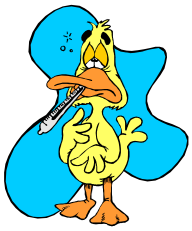
Influenza Virus: A tiny moving target



Level: High School
Time: 2-3 50 minute periods

Overview:
The potential of a new flu pandemic is a frightening idea. This curriculum explains how influenza viruses infect cells and replicate. It also has students explore where influenza viruses come from, how viruses change, and why some become deadly.

Background for Teachers
Influenza viruses are always with us, constantly changing and causing misery each flu season. Most of the time they knock people off their feet for a week or two but sometimes the flu can be dangerous or even fatal. Immunocompromised people, those who are already sick, or those whose immune systems are weak such as young children and older people, are hit hardest by the flu. This version of the flu is with us every year and we are accustomed to it, however the threat of a flu pandemic is a much larger problem and rightfully causes a great deal of concern.

Flu pandemics emerge in a cycle and the world is overdue for the next one. If the avian H5N1 virus doesn't cause the next pandemic, another strain will. There were three pandemics in the 1900's: 1918, 1957, and 1968. In 1918, 30-40 million people died world wide (For more information, see Reference 1). We know much more about influenza now than we did in 1918, and the hope is that we can use this knowledge to prevent or control a serious pandemic (Ref. 2). Pandemics occur when a virus acquires the ability to infect a new host and spread rapidly through the population. How this occurs is more easily understood when the normal replication cycle of the virus is understood.

Influenza viruses come in three types: A, B, and C. All three can infect humans, but the most common infection is by type A. Type A viruses are classified in subtypes based on two proteins that stick out from the surface. These are hemagglutinin (H), which is responsible for initiating entry into the host cell, and neuraminidase (N), which is involved in release of new viral particles from the infected cell. There are 16 varieties of hemagglutinin and 9 of neuraminidase. Subtypes are named by which version of hemagglutinin and neuraminidase, such as H1N2 a common version in humans. The virulence and pathogenicity of the virus depends on the combination of hemagglutinin and neuraminidase.

Influenza viruses are normally found in wild waterfowl. The H5N1 avian version is particularly deadly in its normal host, wild birds. It is easily transferred to domestic birds, and from there has occasionally infected humans, particularly those who came in close contact with infected birds. It appears to be very dangerous in humans as well, but that may be due to reporting. Work in Turkey has revealed people who were infected

with H5N1, but did not get sick enough to require medical attention. At the same time, other people in Turkey have died of the disease.

In order for H5N1 to develop into a pandemic, it must acquire the ability to transfer rapidly between and effectively infect human hosts. Once that occurs, it will be difficult to control the spread of the disease. Our global society ensures wide dispersal of diseases via air travel, and the incubation period for the flu is short. This means that by the time health officials recognize an outbreak, it will already have spread beyond the original area. However, we understand influenza much better now than we have in previous pandemics. We are better equipped to prevent transmission of the virus, have antiviral medications to treat infections, and are working to develop effective vaccines. For more information on influenza viruses and pandemics, see References 3, 4, and 5.

The control of influenza relies on basic evolutionary theory. The development of the annual flu vaccine is based on our understanding of antigenic drift – or change over time. We expect new strains to arise as viral polymerases make mistakes during replication. We can predict the direction of change based on the trends observed in the virus genome over the course of the flu season, and prepare our immune systems with a vaccine. The vaccine is developed with the latest version of the flu so that when the "real thing" shows up in our bodies, the immune system has seen it or something similar and can effectively fight it off. Pandemics occur because our immune system cannot cope with invasion by a completely new viral type and these viruses often infect more cell types than the "normal" flu virus. Pandemic viruses avoid detection by the immune system, cause massive damage in the host, and spread rapidly. Pandemics die down because any pathogen that kills its host before replicating and spreading dies with the host. A milder version of the disease is more likely to allow continued dispersal of the virus and so viral strains causing less intense symptoms will have a selective advantage. In previous pandemics, the virus became less dangerous after 18-24 months. However, incredible damage was done during that brief time.

