HIGHLY PATHOGENIC INFLUENZA A VIRUS (H5N1)

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SUMMARY: Highly pathogenic avian influenza H5N1 previously known to infect only birds was also found to infect human, causing disease and death. Continuous outbreaks of the highly pathogenic H5N1 avian influenza A resulted in an urgent effort to improve treatments, vaccines, and diagnosis to lower the threat of an influenza pandemic. Control measures and continuous surveillance aimed at reducing exposure of human to potential H5N1-infected poultry. Pandemic human influenza viruses have been emerging for many centuries, thrice only in last century. These pandemics were caused by the most common subtypes of circulating viruses in the community at that time. The influenza virus has segmented genome, which undergoes continuous mutations and genetic reassortments. Phylogenetic analyses of the H5N1 viruses isolated from humans showed that these viruses were identical to those circulating in poultry. Any genetic change in H5N1 enabling human to human transmission may lead to a pandemic of human influenza.

Key Words: H5N1, Avian influenza A

INTRODUCTION

Influenza virus infection is considered as a worldwide major public health problem. During the past 90 years, three influenza pandemics occurred. The great pandemic is known as the "Spanish flu" H1N1 occurred during 1918 and 1919 years, killing more than 50 million people. Sequence data of Spanish H1N1 virus genes suggest that this virus emerged from an avian reservoir (1). The first human influenza virus occured in 1933. The second and less virulent influenza pandemic called "Asian flu" H2N2 that occurred later in 1957 and 1958, was first identified in China. The most recent pandemic occurred 10 years later in 1968 to 1969 in the Far East and was known as "Hong Kong flu" H3N2. The latter two pandemics were caused by viruses containing combinations of genes from both human influenza and avian influenza viruses.

In May 1997, the first H5N1 virus was isolated from a 3-year-old boy who died of extensive influenza pneumonia complicated by Reye's syndrome in Hong Kong (2). By the end of 1997, additional 18 cases were reported infected with the avian H5N1 virus and a total of six deaths were confirmed (2). Molecular and antigenic analyses studies of H5N1 viruses isolated from humans showed that these viruses were identical to those circulating in poultry (3-4), indicating direct infection of humans by an avian virus.

Influenza A viruses are of great public concern, because they can undergo gene reassortment or what is called antigenic shift, in which a pandemic virus can

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result from this process during co-infection. This could lead to formation of new combinations of (H) and/or (N) proteins on the surface of virus particle. In addition, point mutations in that genes cause antigenic drift which leads to appearance of new variants. In 2005, World Health Organization pronounced that the world is moving close to another influenza pandemic. The next pandemic is expected to cause clinical disease in 2 billion people. If we translate the death toll associated with the 1918 influenza virus to the current population, there could be 180 million to 360 million deaths globally (6).

Here we review current knowledge on the biological characteristics of influenza A virus, pathology in birds, human and clinical features, pathogenecity, vaccination, immunology, diagnosis, and treatment.

Biological characteristics of influenza A virus

True influenza is caused by small family of the Orthomyxoviridae, which includes the genera of Influenza viruses A, B and C as distinguished serologically on the basis of their matrix (M1) and nucleoprotein (NP). Of these, influenza pandemics are associated with type A and can be categorized into subtypes on the basis of surface antigens: Hemagglutinin (H) and neuraminidase (N). Typical virions are spherical, 100-200nm in diameter, and helical symmetry of nucleocapsid with eight segments of the negative-sense ssRNA of approximately 9 kb in size. Most transcribed mRNAs are monocistronic which encode 10 proteins. The RNA genome is complexed with nucleoprotein (NP) and three subunits of RNA polymerase complex (PA, PB1, and PB2) which is essential for replication process, to form together ribonucleoprotein (RNP) complex. The envelope is lined on the inside by matrix protein (M1) and is spanned by a small number of ion channels M2 proteins. Nonstructural (NS2) protein is thought to play a role in the export of RNP from the nucleus through interaction with M1 protein, while other nonstructural (NS1) protein which has multiple functions, including regulation of splicing and nuclear export of cellular mRNAs, stimulation of translation as well as its major function appears to counteract the interferon activity of the host. There are two types of spikes on the surface of the virus included (H) and (N) molecules. These molecules play an important role in attachment, fusion, and entry to the host cell, release new viruses from infected host cell to determine virulence. Both molecules are considered the major antigenic determinants to which neutralizing antibodies are directed. Influenza A viruses that have been identified in animals including humans are divided into 16 HA subtypes and 9 NA subtypes depending on the antigenicity of these molecules (7). As known, all 16H and 9N subtypes recognized have been currently recorded in birds, while in mammals the number of subtypes present appears to be limited. The most common subtypes that can infect humans and cause serious outbreaks are H1N1, H2N2, and H3N2. It was reported that other subtypes such as H5N1, H7N2, H7N3, H7N7, and H9N2 can also infect humans. The specific influenza strain isolates are identified by a standard nomenclature specifying virus type, host of origin (excluding humans), geographical location where first isolated, sequential number of isolation, year of isolation and antigenic description (subtype) of the (H) and (N) are given last, in parentheses [A/Hong Kong/1/68 (H3N2), A/Hong Kong/156/97 (H5N1), A/Swine/Taiwan/6/70 (H3N2), A/Equine/Prague/ 1/56 (H7N7)].

