

Avian Influenza (Bird Flu)

by Sujata Suri

Introduction

Though man has made impressive advances in the field of medicine over the last century, we remain vulnerable to a host of diseases, ranging from the common cold to those that can only be characterized as frightening. Influenza pandemics (worldwide) have occurred at various times in the 19th and 20th centuries. Several pandemics have been recorded; the first Asiatic (Russian) occurred in 1889-91 when one million people died. In 1918-19 a second pandemic suspected to have originated in Europe, known as Spanish Influenza, took millions of lives worldwide. Pandemics keep occurring regularly, in 1932-33; 1947-48; 1957, when Asian flu killed 1 to 1.5 million people; and the 1968-69 Hong Kong Flu outbreak, which killed 0.75 to 1 million. The possibility of a new type of pandemic influenza—due to mutated strains of Influenza, particularly H5N1—is nightmarish.



Fig. 1 A beautiful wild duck, one possible reservoir of avian influenza virus
http://www.hsus.org/wildlife/a_closer_look_at_wildlife/wild_ducks_of_north_america.html

Bird flu or avian flu is an infection of influenza viruses in birds or other species that can be even fatal. Avian influenza viruses do not normally infect species other than birds and pigs. Migratory aquatic birds, most notably wild ducks, are the natural reservoir of avian influenza viruses that inhabit the intestines of these birds. Infection in domestic poultry is thought to occur due to contact with these aquatic/wild birds. Fifteen subtypes of influenza virus are known to infect birds, providing a large pool of influenza viruses potentially circulating in bird populations.

Avian viruses do not infect humans, but they do tend to exchange genetic material with other influenza viruses infectious to humans and develop into new viral strains. If someone with human influenza is exposed to this avian virus, it would likely transform into a new deadly form that could spread easily from person to person and cause influenza epidemics (or pandemics) that could be even worse than Spanish flu.

Because of their potential to cross the “species barrier,” the great fear is that bird flu viruses currently circulating around the globe might mutate, unleashing a new type of flu virus that could prove even more deadly than Spanish flu, as people's immune systems will not be able to fend off this new strain, and a working vaccine is unavailable.

Viral structure and taxonomy

A virus, (in Latin, toxin or poison), is a sub-microscopic, acellular particle that cannot survive in the absence of a living cell/host cell. Viruses cannot reproduce on their own as they are dependent on host metabolic machinery and ribosomes to replicate and reproduce. Since antibiotics do not harm a virus, treatment for viral diseases such as flu mainly helps ease the symptoms rather than to kill the viruses. Most viruses cause generally mild diseases like the common cold and some even don't cause any symptoms and may go unnoticed, but some cause diseases that can be severe and deadly like Avian influenza, AIDS, and some forms of cancer.

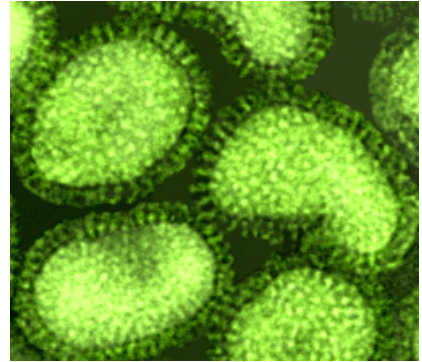


Fig. 2 Electron micrograph of the Influenza virus (~200nm in diameter)
<http://micro.magnet.fsu.edu/cells/viruses/influenzavirus.html>

Influenza viruses are pleomorphic (variable), mostly spherical or ovoid and filamentous, ssRNA (single-stranded RNA) enveloped viruses with a helical symmetry (Fig.2). They are covered over by lipid/lipoprotein envelope. The viral envelope has lipoprotein membranes that enclose nucleocapsids and nucleoproteins.

