

AVIAN INFLUENZA A/H5N1

Milena Veselinovic

The World Health Organization (WHO) regards avian influenza A/H5N1 as a global public health threat with pandemic potential. Between 2003 and October 12, 2007, WHO registered 331 laboratory-confirmed cases (202 fatal) of human H5N1 infection. Human-to-human transmission has not been recorded yet. In the possible future, H5N1 pandemic, primary viral pneumonia would be the dominant clinical feature. Complications include the development of acute respiratory distress syndrome, renal and multiorgan failure. The characteristic laboratory findings are lymphopenia, with the alteration of CD4+/CD8+ index, thrombocytopenia and "cytokine storm". Specimens for laboratory diagnosis include pharyngeal swabs, nasal swabs, tracheal aspirate (or bronchoalveolar lavage) and serum (acute and convalescent). Virus isolation by cell culture is considered the "gold standard" of influenza diagnostics. Identification of infected cells is performed by direct or indirect immunofluorescence (DFA, IFA), enzyme-linked immunoassays (EIA) or PCR-based methods. Mchip, a microarray which enables the detection and subtyping based on M gene segment, is the recent breakthrough in H5N1 diagnostics. WHO Rapid Advice Guideline Panel on avian influenza, formed in 2006, defined the guidelines for chemoprophylaxis and therapy of human H5N1 infection. The most promising primary treatment is oseltamivir. Vigorous antiviral activity against all subtypes of both A and B influenza viruses has been confirmed by in vitro studies. WHO has identified the antigenic and genetic characteristics of the viruses suitable for the development of the vaccine. More than 40 clinical trials have already been carried out or are ongoing. In spite of the promising results, WHO is expressing concern regarding inadequate global capacity for the vaccine production. *Acta Medica Medianae* 2007;46(3): 44-51.

Key words: avian influenza A/H5N1

Faculty of Medicine in Nis

Contact: Milena Veselinović
Faculty of Medicine
82 Dr Zoran Djindjica Blvd.
18000 Nis, Serbia
E-mail: nico7711@hotmail.com

Introduction

Among infectious diseases, avian influenza A/H5N1 presents one of the major hazards to the mankind in the 21st century. Influenza virus A/H5N1 possesses a number of characteristics of the ideal future pandemic pathogen such as the following: the potential for the infection of the greatest number of animal species (compared to other known influenza strains), high mortality rate (30-70%) as well as the continuous evolution towards the increase of virulence, the ability for human transmission and the resistance to the existing therapeutic agents. World Health Organization (WHO) has identified avian influenza A/H5N1 as a disease with pandemic potential, which represents a health risk to the world population as a whole. The mortality rate of the

possible bird flu pandemic, according to the WHO estimations, would be between 10% and 35% of the world population (1,2).

Ethiology

Influenza virus A/H5N1 belongs to the orthomyxoviridae family, with A, B and C types, of which only A and B type are significant for the human epidemiology. Further subtyping is based on two transmembrane glycoproteins – hemagglutinin (HA,H), with 16 serologic subtypes (H1-H16), and neuraminidase (NA,N), with 9 serologic subtypes (N1-N9).

Influenza virus A/H5N1 is a single stranded RNA virus with a viral envelope (average diameter 120 nm). Genome of influenza A and B viruses is comprised of 8 separate gene segments covered by nucleocapsid protein. Together they form ribonucleoprotein (RNP), in which each segment encodes one functionally important protein: polymerase B2 protein (PB2), polymerase B1 protein (PB1), polymerase A protein (PA), hemagglutinin (HA, H), nucleocapsid protein (NP), neuraminidase (NA, N), matrix protein (M) and nonstructural protein (NS). Matrix

protein M has 2 separate components: M1, which builds the matrix of the virus, and M2, which is found to have a role of ionic pump for the regulation pH value of endosomes of influenza A viruses. The active RNA polymerase, which enables replication and transcription, is formed from PB2, PB1 and PA. This enzyme possesses endonuclease activity and is attached to RNP. Proteins NS1 and NS2 have a regulatory function and stimulate the synthesis of viral components in the infected cell. Proteins HA, NA and M2 are a part of the viral envelope. The lipid layer covers the matrix, made of M1 protein.

Hemagglutinin is the major influenza virus antigen with A, B (carries the receptor binding site), C, D and E antigen domains. HA serves as a receptor which binds the sialic acid (N-acetyl neuramic acid), thus inducing the penetration of the inner part of the viral particle through the fusion with the membrane. The body of the HA protein has a loop region and fusiogenic domain, needed for the fusion with the membrane of the infected cell. Low pH values promote the orientation of the fusion peptide towards the inner part of the cell.

Neuraminidase is a transmembrane glycoprotein, presented with its major part as a bud on the viral surface, a middle part in the lipid layer and a little "tail" in the cytoplasm. NA acts as an enzyme which lyses the bond between sialic acid and HA molecule, other NA molecules, glycolipides and glycoproteins of the viral surface. Furthermore, NA is an important antigenic site and seems to be essential for the penetration of the virus through the mucosal layer of the respiratory epithelium (3).