Virus replication starts after binding to scialic acid-containing receptors on the membrane of host cell. Receptor-bound viruses are taken into the cell by clathrin-dependent and independent mechanisms. A high [H+] in the endosome induces conformational changes in HA molecules, resulting in membrane fusion between the viral envelope and the endosomal membrane. The M2 proton channel exposes the viral core to low pH, resulting in dissociation of M1 from RNP and leading to a release of RNP to the cytoplasm, from where it is then transported to the nucleus. The 5/cap from cellular mRNAs is cleaved by a viral endonuclease and used as a primer for transcription by the viral transcriptase. Six of eight RNA segments are transcribed into mRNAs in a monocistronic manner and translated into HA, NA, NP, PB1, PB2, and PA, while each of other two RNA segments are transcribed to two mRNAs by splicing. For both the M and NS genes, these mRNAs are translated in different reading frames, generating M1, M2, NS1 and NS2 proteins, respectively. New viral RNA is encased in the capsid protein, and together with new matrix protein is then transported to sites at the cell surface, haemagglutinin and neuraminadase components have been incorporated into the cell membrane. Progeny virions are formed and released by budding.

It has been shown that human influenza A strains demonstrate a higher binding affinity toward α 2,6 linkage scialic acid-galactose receptors which are predominant in human respiratory epithelia cells. In contrast influenza A strains isolated from avian and equine preferentially bind to α 2,3 linkage scialic acid-galactose receptors. Recently it has been shown that ciliated cells in human respiratory epithelia cells express α 2,3 linkage scialic acid-galactose receptors in sufficient density to allow entry of replication of avian viruses (8). Swine is considered as an intermediate hosts, possess both receptors on their respiratory epithelial cells, and may have been the mixing vessels for genetic reassortment and passage the virus in these animals may well adapt an avian virus to the infection of humans (9). This concept was challenged in 1997 when avian influenza H5N1 virus was demonstrated to be transmitted directly from domestic poultry to humans and raising the concern that chickens may also act as an intermediate host. Other animals might play a role in the transmission of avian viruses to humans (10-11). Studies suggest that the quail is highly susceptible to influenza viruses, including the H5N1 and H9N2 subtypes (12-13). These studies emphasize the role of terrestrial birds especially quail, in generating viruses with pandemic potential and infected birds pass on H5N1 through their saliva, nasal secretions, and feces. Other recent study showed that blow flies could be a mechanical transmitter of H5N1 influenza virus (14).

Waterfowls have showed a very efficient way to transmit viruses through fecal material although the digestive tract is not the main site of H5N1 influenza virus replication in ducks and that the fecal-oral route may no longer be the main transmission path, viruses replicated to higher levels in the trachea than in the cloaca of both inoculated and contact birds (15). These birds are known to carry viruses over great distances. Domestic waterfowls therefore easily contact with both wild birds and domestic animals, and therefore can function as intermediate host to transmit the avian influenza viruses from wild birds to other hosts (13). Recent studies showed that H5N1 virus samples have become progressively more pathogenic for poultry after circulating for short period of time (16-18). Now several different genotypes of the H5N1 lineage have been described (19). The genotype Z is the dominant H5N1 virus and is associated with high pathogenicity in zoo and laboratory mammals including tigers, leopards, domestic cats, mice, cynomolgus monkeys, and ferrets.