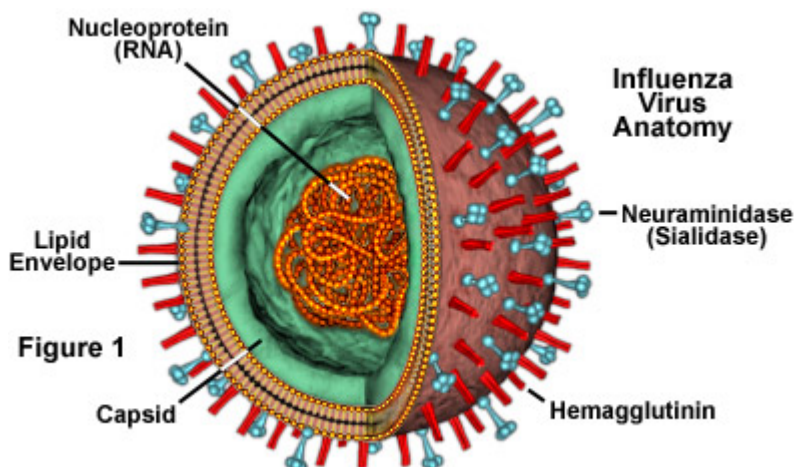


Fig.3 The influenza (flu) virus
<http://micro.magnet.fsu.edu/cells/viruses/influenzavirus.html>

The diameter of each enveloped virus ranges from 50-120 nanometers (nm) and filamentous virions are 20nm in diameter and 200-300nm long (Fig.3). The genome is in the form of eight negative-sense ssRNA fragments (seven for Type C). The total genome length is 12000-15000 nucleotides, the largest segment being 23-25 and the smallest being 800-900 nt. All have terminal repeats at their 5' end and 3' end about 9-13 nucleotides long (Fig. 4). The 5' and 3' terminal sequences of all RNA strands are highly conserved.

The longest RNA strand is closely associated with the nucleoprotein to form helical symmetry.

There are some 500 distinct spike-like surface proteins of the viral envelope, each projecting 10-14 nm from the surface. There are mainly four types of glycoproteins /antigens:

- 1) Hemagglutinin (HA) is a 135Å trimer, a major glycoprotein present on the viral surface as rod-shaped projections, and mediates the attachment of the virus to the cellular receptor. There are 16 types of HA reported.
- 2) Neuraminidase (NA) is a 60Å tetramer, a kind of glycoprotein, and 9 types have been reported. The ratio of HA to NA is about 4-5 to 1.

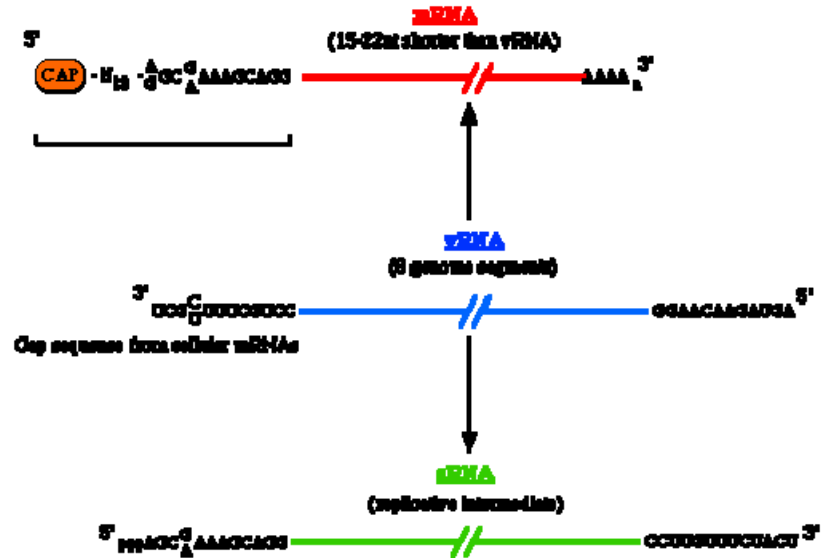


Fig. 4 Different types of mRNAs formed in the host nucleus by influenza viruses
<http://www.microbiologybytes.com/virology/Orthomyxoviruses.html>

- 3) Nucleocapsid protein (NP) It coats the RNA strands.
- 4) Matrix protein (M) The inner side of the viral envelope is lined by the matrix protein.