The characteristic of the orthomyxoviridae viruses is the high mutation potential due to the deficiency of postreplication repair mechanisms. The mutations of antigenic regions inhibit the binding of the neutralising antibodies, as a result of which the spread of a new virus strain is possible in non-immunised population. These changes in the antigenic composition of influenza A viruses are known as antigenic drift. Mutations that result in antigenic drift are an explanation for seasonal influenza epidemics. Immune response to HA antigenic variant induces the synthesis of neutralising antibodies, which represents the basis for the resolution of viral infection and, in some cases, represents a part of cross-immunity found in elderly when a new strain appears.

Antigenic shift, also called genomic rearrangement or reassortment, occurs when viral HA is altered, resulting in the development of mosaic virus. This is possible when a cell is infected with 2 different influenza viruses and a recombination of the segments of their genomes occurs during replication. In A/H5N1 virus genomic reassortment happens in the process of the transmission from poultry to pigs, who are susceptible to infections both with human and avian strains, thus enabling antigenic shift and the creation of the new viral subtype, able to cause infection in humans. This type of co-infection is possible in many poultry species but the dominant way for the occurrence of the antigenic shift is poultry-to-pigs transmission. In addition, direct transmission of avian viruses to humans, without inter-

mediary host, is probable. There are more and more pieces of evidence according to which humans may also serve as a "laboratory" for genome rearrangement of certain circulating avian strains and human seasonal strains, resulting in the development of the new pandemic influenza virus.

Antigenic shift is also possible in NA protein. The mutations of several amino acids in NA can lead to the development of the resistance to the neuraminidase inhibitors. The following mutations are discovered to date: R292K, H274Y, R152K, E119V (the letters represent amino acids: R-arginin, K- lysin, H-histidin, Y-tyrosin, E- glutamic acid, V-valin) When lysin is replaced with arginin on 292 position of NA glycoprotein, complete resistance to neuraminidase inhibitors is probable. The 292 position is crucial, since this mutation can cause resistance not only to oseltamivir but also to zanamivir and two new precursors of active antiviral agents (4).

Pathogenesis

Influenza virus is attached to the cell surface by the binding of the HA to the sialic acid of the glycoproteins and glycolipids of the cell surface. The bond of the sialic acid and galactose, alpha (2,3) (in avian species) or alpha (2,6) (in humans), determines the host specificity of the virus. Since the carbohydrates, which present the sialic acid, can be found in several human cell types, the HA binding to the sialic acid is the explanation for the influenza virus' ability to infect different cell types. Following the binding, the virus enters the cell through the process of endocytosis by clathrin coated receptors. In the cytoplasm, clathrin molecules are released and the vesicle, containing the whole virus, undergoes the process of fusion with endosomes. The content of the vesicle is degraded with the cascade decrease of phagosome pH. When a certain level is reached, the lowering of pH is inhibited by the effect of M2 protein, which also induces the partial disconnection of HA fusiogenic peptide. This enables the fusion of HA with the vesicle membrane and the release of RNP into cytoplasm. Ionic flow from endosomes towards viral particle leads to the lysis of the bonds between different viral proteins; the aggregation of M1 protein is discontinued and RNP is no longer adherent to the M1 protein complex. The release of the virus from the viral envelope is complete 20-30 minutes after the binding of the virus to the cell membrane. RNP is transported to the nucleus, the polymerase complex is linked to the viral RNA and causes the lysis of RNA by its endonuclease activity, accompanied by the elongation. The production of the viral RNA is limited by NP, which enhances the synthesis of mRNA. Both are transported to ribosomes in the cytoplasm, where the protein synthesis occurs. A part of the viral RNA is looped and the production of the viral proteins such as M1 and NS2 is possible. Some of the newly synthesised viral proteins are transported to the nucleus where they form RNP by binding to the viral RNA. Other viral proteins are processed and glycosylated in the endoplasmic reticulum and Golgi apparatus.

These modified proteins are transported to the cell membrane and are incorporated into the lipid dual layer. When a certain high concentration in the membrane is reached, RNP and M1 proteins are aggregated and condensed to form a viral particle. Finally, the particle is released by neuraminidase activity. From the moment of the entry of the virus, approximately six hours is needed to the form the new virus (5).

Immunohistochemical analysis indicate that the focuses of H5N1 producing cells are dominantly located in the lower respiratory tract- in pneumocytes type II, alveolar macrofages and cuboidal epitheloid cells as well as in the intestinal mucosis, endothelial cells, myocard and cerebral tissue. The spread of the virus is enabled by nasal secretion (millions of virions per ml), thus one aerosol particle (0,1 µl) contains more than 100 viruses. Human infectious dose (HID) for the influenza virus ranges from 100 to 1000 particles. In the early phase of the infection, the virus is found in the blood and other bodily fluids (6).

Epidemiology

Genomes of pandemic influenza viruses present a combination of genes of both human and avian viral subtypes. A/H2N2 and A/H3N2 strains, causes of the two out of three flu pandemics in the 20th century, are examples of such a genome. The exception is A/H1N1 strain (Spanish flu virus), for which it is assumed that it resulted from antigene shift solely among avian influenza strains. All bird species are susceptible to avian influenza infection. The reservoirs are migratory wild water species whereas domesticated birds (poultry) are extremely susceptible to highly pathogenic and rapidly fatal avian influenza strains (HPAI), which are characterised by H5 or H7 antigene subtypisation. Conditions favorable for the emergence of antigenic shift are thought to involve humans living in close proximity to domestic poultry and pigs. Because pigs are susceptible to infection with both avian and mammalian viruses, including human strains, they can serve as a "mixing vessel" for the scrambling of genetic material from human and avian viruses, resulting in the emergence of a novel subtype. Additional possible mechanisms for the emergence of a new pandemic strain include antigenic shift among avian strains, direct transmission from poultry to humans as well as recombination between seasonal human and circulating avian strains of influenza A virus in the cases of human coinfection.