About the Lesson

This lesson is structured using the BSCS 5E model. In this model, students are first *Engaged*, in this case by presenting them with a game modeling viral infection. Once the students are interested, they move to combined *Explore* and *Explain* sections where they have an opportunity to learn about viral structure and replication. Students then move to an *Elaborate* section, in which they apply the information they have just learned at a more advanced level to explore how viruses evolve. *Evaluation* can be conducted throughout the inquiry based lesson, but a formal evaluation opportunity is suggested in the form of presentations or written reports in which students are asked to use their understanding of the virus to suggest ways in which to prevent or treat infection.

National Science Standards

Content Standard A. Abilities	Communicate and Defend a scientific argument	• Reviewing information, expressing concepts, constructing a reasoned argument.

Necessary to do Scientific Inquiry		
C: Life Science	The Cell	• Cells have particular structures that underlie their functions. Every cell is surrounded by a membrane that separates it from the outside world. Inside the cells in a concentrated mixture of thousands of different molecules which form a variety of specialized structures that carry out such cell functions as energy production, transport of molecules, waste disposal, synthesis of new molecules and the storage of genetic material.
	Molecular Basis of Heredity	• In all organisms, the instructions for specifying the characteristics of the organism are carried DNA. • Changes in DNA occur spontaneously at low rates. Some of these changes make no difference to the organism, whereas others can changes cells and organisms.
	Biological Evolution	• Species evolve over time. Evolution is the consequence of the interactions of (1) the potential for a species to increase its numbers, (2) the genetic variability of offspring due to mutation and recombination of genes, (3) a finite supply of the resources requires for life, and (4) the ensuing selection by the environment of those offspring better able to survive and leave offspring. • The millions of difference species of plants, animals, and microorganisms that live on earth today are related by descent from common ancestors.
	Interdependence of Organisms	• Organisms both cooperate and compete in ecosystems. The interrelationships and interdependencies of these organisms may generate ecosystems that

		are stable for hundreds or thousands of years. • Human beings live within the world's ecosystems.
Content Standard F: Science in Personal and Social Perspectives	Personal and Community Health	• The severity of disease symptoms is dependent on many factors, such as human resistance and the virulence of the disease-producing organisms. Many diseases can be prevented, controlled, or cured.
Content Standard G: History and Nature of Science	Science as a Human Endeavor	• Scientists are influenced by societal, cultural, and personal beliefs and ways of viewing the world. Science is not separate from society but rather science is a part of society. • Occasionally, there are advances in science and technology that have important and long-lasting effects on science and society. Examples of such advances include the geologic time scale and biological evolution.

Preparation

- Prepare the following for "Gotcha!":
    - 2 tokens per student
    - 1 slip of paper per student stating cell type (virus, respiratory, lung, immune, other organs as needed). Keep a ratio of 1:1:1:1:1, so in a class of 30, there will be 6 viruses, 6 respiratory cells, 6 lung cells, 6 immune cells, and 6 other organs.
    - Write a password on each slip. Give the viruses at least three different passwords (ie. Blue, turtle, apple). Give all the respiratory cells one of the viral passwords (ie. Blue). Give one lung cell a viral password, and give the others completely different passwords. Give the immune cells two out of three passwords in different combinations (ie. Blue and turtle, blue and apple, turtle and apple). Give one of the other cell types a viral password and the others completely different passwords.

- For the viral structure lesson, prepare one set of the following materials for each group:
    - 1 plastic egg
    - 8 one inch strips of black yarn
    - 8 half inch pieces of drinking straw
    - 8 paper clips or buttons that can slide on the yarn
    - 15-20 "dots" of Velcro (soft side), made with a hole punch
    - 15-20 grains of rice.

- If desired, make enough copies of *Master 1.1 Cell Surfaces* for groups of two to three students. You may wish to laminate these if you want to use them repeatedly. Prepare the *Master 1.1 Cell Surface*s by gluing the opposite side of the Velcro "dots" from the virus structure on the "respiratory tract". Glue paper or cloth dots to the other cell surfaces.