Replication, transcription & translation

Transcription & Replication (Fig. 5)

The AI virus which contains negative (-) sense RNA as genetic material enters the host cell by adsorption (attachment) to the cell surface. Its hemagglutinin binds with the sialic acid present on glycoprotein receptors of the host. After adsorption, it is internalized as an endosome due to the acidic environment of the host cell.

In the cell cytoplasm the virus releases its nucleocapsids that further are transported into the nucleus, where mRNA synthesis and replication occurs. Once it enters the nucleus, viral endonuclease snips off the 5' end of the host capped, methylated mRNA about 13-15 bases from the 5' end. This snipped part of the host mRNA is used as a primer by the virus to synthesize its own mRNA. Next, viral RNA polymerase further extends the primer and makes a complementary (mirror image) plus (+) strand mRNA. Transcription results in 8 primary transcripts /pre-mRNA that are further translated in the cytoplasm. The cells treat the viral mRNA like their normal mRNA and uses them to make copies of viral proteins. These are about ten proteins translated

from the 8 mRNA transcripts e.g., hemagglutinin, neuraminidase, PB1, PB2, nucleoprotein, another RNA polymerase complex, 2 matrix proteins and 2 NS proteins.

Replication: RNA replication occurs in the nucleus with the help of viral RNA polymerase (or modified RNA polymerase) that was also involved in transcription. In the same manner, as explained above, the (+) strand of RNA (e.g., cRNA) is synthesized, and is coated with nucleocapsid proteins soon after it is made. This plus strand is then used as a template to synthesize a new negative RNA strand followed by coating with nucleocapsid proteins. These can further serve as templates for replication, mRNA synthesis or packaging into virion particles.

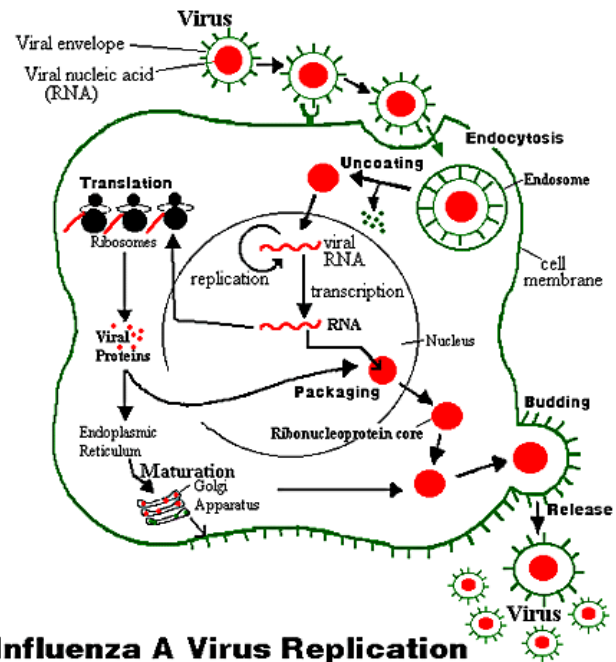


Fig. 5
<http://www.accessexcellence.org/RC/VL/GG/influenza.html>

These (-) strand RNA (vRNA) are transported into the cytoplasm, where other viral proteins assemble together and are packaged into virion particles and, on maturity, buds off from the outer cell membrane and infect new cells.

Classification

AI virus belongs to the Order “Mononegavirales” and Family Orthomyxoviridae, and on the basis of their Nucleocapsid and M protein antigens, they are divided into three distinct immunological types: Influenza virus Type A, Influenza virus Type B, and Influenza virus Type C. IA virus subtypes are classified and named according to the types of HA and NA surface proteins. There are 16 types of HA surface proteins (which are named H1, H2, H3...H16) and 9 types of NA surface proteins (which are named N1, N2...N9). An influenza virus always has one type of HA surface protein and one NA surface protein and it could be of any combination of H and N e.g., H5N1, H7N3, H7N7, H5N2, H5N8 and so on. Within these subtypes, some viruses with slightly different nucleotide sequences are present and are classified into strains. The most virulent form so far reported is H5N1 of Influenza virus.

