

# **Disease Susceptibility and Transmission: The Epidemiologist's Dilemma**

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## **Overview**

This lesson seeks to provide a look at the process of scientific discovery and the basic knowledge that would be applied to a specific, hypothetical epidemiological problem involving the emergence of a new strain of human influenza virus. The lesson covers basic information from numerous scientific disciplines including genetics, cellular biology, population ecology, virology and epidemiology. Students will be epidemiologists for a week, gathering information and posing questions to solve the epidemiological problem. Various activities are incorporated into the lesson plan to engage students in the discovery process and to stimulate questions for solving the problem.

Students should be familiar with the scientific discovery process and use this in the lesson plan. As an added piece of scientific reality students will be asked to keep lab notebooks, just as most scientists do. These notebooks should be filled with information pertinent to solving the epidemiological problem and should include: modes of disease transmission, the host-parasite disease model, the structure of the influenza A virus, the function of the RNA genome of the influenza A virus, the function of the hemagglutinin and neuraminidase surface proteins, the basics of viral reproduction and how this affects evolution and shift of the influenza virus. The notebook should also contain the epidemiological clues given, the question students pose to address the problem culminating in the epidemiological timeline of the problem describing explicitly how events progressed to illustrate knowledge gained from the lesson. The minimum information expected for the notebook will be up to the teacher's discretion and will depend on how in depth the teacher chooses to go.

## **Prior Knowledge**

This lesson will best achieve the goals set forth if it follows a genetic and cellular biology unit. Prior knowledge needed to understand this lesson is as follows: General knowledge of genetics, including the roles of DNA and RNA in coding for proteins; Basic understanding of the cellular machinery used in protein synthesis; and knowledge of the cellular membrane, particularly cell surface proteins and receptors.

## **Possible Continuations/Additions**

This lesson plan could stimulate a number of questions by students about the role of immune response, population dynamics, and wildlife in the emergence of infectious diseases and the possibility for pandemics. The lesson itself is set-up nicely to move directly into the immune system and response to disease. The lesson plan is easily expandable to a two-week lesson or five two-hour lessons.

## **Major Concepts**

- The basics of disease, types of disease and disease transmission

- Basic population ecology.
- Introduction to epidemiology and the numerous sciences that contribute to the study of disease.
- Basic virology focusing on the influenza virus.
- The role of evolution, natural selection, and generation time in producing new viral strains and even new species.
- The role of genetic reassortment in human influenza viruses.
- Application of the scientific discovery process in addressing real world problems.

## **Standards and Benchmarks**

### **Lesson Outline (5, 45 minute lessons)**

- Day 1: Introduction of disease, types of diseases and the epidemiological problem
- Day 2: Disease transmission
- Day 3: Introduction to viral diseases and discussion of Influenza
- Day 4: The Influenza virus and evolution, from ducks to people
- Day 5: Epidemiology end game

**The Epidemiological Problem Clues** (Clues can be presented in a different order as seen fit and after the first clue is given they should be presented after students ask questions that would possibly get at these clues. It will be important to emphasize asking questions useful for solving this problem.)

- Lesson Day 1: A woman in Hicksville, OH is admitted to the emergency room with severe flu-like symptoms. After two days in the intensive care unit she dies from an unknown disease.
- Lesson Day 2: The Centers for Disease Control has recently received reports of large domestic poultry die-offs in Hong-Kong.
- Lesson Day 3: The infectious agent was identified as an unknown Influenza A virus that scientists are attempting to genotype. Additional reports of similar cases have come to the attention of the CDC a businessman in Los Angeles, a woman professional in New York City, a college student in Chicago, and an ER nurse in Hicksville, OH.
- Lesson Day 4: The virus has been genotyped and it is a previously unknown strain of human influenza that most closely resembles a common waterfowl strain of influenza found most recently in Mongolian waterfowl.
- Lesson Day 5: The FBI has gathered travel information for the 5 sick Americans. Turns out all were on the same plane from New York City to Chicago five days before the first woman entered the ER. Additionally, the man from Los Angeles was recently in Hong Kong for business.

### **Lesson Day 1: Introduction to disease, types of diseases and the epidemiological problem**

**Preparation:**

Students will need a lab “notebook” for writing down the important information learned throughout this lesson, to draw diagrams in, and to write their questions and answers to those questions. The “notebook” should be viewed as a mock lab notebook and can be used as the assessment. Have a blackboard or overhead available to write down different types of diseases for the entire class to see and a concise definition of disease and epidemiology.

### **Lesson Objectives:**

- Students begin thinking about different diseases, why they are different and what it means to be a disease.
- Thinking critically about communicable vs. non-communicable diseases why are they different?
- Define disease and epidemiology.
- Introduce the epidemiological problem that will be the focus the week’s lesson and start students thinking about what questions need to be asked and answered.