The dominant way of spreading is both direct and indirect contact with secretions and excretions of wild birds and poultry and also pigs, contaminated water, consumption of infected poultry meat and other products, contact with contaminated gear, clothes, shoes as well as airborne transmission.

The first documented cases of human infection by A/H5N1 virus were registered in 1997. in Hong Kong (18 infected, 6 letal outcomes) during the epizootic caused by the same HPAI strain. The massive destruction of the total poultry population of Hong Kong followed (approximately 1,5 million birds). It is believed that this action

sucesfully prevented the begining of the pandemic. In the South-Eastern Asian countries, since 2003. up to now, the A/H5N1 strain has been present in the bird population and has continued to cause epizootics. Over 140 million birds have been destroyed in the attempt to stop further spread of the virus. Standard anti-epidemic measures include export control and the possible ban on the food products of animal origin, constant supervision over the areas at risk as well as quarantine and disinfection of the contaminated farms and households. Infectious potential of the virus agent depends on the temperature, water pH and salinity and the effect of UV rays. The half-life of viral particle in water at 4°C is between 2 and 3 weeks. Virus is easily inactivated by disinfection agents with alcohol, chlorine or aldehyde basis. Disinfection is also possible in a couple of seconds by the exposure to the temperatures over 70°C.

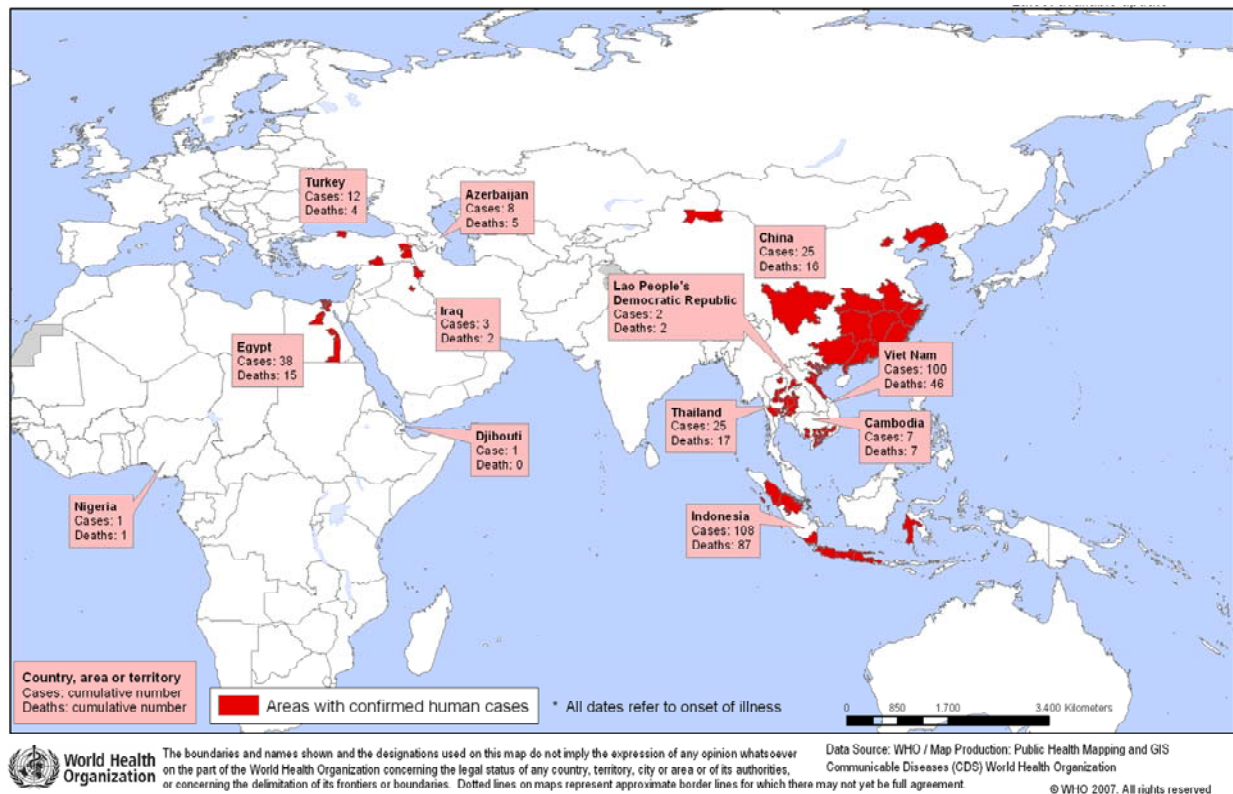
Influenza virus A/H5N1 has up to now spread from Asian continent to other parts of the world, especially Europe and partly Africa, by means of bird migration (supposedly a dominant way) as well as the alternative ways such as the free movement of domesticated poultry and illegal bird trading. In the period between 2003. and 17.10.2007. WHO has confirmed 331 cases (202 letal outcomes) of H5N1 human infection. (Picture 1.)