- Make one copy per group of *Figure 1. Influenza Virus* for the structure exercise.

- Make one copy per student of *Master 1.2 Classifying Influenza Viruses*.

- For each group prepare two sets of 3X5 cards. Label the cards on one side in blue ink with HA, NA, PB1, PB2 PA, M, NP, NS. On the other side of the card write the name of the corresponding protein. For one set write the name in black ink; write the name in red ink on the other set.
    - Hemmagglutinin (HA)
    - Neuraminidase (NA)
    - PB1 (polymerase)
    - PB2 (polymerase)
    - PA (polymerase)
    - M1/M2 (coat protein)
    - NP (nucleocapsid)
    - NS1/NS2 (nonstructural proteins)

- Assure access to computers in teams of two or three students.

Lesson
Engage/Explore
In this section students begin thinking about key components of the viral replication cycle. They will learn that viruses are limited to infecting certain cells based on interactions between viral coat proteins and cell membrane proteins.

1. Find a space that will allow students to walk around (move desks, go outside, use the gym or the stage). Write the following rules on the board, or somewhere where students can see them:

- **Gotcha! First time - give up token. Second time – give up token, count 25 and sit down.**
- **Be discreet!**

2. Tell the students they are going to play "Viral Gotcha". The rules are as follows:
- **Each player takes two tokens and draws a slip assigning a cell type and password. Keep your cell type and password secret!**
- **All players stand up and move slowly around the room.**
- **As you approach other players, clasp hands and whisper your cellular identity (cell or virus) to the other player.**

- **If you are a virus, and you find a cell, state your password. The "cell" player will accept the password or say "no entry". If you are a cell, any virus with a matching password can "infect" you. Viruses with passwords that do not match yours cannot infect you.**
- **If a "virus" finds a "cell" that accepts the password, the cell has to give the virus a token – quietly! This means the "cell" has been infected.**
- **If a "cell" is "infected" twice, then after giving the second virus a token, the cell player keeps moving around while counting to 25 (in his or her head) and then sits down.**
- **Each "immune cell" player will have a list of passwords he or she "recognizes". When an "immune cell" meets a "virus" with a recognized password, the "virus" player has to give his or her tokens to the "immune cell". A "virus" that is caught by an "immune cell" twice must count 25 in his or her head and sit down.**

Allow students to play for 5-10 minutes, depending on how many are sitting down.

3. At the end, tally how many viruses and cells are left standing or "alive". Ask the "live" viruses how many tokens they each acquired. Group cells together by type and password, and determine how many times each cell type was infected. Ask the "immune cells" how many viruses each of them caught. Write the answers on the board.

4. Ask the students what they notice about the results.

Guide the students to find answers to the following questions:
- Which did better, cells or viruses?
- Did a particular type of cell get infected more often?
- Did a particular type of virus infect more cells?
- Did a particular type of virus get caught by the immune cells more often?

Let students explore the results as independently as possible. The goal is for students to come to understand that viruses do not infect cells randomly, and that it is possible for a virus to avoid detection by the immune system.

One option, if time allows, is to run the game multiple times and see if the results vary.

5. Explain to the students that viruses cannot infect cells without the correct "password" although in real life, the password is actually a coat protein on the virus interacting with a membrane protein on the cell. Usually passwords are cell type specific, for example, HIV targets T cells, but some viruses have a broader range of host cells.

Explore/Explain
In this section, students explore viral structure and how the virus replicates.

6. Have students work in groups of two to three to build their own influenza virus. Give each group one plastic egg, eight strands of yarn, enough modeling clay to cover the egg, 8 paper clips or buttons, 8 pieces of drinking straw, 15-20 velcro "dots", and 15-20 grains of rice.

