

Early Diagnosis: A Critical Step in Bird Flu Prevention

If avian influenza (bird flu) mutates sufficiently to jump from chickens and migratory birds to people, early diagnosis and identification of the viral strain that has developed the capability to infect humans will be a critical step in preventing a human pandemic.

According to researchers at Cornell University in New York, successful containment will depend on getting a start on creating a vaccine. To create a vaccine, researchers will have to learn more about how the flu virus enters human cells.

The influenza virus is one of the most extensively studied and best-understood viruses, but it is also one of the most adaptable. The way flu virus works has been studied in great detail – but parts of the puzzle remain missing.

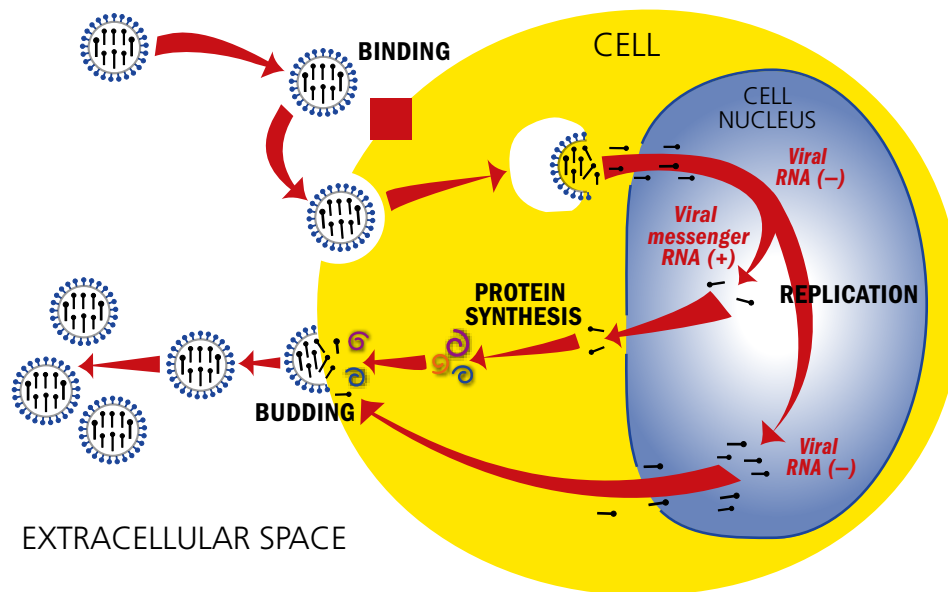
How the Flu Virus Works

A virus is a package of genetic material – DNA or RNA – surrounded by a shell of protein and fat (lipid).

The type A influenza virus – the family to which bird flu belongs – consists of 10 proteins and eight strands of RNA that carry the code for making the proteins.

To invade a host, the virus shell uses certain proteins that bind to receptors on the outside of cells in victims' airways and lungs.

This so-called binding draws the virus into the normally protective cell membrane. The virus shell fuses with membrane and moves through it, emerging into the cell's cytoplasm, where the shell opens and releases its RNA.



After binding to a receptor on the cell membrane, the influenza virus moves into the cytoplasm where the virus shell opens, releasing RNA. In the nucleus, viral RNA is copied to messenger RNA, which moves back to the cytoplasm as a template to make more viral proteins. Copies of the viral RNA join with the viral proteins to make more viruses, which bud on the outer surface of the cell and seek new cells to infect. (Kathryn Seely/Cornell University)

The cytoplasm is a gel-like substance that helps maintain the cell's shape and consistency, stores chemicals critical to life, and is home to the organelles, which produce proteins and energy for the cells.

The viral or infected RNA is called “negative sense” RNA – it is a mirror image of the messenger RNA the cell uses to make proteins.

The viral RNA then moves into the cell nucleus, where the cell's machinery makes “positive” copies that travel back out into the cytoplasm.

Bird Flu Hijacks the Cell's Protein Factory

The cell treats the viral RNAs like any other messenger RNAs and uses them to make copies of the viral proteins – essentially hijacking the cell's own protein-making machinery.