The first suspected cases of interhuman transmission were registered in Thailand (2004, child to mother transmission) and in Indonesia (2006, transmission in eight member family) (7,8,9). According to the WHO Global Influenza preparedness plan for the possible pandemic, phase 3 of the new viral strain evolution is ongoing, with the limited and low level of interhuman transmission registered in the South-Eastern Asian countries (Vietnam, Indonesia), in which the highest number of infected is documented on the global level (10) Transplacental transmission of the A/H5N1 virus has been confirmed (11).

Clinical manifestations

Clinical presentation of the new avian flu strain, which would be capable of interhuman transmission and would lead to the outbreak of the pandemic, cannot be precisely determined. It is assumed that in the potential future bird pandemic, the dominant clinical picture would be that of the primary viral pneumonia. This assumption is based on the analysis of registered cases of human H5N1 infection.

Incubation ranges from 2 up to 4 days, as is the case in seasonal influenza infections. The clinical presentation includes temperature over 38°C with fever, dispnea and dry cough, viral pneumonia, followed by secondary bacterial infection as well as the development of acute respiratory distress syndrome (ARDS). Gastro-intestinal symptoms, including abdominal pain, sickness, vomiting and diarrhea, may be subsequent clinical manifestations; nevertheless, in some cases they can precede other symptoms. Conjunctivitis is rare in comparison to the clinical presentation of seasonal influenza.



Picture 1. WHO confirmed cases of human A/H5N1 infection

Complications include the development of renal and multiorgan failure.

Radiological findings show extensive bilateral infiltrations, lobar collapse and focal consolidations.

Laboratory analysis are characterised by lymphopenia, with a change in CD4/CD8 index and thrombocytopenia, in some cases slightly or moderately elevated aminotransferase levels or the development of disseminated intravascular coagulation. "Cytokine storm", with persistently high levels of cytokines and chemokines, is also a characteristic of H5N1 influenza (12,13).

One case in Vietnam (2004) was characterized by the development of fulminant viral encephalitis and the absence of respiratory manifestations. This indicates a potential evolution or already existing variability of A/H5N1 virus tissue tropism (14).

Diagnosis

The diagnosis of avian influenza A/H5N1, due to the severity of the disease and the nonspecific clinical picture, cannot be based solely on the appearance of clinical manifestations. In the cases with positive epidemiological data and clinical presentation, it is crucial to perform urgent laboratory diagnostic procedures for the identification and typisation of the H5N1 virus.

In contrast to seasonal influenza, pharyngeal swabs provide a more accurate basis for the virus detection (in rRT-PCR, higher level of viral RNA), when compared to nasal swabs. During the Vietnam epidemic, the detection of the viral RNA was possible 2 to 15 days following the onset of the disease and the level of the virus isolate was 10 times higher in comparison to the specimens of influenza A/H3N2 as well as A/H1N1 patients.

According to the WHO recommendations, the necessary material for laboratory diagnostics

in suspected patients includes, besides pharyngeal and nasal swab, tracheal aspirate (or bronchoalveolar lavage specimen) and serum (in the acute phase-7 days after the onset of the disease, and in the convalescence stage -3 to 4 weeks after the beginning of the illness, if possible). Ideally, the specimens should be taken before the administration of antiviral drugs; nevertheless, the treatment must not be delayed because of the collection of the samples. Plasma in EDTA (7 to 9 days after the onset), rectal swab, spinal fluid (if meningitis is suspected), pleural fluid and autopsy materials are taken as secondary specimens.

Virus isolation is considered the "gold standard" of influenza testing (15). A positive result is considered definitive for the diagnosis. Specimens for culture should optimally be collected within 3 days after illness onset. Cell lines suitable for the cultivation include Madin-Darby canine kidney (MDCK) and primary rhesus monkey kidney (PRMK). Other cell lines, such as Vero, mink lung, and MRC-5, also support growth of influenza virus if trypsin is incorporated into serum-free medium. Hemagglutination inhibition (HI or HAI) is used to identify the viral subtype. Identification of infected cells is performed by direct or indirect immunofluorescence (DFA, IFA), enzyme-linked immunoassays (EIA), or PCR-based methods. The time to detection in culture ranges from 5 days (>90% of positive specimens) to 7 days (100% of positive specimens) (16).

Direct detection methods do not result in production of an isolate and would be inadequate for surveillance or definitive characterization of pandemic strains. Nevertheless, owing to their relatively rapid orientational diagnosis, safety and stability, these methods play an important role in global pandemic influenza preparedness. RT-PCR

assays use conserved targets such as the matrix (M) protein for genus-level identification as well as hemagglutinin and neuraminidase targets for specific identification of avian subtypes. The sensitivity of RT-PCR has been reported to be in the range of 90% to 100% when compared with cell culture (17,18). Commercial tests for rapid detection showed lower sensitivity level for A/H5N1 virus detection compared to rRT-PCR based on primers complementary to H5 sequences of avian viruses (GenBank) (12,18). The development of A/H5 RT-PCR set i multiplex real-time RT-PCR assay presents a new step towards specific diagnostic techniques for A/H5N1 influenza.