Influenza virus Type A can be divided into 2 distinct groups on the basis of their ability to cause disease. Highly pathogenic avian influenza (HPAI) can cause up to 100% mortality in birds (Alexander). To date, all outbreaks of the highly pathogenic form have been caused by influenza A viruses of subtypes H5 and H7. I will mainly focus on Influenza virus Type A, the most virulent human pathogen and cause of all flu pandemics.

Generally, Avian viruses do not infect humans, but they do have potential to cross the “species barrier” and develop into new viral strains that are infectious to humans. Several theories have been put forward to explain the origin of new strains. A few are as follows:

Antigenic Shift

Viruses isolated in the years 1933-46, 1947-56, 1957-67 and from 1968 onwards demonstrated wide antigenic variation, so it is apparent that pandemics are due to the appearance of new influenza A subtypes against which the human population has no immunity. This phenomenon is known as antigenic shift. As immunity to a particular new subtype builds up in the population at large, further epidemics are more limited.

Reassortment

This theory is based on the view that the new virus subtypes are reassortant viruses resulting from dual infection. The eight ssRNA segments of each strain reassort with each other, producing a new subtype. IA viruses can cross the “species barrier,” and pigs are postulated as the most likely “mixing vessel.”

Antigenic Drift

Though pandemics arise due to antigenic shifts every 10-12 years, smaller epidemics can occur regularly in the intervening years. The viruses isolated from such epidemics showed strain differences when compared in the HAI tests, i.e. although the viruses belong to the same subtype, they do not crossreact completely. These lesser antigenic changes are known as antigenic drift. Antigenic drift can arise due to natural mutation or selection over time.

Pathology and epidemiology

Typically, the Avian Influenza (AI) virus refers to Influenza A, found chiefly in birds. Infected birds show clinical symptoms like a sudden drop in egg production, brittle or soft-shelled and even shell-less eggs, swollen wattles and combs, congestion, and swollen skin under the eyes.

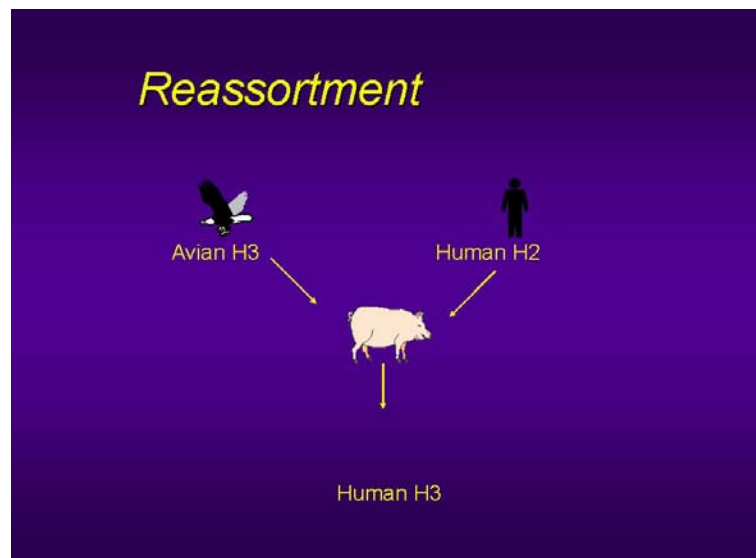


Fig. 6 Reassortment of human H2 with avian H3 virus: e.g., emergence of the H3N2 pandemic virus in 1968.

