



Center for Infectious
Disease Research & Policy

UNIVERSITY OF MINNESOTA

MCEIRS PANDEMIC H1N1 2009 INFLUENZA TRAINING

Individual Study Guide

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Origin of the Pandemic H1N1 2009 Influenza Virus

**Minnesota Center of Excellence for Influenza
Research and Surveillance**

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INTRODUCTION

Origin of the pH1N1 2009 Influenza Virus

This module will familiarize you with what is known and what is not known about the origin of the pH1N1 2009 influenza virus. Viral structure and evolution, historical context of pandemic influenza virus strains, and key uncertainties related to the emergence of the pH1N1 virus in 2009 are topics that will be covered.

LESSON 1: INFLUENZA VIRUS BASICS

In this lesson, we will cover:

- Viral Structure
- Viral Nomenclature
- How Influenza Viruses Evolve

VIRAL STRUCTURE

The overall structure of the virus includes a lipid membrane that has three integral membrane proteins: hemagglutinin (HA), neuraminidase (NA), and the matrix 2 protein (M2). There is also a matrix 1 protein (M1) that serves as a bridge between the lipid membrane and the viral core (not shown on Figure 1 since it is not visible on the surface of the virus).

The HA protein appears as spikes on the lipid membrane and the NA protein forms globular structures that extend outward from the viral surface.

The HA protein is critical for pathogenesis; it contains the receptor for binding to the host cell and allows fusion of the virus membrane to the host cell membrane, which allows the viral contents to enter the host cell. Cleavage of the HA protein is essential for fusion to occur; this happens at the proteolytic cleavage site (PCS). The PCS is the primary virulence factor for Avian Influenza viruses; alterations at this site affect pathogenesis.

The NA protein also is important in pathogenicity.

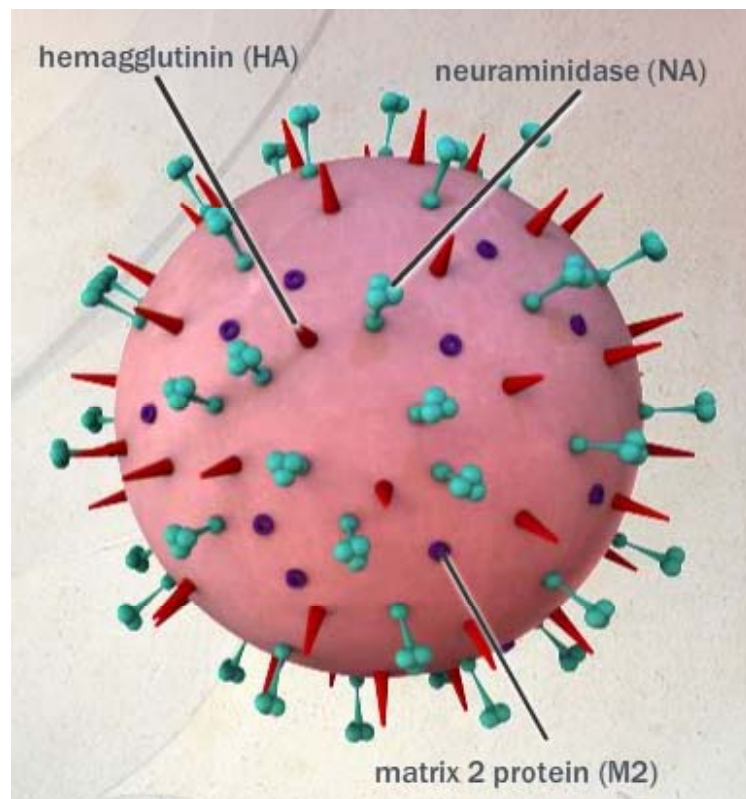


Figure 1: Virus Structure

The lipid membrane surrounds the nucleocapsid, which contains eight different segments of negative-sense single-stranded RNA. Each segment of RNA is part of a ribonucleoprotein complex that contains the RNA segment, three polymerase proteins, and the nucleoprotein.

The eight RNA segments code for a total of 10 genes.