Meanwhile, inside the nucleus, other positive copies of the viral RNA act as templates to churn out more negative viral RNA. The new viral RNA then moves back into the cytoplasm where it joins with the newly made viral proteins to form new copies of the complete virus.

The assembly occurs inside the cell membrane and, as the process is completed, the new flu virus moves out through the cell wall and is released into the airway to find another cell to infect — or it is ejected from the body in a cough or sneeze and launched to find a new host.

Eventually, the virus replication takes over so much of the cell's machinery that the cell dies. Dead cells in the airways result in a runny nose and scratchy throat. Too many dead cells in the lungs result in death.

Moving From Species to Species

The shape of receptors in the cell wall is a little different from one species to another, so a virus that can latch onto a chicken cell usually cannot infect a human. But the process is not exact and there are minor variations from one organism to another, even within a species.

The 107 people who have died of avian flu infections from December 2003 to April 2006 may have had just enough variation in their cell structure to let the avian virus attach. Or a few copies of the avian virus may have mutated enough to infect a person. Some species have receptors whose shapes are about halfway between birds and humans.

Avian and human flu strains both can infect pigs, for example. Scientists fear that a pig somewhere could be infected with both viruses at the same time. With proteins and RNA strands from both viruses inside the cell, new viruses might assemble, perhaps with the proteins that attach to a human cell but with features that give it the virulence of the avian virus, including the ability to infect cells outside the respiratory tract.



Research continues, but development of a vaccine effective against all flu viruses is a long way off. (© AP/WWP)

Another Step

The Cornell University researchers found that attaching to a single receptor is not sufficient to allow the flu virus to enter a cell. Another receptor or another process must be involved.

The primary receptor, already extensively studied, varies from one virus to another, but whatever the additional step is, it seems to be the same for many different flu viruses and perhaps for all.

The researchers believe that understanding the process could lead to the development of new anti-viral drugs or even a vaccine effective against all flu viruses, but they caution that such a result is a long way off.

Global Surveillance Yields Annual Vaccines

Scientists around the world are working to develop a human vaccine against the H5N1 strain of bird flu that has moved steadily westward since its 2003 appearance in Asia, but experts are not sure how well such a vaccine will work if a pandemic strikes. Meanwhile, the World Health Organization (WHO) conservatively estimates that such a pandemic could cause a “large number of deaths” – on the order of 2 million to 8 million deaths.

The problem is that flu viruses, including the avian influenza virus, mutate so rapidly that a vaccine created one year is not effective the next year.

As of April 2006, the WHO has confirmed 191 human cases of H5N1 infection in nine nations, resulting in at least 107 deaths. Because access to medical care is poor in some rural areas and cases may go undiagnosed, the human count could be higher.

Since the first confirmed appearance of the H5N1 virus in European flocks October 13, 2005, and subsequently in the Near East and Africa concern and vigilance has been heightened in many more capitals. Human cases were reported in Azerbaijan, Egypt, Iraq, Turkey by early 2006.

Global Flu Surveillance

Surveillance is the close observation of someone or something – in this case, influenza. Because viruses do not respect national boundaries, such surveillance is international and is coordinated by the WHO Global Influenza Surveillance Network, established in 1952.

The WHO network is made up of four collaborating centers – in the United States, Australia, Japan and the United Kingdom – and 112 institutions in 83 countries that are called WHO National Influenza Centers. The national influenza



Annual flu shots are highly recommended for the elderly and other vulnerable age groups. (© AP/WWP)

centers collect specimens in their countries and isolate and scientifically characterize the viruses. The centers then ship the newly isolated strains to WHO collaborating centers for more scientific and genetic analysis. WHO flu experts use this analysis to recommend the formulation of each year’s flu vaccines for the Northern and Southern Hemispheres, then prepare and distribute the candidate vaccine strain to manufacturers.