A recent advancement in the avian flu diagnostic is MChip, a microarray which enables the detection and subtyping based on M gene segment of H5N1 virus. M gene segment, due to its stability in comparison to rapidly mutating H and N segments, allows more reliable diagnosis of H5N1 infection. Preliminary testing of the MChip, using H5N1 isolates from cats, a range of birds, and human beings found the technology accurately identified 24 different isolates and gave no false positives (clinical specificity 100%) It gave complete information about subtypes in 21 of 24 cases (clinical sensitivity 97%). Precise, economical and rapid diagnostics by application of MChip presents the ideal testing tool in the possible future pandemic (19).

For the definite diagnosis of avian influenza A/H5N1, according to the WHO recommendations, a confirmation of WHO and World Veterinary Organization (WVO) reference laboratories is required (20).

Therapy

Two medication groups are currently available for the treatment and prophylaxis of influenza infections: the adamantanes, M2 inhibitors (amantadine and rimantadine) and the newer class of neuraminidase inhibitors (zanamivir -Relenza and oseltamivir -Tamiflu) (21).

Amantadine and rimantadine interfere with viral uncoating inside the cell and are effective only against influenza A. The use of adamantanes is associated with several toxic effects and with rapid emergence of drug-resistant variants. Adamantane-resistant strains are genetically stable, which enables easy transmission, circulation in natural reservoirs (bird species) and continuing virus evolution. Likewise, a prolonged, latent infection in immunocompromised patients, after the application of adamantanes, is probable. The data and the experiences from the epidemics in the South-Eastern Asian countries indicate that the potential for the development of resistance excludes the use of adamantanes in influenza therapy. As a result, this group of drugs has been eliminated as the therapeutic agent for bird influenza. 22 It should be emphasized that this class of therapeutics still has a place in prophylaxis during a potential epidemic or pandemic (21).

The neuraminidase inhibitors zanamivir and oseltamivir hinder the release of new influenza virus particles from infected host cells, a process

that prevents the spread of infection in the respiratory tract. Since replication of influenza virus in the respiratory tract reaches its peak between 24 and 72 hours after the onset of the illness, neuraminidase inhibitors must be administered as early as possible. In contrast to the adamantanes, the NA inhibitors are associated with little toxicity and are less likely to induce the development of resistant influenza strains. The neuraminidase inhibitors are effective against all NA subtypes of influenza virus, which represents a key advantage of their administration during avian flu pandemic (21).

WHO Rapid Advice Guideline Panel on Avian Influenza, formed in March 2006, has designed the guidelines for the prophylaxis and therapy of human influenza A/H5N1 infection (Table 1). The recommendations are applicable to sporadic cases of human infection or small, contained family epidemics and are not relevant for the potential pandemic. The group developing the guidelines included clinicians with experience in treating H5N1 patients, infectious disease experts, influenza specialists, molecular biologists, epidemiologists and other public health specialists. The evidence summaries were based on systematic reviews and randomized trials for the treatment and chemoprophylaxis of seasonal influenza virus infections, case studies of H5N1 patients, animal as well as in-vitro studies for the treatment or chemoprophylaxis of H5N1 virus infection. 23 Further international collaboration and the formation of data basis of clinical manifestations and management in human cases with A/H5N1 infection are essential to improve the understanding of this disease and to standardize case management (24).

In the therapy of avian flu, the most promising treatment is oseltamivir in the first 48 hours after the onset of the disease. If administered in the later stages of the illness, due to the development of the resistance, the therapeutic effects are not satisfactory. 25 Vigorous antiviral activity against all subtypes of both A and B influenza viruses, including avian strains H5N1 and H9N2, the agents of Hong Kong epidemics, has been confirmed by in vitro studies. The revision of the confirmed cases by WHO has shown that the release of virus and the infectiousness can be reduced. Nonetheless, the clinical benefit of oseltamivir in the human H5N1 infection remains poorly defined. Recent studies have showed that in some patients, the replication of the virus is not diminished entirely following oseltamivir application, allowing the development of the drug resistance. 25 Modified regimens of oseltamivir treatment, including two-fold higher dosage, longer duration and possibly combination therapy with amantadine or rimantadine (in countries where A(H5N1) viruses are likely to be susceptible to adamantanes) may be considered on a case by case basis, especially in patients with pneumonia or progressive disease (25). Another important issue to consider is the late treatment, when there is already evidence of viral replication. Very limited evidence suggest that late therapy lowers the viral load and might be beneficial for the survival in some cases (25). These findings are in

accordance with the data from the studies on mice inoculated with H5N1 virus. The five day regime (10 mg/kg/day) showed protective effects in 59% of the mice and in eight day long application the survival rate was 80%. 26 In another research, the survival rate was raised from 0% to 75% after the application of oseltamivir, even when the therapy was prolonged for 5 days after the onset of the flu. The higher doses of oseltamivir can be safe in human cases of H5N1 infection. The data from the studies for the determination of the maximal nad minimal doses indicate that 5 weeks long therapeutic application of 150 mg/2 times a day and prophylactic 6 weeks long treatment with 75 mg/2 times a day are equally well tolerated as the approved dosage regimens (27,28).