The eight RNA segments are shown in Figure 2 below and include:

- PB1 (codes for basic polymerase 1 protein)
- PB2 (codes for basic polymerase 2 protein)
- PA (codes for acidic polymerase protein)
- HA (codes for the hemagglutinin glycoprotein)
- NA (codes for the neuraminidase glycoprotein)
- NP (codes for the nucleoprotein)
- M (codes for matrix proteins 1 and 2)
- NS (codes for non-structural proteins 1 and 2)

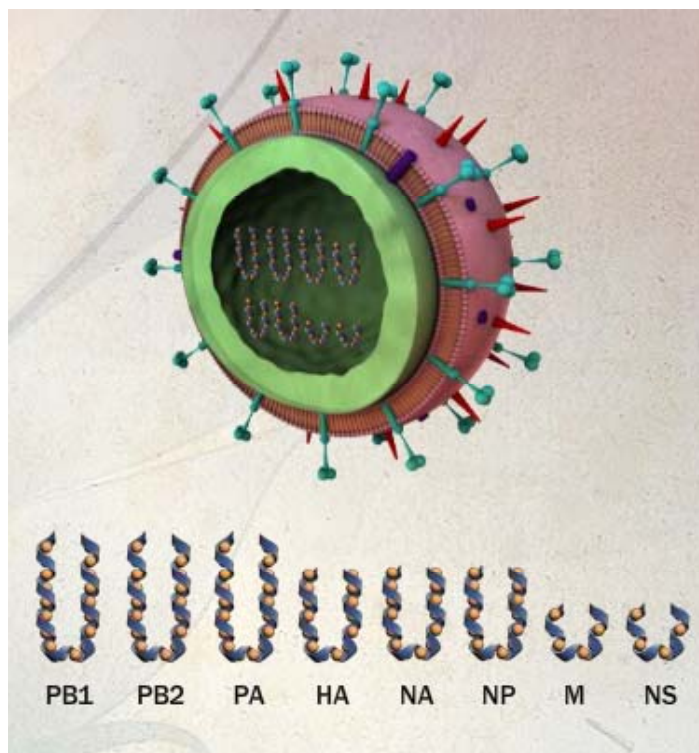


Figure 2: Eight RNA Segments

VIRAL NOMENCLATURE

Influenza viruses belong to the Orthomyxoviridae family of segmented negative-sense RNA viruses.

The genus influenza A consists of a single species: influenza A virus, which is the cause of type A influenza.

All avian influenza is caused by influenza A virus. Influenza A virus also causes illness in a variety of mammals and is the most common cause of influenza in humans.

There are 16 different HA antigens (H1 to H16) and nine different NA antigens (N1 to N9) for influenza A. These antigens give rise to the subtype designation.

There is only one H and one N in each viral subtype (as shown in Figure 3).

- H1N1 subtype has H1 antigen and N1 antigen.
- H5N1 subtype has H5 antigen and N1 antigen.
- H3N2 subtype has H3 antigen and N2 antigen.

Combinations of HA and NA antigens can result in up to 144 different unique subtypes.

The vast majority of these subtypes occur only in wild birds.

Human illness historically has been caused by H1, H2, and H3 subtypes.

Several other avian subtypes, including H5 (i.e., H5N1), H7 (i.e., H7N7), and H9 (i.e., H9N2) have also caused sporadic illness in humans.

Each influenza virus is named on the basis of the following features:

- Type of influenza strain
- Host of origin (if other than human)
- Geographic origin
- Laboratory number
- Year of isolation
- HA and NA subtypes