In this model, the Velcro represents the hemagglutinin, and the rice represents the neuraminidase. The egg represents the coat proteins, M1 and M2, and the yarn represents the eight strands of the genome with the sliding buttons or paper clips as the polymerase. The pieces of straw represent the nucleocapsid protein. The two nonstructural proteins are not present in the virus; they are found only in infected cells.

You may wish to provide students with additional materials to allow them more creativity in building their virus.

7. Show students Figure 1. Influenza Virus, and have them watch the animation of the viral life cycle. This figure and animation will serve as their guide in constructing their own virus. In addition, they may wish to explore the following site:
http://www.bact.wisc.edu/Microtextbook/index.php?name=Sections&req=viewarticle&artid=126&page=1.

The influenza virus is fairly simple. It is composed of a protein coat, with a membrane covering, which is punctuated by two proteins, hemagglutinin (H1-16) and neuraminidase (N1-9). These two proteins determine which host cells the virus can infect. Inside are 8 pieces of RNA, the coat proteins (M1 and M2), two nonstructural proteins (NS1 and NS2), a nucleic acid binding protein, also called the nucleocapsid (NP), and a polymerase composed of three proteins (PA, PB1, and PB2). The three polymerase genes, hemagglutinin, neuraminidase, and nucleocapsid protein are encoded on single strands of RNA. M1 and M2 are produced by different spicing patterns off of one piece of RNA, as are NS1 and NS2.

To infect a cell, the hemagglutinin must first bind a host protein, causing the virus to be phagocytized. Once inside the cell, M2 is involved in releasing the virus genome whereupon the genome moves to the host nucleus. In the nucleus, viral protein production is initiated and the genome is replicated. PA, PB1, and PB2 form the polymerase. The polymerase, nucleocapsid protein (NP), NS2 and M1 bind the pieces of RNA. Hemagglutinin, neuraminidase, and M2 are produced and localized to the host membrane. The nonstructural protein NS1 inhibits the host defense response. When enough components are prepared, M1 and NS2 guide the export of the genome to the cell membrane where the viral coat has assembled. The virus forms and leaves the cell. Neuraminidase plays a critical role in viral exit.

8. If desired, provide teams with *Master 1.1 Cell Surfaces*. Have students demonstrate to themselves that their virus will only interact with one type of cell.

Point out to students that this is very much not to scale. Another option is to make a similar display, closer to scale, on poster board and have students stick their viruses on the large display.

Explain/Elaborate
In this section, students will consider how a virus mutates, acquiring the ability to infect different cells.

9. Have students fill out *Master 1.2 Classifying Influenza Viruses* using the CDC web site: http://www.cdc.gov/flu/avian/gen-info/flu-viruses.htm. Have students go over their results in teams or collect student responses and have a brief class discussion.

This is a Type A virus because only Type A is classified in subtypes. Subtypes of influenza virus are named for the version of hemagglutinin and neuraminidase they carry. H7N2 carries hemagglutinin version 7, and neuraminidase version 2. Usually, only H1N1, H1N2, H3N2 influenza viruses are found in humans. H7 is a subtype that is found in humans and wild birds. The most likely source of the H7N2 virus was the wild geese at the pond.

Hemagglutinin and neuraminidase change by antigenic drift – changes due to mutations caused by the influenza virus' error prone polymerase. This generates different strains of virus which the immune system may not recognize. A new vaccine must be developed each year because of antigenic drift. The early sample was a similar strain to the one used for the vaccine. By the end of the flu season in 2005, enough antigenic drift had occurred to generate a new strain which was not included in the vaccine.

10. Tell students that a cell can be infected by more than one virus. Explain that in this exercise they will model what could happen when two influenza viruses infect a cell simultaneously.

11. Give each group a set of red viral genome cards and a set of black viral genome cards. Have one student in each group shuffle the cards and hold them face down so that the blue labels show. Another team member should choose 8 cards to assemble a complete genome including one each of the cards labeled HA, NA, M, NP, PB1, PB2, PA, and NS.