### **Lesson:**

#### **Background Information:**

Disease is defined as “**an impairment of the normal state of the living animal or plant body or one of its parts that interrupts or modifies the performance of the vital functions** and is a response to environmental factors (as malnutrition, industrial hazards, or climate), to specific infective agents (as worms, bacteria, or viruses), to inherent defects of the organism (as genetic anomalies), or to combinations of these factors” –*from Merriam-Webster’s Medical Dictionary, 2002*. Basically any state of being outside of what is normal for that organism. Diseases can generally be divided into communicable and non-communicable diseases. Communicable diseases are those transmitted through direct contact with an infected individual or indirectly through a vector and generally include bacterial, viral, fungal, parasitic and prion diseases. Some examples can be found at <http://kidshealth.org/parent/infections/> and particularly <http://www.cdc.gov/page.do> and include:

- **Bacteria** – the plague (*Yersinia pestis*) streptococcus (strep throat), staph infection, *Campylobacter*, *Escherichia coli*, tetanus, tuberculosis, pinkeye (conjunctivitis) caused by many different types of bacteria including those that cause gonorrhea and Chlamydia, diarrhea is often caused by bacteria in the gastrointestinal tract, diphtheria, *Helicobacter pylori* can cause gastritis and peptic ulcers, botulism, Cat scratch disease, Rocky Mountain spotted fever, Brucellosis, salmonella, Lyme disease is caused by the bacteria *Borrelia burgdorferi*, Scarlet fever is caused by a *streptococci* bacteria, Syphilis, and Whooping cough to name a few
- **Virus** – the flu (influenza virus), colds (rhinoviruses), chickenpox (varicella-zoster virus), polio, hepatitis, West Nile virus, warts, AIDS, Herpes, measles, Mononucleosis, mumps, rabies, Hantavirus, Dengue fever, hemorrhagic fevers, smallpox, yellow fever and many others.
- Many common diseases are caused by both bacteria and viruses – hepatitis (although mostly viral), strep throat (although mostly bacterial), pneumonia
- **Fungus** – ringworm, jock itch, athlete’s foot, many fungal diseases of plants
- **Prion (mal-folded infectious protein)** – mad cows disease, chronic wasting disease, scrapie, Creutzfeldt-Jakob disease

- Parasite – malaria, tapeworm, Giardia, roundworm, toxoplasmosis, hookworm, lice, intestinal amebic infections (often cause severe diarrhea), scabies, African sleeping sickness

Non-communicable diseases would be those that are not transmitted from one individual to another and include:

- Genetic diseases, caused by abnormalities in the individual's genetic material illustrating the role of mutation and inheritance in disease. Also, important to note that an individual's genotype and environment will affect the outcome and progression of every disease (including infectious diseases).
  - Examples: cerebral palsy, down syndrome, sickle cell anemia, cystic fibrosis, dwarfism, cleft lip and palate, Tay-Sach's disease, Marfan syndrome, muscular dystrophy, spina bifida, Jacob's syndrome (XYY syndrome).
- Environmental diseases, caused by the biotic (living) and abiotic (non-living) factors surrounding us.
  - Examples: asthma and allergies, dermatitis, emphysema, goiter (not enough iodine), scurvy (not enough Vit. C), lead poisoning, mercury poisoning, malnutrition, vitamin deficiencies, sunburn, skin cancer (in fact many other cancers are caused by environmental carcinogens), radiation exposure, etc.

It is important to not that some students will either have or know of someone who has a genetic disease or condition such as Down's syndrome. This highlights a kind of grey area in that to be politically correct one may not want to say that a person with a genetic condition such as Down's syndrome is diseased, since disease has a very negative connotation. This would be a good point to have students think about.

Epidemiology is defined as “**1:** a branch of medical science that deals with the incidence, distribution, and control of disease in a population **2:** the sum of the factors controlling the presence or absence of a disease or pathogen.” –*from Webster's-Webster's Medical Dictionary, 2002*. Like many words it has two general but related meanings. An epidemiologist is someone who studies the “incidence, distribution, and control of disease in a population” which implies the study of the “factors controlling the presence or absence of a disease or pathogen.” It is a science, like many others, that draws heavily from a number of different disciplines including virology (study of viruses), bacteriology (study of bacteria), genetics, molecular biology, medical and veterinary sciences to name a few as well as sociology, behavior, political science, anthropology and even religion. Epidemiologists are basically disease detectives, their goal/problem is to discern where an infectious disease originated from, who it has and will infect, what may be the outcome, and how best to deal with the potential outcomes. Epidemiologists deal with *epidemic* (Spreading rapidly and extensively by infection and affecting many individuals in an area or a population at the same time) and *pandemic* (occurring over a wide geographic area and affecting an exceptionally high proportion of the population) disease outbreaks.

### **Activities:**

10 min. – In groups or as a class, have students brainstorm as many different diseases as they can. These should be written in the students “lab notebook.” Encourage students to think about diseases affecting any animal, not just human diseases, as many students may know of livestock or wildlife diseases, these should be encouraged as it will provide a stepping stone to introduce

viral diseases such as Influenza that can infect different vertebrates, including humans. You will likely get a wide range of different diseases but most will probably be bacterial, viral, prion, parasitic, genetic or environmental ailments.