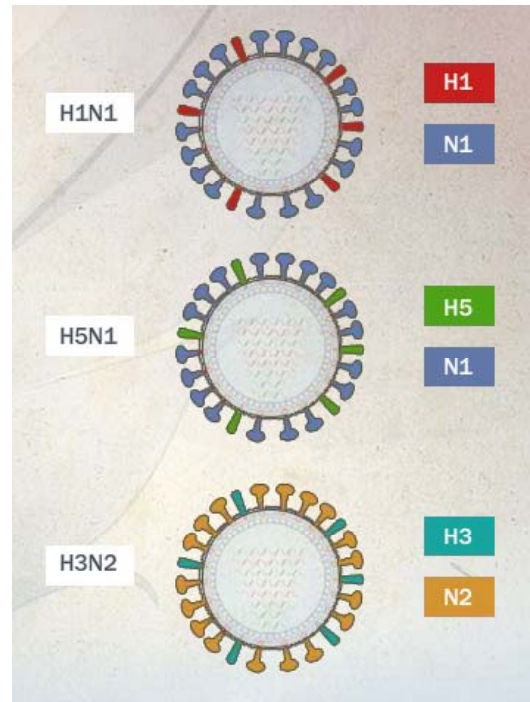


Figure 3: Viral Subtypes

Examples include the following:

HUMAN STRAIN

A/California/04/2009/H1N1

This strain was originally isolated from a human in California (US) in April 2009 and is responsible for the influenza pandemic that began that year.

A/Vietnam/1203/2004/H5N1

This strain was isolated in 2004 from a patient in Vietnam with H5N1 influenza.

AVIAN STRAIN

A/bar-headed goose/Qinghai/1A/2005/H5N1

This strain was isolated in 2005 in Qinghai province, China, from a bar-headed goose. A mass die-off of wild migratory birds occurred at Qinghai Lake in that year.

AVIAN STRAIN

A/chicken/Chile/4977/2002/H7N3

This strain caused an outbreak of highly pathogenic avian influenza (HPAI) in Chile in 2002.

SWINE STRAIN

A/Swine/Minnesota/00395/2004/H3N1

This strain was isolated in Minnesota in 2004 and is an example of a triple reassortant swine influenza virus (i.e., a strain that contains genes from viruses of human, avian, and swine origin).

HOW INFLUENZA VIRUSES EVOLVE

Influenza viruses evolve in three ways:

- Antigenic Drift (Mutation)
- Antigenic Shift (Reassortment)
- Gradual Adaptation

ANTIGENIC DRIFT

This process refers to the small genetic mutations that influenza viruses continuously undergo from year to year and is illustrated in Figure 4.

Antigenic drift is an important determinant of influenza epidemiology in humans. Because of antigenic drift, new influenza vaccines for humans are developed each year. Partial immunologic cross-reactivity between new strains and those they are replacing limits morbidity, mortality, and spread in the human population.

Antigenic drift also occurs in influenza viruses in domestic and wild animals. The implications of these genetic changes for the management of influenza in animals are not clear.

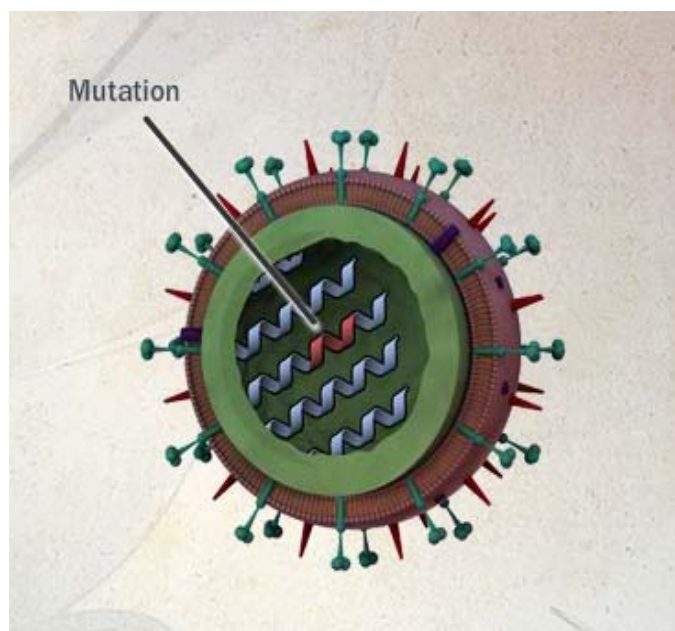


Figure 4: Small Genetic Mutations

ANTIGENIC SHIFT

This process refers to substantial genetic changes in the HA or NA, or a shift from one subtype to another in a given population. Antigenic shift is important for emergence of pandemic strains in humans and also has been seen in swine; however, this process has not been important in poultry.