12. Have students flip the cards over and see if they were able to build a virus from a single source. Ask students to figure out the probability of selecting a genome from either the black set or the red set.

The odds of generating a genome that is completely red or completely black is 1/256. There is a ½ chance each time a card is picked, since each protein must be represented, or ½ x ½ x ½ x ½ x ½ x ½ x ½ x ½ = $1/2^8$ =1/256.

13. Ask the students what the outcome of this exercise is in terms of viral genomes. If mixed viruses are generated, will they all be able to infect the same cell types? What happens if they can infect fewer cell types? More? What are some other possible outcomes?

The students should recognize that at many viruses produced under these circumstances will have mixed genomes. In some cases these mixed genomes are not viable, but in some cases they are. Sometimes the virus will be released, but be unable to replicate because it lacks compatible proteins. The mixed viruses might also acquire new traits, such as the ability to infect different cells or replicate faster. If the hemagglutinin or neuraminidase changes, this is called "antigenic shift". This is the scenario scientists fear could lead to a pandemic – an H5N1 (avian flu) and some other influenza Type A virus infecting a human cell, allowing the H5N1 subtype to acquire the ability to pass from human to human. For more information go to: http://www.cdc.gov/flu/avian/gen-info/transmission.htm.

14. Tell students one way for H5N1, the avian flu virus, to develop into a pandemic is if an H5N1 virus infects a human cell simultaneously with a normal human Type A virus. The two viruses might shuffle components creating a deadly virus with the ability to spread rapidly in humans.

Sometimes this antigenic shift occurs in an intermediary host, such as pigs. It's possible that this chance event will never occur for H5N1, but it will occur sometime for some other similar virus.

Evaluate

Students should understand the basics of viral infection and replication. Evaluate their understanding by asking them to apply this knowledge to design ways to prevent infection or replication, or by asking them to determine how a virus could develop resistance to anti-viral medications.

14. Tell students that they are members of the Centers for Disease Control Division of Viral and Rickettsial Disease. Their task is to design two ways to prevent influenza virus from causing disease in humans. Working in teams of three to four students, they will prepare an oral report or poster that explains their methods for prevention of disease. They must justify their methods based on what they know about viral structure and replication cycle.

This question is deliberately left open-ended to allow students to design various methods. All answers should be considered valid as long as they use the information they have learned about viral structure and replication. For example, "Thoroughly wash your hands" is valid if it is based on keeping the virus away from pathways into the body or the concept that the virus capsule can be damaged by soap. More technical approaches would include interfering with the viral-cell recognition step, inhibiting entry into the cell, or interfering with viral replication.

You may wish to have students develop a public awareness campaign and put up posters of their prevention and treatment suggestions. They may wish to create a flyer, a web site, or an article for the school paper. Make sure student suggestions are valid before allowing students to post them.

15. OR have students visit http://www.niaid.nih.gov/factsheets/fludrugs.htm where they will learn about the four antiviral drugs currently available. This page explains how each antiviral medication works. Have students explain in evolutionary terms how a virus develops resistance to a medication. What is the variation? What is the selective pressure? Do viruses evolve more or less quickly than other species, vertebrates for example? Why?

This is an example of microevolution, in which small changes enhance the ability of the organism to survive. Virus populations have high levels of variation first because the RNA polymerase is not very accurate, and secondly because the replication cycle is fast and third because each virus generates many offspring. The selective pressure is ability to enter cells before the immune system detects and destroys the virus, survive in hostile environments between hosts, and replicate quickly and effectively once inside the cell. Successful viruses will replicate more often than unsuccessful viruses, increasing the number of viruses in the population with the "successful" characteristics.

Evolution of viruses leads to some interesting questions as there is ongoing debate regarding whether viruses are alive. Computer programs that model evolution may be considered the equivalent of viruses, since they cannot survive outside the host (computer program), and they evolve rapidly. For further exploration of this idea, try the Avida site: http://dllab.caltech.edu/avida/ .