WHO recommendations point out that corticosteroids should not be used routinely, but may be considered for septic shock and suspected adrenal insufficiency. Prolonged or high dose corticosteroids can result in serious

adverse events, including opportunistic infection. Antibiotic therapy in accordance with antibiogram is needed in suspected bacterial co-infection in patients with primary viral pneumonia. Monitoring of oxygen saturation should be performed whenever possible at presentation and routinely during subsequent care and supplemental oxygen should be provided to correct hypoxemia. Therapy for A (H5N1) virus-associated ARDS should be based upon published evidence based guidelines for sepsis-associated ARDS, including lung protective mechanical ventilation strategies (29).

Antiviral chemoprophylaxis is implemented with regard to the level of the risk from infection. The potentially exposed population is divided into groups with high, moderate and low exposure risk. In this phase of the virus evolution, characterized by small clusters of the infected and low and limited human-to-human transmission, it is considered that the general population, outside the risk groups, is not at danger of exposure to the infection (24).

Table 1. WHO recommendations for the prophylaxis and therapy of human influenza A/H5N1 infection

Duration	Age groups (years)					
	1-6	7-9	10-12	13-64	≥65	
Oseltamivir						
Treatment	5 days	Weight adjusted doses: • 30 mg twice daily for ≤15 kg • 45 mg twice daily for >15-23 kg • 60 mg twice daily for >23-40 kg • 75 mg twice daily for >40 kg	Weight adjusted doses: • 30 mg twice daily for ≤15 kg • 45 mg twice daily for >15-23 kg • 60 mg twice daily for >23-40 kg • 75 mg twice daily for >40 kg	Weight adjusted doses: • 30 mg twice daily for ≤15 kg • 45 mg twice daily for >15-23 kg • 60 mg twice daily for >23-40 kg • 75 mg twice daily for >40 kg	75 mg twice daily	75 mg twice daily
Prophylaxis	Begin as soon as exposure identified and continue for 7-10 days after last known exposure	Dose varies by child's weight as for treatment but administered once daily	Dose varies by child's weight as for treatment but administered once daily	Dose varies by child's weight as for treatment but administered once daily	75 mg/day	75 mg/day
Zanamivir						
Treatment	5 days	Not licensed for use	10 mg (two inhalations) twice daily	10 mg (two inhalations) twice daily	10 mg (two inhalations) twice daily	10 mg (two inhalations) twice daily
Prophylaxis	Begin as soon as exposure identified and continue for 7-10 days after last known exposure	1-4 years: NA; 5-6 years: 10 mg (two inhalations) once daily	10 mg (two inhalations) once daily	10 mg (two inhalations) once daily	10 mg (two inhalations) once daily	10 mg (two inhalations) once daily
Amantadine						
Treatment	5 days	5 mg/kg per day up to 150 mg in two divided doses	5 mg/kg/day up to 150 mg in two divided doses	100 mg twice daily	100 mg twice daily	≤100 mg/day
Prophylaxis	Begin as soon as exposure identified and continue for 7-10 days after last known exposure	5 mg/kg per day up to 150 mg in two divided doses ⁷	5 mg/kg per day up to 150 mg in two divided doses	100 mg twice daily	100 mg twice daily	≤100 mg/day
Rimantadine						
Treatment	5 days	Not licensed for use	Not licensed for use	Not licensed for use	100 mg twice daily	100 mg/day
Prophylaxis	Begin as soon as exposure identified and continue for 7 days after last known exposure	5 mg/kg per day up to 150 mg in two divided doses ⁷	5 mg/kg per day up to 150 mg in two divided doses ⁷	100 mg twice daily	100 mg twice daily	100 mg/day

NA=not applicable.

According to the WHO recommendations, the application of seasonal influenza vaccine is necessary in the population with high risk for the exposition to circulating H5N1 strains, with the aim of reduction of the possibility for the mutation of humane circulating avian viruses and the creation of the new pandemic strain. Obligatory vaccination is advised in the countries with the ongoing or expected epizootics of HPAI H5N1 influenza. The risk groups include workers professionally in contact with poultry, countryside residents of the potential risk areas as well as healthcare staff exposed to infected patients during the potential outbreak of epidemic or pandemic. Current epidemiological situation does not require mass immunization of the population in the endangered regions (30).

Due to the high pandemic potential of H5N1, WHO has defined as a priority the development of a vaccine effective against avian influenza H5N1. Three main barriers exist to actually having effective vaccines against H5N1 influenza available for practical use. These include: 1) time factor – further research and development of vaccines that are "market ready" will likely take several more years, 2) limited global vaccine production capacity - currently only about 500 million doses of influenza vaccine can be produced annually, and 3) developing an effective vaccine may require having the pandemic strain, which will mean that a vaccine cannot be produced until the onset of the pandemic and not sooner.

On the basis of human and animal isolates collected in the regions affected by H5N1 epidemics in Asian countries in 2004/2005, WHO has defined antigenic and genetic characteristics of the viral strains suitable for the creation of the vaccine and recommended the following subtypes: A/Indonesia/5/2005, A/Bar-headed goose/Qinghai/1A/2005, A/Anhui/1/2005, A/turkey/Turkey/1/2005 and A/Whooper swan/Mongolia/244/2005. Furthermore, several recombinant H5N1 strains for the production of the vaccine have been designed in WHO referent laboratories, including i A/Vietnam/1194/04, A/Vietnam/1203/04 i A/Hongkong/213/03 strains. These strains are available to a number of institutions and companies for the clinical testing of several different variants of the candidate vaccine (30).