Antigenic shift can occur due to reassortment between human and animal strains, which appears to be what caused the pandemic strains of the 1957 and 1968 pandemics to emerge in the human population; these strains involved genes from viruses of avian and human origin.

The 2009 H1N1 pandemic strain is a triple reassortant virus that contains genes from viruses of avian, human, and swine origin.

Figure 5 illustrates an example of a triple reassortant virus. The blue genes are from the avian reservoir, the red genes are from the swine reservoir, and the green genes are from the human reservoir.

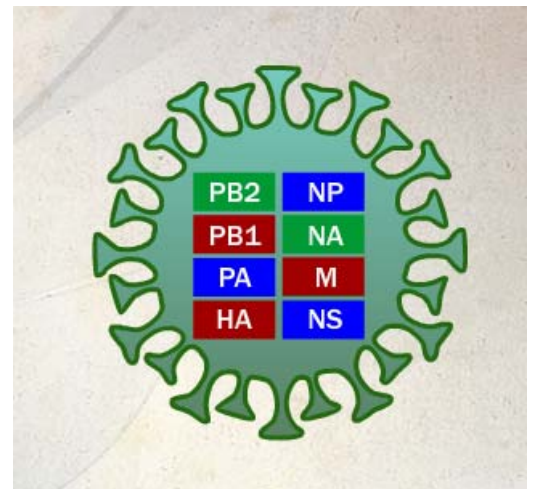


Figure 5: Triple Reassortant Virus

GRADUAL ADAPTATION

Not all pandemic strains arise from genetic reassortment.

For example, the 1918 H1N1 strain may have originated in the avian reservoir and then adapted gradually to the human population over time through a series of genetic mutations to become a global pandemic strain. This process of gradual adaptation with multiple small genetic mutations is illustrated in Figure 6.

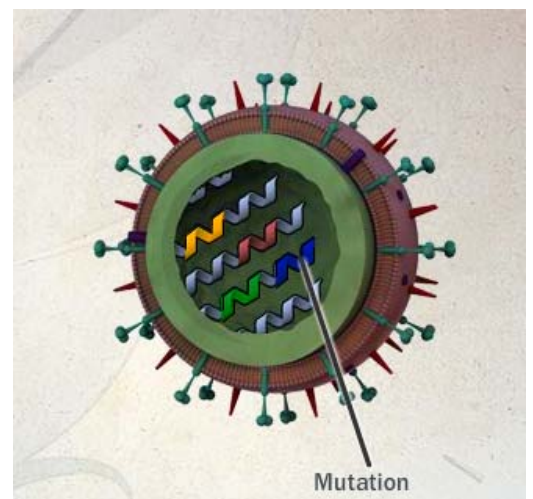


Figure 6: Gradual Adaptation with Small Genetic Mutations

LESSON 2: BACKGROUND ON PANDEMIC INFLUENZA STRAINS

In this lesson, we will cover:

- Background on Pandemic Influenza Virus Strains
- History of H1N1 Influenza Viruses in Humans

BACKGROUND ON PANDEMIC INFLUENZA VIRUS STRAINS

Influenza viruses are continually circulating in birds, swine, and humans (as shown in Figure 7). Influenza viruses sometimes cause disease in birds and often cause disease in humans and swine. Several other mammalian species also are susceptible to influenza viruses and illness is occasionally seen. Influenza viruses tend to be species specific.

Because swine are susceptible to both avian and human influenza viruses they are considered a potential "mixing vessel" where the genes of unrelated influenza viruses can reassort to produce novel viral subtypes.

Influenza pandemics occur when an influenza virus, against which there is little or no existing immunity, emerges in the human population and efficiently transmits from human to human.

Typical disease severity in a pandemic is unpredictable, ranging from mild to severe, and depends upon the characteristics of the specific virus involved. Disease severity in individuals depends upon the status of their immune system and presence of co-infections or pre-existing conditions.

Genetic studies can provide insight, if not definitive answers, about the history and origins of pandemic influenza viral strains.