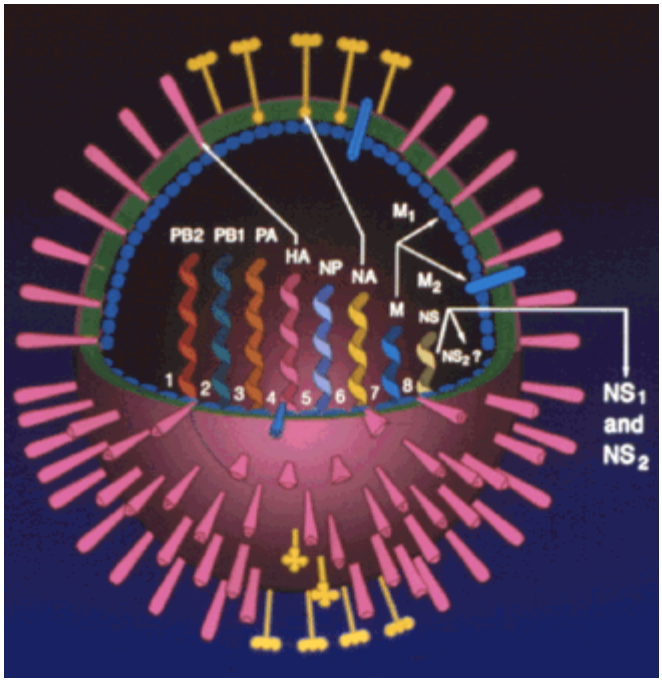
Transparencies/Handouts

Figure 1. Influenza Virus

Master 1.1 Cell Surfaces

Master 1.2 Classifying Influenza Viruses

Figure 1. Influenza Virus



Eight strands of RNA encoding:

Polymerase(PB2, PB1, PA)

Hemagglutinin (HA)

Nucleocapsid (NP)

Neuraminidase (NA)

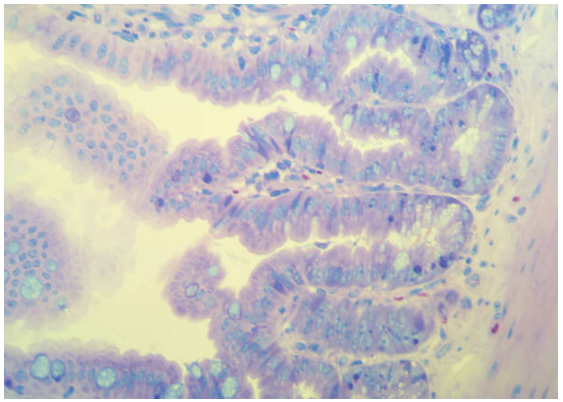
Coat proteins (M1 and M2)

Nonstructural proteins (NS1 and NS2)

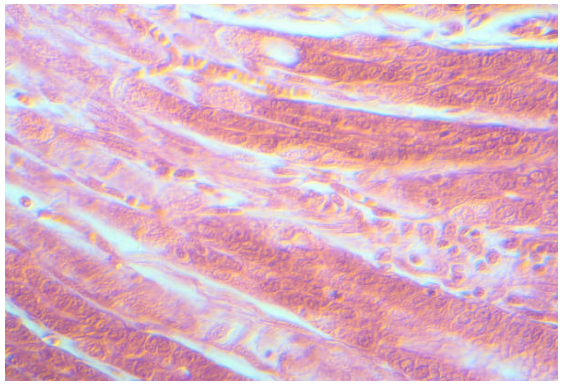
Arrows indicate location of proteins in virus. The polymerase, NS1 and NS2 are not found in the virus particle. They are only produced in the host. NP coats the RNA strands.