Pathology in birds

Outbreaks of highly pathogenic avian influenza can be catastrophic for farmers and for the poultry industry especially in developing countries. Since 2003, H5N1 has had a devastating impact on domestic or wildbirds in many parts of South East Asia, Europe, Russia, the Middle East and in some parts of Africa. In birds, the spectrum of disease ranges from asymptomatic infection, mild to severe and rapidly fatal systemic disease. In the mild form, signs of illness may be expressed only as ruffled feathers, reduced egg production, or mild effects on the respiratory system. In contrast, the second severe and rapidly fatal systemic form is characterized by sudden onset of severe disease, rapid contagion, and a mortality rate that can approach 100% within 2 days. In this form of the disease, there is a progressive decline in water and food consumption, a cessation of egg production is apparent, individual birds affected by highly pathogenic strains often reveal little more than severe apathy and immobility, swollen head and face, cyanosis of unfeathered skin, watery diarrhea that begins as bright green and progresses to white, respiratory signs are dependent on tracheal involvement, nasal and ocular discharge, mucus accumulation, sinusitis, also invades multiple organs and tissues, resulting massive internal hemorrhage (18,20). The gross and histological changes are quite similar in chickens inoculated experimentally with highly pathogenic viruses. Variations among reported cases may reflect differences in experimental conditions, the route of inoculation, the breed and age of the chickens, the dose of virus, strain variation and accompanying bacterial infections. But in general, involvement of the cardiovascular system plays an important role in the pathogenesis of avian influenza virus infection. Viral antigens can be detected in tissues with necrotic and inflammatory changes included heart, spleen and brain (21,22). Ducks developed acute disease, including severe neurological dysfunction and death (16).

Human and influenza A virus subtype H5N1- clinical features

In recent years, it has become clear that human infections with highly pathogenic influenza H5N1 viruses

are associated with severe, often fatal disease (23). Increasing and continuing occurrences of bird-to-human transmissions increase the opportunity of the virus to adapt to humans and acquire the ability to spread among humans. The virus can improve its transmissibility among humans via reassortment and/or adaptive mutation.

The modes of transmission of influenza A virus to human can be either direct or indirect, these include contact with infectious aerosol and nuclei, virus-contaminated materials, self-inoculation of the upper respiratory tract or conjunctival mucosa. For human influenza A (H5N1) infections, evidence is consistent with bird-to-human, possibly environment-to-human, and limited, nonsustained human-to-human transmission to date. Highly pathogenic viruses can survive for long periods in the environment, especially when temperatures are low.

The clinical course in human is influenced by the patient's age, the degree of pre-existing immunity, properties of the virus, immunosuppression, and pregnancy. The spectrum of clinical outcome varies from asymptomatic to mild upper respiratory illness to severe pneumonia and death. The incubation period is 2 to 4 days in most cases. Current data for H5N1 infection indicate an incubation period ranging from 2 to 8 days and possibly as long as 17 days (18). The patient's illness typically lasts 5 to 24 days. At time, most cases of human H5N1 infections were characterized by a severe influenza syndrome, clinically indistinguishable from severe human influenza, with symptoms of fever, asthenia, watery nasal discharge, nonproductive cough and shortness of breath, malaise, sore throat, chest crackles, and radiological evidence of pneumonia, watery diarrhea without blood appears to be more common in H5N1 avian influenza than in normal seasonal influenza, vomiting, abdominal pain, chest pain and bleeding from the nose and gums have also been reported as early symptoms in some patients (18,24-28). Abnormalities on chest radiographs included extensive, usually bilateral infiltration, lobar collapse, focal consolidation, and bulla formation. Radiological evidence of pulmonary damage could still be observed in surviving patients several months after the illness (18,24,28). Beside respiratory symptoms, patients with avian influenza A (H5N1) rarely have conjunctivitis, unlike human cases of infection by the H7 viruses. In some cases, an encephalopathic illness and diarrhea without apparent respiratory symptoms have been reported. The clinical course of the illness in severe cases was characterized by rapid development of severe bilateral pneumonia necessitating ventilatory support within days after onset. Blood examination showed mild to moderate lymphopenia and some had mild to moderate thrombocytopenia and slightly or moderately elevated aminotransferase levels, some instances, disseminated intravascular coagulation, and elevated creatinine levels also occur (18,24). Complications included acute respiratory distress syndrome (ARDS) multiorgan failure causing a high fatality rate and associated with cytokine imbalance. Deaths occur at an average of 9 or 10 days after the onset of illness (range, 6 to 30).