Viruses that go into flu vaccines have been grown the same way for more than 50 years – in fertilized (embryonated) chicken eggs. Eleven days after the eggs are fertilized, each of three flu virus strains is injected into an egg and accumulates in the fluid around the embryo. The virus infects the embryo and multiplies.

After several days of incubation, a machine opens the egg and harvests the virus. The virus is then purified, chemically killed and used to produce the vaccine. On average, it takes between one and two eggs to produce one dose of annual flu vaccine. The process takes six to nine months and uses 100 million eggs in the United States alone.

The egg-based method is problematic for a potential H5N1 vaccine because the virus kills chicken embryos before much of the virus can grow. Thus

there is an urgent need for alternative methods of vaccine production which the U.S. National Strategy is trying to address.

The WHO Influenza Surveillance Network serves also as a global alert mechanism for the emergence of influenza viruses with pandemic potential, like the H5N1 bird flu strain.

U.S. Flu Surveillance

In the United States, the Centers for Disease Control (CDC) is one of the four WHO collaborating centers. As part of its local surveillance process, the Influenza Branch collects and reports information on flu activity in the United States each week from October through May.

The U.S. flu surveillance system has seven components that tell CDC when and where flu activity is occurring, which flu viruses are circulating, how the flu viruses are changing, where flu-related illness is occurring and the effect of flu in deaths.

The surveillance system includes reports from more than 120 laboratories, 2,000 “sentinel” health care providers, vital statistics offices in 122 cities, research and health care personnel at specific surveillance sites, and flu surveillance coordinators and state epidemiologists from all state health departments. All flu activity reporting by states and health care providers in the United States is voluntary.

Such surveillance is critical to keep up with mutating influenza A viruses and ensures each year’s flu vaccine protects against the currently circulating strains.

The influenza virus contains eight interior “genetic segments.” The outside is lined with H and N receptors that vary from strain to strain. (Los Alamos National Laboratory)

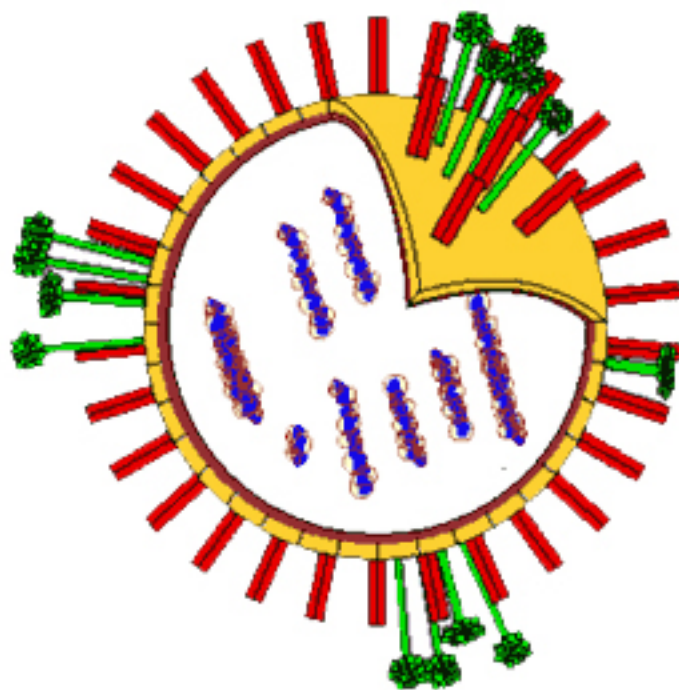
Influenza Viruses: Types, Subtypes, and Strains

There are three types of influenza viruses: A, B, and C.

Influenza Type A

Influenza type A viruses can infect people, birds, pigs, horses, seals, whales, and other animals, but wild birds are the natural hosts for these viruses. Influenza type A viruses are constantly mutating and are divided into subtypes based on two proteins on the surface of the virus. These proteins are called hemagglutinin (HA) and neuraminidase (NA). There are 15 different HA subtypes and nine different NA subtypes. Many different combinations of HA and NA proteins are possible. Only some influenza A subtypes (H1N1, H1N2, and H3N2) are currently in general circulation among people. Other subtypes are found most commonly in other animal species, for example, H7N7 and H3N8 viruses affect horses.