5 min. – Based on the different diseases mentioned have the students try to define “disease” in their own words. Be sure to point out similarities and differences between the classes of disease (i.e. infectious vs. genetic vs. environmental)

10 min. – Provide actual definition for disease and discuss why disease is defined in this manner. Compare to what students defined disease as. Pose the question, are genetic disorders considered diseases? This is the grey area for the definition of disease. The right answer may be a simple following of the definition or may be dictated by more politically correct thinking. This is more of a thought exercise as there really is no right or wrong answer

10 min. - Discuss the different types of diseases, i.e. communicable vs. non-communicable and further categorize into bacterial, viral, genetic, environmental, etc. Have students place the diseases from the brainstorm session into different categories.

10 min. – Introduce Epidemiology, define and discuss. Introduce the Epidemiological problem emphasizing that students will be government epidemiologists (employed by the Centers for Diseases Control) for the rest of the week using their lab notebooks to record information pertinent to their epidemiological problem. Introduce the problem by providing the first clue: The CDC was contacted by a doctor from Hicksville, OH (a real town) who treated a woman in the emergency room that presented with severe flu-like symptoms. After two days in the intensive care unit she died of unknown causes. An autopsy is being performed but no other information is available. As epidemiologists what questions should be asked and how will they be answered?

Questions that will need to be answered first include: Are there any other similar cases being reported? If so, where? How long was she sick before being admitted to the hospital? What were the signs and symptoms? Did she travel recently? Was she in contact with other people? Was she in contact with animals, wild or domestic? Are hospital workers that treated her getting sick? This is a good start, there will probably be lots other questions students pose many of which will be pertinent. In following lessons prompt students to provide some of these pertinent questions in order to receive further clues to the epidemiological puzzle.

## **Lesson Day 2: Disease Transmission**

### **Preparation:**

Teacher will need one 50ml beaker for each student, a 125ml flask for each student and enough distilled water to provide 50ml for each student. Dilute hydrochloric acid (HCl), bromthymol blue, and a dropper or pipette. The use of a chalkboard or overhead will be necessary for the disease model.

### **Lesson Objectives:**

- Introduce different modes of disease transmission

- Introduce the idea that disease and epidemiology is basically a specific facet of population ecology
- Provide a basic model of disease within in the context of population ecology

## **Lesson:**

### **Background Information:**

For disease and infection to occur and spread three elements must be present: 1) a source of infecting microorganisms; 2) a means of transmission for the microorganism; 3) a susceptible host. A source of infecting microorganisms can be just about any living organism that carries and sheds the microorganism. Modes of transmission are varied, however, and generally fall into three categories:

- Contact transmission including direct contact like a handshake, kiss, sex or bite and indirect contact such as sharing drinking glasses, toothbrushes, toys, punctures, or droplets from sneezing or coughing (within one meter).
- Vehicle transmission including airborne particles such as dust, waterborne in streams and swimming pools, or food borne by eating improperly prepared poultry, meat or seafood.
- Vector transmission including mechanical via insect bodies or inanimate objects and biological such as biting insects like lice, mites, mosquitoes and ticks.

Finally a host needs to be susceptible in order for the microorganism to cause disease. If for example a virus that causes disease in the respiratory tract of an animal is swallowed, then the host organism will not be susceptible since the virus will have no way to enter cells and replicate since it is in the GI tract and only has receptors for cells in the lung.

For influenza in humans the infecting microorganism is the influenza A virus, the original source being wild waterfowl (transmitting the virus to domestic fowl/poultry). The means of transmission from bird to bird and bird to human (or other mammals) would be contact transmission most likely due to contact with infected birds and their excrement (since in birds influenza is usually a gastrointestinal infection with virus particles shed in feces). Transmission from human to human would also be contact transmission but via saliva and mucous expelled by sneezing or coughing since the influenza virus affects the respiratory system in humans. Finally, because of the nature of the influenza virus, humans are most definitely susceptible.

Since students will be acting as epidemiologists and most infectious diseases are for all intents and purposes host-parasite systems (the influenza virus would be the parasite and the infected human the host), it is important to understand how host-parasite interactions may affect a population. Basic population dynamics focuses on birth and death rates to determine whether a population is growing, shrinking or stable. The basic disease model is in fact a host-parasite interaction model and assumes certain birth and death rates specific to a population. The introduction of a parasite into a population will affect the birth and death rates of that population and is therefore tied to the basic tenants of population dynamics. The basic disease model will be useful for predicting what has, is, and will happen once a disease enters a population and is an extremely important aspect of epidemiology.