A recently published report involving a Sanofi Pasteur H5N1 vaccine found that 54% of 99 subjects had neutralization antibody titers that reached 1:40 or greater (31). In April 2007, Food and Drug Administration (FDA) approve the inclusion of this vaccine in the federal Strategic National Stockpile and distribution to public health officials as needed.

In GlaxoSmithKline (GSK) clinical trial for adjuvant H5N1 vaccine with inactivated viral strain from Vietnam 2004. epidemic, preliminary results showed that 80% of volunteers who received two vaccine doses containing at least 3.8 mcg of antigen with an adjuvant had a strong immune response (HI titer of 40).

A universal vaccine that would be effective against all types of influenza, including emerging pandemic strains, is the object of intensive research. British company Acambis announced in 2005 that it has had successful results in animal testing. The vaccine focuses on the M2 viral protein, which is stable and not susceptible to mutations, rather than the surface hemagglutinin and neuraminidase proteins targeted by traditional flu vaccines. The universal vaccine is made through bacterial fermentation technology, which would greatly speed up the rate of production over that possible with the traditional viral cultivation in chicken embryos. Furthermore, the vaccine could be produced constantly, since its formulation would not have to be adapted to seasonal influenza strains (32).

The development of the candidate prototypes of the pandemic influenza vaccine is ongoing in 10 countries and involves 16 companies. In addition, five of these companies are developing the vaccines for other avian influenza strains (H9N2, H5N2 and H5N3). More than 40 clinical trials are concluded or in progress. Even though these findings are encouraging, WHO also expresses the concern about inadequate global vaccine production capacity.

The government of the Republic of Serbia has outlined the Program for the control, prevention, elimination and eradication of avian influenza, which contains the necessary actions in the case of avian influenza A/H5N1 outbreak in the region or the country itself (33).

References

1. Webster RG, Govorkova EA. H5N1 influenza- continuing evolution and spread. *N Engl J Med* 2006;335:2174-7.
2. Webster RG, Walker EJ. "The world is teetering on the edge of a pandemic that could kill a large fraction of the human population". *American Scientist* 2003;91 (2): 122-3.
3. Wright PF, Webster RG. Orthomyxoviruses. In: Knipe DM, Howley PM, editors. *Fields virology*. Philadelphia: Lippincott & Wilkins 2001; p. 1533-79.
4. Gong J, Xu W, Zhang J. Structure and functions of influenza virus neuraminidase. *Curr Med Chem* 2007;14:113-22.
5. Cinatl J Jr, Michaelis M, Doerr HW. The threat of avian influenza a (H5N1): part II: Clues to pathogenicity and pathology. *Med Microbiol Immunol* 2007;196(4): Epub ahead of print.
6. Uiprasertkul M, Puthavathana P, Sangsiriwut K, Pooruk P, Srisook K, Peiris M, et al. Influenza A H5N1 replication sites in humans. *Emerg Infect Dis* 2005;11:1036-41.
7. Cinatl J Jr, Michaelis M, Doerr HW. The threat of avian influenza A (H5N1) Part I: epidemiologic concerns and virulence determinants. *Med Microbiol Immunol* 2007;196(4): Epub ahead of print.
8. Writing Committee of the World Health Organization (WHO) Consultation on Human Influenza