Pandemic viruses tend to originate, at least in part, from nonhuman reservoirs. The HA genes of the previous two pandemic influenza viruses (1968 - H3N2 and 1957 - H2N2) originated from avian influenza viruses.

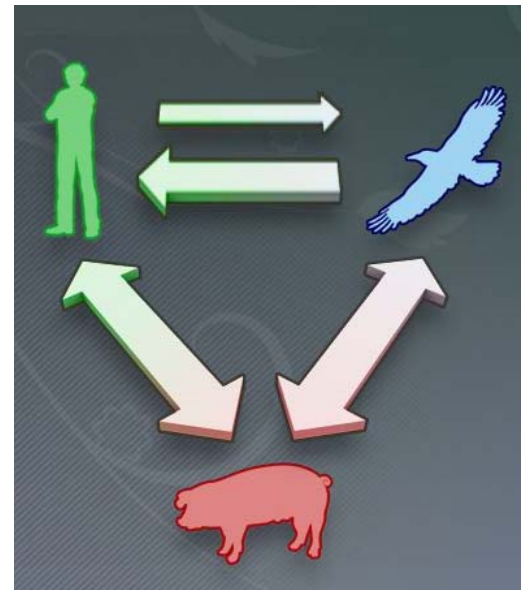


Figure 7: Swine as "Mixing Vessel"

The origin of the 1918 H1N1 pandemic strain is not clear. Some researchers have suggested an avian source that circulated in another species before the pandemic. Other research suggests that the virus was from an avian source and gradually adapted to the human population without an intermediary host.

The pH1N1 2009 virus contains a combination of gene segments that previously has not been reported in swine or human influenza viruses in the United States or elsewhere.

HISTORY OF H1N1 INFLUENZA VIRUSES IN HUMANS

H1N1 viruses are thought to have been introduced from the avian reservoir to humans and swine in, or not long before, the 1918 pandemic (which was caused by an H1N1 strain). Figure 8 illustrates the history of H1N1 influenza viruses in humans.

After the 1918 pandemic, H1N1 viruses continued to circulate among humans (as a major cause of seasonal influenza) until the H2N2 influenza pandemic of 1957. Between 1918 and 1957, there was substantial antigenic drift of the H1 viruses in humans.

In the years following the introduction of H1 influenza viruses to human populations in or before 1918, substantial genetic drift occurred. In contrast, classical H1 influenza viruses in swine exhibited relative antigenic stability until 1998 when a triple reassortant strain emerged. The different pace of viral evolution created substantial antigenic differences between human seasonal H1 and classical swine H1 viruses.

H1N1 viruses from the early 1950s reemerged in humans in 1977. From 1977 to 2009 further antigenic changes occurred that were sufficient to warrant eight updates of the H1 component of the seasonal influenza vaccine.

Over the course of time, classical swine influenza viruses have been isolated from and caused disease in humans, but no or only limited human-to-human transmission has been documented in those cases.

In 1976, H1N1 swine influenza caused severe respiratory illness in 13 soldiers with 1 death at Fort Dix, New Jersey. The virus was detected only from January 19 to February 9 and did not spread beyond Fort Dix.

During the past few decades, triple reassortant swine influenza viruses have been isolated from and caused disease in humans, but no or only limited human-to-human transmission has been documented in those cases.

Because of the divergence in human and swine H1 strains, swine are a reservoir of H1 viruses with the potential to cause disease in humans. Very little is known about whether or not other domesticated animals (e.g., horses) could also serve as reservoirs of novel influenza viruses for humans.

The pH1N1 2009 influenza virus has genetic origins similar to swine influenza viruses. However, this strain represents a new genetic reassortant, and it had not been identified in swine or humans before being detected in April 2009.