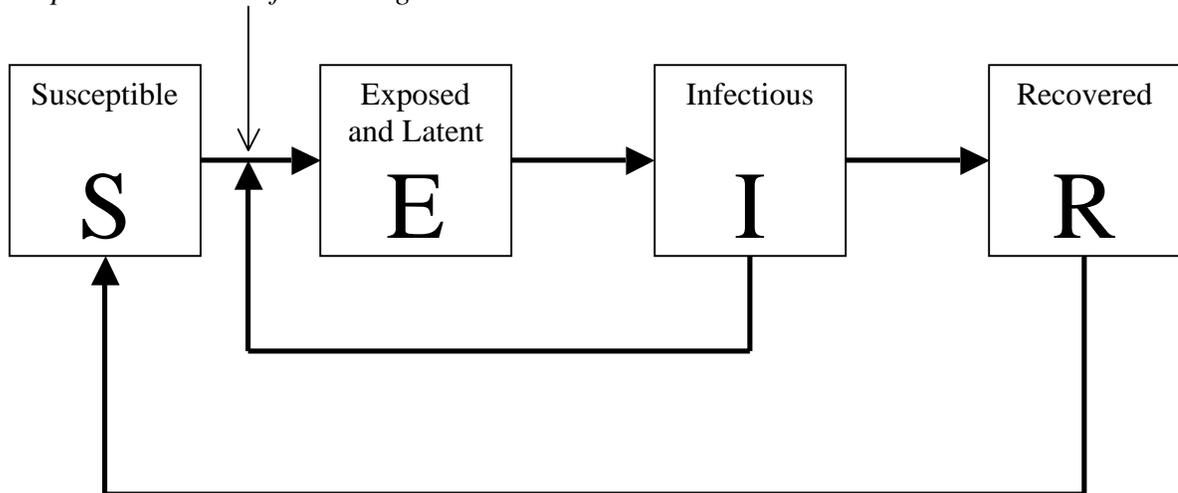
Models are important scientific tools because they take a complex system and break it down to more manageable parts. There are many factors involved in a host-parasite interaction including the basic biology of the parasite (or virus), the physiology and condition of the host, and the behavior of the host within the population. Since the model is basically a host population

model the complex system is broken down into four categories of individual hosts. These include:

- the susceptible population – only those individuals able to be infected by the parasite are considered
- the exposed but latent population – once exposed there is often a latent period (a time where the host is infected with the virus but is not yet shedding the virus)
- the infectious population – those infected and actively shedding the virus, this is the population that can infect others that are susceptible
- the recovered population – those individuals that have survived the infection (remember depending on the parasite some infected individuals may die and are removed from the population), there are two recovered outcomes; recovered and immune or recovered and susceptible (depending on type of infection and the host's immune system), the latter case move back into the susceptible population

The basic model therefore looks something like this:

*Some portion of susceptibles will be exposed, and some portion of these will develop the disease. This is determined by a simple equation using an infectivity coefficient dependent on the infectious agent.*



The arrows indicate potential pathways. In general a susceptible is either exposed or not, then becomes infectious and then recovers. The arrow from infectious to the arrow between susceptible and exposed indicates that infectious individuals are a source for exposing susceptible individuals. The arrow from recovered to susceptible indicates that at least some of the population once recovered are susceptible again. The model provides a simplified view of a complex problem and a place to begin answering questions about how the disease may affect the population (given what is known about the disease and the population) and where best to intervene to control any pending outbreak. In most cases the best place to act is keep the susceptible population from moving to exposed and infectious. This is generally accomplished in three ways: vaccinate the susceptible so they can mount an immune response, quarantine the exposed and infectious so they cannot infect the susceptible, or cull the exposed and infectious. This brings up certain ethical questions when dealing with human populations, one certainly

cannot cull all the exposed and infected individuals, forced quarantine can be an unsavory prospect, and due to some religious or social beliefs vaccination may not be an option either.

### **Activities:**

10 min: Discussion on disease transmission. Present the question, how are diseases passed from one person to another? The teacher should direct discussion towards the most common modes of disease transmission that are as follows:

- contact transmission
- vehicle transmission
- vector transmission
- End discussion with an in depth look at contact transmission, which group does the influenza virus fall in?
- Does this have repercussions for the progression of the disease? Use as a lead into the disease model.

20 min: Contact transmission exercise. Provide each student with a 125ml flask filled with 50ml of the student's stock solution and a 50ml beaker filled with 10-20 ml of the stock solution. Every stock solution will be distilled water, but in one student's stock solution add some dilute HCl. Be sure to record the student with the acidic stock solution and make sure students do not actually drink their stock solution. The acidic stock solution will be the "infected" individual. Have students "roam" and meet other students, make sure this is relatively random. At defined intervals (15-30 seconds will do) have students exchange fluid. If enough plastic pipettes are available this may be the easiest way to "exchange" fluids. Have students record each individual they exchange fluids with and make sure students are exchanging fluids with different people each time. After 10 or so exchanges (can adjust by number of students and time available, but at least 5 exchanges) have the students stop and add a good squirt of bromthymol blue to each student's 50ml beaker. If the solution turns from blue to yellow or orange then there is some acid present and that individual has been infected. Take this data and combine with the data of who exchanged fluids with whom and have the class try to figure out who was the original infection. This is a good example of what an epidemiologist would be expected to do in the case of an outbreak and is partly what students will be expected to do for the epidemiological problem.

15 min: Introduce and discuss the host-parasite interaction model. Guide student discussions towards how a model of this kind can help in solving the epidemiological problem. Be sure to:

- Introduce the basics of population dynamics, namely death and birth rates and what might affect these.
- Outline the parts of the model including what it means to be susceptible, exposed, infectious and recovered.
- Discuss how this model would change for different diseases with different transmission rates and virulence, think about the arrows. Visit the website <http://science-education.nih.gov/supplements/nih1/diseases/activities/activity4.htm> for a web-based disease simulation program.