- A/H5. Avian influenza A (H5N1) infection in humans. *N Engl J Med* 2005;353:1374-85.
9. Chan PK. Outbreak of avian influenza A (H5N1) virus infection in Hong Kong in 1997. *Clin Infect Dis* 2002;34(2):S58-S64.
 10. http://www.who.int/csr/disease/avian_influenza/phase/en/
 11. Gu J, Xie Z, Gao Z, Liu J, Korteweg C, Ye J, et al. H5N1 infection of the respiratory tract and beyond: a molecular pathology study. *Lancet* 2007;370:1137-45
 12. Yuen KY, Chan PK, Peiris M, Tsang DN, Que TL, Shortridge KF, et al. Clinical features and rapid viral diagnosis of human disease associated with avian influenza A H5N1 virus. *Lancet* 1998;351:467-71.
 13. Angelava NA, Angelava AV. Epidemiology, clinical picture, prevention and treatment of Avian influenza. *Georgian Med News* 2006;131:69-76.
 14. De Jong MD, Simmons CP, Thanh TT, Hien VM, Smith GJ, Chau TN, et al. Fatal outcome of human influenza A (H5N1) is associated with high viral load and hypercytokinemia. *Nat Med* 2006;12:1203-7.
 15. Newton DW, Mellen CF, Baxter BD, et al. Practical and sensitive screening strategy for detection of influenza virus. *J Clin Microbiol* 2002;40(11):4353-6.
 16. Herrmann B, Larsson C, Zweggberg BW. Simultaneous detection and typing of influenza viruses A and B by a nested reverse transcription-PCR: comparison to virus isolation and antigen detection by immunofluorescence and optical immunoassay (FLU OIA). *J Clin Microbiol* 2001;39(1):134-8.
 17. Pachucki CT, Khurshid MA, Nawrocki J. Utility of reverse transcriptase PCR for rapid diagnosis of influenza a virus infection and detection of amantadine-resistant influenza a virus isolates. *J Clin Microbiol* 2004;42(6):2796-8.
 18. Senne DA, Pedersen JC, Suarez DL, Panigrahy B. Rapid diagnosis of avian influenza (AI) and assessment of pathogenicity of avian H5 and H7 subtypes by molecular methods. *Dev Biol* 2006;126:171-7.
 19. Harris Cheng M. Rapid bird flu detection. *Lancet* 2007; 7(1):13
 20. http://www.who.int/csr/disease/avian_influenza/guidelines/referencelabs/en/
 21. Moscona A. Neuraminidase inhibitors for influenza. *N Engl J Med* 2005;353:1363-73.
 22. Hayden GF. Antiviral Resistance in Influenza Viruses — Implications for Management and Pandemic Response. *N Engl J Med* 2006;354:785-8.
 23. Schunemann HJ, Hill SR, Kakad M, Bellamy R, Uyeki TM, Hayden FG, Yazdanpanah Y, Beigel J, Chotpitayasunondh T, Del Mar C, Farrar J, Tran TH, Ozbay B, Sugaya N, Fukuda K, Shindo N, Stockman L, Vist GE, Croisier A, Nagjdaliyev A, Roth C, Thomson G, Zucker H, Oxman AD- WHO Rapid Advice Guideline Panel on Avian Influenza. WHO Rapid Advice Guidelines for pharmacological management of sporadic human infection with avian influenza A (H5N1) virus. *Lancet Infect Dis* 2007;7(1):21-31.
 24. World Health Organization (May 2006) WHO Rapid Advice Guidelines on pharmacological management of humans infected with avian influenza A (H5N1) virus. http://www.who.int/csr/disease/avian_influenza/guidelines/pharmamanagement/en/index.html
 25. de Jong MD, Tran TT, Truong HK, et al. Oseltamivir resistance during treatment of influenza A (H5N1) infection. *N Engl J Med* 2005;353:2667-72.
 26. Yen HL, Monto AS, Webster RG, Govorkova EA. Virulence may determine the necessary duration and dosage of oseltamivir treatment for highly pathogenic A/Vietnam/1203/04 influenza virus in mice. *J Infect Dis* 2005;192:665-72.
 27. McCullers JA. Effect of antiviral treatment on the outcome of secondary bacterial pneumonia after influenza. *J Infect Dis* 2004;190:519-26.
 28. Ward P, Small I, Smith J, Suter P, Dutkowski R. Oseltamivir (Tamiflu) and its potential for use in the event of an influenza pandemic. *J Antimicrob Chemother* 2005;55(1):1-21.
 29. www.who.int/csr/disease/avian_influenza/guidelines
 30. http://www.who.int/csr/disease/avian_influenza/guidelines/seasonal_vaccine/en/index.html
 31. Treanor JJ, Campbell JD, Zangwill KM, et al. Safety and immunogenicity of an inactivated subvirion influenza (H5N1) vaccine. *N Engl J Med* 2006;354:1343-51.
 32. <http://www.acambis.com/default.asp?id=1944>
 33. <http://www.minpolj.sr.gov.yu/article.php?sid=690>

PTIČIJI GRIP A/H5N1

Milena Veselinović

Avijarna influenza A/H5N1 označena je od strane Svetske Zdravstvene Organizacije (SZO) kao bolest koja predstavlja zdravstveni rizik za svetsku populaciju u celini i poseduje pandemijski potencijal. U periodu od 2003. do 12.10.2007. godine, SZO je laboratorijski potvrdila 331 slučaj (202 letalna ishoda) H5N1 infekcije kod ljudi. Interhumana transmisija nije dokazana. Smatra se da bi u potencijalnoj budućoj pandemiji ptičijeg gripa A/H5N1 dominantna bila klinička slika primarne virusne pneumonije. Komplikacije uključuju razvoj akutnog respiratornog distres sindroma, bubrežne i multiorganske insuficijencije. U laboratorijskim analizama karakteristična je limfopenija, sa promenom CD4+/CD8+ indeksa, trombocitopenija i "citokinska oluja". Materijal za laboratorijsku dijagnostiku uključuje: nazalni i faringealni bris, trahealni aspirat (ili bronhoalveolarni lavat) i serum (u akutnom i rekonvalescentnom stadijumu). Izolacija virusa u ćelijskoj kulturi predstavlja "zlatni standard" u dijagnostici avijarne influence. Identifikacija inficiranih ćelija vrši se indirektnom (IFA) ili direktnom imunofluorescencom (DFA), enzyme-linked imunoesejima (EIA) ili PCR metodama. Napredak u dijagnostici predstavlja MChip, microarray koji omogućava detekciju i subtipizaciju na nivou M genskog segmenta virusa. Savetodavni panel, sastavljen od strane SZO 2006. godine, formirao je vodiče za profilaksu i terapiju humane A/H5N1 infekcije. U terapiji ptičijeg gripa najviše obećava farmakološki tretman oseltamivirom. In vitro ispitivanja su potvrdila snažnu antivirusnu aktivnost oseltamivira protiv svih sojeva influence A i B. SZO je definisala antigene i genetske karakteristike virusa pogodnih za produkciju vakcine. Više od 40 kliničkih trajala završeno je ili je u toku. I pored ohrabrujućih rezultata, SZO naglašava zabrinutost usled neadekvatnih globalnih kapaciteta za produkciju vakcine. *Acta Medica Medianae* 2007;46(3):44-51.

Ključne reči: ptičiji grip A/H5N1