As of February 16, 2007, 274 laboratory confirmed human cases of H5N1 infections with 60.95% of these cases have been fatal (167/274) were reported from 11 countries viz., 93 from Vietnam (42 deaths), 81 from Indonesia (63 deaths), 25 from Thailand (17 deaths), 22 from China (14 deaths), 12 from Turkey (4 deaths), 6 Cambodia (6 deaths), 3 from Iraq (2 deaths), 8 from Azerbaijan (5 deaths), 22 from Egypt (13 deaths), 1 from Neigeria (1 death), and 1 from Djibouti (0 death) (29).

Pathogenecity

Influenza A virus subtype H5N1 is of particular interest because of its increasing pathogenicity and ability to form a new subtype to which there is no native immunity in human hosts. Because the number of well-characterized human cases is too small, studies of influenza A virus subtype H5N1 in different animal models have been useful to certain degree in elucidating the pathogenesis of infection in humans. The pathogenicity of Influenza A virus H5N1 has been studied extensively in different mammalian models, such as mice (22,30), ferrets (22,31-32), pigs (5,9), and primates (33) but the results obtained from these studies are conflicting. The H5N1 isolates are categorized as viruses that either of high pathogenicity and replicate systemically or extrapulmonary sites including the brain, these particles showed neurotopism or that of low pathogenicity and were restricted to the respiratory tract of the mice (31,34). Differences in influenza A virus H5N1 subtype virulence, data suggest that virulence is likely to be polygenic. Studies conducted on influenza A virus H5N1 subtype showed that multiple different amino acid

mutations seem to be associated with virulence, but the exact mechanisms for the emergence of virulent viruses from avirulent viruses are not completely understood. Although it is certain that the acquisition of HA cleavability, together with molecular changes in other viral genes (NA, M1, PB1, PB2, PA, NP and NS1), are among the factors seem to be associated with virulence. Virulent and avirulent avian viruses differ in amino acid sequence at the HA cleavage site, localized or systemic infection depends on the amino acid sequence at the cleavage site of the precursor hemagglutinin. It has also been shown that the non-structural (NS) gene of H5N1 viruses can increase the pathogenecity by conferring the resistance to the antiviral effects of interferons and tumour necrosis factor alpha (34,37).

Finally, USDA-APHIS defines an influenza A virus as highly pathogenic if it meets one of the following criteria: first: kills at least 75% experimentally inoculated susceptible four to six week old chickens; second: any H5 or H7 subtype that kills less than 75% experimentally inoculated chickens, but has an amino acid sequence at the HA cleavage site that is compatible with high pathogenic influenza A viruses; third: any other HA subtype (neither H5 nor H7) which kills 12.5-75% experimentally inoculated chickens and grows in cell culture in the absence of trypsin (37).