Master 1.1 Cell Surfaces/Nucleus

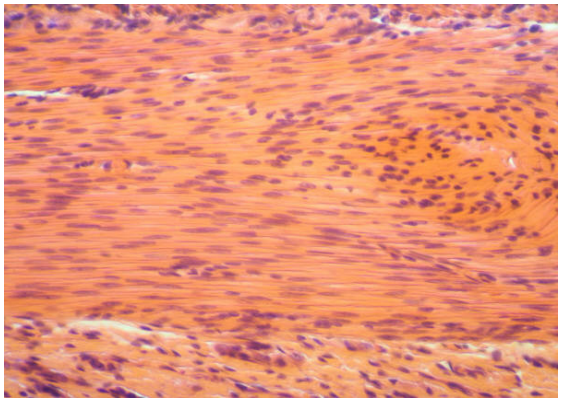
Cell Surfaces



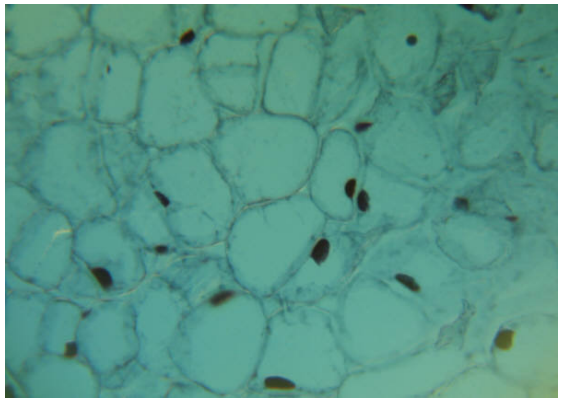
Lung Tissue



Respiratory Tissue



Muscle Tissue



Epithelium

Master 1.2 Classifying Influenza Viruses

You are applying for a job as a lab technician at the Centers for Disease Control in the Viral and Rickettsial Division. One task you must be able to complete is to gather information from reliable sources and apply that information in your work. Demonstrate your ability to do this by answering the following questions using the CDC web site as a guide: http://www.cdc.gov/flu/avian/gen-info/flu-viruses.htm.

1) You are given a virus sample labeled "H7N2". Answer the following questions about this virus.

Is it Type A, B, or C?

What does "H7" indicate?

What does "N2" indicate?

This sample was isolated from a park caretaker in Turkey. The park has a golf course, playground, duck pond, and petting zoo. The caretaker is responsible for maintaining the lawn in the park, the playground equipment, and the pond area. There are many wild geese, as well as a steady population of ducks at the pond. The petting zoo includes goats, sheep, potbellied pigs, rabbits, and a pony. The man keeps a cat and an aquarium. He has no children, but does work with groups visiting the petting zoo. He has not traveled recently, but his sister and her family visited two weeks ago. What is the most likely source of his infection and why?

2) You are given a set of 25 virus samples from a community. The first sample was collected in December, 2004 and the last sample is from May, 2005. In lab tests, the 2004 influenza vaccine was found to be effective against the 2004 sample, but not as effective against the 2005 sample. What could cause this difference?

References

1. American Experience "Influenza 1918"
The story of the 1918 pandemic.
http://www.pbs.org/wgbh/amex/influenza/

2. Secrets of the Dead "Case File: Killer Flu"
Modern scientists study the 1918 virus to understand the source of its virulence.
http://www.pbs.org/wnet/secrets/case_killerflu/

3. Centers for Disease Control
Basic Q&A, information for specific groups, and specific topics such as prevention and outbreaks.
http://www.cdc.gov/flu/avian/

4. World Health Organization (WHO)
Concentrates on the potential pandemic, with information about how a pandemic will affect the world.
http://www.who.int/csr/disease/avian_influenza/en/

5. BBC News In Depth Bird Flu
Includes an interactive site for tracking bird flu, video reports, Q&A on bird flu, and various articles on "Background and Features" and "Fighting the Virus".
http://news.bbc.co.uk/1/hi/in_depth/world/2005/bird_flu

6. National Institute on Allergies and Infectious Diseases
Information about flu drugs.
http://www.niaid.nih.gov/factsheets/fludrugs.htm

This material was developed by Kristin Jenkins for NESCent. For questions or comments on this material, please contact the developer at kjenkins@nescent.org.

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