## **Lesson Day 3: Introduction of Viral Diseases and Influenza**

**Preparation:**

The teacher will need a blackboard, chalk, overhead and blank overhead sheets. The students should have scrap paper and coloring utensils (color pencils).

**Lesson Objectives:**

- Continue discussing the epidemiological problem presented Day 1 and add another piece of the puzzle.
- Introduce viruses and present the basics of virology needed to address the epidemiological problem.
- Provide a focus on influenza viruses, ending with an understanding of the genetic composition of Influenza A viruses and a diagram of the structure of Influenza A viruses.

**Lesson:****Background Information:**

A virus is defined as a tiny, millions can fit on the head of a pin, infectious particles consisting of a nucleic acid (DNA or RNA) enclosed in a protein coat and, in some cases, a membrane envelope. Viruses use the host cells' own molecular machinery to replicate and therefore do not persist outside of the host for long. There are many types of viruses differing in the size and type of genetic material, their structure, and whether enveloped or not (see Figure 1 for some examples and Table 1 for classification of viruses). At the very least students need to understand that: viruses are parasitic they need the host cell to replicate, hijacking the host cell's machinery to produce more virus particles; the cell's machinery includes mRNA, ribosomes and other proteins needed to produce proteins used by the cell; there are many different types of viruses; and each virus has some way of binding to the cell surface and injecting its genetic material into the cell.

We generally think of genomes as double-stranded DNA molecules containing the genetic information. In viruses the genome may be either single or double-stranded DNA or RNA, depending on the virus (Table 1 for examples). In any case the viral genome is usually organized as a single linear or circular molecule or molecules of nucleic acid containing the genes that code for the various viral proteins that make up a particular virus. The smallest viruses only have 4 genes where as the largest have several hundred genes. The viral genome is encapsulated in a protein shell called a capsid. Depending on the virus the capsid may be rod-shaped, a polyhedral or even more complex in shape. Capsids are built, using the host cell's machinery, from a large number of individual protein subunits called capsomeres, though only a few different kinds (see Figure 1 for examples). Capsids of bacteriophages tend to be the most complex. Viral envelopes are secondary structures derived from the host cell's membrane, which contains host cell phospholipids and membrane proteins. The membrane envelope help viruses infect host cells with surface proteins providing a means to bind to host cell membranes, evade the host cell's immune system since each enveloped virus is surrounded by a membrane derived from the hosts own cells, and may allow these viruses to persist longer in their environment. Figure 1 and Tables 1 and 2 provide some examples of different viruses. Note that there are different structures and morphologies for different viruses. These differences are a quick way to narrow down the type of virus if seen under a microscope and often affect the types of cells infected and the manner to which a host cell is infected.

There are three types of influenza viruses, Influenza A, B and C. They are all generally the same in structure, genome, function and we see all three types in humans. Influenza B and C, however, cause very mild illness and are not causes of pandemics. Influenza A, however, is the primary culprit responsible for seasonal flu outbreaks in humans and bird flu outbreaks in humans, domestic poultry and wild birds and will be the focus of this lesson. The Influenza A virus is an enveloped, single-stranded RNA (ssRNA) virus of the Orthomyxovirus family (other ssRNA viruses include the rabies, measles and mumps viruses, Table 1). The genomic RNA basically acts as a template for mRNA in the host cell (where mRNAs interact directly with cellular protein synthesizing machinery to direct the production of polypeptides or proteins). The influenza viral genome consists of 8 ssRNA segments, which code for at least 10 different viral proteins (from Wikipedia Influenzavirus A article

[http://en.wikipedia.org/wiki/Influenzavirus\\_A#\\_note-5](http://en.wikipedia.org/wiki/Influenzavirus_A#_note-5) accessed 4 July 2006):

- HA encodes **hemagglutinin** (about 500 molecules of **hemagglutinin** are needed to make one **virion**) "The extent of infection into host organism is determined by HA. Influenza viruses bud from the apical surface of polarized epithelial cells (e.g. bronchial epithelial cells) into lumen of lungs and are therefore usually pneumotropic. The reason is that HA is cleaved by trypsin which is restricted to lungs. However HAs of H5 and H7 pantropic avian viruses subtypes can be cleaved by furin and subtilisin-type enzymes, allowing the virus to grow in other organs than lungs." - <http://ca.expasy.org/uniprot/P09345>
- NA encodes **neuraminidase** (about 100 molecules of **neuraminidase** are needed to make one **virion**).
- NP encodes **nucleoprotein**.
- M encodes two **matrix proteins** (the **M1** and the **M2**) by using different **reading frames** from the same RNA segment (about 3000 matrix protein molecules are needed to make one **virion**).
- NS encodes two distinct non-structural proteins by using different reading frames from the same RNA segment.
- PA encodes an **RNA polymerase**.
- PB1 encodes an **RNA polymerase**.
- PB2 encodes an **RNA polymerase**.