Immunology

Immunity to Influenza A virus is mediated by both humoral and cell-mediated immune responses. Influenza infection results in the production of antibody mainly to influenza surface glycoproteins HA and NA. Immunoglobulins, including IgM, IgA and IgG, appear within 2 weeks of inoculation and the peak of antibody titers have been seen between 4-7 weeks after infection. Antibodies remain detectable for years after infection even without re-infection. Antibodies are produced against the surface viral proteins HA, NA play the main role for protection and called neutralizing antibodies. The mucosal immune response against influenza, as measured in nasal secretions, is characterized by the presence of IgA and IgG1 against HA. Either mucosal or systemic antibody alone can be protective if present in sufficient concentrations, and optimal protection occurs when both serum and nasal antibodies are present (38). Antibodies act in immunity against influenza by neutralization of the virus or lysis of infected cells via complement or antibody-dependent cellular toxicity. NS1 mutant influenza A virus strains, which could not replicate in murine lungs, were capable of inducing Th1-type immune response resulting in significant titers of virus-specific serum and mucosal IgG2 and IgA, but with lower titers of IgG1. In contrast, the strains which could replicate showed high titers of serum and mucosal IgG1 but less serum IgA (39). In addition to antibody-mediated immunity, cytotoxic T-cell responses directed against conserved core proteins such as the matrix (M) and nucleoprotein (NP) play a significant role in recovery from illness and viral clearance and a minor role in protection against illness. Humans have no pre-existing immunity against influenza A (H5N1). During influenza infection, both $CD4^+$ and $CD8^+$ memory T cell subsets respond to, and mediate control of an influenza virus re-infection, which is in contrast to the primary infection where viral clearance depends on $CD8^+$ T lymphocytes (40). There is evidence in favor of a site specific accumulation of influenza-specific $CD8^+$ memory T cells in human lungs for the immediate immunological protection against. Mice infected with lethal H5N1 virus showed lymphopenia ($CD4^+$ and $CD8^+$ T cells) (41-42). The possible mechanisms behind lymphopenia induction with influenza A viruses, the potential induction of apoptosis of lymphocytes by this virus and this may be playing an important role in the disease aggression. Both HA and NA stimulate T cell-mediated immunity. Whereas the cytotoxic T lymphocyte response can reduce viral shedding of low pathogenic influenza A viruses (43). The innate immunity to this virus may contribute to disease pathogenesis. It included increasing levels of different cytokines (23,44-45). H5N1 influenza A virus has developed strategies for evading innate immunity by the viral NS1 polypeptide acts as an antagonist of interferon induction in infected cells. Studies of the H5N1_97 viruses proposed that the NS gene contributes to the high TNF-α-inducing phenotype of these viruses (46). But production of interferon also appears to contribute to protection from influenza, particularly during the early stages of infection.

H5N1 influenza A diagnosis

Samples taken from dead birds should include intestinal contents or cloacal swabs and oropharyngeal swabs.

Samples from trachea, lungs, air sacs, intestine, spleen, kidney, brain, liver and heart may also be collected and processed either separately or as a pool. Samples from live birds should include both tracheal and cloacal swabs although swabs of the latter site are the most likely to yield virus. Collection of fresh faeces from small delicate birds may serve as an adequate alternative.

In human patients, nasopharyngeal aspirate or bronchoalveolar lavage followed by nasopharyngeal swab or throat swab placed in isotonic phosphate buffered saline containing antibiotic and should be collected with airborne precautions in patients suspected to have this infection. A stool or rectal swab placed in viral transport medium should also be considered in suspected cases presenting with diarrhea. At autopsy, carried out under safe conditions and avoiding spread of disease, unpreserved specimens of brain, trachea, lung, spleen and intestinal contents are collected for isolation of the virus. Serum and cerebrospinal fluid should be collected in those with encephalitis. Avian influenza A (H5N1) infection may be associated with a higher frequency of virus detection and higher viral RNA levels in pharyngeal than in nasal samples.

Classical diagnosis involves virus isolation in embryonating chicken eggs or tissue culture including Madin-Darby canine kidney (MDCK) cells, mink lung epithelial cell line (Mv1Lu), primary monkey kidney cells or St. Jude porcine lung cells (SJPL), subsequent hemagglutinin and neuraminidase subtyping by serological and/or molecular techniques considered the gold standard for the definitive diagnosis of this disease (47). Although virus isolation using embryonating eggs or tissue culture is a sensitive method, it is tedious and time consuming to obtain results. Virus can be cultured from the serum, cerebrospinal fluid, and stool in addition to the respiratory secretions.

More rapid antigen detection methodologies have been developed and evaluated. Several immunofluorescence assays (IFA), optical immunoassays, and enzyme-linked immunosorbant assays (ELISA), have been applied for rapid detection of influenza virus. However, these assays lack the sensitivity required for the rapid detection of these viral pathogens. Moreover, specimen integrity and the presence of sufficient numbers of intact cells in the specimen are crucial for reliable direct immunofluorescence assay results. The results of microneutralization or ELISA confirmation by Western blot gave a high degree of sensitivity and specificity. A modified single radial haemolysis offers an alternative serological technique for detecting antibody to H5 haemagglutinins, this developed technique does not depend on use of live pathogenic H5N1 virus (48).

