inhalation of aerosolized infectious excreta. Oral ingestion of contaminated water during swimming and direct intranasal or conjunctival inoculation during exposure to water is other potential modes (55, 64).

Transmission of (H5N1) viruses from human to human
Human-to-human transmission of influenza A (H5N1) has been suggested in several household clusters (26). Most persons in case clusters probably acquired infection from common-source exposures to poultry, but limited, nonsustained human-to-human transmission has probably occurred during very close, unprotected contact with a severely ill patient (31, 50, 63).

Clinical features of H5N1 virus’s infection in humans

Incubation
The incubation period of avian influenza A (H5N1) may be longer than for other known human influenza. In 1997, most cases occurred within two to four days after exposure (47), other reports indicated similar intervals but with ranges of up to eight days (13). The case-to-case intervals in household clusters have generally been 2 to 5 days, but the upper limit has been 8 to 17 days, possibly owing to unrecognized exposure to infected animals or environmental sources (73).

Initial symptoms
Most patients have initial symptoms of high fever (typically a temperature of more than 38 C°) and an influenza-like illness with lower respiratory tract symptoms, upper respiratory tract symptoms are present only sometimes (7). Diarrhea, vomiting, abdominal pain, pleuritic pain, and bleeding from the nose and gums have also been reported early in the course of illness in some patients (1,67).
Clinical course

Avian influenza A infections in humans differ from seasonal influenza in several ways. The presence of conjunctivitis is more common with avian influenza A infections than with seasonal influenza. Gastrointestinal symptoms, as seen with HPAI (H5N1), reports of primary influenza pneumonia, development of acute respiratory distress syndrome (ARDS), tachypnea, and inspiratory crackles are also more common with avian influenza A infections. Sputum production is variable and sometimes bloody (65,15).

Multiorgan failure with signs of renal dysfunction and sometimes cardiac compromise, including cardiac dilatation and supraventricular tachyarrhythmias, has been common (8). Other complications have included ventilator-associated pneumonia, pulmonary hemorrhage, pneumothorax, pancytopenia, Reye’s syndrome, and sepsis syndrome without documented bacteremia(62). Finally, the rapid progression to multiorgan failure and eventually death occurs at a much higher rate with avian influenza A infections (24).

Host immune responses:

The innate immune responses to influenza A (H5N1) may contribute to disease pathogenesis. In the 1997 outbreaks, elevated blood levels of interleukin-6, TNF \( \alpha \), interferon \( \gamma \) and soluble interleukin-2 receptor were observed in individual patients (12) and in the patients in 2003, elevated levels of the chemokines interferon-inducible protein 10, monocyte chemoattractant protein 1, and monokine induced by interferon \( \gamma \) were found three to eight days after the onset of illness (48,25).

Plasma levels of inflammatory mediators (interleukin-6, interleukin-8, interleukin-1 \( \beta \), and monocyte chemoattractant protein 1) were found to be higher among patients who died than among those who survived, and the average levels of plasma interferon \( \alpha \) were about three times as high among patients with avian influenza A who died as among healthy controls. Such responses may be responsible in part for the sepsis syndrome, ARDS, and multiorgan failure observed in many patients (16).

Tissue damage in human influenza A (H5N1) disease probably results from the combined effects of unrestrained viral infection and inflammatory responses induced by influenza A (H5N1) infection. Among survivors, specific humoral immune responses to influenza A (H5N1) are detectable by microneutralization assay 10 to 14 days after the onset of illness. Corticosteroid use may delay or blunt these responses (61).

Fatality

The fatality rate among hospitalized patients has been high (4). In contrast to 1997, when most deaths occurred among patients older than 13 years of age, recent avian influenza A (H5N1) infections have caused high rates of death among infants and young children (13). The case fatality rate was 89 percent among those younger than 15 years of age in Thailand. Death has occurred an average of 9 or 10 days after the onset of illness, and most patients have died due to progressive respiratory failure (24).
Laboratory diagnosis of avian influenza A (H5N1) viruses:

Virus isolation

Similar to human influenza viruses, avian viruses can be isolated in embryonated eggs or in cell culture, using permissive cells such as Madin Darby canine kidney (MDCK) cells or rhesus monkey kidney (LLC-MK2) cells (20). For safety purposes, the isolation of highly pathogenic avian influenza virus requires biosafety level 3 laboratory facilities or higher (65). In human infections, avian influenza viruses have mostly been isolated from conjunctival swabs and respiratory specimens such as throat or nasal secretions or washings. In one reported case of (H5N1) infection, virus was also isolated from serum, cerebrospinal fluid, and a rectal swab (15).

Antigen detection

Detection of influenza A viral antigens in clinical specimens by direct immunofluorescence or by rapid immunochromatographic assays are widely used for diagnosis of human influenza because of their ability for rapid diagnosis (32, 5). However, in patients with avian influenza, the usefulness of these assays seems limited due to low sensitivity (48). In addition, some rapid antigen detection kits do not distinguish between influenza types A and B, and none of the currently available immunofluorescent and immunochromatographic assays distinguish between influenza A subtypes. However, developments of H5N1-specific rapid antigen detection tests are ongoing (6).

Reverse transcriptase PCR (RT-PCR)

Reverse transcriptase PCR (RT-PCR) methods allow for sensitive and specific detection of viral nucleic acids and have shown to increase the diagnostic sensitivity for many viral pathogens when compared to culture or antigen detection methods, Especially when using RT-PCR technology, a reliable subtype-specific diagnostic result can be generated within a few hours after specimen collection (68,57).

Serology

During outbreaks of avian influenza, the detection of subtype-specific antibodies is particularly important for epidemiological investigations. Hemagglutination inhibition (HI) assays are the gold standard for detection of antibodies against human influenza viruses (3). However, their usefulness for detection of antibodies against avian viruses in mammalian species, including humans, seems limited (71). Several studies have shown a failure to detect HI antibodies against avian viruses in mammals, even in cases where infection was confirmed by virus isolation. Possible reasons for this failure include poor immunogenicity of some avian viruses and lack of sensitivity to detect low tittered or less avid antibodies induced by avian viruses (53).
Treatment
Currently, two classes of drugs are available with antiviral activity against influenza viruses: inhibitors of the ion channel activity of the M2 membrane protein, amantadine and rimantadine, and inhibitors of the neuraminidase, oseltamivir, and zanamivir (45,44) figure(4). These two classes of drugs have different mechanisms of action on the replication cycle of the virus. Amantadine and rimantadine inhibit virus replication during the early stage of infection by blocking the ion channel formed by the transmembrane domain of the M2 protein (49), and NA inhibitors interrupt the established replication cycle by preventing virus release and allowing virus to clump on the cell surface (41).

Vaccination
The bulk of human influenza vaccines are produced from inactivated viruses grown in embryonated eggs. Vaccine production against highly pathogenic avian influenza viruses is complicated because of the requirement for high biosafety containment facilities, and the difficulty, in some cases, to obtain high virus yields in embryonated eggs because of the virus’ pathogenicity (59). Several other approaches have been used in an attempt to overcome these obstacles, including the use of reverse genetics techniques, generation of recombinant hemagglutinin, DNA vaccination and the use of related a pathogenic H5 viruses with and without different adjuvant (37).
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