Associated with the membrane envelope are some viral glycoproteins (cell surface proteins), which are very important for the infectivity and pathogenicity of the influenza virus. The hemagglutinin (HA) proteins are cell surface proteins necessary for host cell recognition and binding. They function as a lock-and-key, binding with the host cell receptor sites (Figure 4). There are at least 16 different forms of HA. The neuraminidase (NA) are cell surface proteins necessary for the packaged release of virus particles from infected cells. There are at least 9 different forms of NA. These two glycoproteins are what further sub-type Influenza A viruses, i.e. H5N1 (the "bird flu") refers to HA type 5 combined with NA type 1 forming the cell surface proteins of that particular virus, compared to H3N2 or H1N1 which are the current circulating seasonal flu strains. Changes in these two proteins, particularly the HA protein are what allow the virus to jump hosts. Originally influenzas viruses evolved in wild waterfowl where through millions of years of coevolution the viruses cause little to no illness. In novel hosts such as humans, pigs, dogs, horses and other mammals the influenza viruses can cause severe illness and even death since the new hosts' immune systems have little or no historical experience with influenza viruses. It is important to understand that the HA and NA proteins are the means by

which the influenza virus can infect humans and even infect different areas of the human respiratory system (i.e. the seasonal flu strains bind to cells in the upper respiratory tract and can therefore be aerosolized and easily passed from person to person, the H5N1 bird flu virus binds preferentially to cells in the lower respiratory tract and therefore cannot be easily aerosolized but may be the reason for the enhanced ill effects; Shinya et al 2006).

### **Activities:**

10 min: Discuss questions students came up with for the epidemiologist's problem.

- Distill these questions down to the most pertinent ones.
- Can any of the questions be answered by what has already been reported?
- **Add one more piece of information to the epidemiological puzzle: Scientists have identified the pathogen as an influenza virus never seen in humans before. It has not been fully genotyped so the origin is still unknown.**
- Discuss any additional questions this information brings up? One should be, what do we know about viruses in general and the influenza virus in particular?

5 min: Brainstorm what students know about viruses in general and the influenza virus in particular. This can be done in groups or as a class.

10 min: The basics of viruses.

- Definition of a virus
- The Viral Genome
- Capsids and Envelopes
- See Figure 1 and Tables 1 & 2 for different types and classification of animal viruses. What can students note about these four different viruses? Are any familiar to the students?

10 min: The Influenza virus (Figure 4) – The absolute minimum information includes:

- Enveloped ssRNA virus with 8 individual ssRNA segments encoding for at least 10 different proteins.
- The M1 matrix protein encapsulates the viral genome and this is further enveloped by the host cell's membrane.
- The HA glycoprotein is responsible for host cell binding, there are at least 16 different versions and likely more able to evolve.
- The NA glycoproteins are necessary for the packaged release of virus particles from the host cell, and there are at least 9 different versions with more likely able to evolve
- Have the students calculate the different possible combinations of Influenza A viruses based on 16 HA and 9 NA. Be aware that this number does not include the fact that you can also have different combinations of the remaining 6 ssRNA segments coding for other proteins.

10 min: Draw an Influenza virus labeling the most important structures of the virus particularly the RNA strands, the glycoproteins HA and NA, the M1 capsid protein, and the membrane envelope. Use Figures 2 and 3.

## **Lesson Day 4: The Influenza Virus and Evolution, from ducks to people**

### **Preparation:**

The teacher will need an overhead projector and chalkboard. For the activity at the end of there will be 8 bowls (or any container). Each individual bowl will be filled with pipe cleaners of the same size but two different colors. Different size or shaped pipe cleaners will be in the different bowls so there are two different color pipe cleaners, representing two different viral genomes, and eight different sized or shaped pipe cleaners representing the eight different ssRNA segments.

### **Lesson Objectives:**

- Introduce the basics of viral reproduction focusing on the influenza virus
- Provide information on genetic mutation, evolution, natural selection and short generation time as a means for relatively rapid viral evolution
- Introduce antigenic shift (genetic reassortment) as the most likely candidate for the formation of new pandemic flu strains

### **Lesson:**

#### **Background Information:**

It is important for students to learn the basics of viral reproduction in order for them to understand how the influenza virus can jump from its natural host, wild waterfowl, to novel hosts such as humans. Viruses lack metabolic enzymes, ribosomes, and other equipment for making proteins so they are unable to replicate by themselves and are therefore obligate intracellular parasites, being able to reproduce within a host cell using its machinery to replicate. Generally viruses have a specific host range, a limited range of hosts it can infect. This specificity is due to the evolution of specific recognition systems by the virus, a lock-and-key fit between proteins on the outside of the virus and receptors on the host cell membrane. For example a triangle shaped key can only fit into a triangle shaped lock, it would be ineffective unlocking a round or square lock. The basic reproductive cycle is as follows (see Figure 5):

1. A virus binds to the surface of the host cell and enters. Any envelope is removed releasing capsids and viral genome into the cell. The mechanism by which the nucleic acid enters the cell varies among the different viruses (phages will actually inject their nucleic acids where as enveloped viruses enter the cell via a form of endocytosis).
2. Once inside the cell, the virus genome hijacks the host cells machinery and host enzymes begin replicating the viral genome.
3. At the same time, host enzymes transcribe the viral genome into viral mRNA, which other host enzymes use to make more viral proteins.
4. Viral genomes and capsid proteins spontaneously self-assemble within the cell into new virus particles, which then exit the cell as newly packaged virus particles. Influenza viruses are unique in that there is no mechanism for assembling the genome of new virus particles, which means that if two different influenza viruses infect the same host cell there is a pretty good chance for a swapping of genetic material and the emergence of a novel virus (see following section on antigenic shift).