Rapid and sensitive molecular diagnostic techniques of reverse transcriptase PCR for the detection of influenza viruses have been developed and evaluated (49). An efficient, simple, specific and sensitive one-step reverse-transcription PCR assay expected to make it very useful for the clinical diagnosis of influenza A H5N1 isolates (50-51). The increased usage of PCR technology in the detection of influenza type A has also been stimulated by the advent of real-time PCR (52-53) and single step multiplex real-time PCR assay (54). These techniques provide a rapid, specific, more sensitive than the traditional techniques, less expensive than virus isolation, do not require viable virus for detection or the presence of intact, infected cells within the specimen, and can detect a broad range of influenza A subtypes, including H5N1. Clinically, the rapid and accurate diagnosis of influenza A is important to improve patient management. This can limit unnecessary antibiotic usage, help in the prevention of spread of virus, and also may enable the timely implementation of appropriate antiviral treatment. Diagnosis of influenza A (H5N1) has been confirmed by either one or more of the following tests: viral isolation, detection of H5-specific RNA, and immunofluorescence test for antigen with the usage of monoclonal antibody against H5 (24,55). Recently a unique gene amplification method is being used to detect H5N1 called reverse transcriptase loop-mediated isothermal amplification (RT-LAMP), it is a rapid and sensitive method and considered as a useful diagnostic tool for surveillance of recent outbreaks of H5N1 (56).

Molecular techniques such as microarray-based method, simple restriction fragment length polymorphism (RFLP) and a combined reverse transcription (RT)-PCR/heteroduplex mobility assay (HMA) were designed to differentiate between different subtypes of influenza A viruses (57-58). These techniques are effective and sufficiantly sensitive to detect unusual influenza viruses.

Treatment

Vaccines and antiviral agents are the most promising for controlling a potential influenza epidemic. Currently, there are two classes of antiviral drugs that have different mechanisms of action on the life cycle of the

virus: the adamantane derivatives and the neuraminidase inhibitors.

The adamantane derivatives, amantadine and rimantadine, which are inhibit the early stages of viral replication by blocking the ion channel formed by the M2 protein. These drugs have been shown to be therapeutically and prophylactically effective against human influenza A viruses, but have limited utility because of their side effects, rapid emergence of resistant mutants, and ineffectiveness against influenza B virus infection (59). Viruses become resistant to amantadine through a single amino acid substitution at position 26, 27, 30, 31, or 34 in M2 protein (60). Resistant variants vary among the countries recently reported, more than 95% of the viruses isolated in Vietnam and Thailand contained resistance mutations, but were less commonly isolated 8.9%, 6.3% from China and Indonesia, respectively (61). The emergence of adamantane derivatives resistance makes the development of new anti-influenza molecules a priority; investigators have targeted neuraminidase in the design of new antiviral drugs. These inhibitors are zanamivir, oseltamivir, and peramivir. The efficacy of Zanamivir has been demonstrated in cell culture, animal models, and experimentally infected human volunteers. The results indicate that neuraminidase inhibitors may be used as prophylactic agents (62). Neuraminidase inhibitors are potent and specific against all subtypes of influenza A and B viruses, and have a better safety profile. Unlike M2 blockers, they appear to be associated with a lower emergence of resistant viruses during treatment (63). The neuraminidase substitutions in drug-resistant viruses include amino acid residues 116, 117, 119, 152, 274, 292, and 294 (64-66). Recently, resistant viral variants have been detected in up to 25% of patients who receive oseltamivir (27,64,67). These oseltamivir-resistant mutants showed reduced infectivity and virulence with low risk of transmission in a ferret model.

Recently, a new class called nucleic acid-based antiviral drugs in development that could be used in antiviral prevention and treatment. These antiviral agents are versatile in their mode of action in that they can be designed to elicit broad-spectrum antiviral immunity, interfere with viral replication, suppress viral gene expression or cleave viral mRNAs (68-69).