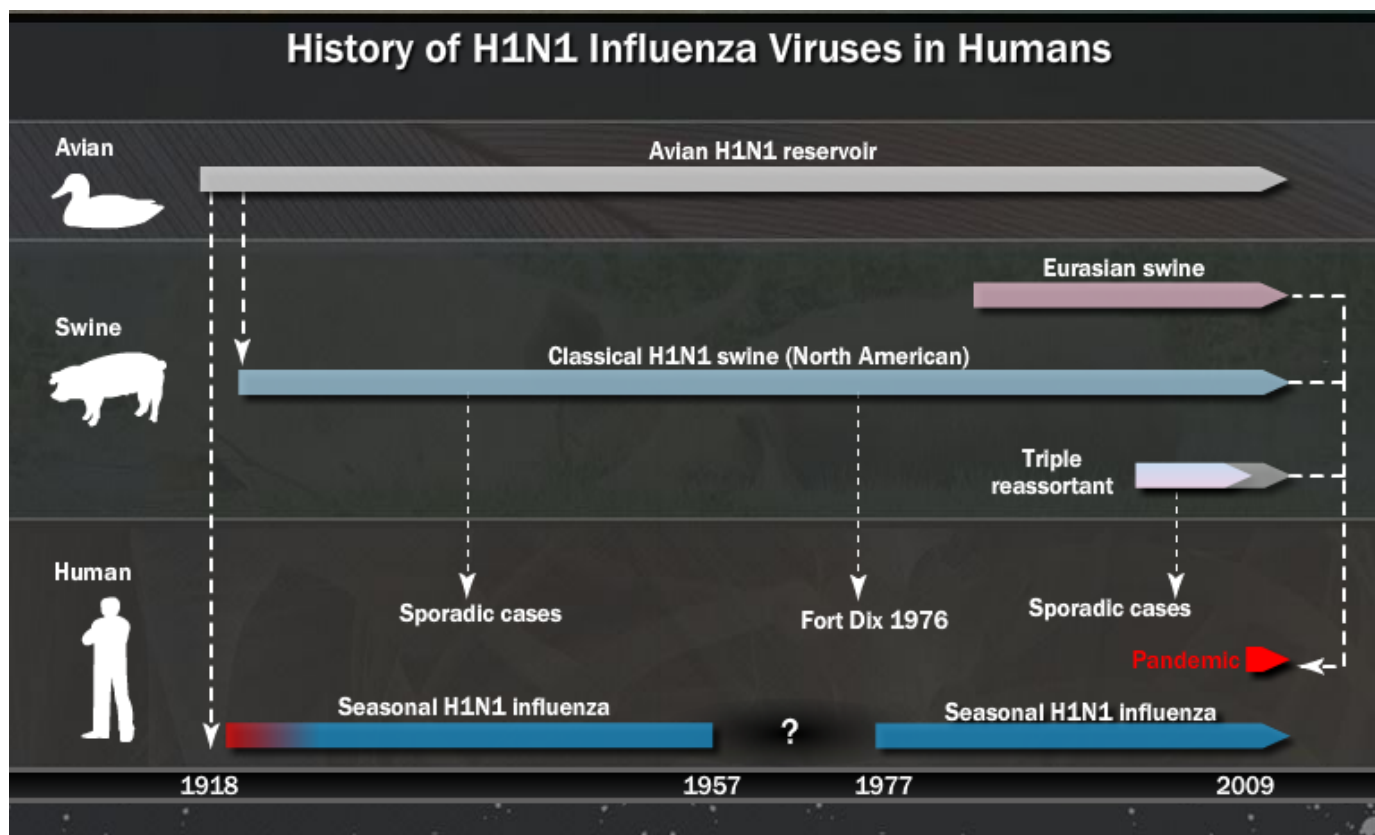


Figure 8: H1N1 Influenza in Humans Timeline

LESSON 3: GENETIC ORIGINS OF THE pH1N1 2009 INFLUENZA VIRUS

In this lesson, we will cover:

- Hosts and Lineages by pH1N1 2009 Viral Gene Segment
- History of the pH1N1 2009 Viral Gene Lineages
- Key Genetic Unknowns

GENE SEGMENTS, HOSTS, AND YEARS OF INTRODUCTION

The PB2 and PA gene segments are in the swine triple reassortant lineage.

The PB1 gene segment is also in the swine triple reassortant lineage, but became a part of this lineage through a different path than the PB2 and PA gene segments.