<http://virology-online.com/viruses/Influenza.htm>

Usually the risk of human infection from birds is through coming in close contact with bodily fluids or with contaminated surfaces. Infection can be transmitted from infected bird droppings, saliva, nasal secretions, feces, or blood. These viruses can remain infectious for about 1 week at human body temperature or a month at 32 degrees F, and can survive at very low temperatures indefinitely. Symptoms of AI in infected humans are mild fever, myalgia, sore throat, cough, conjunctivitis, myositis, and myoglobinuria. However, some people develop life-threatening complications like respiratory distress syndrome, pneumonia, and multiorgan failure.

In 1997, the first documented infection of humans with an avian influenza virus occurred in Hong Kong. At the same time, the poultry population in Hong Kong was also found to be infected with avian influenza caused by the same pathogenic strain. Studies determined that the infection occurred when the virus jumped directly from birds to humans due to close contact with infected poultry. A pandemic was averted by rapid mass killing/burning of over a million birds – the entire poultry population of Hong Kong (Chan, 2002; Yuen et al., 1998).

In Dec 2003, a highly pathogenic form of H5N1 caused another outbreak in poultry in The Republic of Korea (Lee et al., 2005). Another human infection was confirmed in February 2004 when two fatal cases were reported in Hong Kong due to H5N1 (Peiris et al., 2004), followed by 112 cases (57 fatal) from Thailand, Cambodia, Indonesia, and Viet Nam. Until now the cumulative number of confirmed human cases is 317 (191 fatal) (WHO, 2007). RnH5N1 viruses also have been isolated from ducks in Southern China (Chen et al., 2004) and antiviral antibodies have been found in pigs in Viet Nam (Choi et al., 2005). These cases could be the result of new strains due to reassortant viruses, antigenic shift or antigenic drift, as explained earlier. People are not immune to these different strains. Generally speaking, an individual has immunity to only those microbes or viruses to which they are earlier exposed. The possibility of dreadful new strains is thus worrying, as people either have no immunity or extremely delayed immunity depending upon the individual's health and age. However, many new harmless strains causing symptomless infections go unnoticed. It was noticed that Spanish flu was most lethal in young adults, who generally are most able to fight off severe infections. One theory for why Spanish flu preferentially killed young people is because they are the one with robust and reactive immune systems and therefore were most likely to mount a self destructive response (Brown, 2007)

Treatment

The following few antiviral compounds have been recommended:

Amantadine

This antiviral compound is only effective against influenza, though some influenza viruses are resistant. It inhibits the growth of influenza viruses in vitro and in some experimental animals; the only problem associated with it is potential toxicity, which can induce mild neurological problems like insomnia and lack of concentration. The therapeutic efficacy of amantadine in humans is unclear due to a scarcity of clinical studies, but reductions of fever or illness after one day have been observed in adults and children (Nicholson et al., 2003)

Rimantidine

This drug is approved by the FDA and has fewer side effects than amantidine. Some viruses have shown resistance linked to changes in the M2 protein. Rimantidine, like amantidine, works by inhibiting the ion channel activity of M2 membrane proteins.

Zanamivir

This acts as a neuraminidase inhibitor that is administered by inhalation. It is also approved by the FDA for treatment of persons more than 12 years of age. Clinical studies do not show any side effects. (Wong)

Oseltamivir

An FDA approved drug, Oseltamivir, is recommended for persons 13 years or older. No side effects are reported so far. Oseltamivir is also an inhibitor of neuraminidase.

Vaccines

Immunity to viruses is induced by the host responses to the virus hemagglutinin (HA) and to neuraminidase (NA). Antibodies against HA are the most important component in the protection against AI viruses, followed by antibodies against NA, which not only confer protection against infection, but also reduce the severity of infection and decrease virus spreading in an infected person. Thus an influenza vaccine must contain both HA and NA antigens in a form which will stimulate the production of neutralizing antibodies, local IgA antibodies and possibly cellular immunity. Partial protection has been reported due to the antibodies (serum anti-neuraminidase Ab) and to the surface glycoprotein NA in the chickens (Suarez et al; 2007).