A few studies demonstrated that the combination of two drugs produced synergistic or additive effects *in vitro* and *in vivo* reduced the emergence of drug-resistant influenza variants (70). This strategy could be an option for the control of influenza virus infection. Chemoprophylaxis has been recommended for health care workers or other personnel who might have been exposed to the highly pathogenic avian influenza viruses. The antiviral therapy should be started as soon as possible within 48 hours following symptom onset to maximize its therapeutic benefits, without waiting for laboratory reports (59).

Vaccine

Vaccination is considered the most-effective preventive measure to control influenza epidemic or pandemic. Inactivated vaccines are the main stream of influenza prophylaxis. Currently two types of influenza vaccines are licensed: trivalent inactivated influenza vaccine and live attenuated influenza vaccine (71). Currently no vaccine is available to protect humans against avian influenza H5N1, but production of an effective vaccine for influenza virus A H5N1 is in progress. Several types of vaccines against influenza A virus H5N1 prepared by different strategies are in evaluation, including inactivated vaccines, DNA vaccines, vaccines based on reverse genetics, viral-vectored based vaccine, RNA interference vaccine, M2 based universal vaccine and liposome-based vaccine technology (72-75). Traditional strategies, as yet, have failed to produce a suitable immunogenic vaccine against the high pathogenic H5N1 viruses. Inactivated homologous vaccines induce proper protection but can not differentiate serologically between vaccinees and infected animals. However, inactivated heterologous vaccines, vaccinees and infected animals can be distinguished easily. Reverse genetics will greatly aid in producing vaccines both for veterinary and medical use with the desired hemagglutinin and neuraminidase combinations in a favourable genetic background using (PR8) virus (76-77). Several candidate vaccines produced by reverse genetic are under study however not yet available in commercialised form. These have been shown to be safe and effective in animal models (78). Approaches to the vaccines devolepments with dual specificity against influenza A viruses and Newcastle disease virus using a single immunization or construction a chimeric avian influenza A virus using a reverse genetics have been reported (79-80). Recombinant plasmids or fowlpox virus expressing

HA proteins of H7 and H5 subtypes have also shown to confer protection against highly pathogenic influenza A viruses of both subtypes (81-82). DNA vaccine is highly immunogenic and can provide protection from lethal H5N1 infection in animals (83), while other results showed ineffective results (84). The available evidence indicates that the immunogenicity of HA is the most important factor in protecting against human infection. Live attenuated vaccines may be useful as they induce humoral, cellular, and mucosal immune responses. Mammals can be immunized against lethal H5N1 viruses with a strategy that emphasizes cellular immunity (42). Recently, new vaccine has been developed (85), composed of recombinant viruses that included genetically altered avirulent-type HA and intact NA genes, these are derived from the H5N1 human strain isolated in 2003, and the genes derived from viruses capable of rapid growth in eggs, this virus is highly attenuated, unlikely to revert to a virulent form and considered one of best vaccine candidate for H5 influenza.

The efforts for pandemic vaccine production are focused on the development of vaccines derived from inactivated virus and HA or NA proteins. However, the efficacies of these vaccines are suboptimal due to a lack of mucosal immunity in the respiratory epithelium, which is the main portal for influenza viruses. Antigenic studies using monoclonal and polyclonal antibodies have demonstrated that H5 antigen has high antigenic drifts since the emergence H5 virus and that H5 viruses with different antigenicities are co-circulating in hosts (86). For this reason, A panel of H5 vaccine candidates covering a variety of antigenicities could be produced and used in the event of an H5 virus pandemic, also H5 vaccine candidates must be reformulated annually to keep pace with antigenic changes to match and include the antigenecity of circulating strains (85). An alternative way for vaccine research can be used in a recent article characterizing polymerase genes of the 1918 influenza virus (87). Specific mutation in the polymerase B2 (PB2) gene strongly influences the virulence of H5N1 (88). This mutation could be used to start developing a vaccine instead of waiting new mutations. Available data bases were used to generate hypothesis about peptide based vaccine targeted at specific mutation in PB2 avian influenza virus. This hypothesis suggests that this peptide capable of stimulating protection by evoking cytotoxic T lymphocytes. This vaccine approach would have the advantage of avoiding the use of dangerous, live, avian influenza virus during mass production (89). However, any vaccine that is developed must closely match the pandemic virus, and the vaccine production will not start until the new virus has emerged and a pandemic has been declared.