This leaves the question of how exactly does an influenza virus jump between species? There are two general mechanisms that can accomplish this feat. The first is a good example of evolution and natural selection at work and is a relatively slow process. On average there is one mutation every generation for the influenza virus. Considering a generation for an influenza virus is mere days, mutations can accumulate rapidly. A mutation is any inheritable change in the nucleotide sequence of a chromosome. In the case of the influenza virus it would be a change in the nucleotide sequence of one of the single-stranded RNA fragments that make up the genetic material of the virus.

The more common mutations expected include point mutations, which are an exchange of a single nucleotide for another. Most tend to be silent mutations in that the changed nucleotide does not alter the amino acid that is coded. The genetic code has a built in safe guard against mutations. Coding regions consist of nucleotide triplets that code for specific amino acids, the third nucleotide in the triplet is often non-specific and can be changed without altering the amino acid the triplet codes for (see Table 3 for examples). Missense mutations occur when the affected triplet codes for a different amino acid and usually involves a point mutation occurring in one of the first two positions of the codon. Nonsense mutations occur when the point mutation changes the codon to a stop codon. Other relatively common mutations include insertions, which are the addition of one or more nucleotides into the genome and deletions, which are the removal of one or more nucleotides from the genome. Both insertions and deletions can have large effects in that they completely shift the reading frame of the gene. That is if you insert or delete 2 nucleotides then every coding region following is shifted by 2 as well. Mutations are usually thought of as being bad for the organism, and most tend to be. Since they are detrimental, however, these mutations tend to be removed from the population via natural selection and therefore do not persist. A small percentage of mutations are not detrimental and, if they provide greater fitness for the organism, will be selected for and passed on. Simply speaking this is how new species form through time.

For organism with dramatically shorter generation times than their host, including the influenza A virus, the rapid accumulation of mutations can have a significant effect on the host-parasite interaction. With the immune system of the host acting as the selective force, only the most fit viruses will survive the immune response and be available to infect others. Therefore we expect to see different influenza viruses over time, potentially with different surface proteins that recognize host cells. If these HA and NA surface proteins change, then the virus could jump host species or affect different organ systems. Additionally, the surface proteins may remain the same but the RNA fragments coding for the other proteins may change and the virulence of the virus could be affected. There are a multitude of different outcomes that could happen. Generally speaking, however, the longer a particular virus coevolves with a host species the less virulent the virus becomes since it is not good for the virus if the host dies or becomes too sick to transmit the virus (the history of AIDS in the human population is a good example of this). Having said this, one must remember that even though humans have likely been in contact with influenza viruses for a long time (probably since we began domesticating wild waterfowl) each new influenza virus is basically a new virus. For instance, the two currently circulating seasonal influenza viruses, the H3N2 and H1N1 influenza A viruses, are both descended from the H1N1 strain that caused the 1918 flu virus (aka the Spanish Flu), which killed millions of people with a mortality rate of at least 2.5% compared to <0.1% for the seasonal flu (Taubenberger and Morens 2006) illustrates how as a virus persists in a population it tends to become less virulent through coevolution with the host species.

A second, somewhat more sinister way influenza viruses can change is a process called antigenic shift and is more or less unique to the influenza A virus (Figure 6). This is what has epidemiologists constantly worried about a pending influenza pandemic. Antigenic shift involves a reassortment of the genetic material packaged into a virus particle exiting a cell infected with two different strains of influenza A virus. The influenza A virus has no mechanism to control the repackaging of genetic material when viruses exit the host cell. The packaging of RNA segments is spontaneous and random. This means that if a single cell is infected with two different influenza A viruses, there is a chance the RNA segments will reassort producing a novel influenza virus.

For example, influenza A viruses can infect a number of different species and swine can be infected by both bird influenza viruses and human influenza viruses (which are basically the same viruses differing mostly in the HA and NA surface proteins). In a pig infected at the same time with these two viruses, there is a chance that both viruses will infect a single cell at the same time. If this happens then due to the random repackaging of the viral genome, the RNA segments from the two different viruses could be repackaged together in different combinations, genetic reassortment, producing a novel influenza virus (Figure 6). If the new virus retains the hemagglutinin proteins of the original human virus then we could see a new human influenza virus. Depending on a number of factors like pathogenicity, communicability, and organ specificity of the new virus (to name a few) this new virus could cause a pandemic (i.e. affecting a wide geographic area). Additionally, since the new virus has aspects of the bird flu virus originally infecting the swine, the human immune system will have had no prior experience with this virus and therefore not be able to mount a strong and timely response. It is for this reason that the H5N1 bird flu virus is such a concern and in fact all influenza viruses have this ability for reassortment and shift.