Whole inactivated virus vaccines:

Whole inactivated virus vaccines were the first influenza vaccines to be produced. They are made from whole viruses that have been killed so that they are non-infectious but retain the ability to induce a protective immune response. These vaccines confer 60-90% protection that lasts for one to five years, depending on type of viral strain, but due to antigenic drift the vaccine-induced antibodies will be less effective in conferring protection against new strains.

Subunit virus vaccines:

Subunit vaccines can be produced by identifying and purifying the major antigenic sites (HA & NA) of viral antigen. Examples of purified subunit vaccines include the HA vaccines for influenza A and B. These cause fewer negative reactions and seem to be a good option

Live attenuated vaccines:

Live attenuated vaccines are produced by modifying (attenuating or weakening) a disease-producing ("wild") virus, so that the virus (vaccine) retains the ability to replicate (grow) and produce protective immunity, but usually does not cause illness. Generally, the attenuated form

of the virus is obtained by serial passages of the active organism in culture media or cells. These are supposed to induce more solid immunity than inactivated vaccines, but again antigenic drift can interfere. To circumvent antigenic drift, already attenuated strains have been mixed with wild-types of virus to produce recombinants, that code for both attenuated and wild-type HA and NA.

DNA vaccines:

The first DNA vaccine was designed by scientists from the Vaccine Research Center (VRC) at the National Institute of Allergy and Infectious Diseases (NIAID). This candidate vaccine was synthesized by using a modified version of the hemagglutinin gene from the H5N1 Avian Influenza virus. It contains no infectious material from the H5N1 A1 virus and contains only a portion of the DNA. Currently it is under clinical trials. (<http://www.nih.gov/news/pr/jan2007/niaid-02.htm>)

Use of influenza virus-like particles (VLPs) as vaccine:

Pushko et al. (2005), have shown that influenza virus-like particles (VLPs) comprised of HA, NA, and M1 proteins represent a candidate vaccine for avian influenza H9N2 virus. Recently Pushko et al. evaluated an H9N2 VLP vaccine and recombinant HA (rH9) vaccine in three animal models. The H9N2 VLP vaccine protected mice and ferrets from challenge with A/Hong Kong/1073/99 (H9N2) virus.

Use of Adjuvants for improving the efficacy of vaccine:

Pushko's group further observed that adjuvant novasome increased the immunogenicity and protection of the rH9 vaccine. Their results have implications for the development of safe and effective vaccines with pandemic potential for avian influenza viruses (Pushko et al., 2007).

Human Monoclonal antibodies:

Recent studies have reported that blood taken from four Vietnamese survivors of the H5N1 bird flu virus protected mice from several strains of the virus. This finding may offer a new way to treat avian influenza. The human monoclonal antibodies were engineered to neutralize H5N1 virus. "We have shown that this technique can work to prevent and neutralize infection by H5N1 bird flu virus in mice," said Dr. Cameron Simmons of the Hospital for Tropical Diseases in Ho Chi Minh City, Vietnam. So this technique may offer a weapon to stockpile ahead of a feared pandemic of bird flu. (Human)

Prevention

The first and major control measure should be to take precautions so that an AI virus does not cross the "species barrier" and infect humans. People dealing with poultry or poultry products should take the following prevention measures:

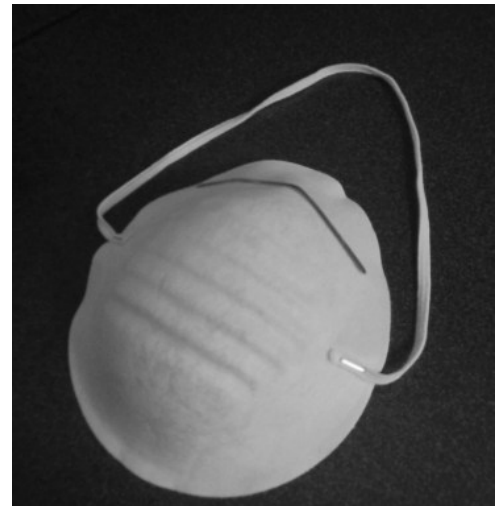
- practice proper sanitation and good hygiene during handling of poultry and poultry products.
- wear masks, safety glasses and gloves