Control and prevention

Measures taken to prevent, control and eradicate influenza A virus vary from no action as in the case of low pathogenic influenza A virus, to high costly programmes focusing on surveillance, strict quarantine and massive slaughter of infected birds or potentially exposed, proper disposal of carcasses, wholesale and retail outlets for poultry, supervised cleaning and disinfection procedures of poultry farms, equipment and vehicles entering and leaving the farm is enforced, keeping incoming poultry separate from unsold birds, especially if birds are from different lots, restrictions on the movement of live poultry and products that may contain virus both within and between countries in the case of highly pathogenic influenza A virus. Poultry cullers wear protective clothing and take antiviral drugs as a precaution, vaccination against normal seasonal influenza was also recommended as a way to reduce chances that this high-risk group might be co-infected with an avian and a human virus, which give the viruses an opportunity to exchange genes (90-95). These standard control measures aimed at preventing spread to other farms and eventual establishment of the virus in a country's poultry population. Such measures successfully controlled the outbreak and continuous surveillance of the poultry in H5N1 infection maintained to minimize future human exposure. Other measures must be taken to reduce the risk of H5N1 avian influenza spreading from poultry to humans. That includes educating the farmers and their families concerning the danger of high-risk behaviors and how to change their farming practices, avoid contact between domestic poultry and wild birds, and eliminate intermingling between these animals and humans, and rewards for farmers to encourage them to report suspected avian influenza outbreaks in their flocks and to apply control measures and pursue the vaccination of poultry flocks as part of a multi-element response to the avian influenza threat in high-risk areas (92).

Education on personal hygiene and infection control measures should be given to family along with the advice that children should not attend school to return to health facility immediately if fever recurs. All the patients coming to a hospital or emergency room had contact with patients who possibly have or are proven to have avian flu or with fever and respiratory symptoms should be managed according to the recommendations for respiratory hygiene and cough etiquette. CDC recommends that all patients with such symptoms and a history of travel to a country where avian influenza activity has been reported should be managed using isolation precautions similar to those recommended during the SARS epidemic. These measures include standard precautions, contact precautions, eye protection and airborne precautions (93). Other measures that may control an outbreak include vaccination, good surveillance and monitoring of health care workers. Control human traffic, both within and between countries during outbreaks. In the pandemic alert period, recommendations include isolation of patients and quarantine of contacts, accompanied by antiviral therapy. Persons should remain home when they first became symptomatic. If the pandemic is severe, social distancing measures such as school closures should be considered. Nonessential domestic travel to affected areas should be deferred, screening of travelers. Hand and respiratory hygiene should be routine; mask use should be based on setting and risk, and contaminated household surfaces should be disinfected (90,94-98). Vaccination against the prevalent wild-type influenza virus is recommended for all individuals in high-risk groups and antiviral drugs may be a useful option in those not covered or inadequately protected by vaccination. However only prevention based on large epidemiological studies, and research and development of new vaccines may be able to control and eventually eradicate this deadly viral infection.

CONCLUSION

A major influenza pandemic will have devastating consequences, with uncalculable risks for human health, global economy and political and social stability in most countries. Infected birds have been the primary source of influenza A (H5N1) infections in humans. Transmission among humans is very limited at present, but continued monitoring is required to identify any increase in viral adaptation to human hosts. Southern China is considered to be an influenza epicenter based on its role as the site of two previous pandemics (Asian and Hong Kong influenza). The large mix of people, pigs, and ducks in this region affords an ideal environment for the generation of reassortant influenza viruses. The high case-fatality rate of the present avian influenza epidemic this, together with the propensity of influenza viruses to undergo genetic reassortment and mutation, makes the avian influenza virus a likely candidate for the next human influenza pandemic. Preparedness for the next influenza pandemic requires support and collaboration from world-wide nation. Global and domestic laboratory and disease surveillance must be strengthened to increase the likelihood of early detection and tracking of pandemic influenza. There was an urgent need to develop an H5N1 vaccine, which could be used to combat possible pandemic activity.

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