Subtypes of influenza A virus are named according to their HA and NA surface proteins. Thus an “H5N1” virus has an HA 5 protein and an NA 1 protein.



Influenza Type B

Influenza B viruses are normally found only in humans. Unlike influenza A viruses, these viruses are not classified according to subtype as these do not mutate rapidly. Although influenza type B viruses can cause human epidemics, they have not caused pandemics.

Influenza Type C

Influenza type C viruses cause mild illness in humans and do not cause epidemics or pandemics. Influenza Type C viruses are not classified according to subtype.

Strains (subgroups)

Influenza B viruses and subtypes of influenza A virus are further characterized into strains. There are many different strains of influenza B viruses and of influenza A subtypes. New strains of influenza viruses appear and replace older strains. This process occurs through a type of change called “drift.”

When a new strain of human influenza virus emerges, antibody protection that may have developed after infection or vaccination with an older strain may not provide protection against the new strain. Thus, the influenza vaccine is updated on a yearly basis to keep up with the changes in influenza viruses.

Human Influenza Viruses Versus Avian Influenza Viruses

Humans can be infected with influenza types A, B, and C. However, the only subtypes of influenza A virus that are circulating now among people are influenza A subtypes H1N1, H1N2, and H3N2.

Only influenza A viruses infect birds. Wild birds are the natural host for all subtypes of influenza A virus. Typically, wild birds do not get sick when they are infected with influenza virus. However, domestic poultry, such as turkeys and chickens, can get very sick and die from avian influenza, and some avian viruses also can cause serious disease and death in wild birds.



These baby geese in Poland are kept indoors to prevent infection from wild birds. (© AP/WWP)

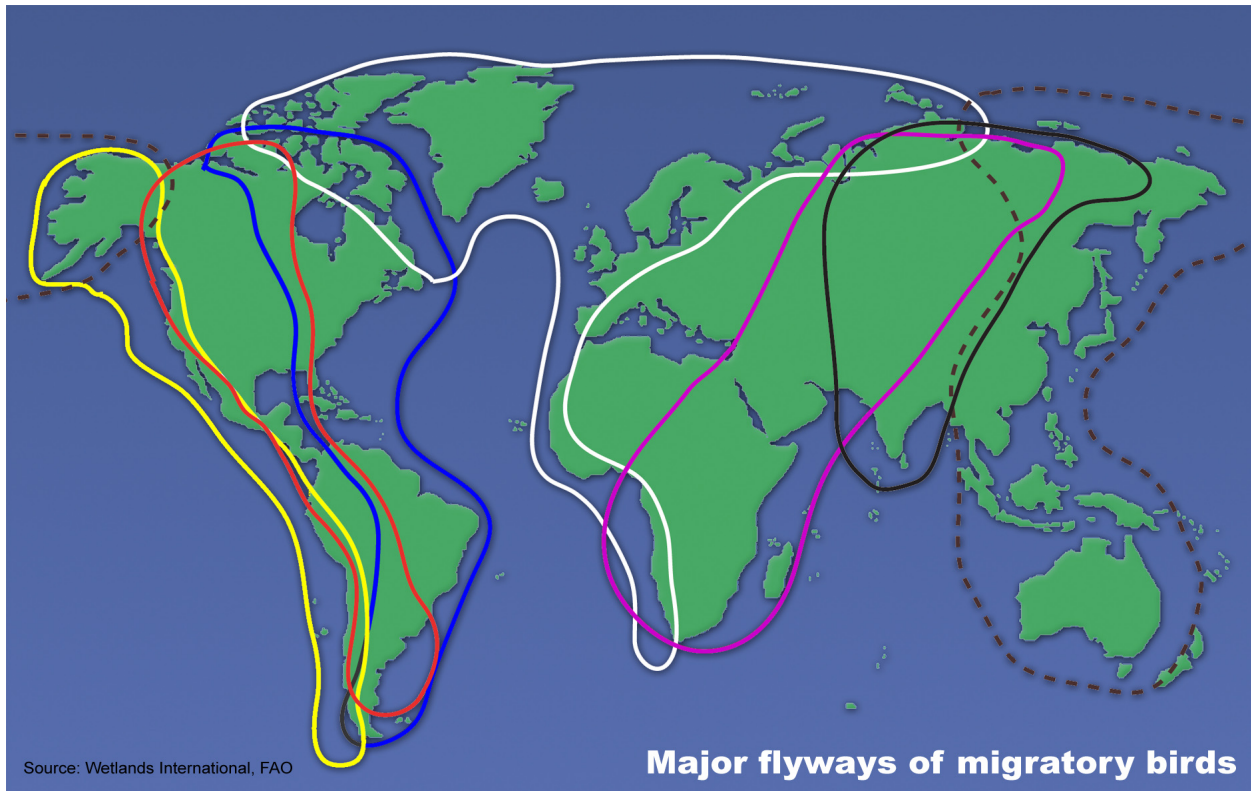
Low Pathogenic Versus Highly Pathogenic Avian Influenza Viruses