- avoid unnecessary contact with live, sick or dead birds
- clean kitchen utensils & surfaces before and after use
- cook chicken until boiling temperature or when cooking temperature exceeds 71 degrees C, because H5N1 viruses are killed at this temperature (Bhatia & Narain, 2006)
- wash eggs thoroughly with soapy water, rinse, and thoroughly cook.
- store and prepare raw chicken and eggs separately from other food items to avoid cross-contamination

Also there should be routine tests for AI virus in poultry and if any positive case is seen, the entire poultry flock and poultry products should be destroyed and should not be consumed by the public at any cost.

If, unfortunately, AI crosses the “species barrier” and infects people, then the medical personnel, or people close to patients should take following precautions.

- Stay at least 1-2 meters away from the infected patient, as cough droplets are full of flu viruses (>5micrometer in diameter). Coughing or sneezing is the predominant direct mechanism of transmission (Bridges et al, 2003).
- Wash hands frequently and avoid touching mouth or eyes. Indirect contact with door knobs, toilet knobs, taps, stair railings, hand shaking etc, is another possible method of transmission (Bean et al., 1982).
- Wear masks and safety glasses.



A nanoshield mask designed to stop the spread of avian influenza
<http://nxtrx.com/>

What is the government doing?

Governments over the Globe need to cooperate and develop detection systems and control measures to prevent the spread of AI and other potential pandemics. Many important steps have already begun, but an even more extensive response is needed.

The World Health Organization (WHO) has a network of around 110 Influenza centers worldwide that regularly submit new influenza isolates to the four WHO collaborating centers (US,

Australia, Japan, and UK). Their aim is to detect new and potentially dangerous strains of influenza at the earliest moment so that control measures can be enacted in the event of a pandemic.



Poultry must be watched closely for avian influenza

<http://www.the-ba.net/the-BA/CurrentIssues/ReportsandPublications/ScienceAndPublicAffairs/SPAArchive/SPADec05/AvianFlu.htm>

On February 1, 2007, U.S. health officials announced an early-warning system similar to that for hurricanes to protect the country against the avian influenza pandemic. The community-based response system will categorize flu pandemics by using a "Pandemic Severity Index" (PSI) on a scale of 1 to 5, with 5 being the deadliest. Each PSI level will carry a set of recommendations, ranging from hand washing to closing schools, which are intended to slow the spread of the virus while a vaccine is being prepared.

A viral strain/infection that does not move rapidly from person to person would likely cause a fairly mild pandemic. On the other hand, a strain that not only moved with extraordinary speed but also had an unusually high mortality rate would be categorized as a category 5 (pandemicflu.gov.).

The U.S. Department of Health and Human Services (HHS) on June 13, 2007 hosted The *Pandemic Influenza Leadership Forum* to help Americans become more prepared for an influenza pandemic. This forum, sponsored by HHS, encouraged people to prepare for a possible pandemic and provided the public with the essential steps for personal pandemic flu preparedness, including:

- Communicate to your community that it is critical for everyone to prepare for possible pandemic flu.
- Use tools and ideas provided by HHS to help reach your audience.
- Encourage people to: (1) Store extra food and other daily supplies to make it easier to stay home for a prolonged period of time; (2) Learn and practice proper hand washing; (3) Use safe cough and sneeze techniques to limit the spread of illnesses; and (4) Stay home and avoid others if you are sick. (<http://www.hhs.gov/news/press/2007pres/06/pr20070613a.html>)

“Preparing for an influenza pandemic is a shared responsibility,” HHS Secretary Mike Leavitt said. “By preparing now, individuals will be better able to deal with a pandemic, slow the spread of illness, and lessen the overall impact to themselves and to society.”

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