### **Activities:**

20 min: Introduction to viral reproduction, include:

- Lock-and-key recognition and binding of virus particles to the surface receptors of the host cell.
- Endocytosis of virus into the host cell releasing the virus' genetic material into the cell.
- The virus genome takes over the cell machinery of the host cell and begins replicating itself producing more copies of the viral genome and producing the necessary viral proteins.
- The viral proteins and genetic material are assembled and exit the host cell as whole virus particles.

Class activity: Divide the class into virus particles and parts of a cell (including cell membrane, surface receptors, and cell machinery). Provide viruses and cell surface receptors with different "keys" you could use playing cards or anything that can be matched. Have the cell surface receptor students circle around the cell machinery students facing out towards the "environment." The virus students then must find a match for their key, e.g. queen of spades and queen of spades. Only those viruses that find a match can enter the "cell." Once inside the virus students will ask the cell machinery students to start making copies of the virus. You can recruit the virus students that did not have the right "key" to be the newly made virus particles which will then bust our of the cell ready to infect other cells.

10 min: How do we get different strains of influenza emerging?

- The role of evolution and natural selection
  - Discuss mutations and how they can create new species or virus strains
  - Introduce the idea of short virus generation times and how they affect host-parasite interactions
  - What does this mean for influenza viruses?
- Antigenic shift and genetic reassortment (Figure 6)
  - When packaging new virus particles there is no mechanism to keep the original RNA segments together
  - The random packaging of genetic material can create new influenza viruses exiting from host cells infected with two different strains of influenza
  - What does this mean for influenza viruses? Is this a good way to provide genetic variability allowing for “shifts” in the influenza virus.

15 min: Antigenic shift exercise

- Place eight containers in the middle room or on the teacher’s desk and surround with something to represent the host cell’s membrane.
- Each individual bowl will be filled with pipe cleaners of the same size but two different colors. Different sized or shaped pipe cleaners will be in the different bowls so there are two different color pipe cleaners, representing two different viral genomes, and eight different sized or shaped pipe cleaners representing the eight different ssRNA segments.
- Have each student randomly grab one pipe cleaner from each bowl and take them back to their desks.
- Once every student has taken eight pipe cleaners compare the “viral genomes” of each student. There will be many different combinations and hopefully a few that have pipe cleaners all of one color. Illustrate how this is similar to the random repackaging of viral RNA segments for the influenza A virus and how with two different viruses infecting the same cell we can potentially get many different new viruses emerging.

## **Day 5: The Epidemiological End Game**

### **Preparation:**

Provide color pencils for students to use in creating their timelines.

### **Lesson Objectives:**

- Wrap up the week’s major topics and use them to solve the epidemiological problem and fill in the blanks.
- Provide the time and environment for students to create a timeline of events for the epidemiological problem.
- Have students provide the links between one event in the timeline and the subsequent events.
- Discuss how, as epidemiologists, the class would handle this situation of an emergent infectious disease and the possibility of a pandemic
- Discuss how many different scientists would have collaborated to provide the necessary information to tackle this problem

## **Lesson:**

### **Activities:**

**10 min:** Review the clues provided throughout the week and discuss the next question to answer. Lead students to asking if there is a connection between the infected individuals. Once prompted provide the final clue: **The FBI has gathered travel information for the 5 sick Americans. Turns out all were on the same plane from New York City to Chicago five days before the first woman entered the ER. Additionally, the man from Los Angeles was recently in Hong Kong for business.**

**25 min:** The clues provided should be enough for the students to connect the dots. In their lab notebooks have them create a timeline of events, keeping in mind that the order clues were presented was not indicative of the actual order of events. Students need to provide support for each event, i.e. how could one thing happen following another event, inferences should be encouraged. Students should draw upon what they learned in the lesson and any outside sources students may have consulted (e.g. the CDC website) to complete this task.

**10 min:** Once the events have been hashed out, the students should discuss what went into addressing this problem. That is, many different scientists with different skills collaborated to provide the information gathered by the students. To name a few: geneticist, virologists, microbiologists, medical doctors, veterinarians, and other epidemiologists. Finally, the students should address the question of how to control the outbreak. This can be done in groups or as a class discussion. Some viable options include vaccinations (however current H5N1 vaccinations are not entirely effective, in short supply and take a long time to manufacture), anti-viral medications like Tamiflu (these are expensive and in short supply as well, additionally some strains of influenza are resistant to certain anti-virals) these two options are directed towards the susceptible and exposed. You can focus on the infectious group as well by enacting quarantine measures (or culling if it was a wildlife population). Some ethical questions may be raised by this discussion, such as quarantining infected humans against their will or religious and cultural fears of vaccinations or the like. This can be pursued and will allow students to think a little about bioethical questions.

## **Assessment:**

The whole lab notebook or parts can be used as an assessment. The final product is the timeline of events for the epidemiological problem and formulating questions on how to tackle the problem based on what is known about the virus, susceptible population and previous events.