H5 and H7 subtypes of avian influenza A viruses can be further classified as either highly pathogenic avian influenza (HPAI) or low pathogenic avian influenza (LPAI). This distinction is made on the basis of genetic features of the virus. HPAI is usually associated with high mortality in poultry. It is not certain how the distinction between “low pathogenic” and “highly pathogenic” is related to the risk of disease in people. HPAI viruses can kill 90 to 100 percent of infected chickens, whereas LPAI viruses cause less severe or no illness if they infect chickens. Because LPAI viruses can evolve into HPAI viruses, outbreaks of H5 and H7 LPAI are closely monitored by animal health officials.

Avian Influenza Viruses in Birds

Bird flu outbreaks among poultry occur worldwide from time to time. Domesticated birds may become infected with the avian influenza virus through direct contact with infected birds, or through contact with surfaces (such as dirt or cages) or materials (such as water or feed) that have been contaminated with the virus. People, vehicles, and other inanimate objects, such as shoes or equipment, can help spread the virus. When this happens, bird flu outbreaks can occur among poultry.

Whether outbreaks cause much havoc depend on whether the avian influenza viruses are of low or high pathogenic forms. Low pathogenic forms of bird flu viruses are responsible for most out-



breaks in poultry but these outbreaks usually result either in no illness or mild illness (e.g. chickens producing fewer or no eggs), or low levels of mortality. However, when outbreaks involve highly pathogenic forms of H5 and H7 viruses, mortality is close to 100 percent among infected poultry, leading to massive culling in efforts to keep the virus from spreading.

Annual Bird Migration: Facilitating the Spread?

Scientists do not fully understand how the H5N1 virus has spread across Asia and into Europe since its first appearance in 2003 in China. They do know that wild migratory birds serve as nature's reservoir for the highly pathogenic virus, and can pass it to domestic birds through direct contact. Wild birds also shed the virus, so domestic birds may be exposed through contact with contaminated dirt, water, or feed.

Thus, the flyways of seasonally migrating flocks are under great scrutiny, particularly during the time of year when various bird populations fly from continent to continent. As shown above, certain wild flocks from Asia make a seasonal trip

to North and South America while other migrating birds from Northern Asia and Northern Europe make a yearly journey to Africa. Because of that, attention on the possibility of a pandemic has heightened monitoring in all these areas.

No definitive proof confirms that migrating birds have carried the H5N1 virus as far as it has traveled in the two years since its reappearance. The virus does have other means of travel. The virus can survive outside a host at moderate temperatures for long periods and can survive indefinitely in frozen material. The H5N1 virus can travel from farm to farm in the mud of a farmer's truck or in the dust on his shoes. It can survive on the bars of cages that may be used in the commercial transport of live animals. For these reasons, animal health experts are calling for increased attention to biosecurity, and some nations are barring the import of live poultry.

The great distance encompassed by the nations so far affected demonstrates the capability of the H5N1 virus to survive and spread.