The HA, NP, and NS gene segments are in the classical (North American) swine lineage.

The NA and M gene segments are in the Eurasian swine genetic lineage as shown in Figure 9.

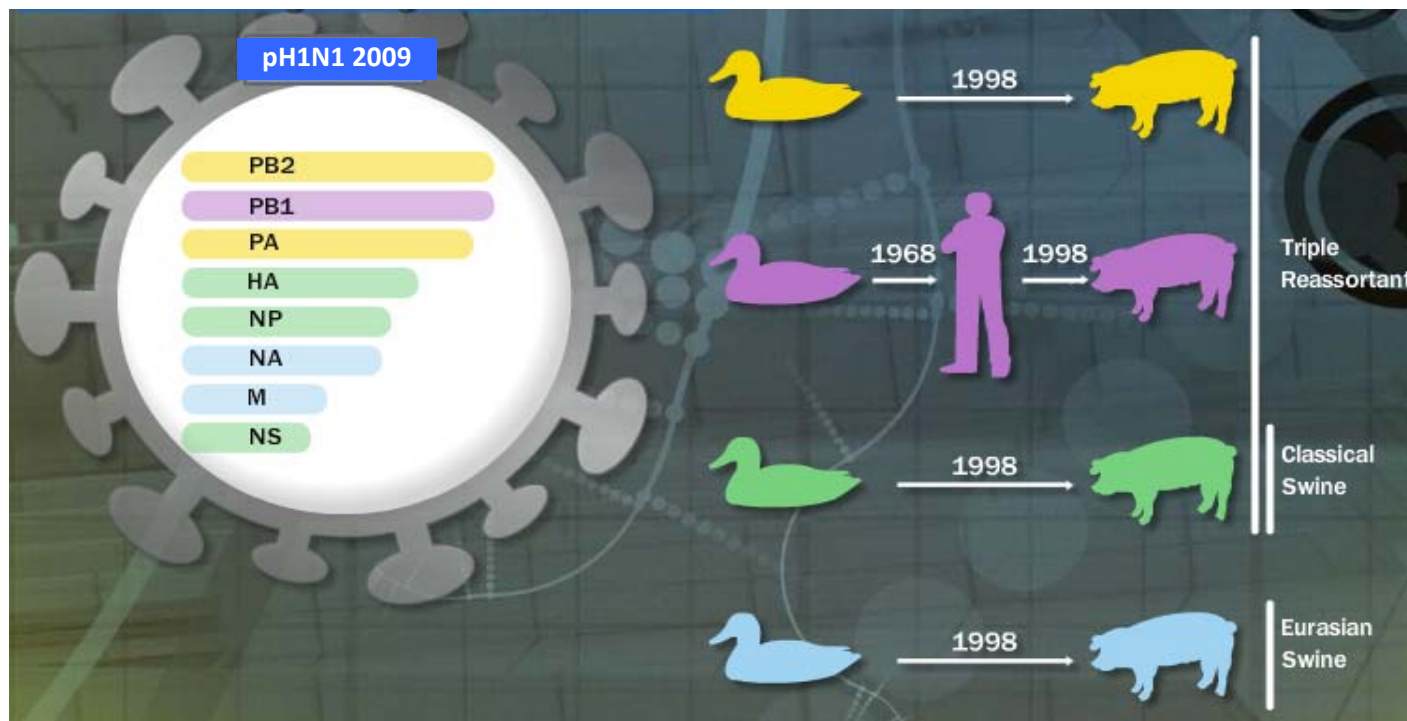


Figure 9: Gene Segments, Hosts, and Years of Introduction

HISTORY OF THE pH1N1 2009 VIRAL GENE LINEAGES

PB2, PA / Triple reassortant: Viral genes that seeded the lineage of these specific gene segments were originally of avian origin and entered swine in North America around 1998. Soon after, these viral genes combined with others circulating among swine to produce the "triple reassortant" lineage. During the past few decades, triple reassortant swine influenza viruses have been isolated from and caused disease in humans, but no or only limited human-to-human transmission has been documented.

PB1 / Triple reassortant: Viral genes that seeded the lineage of this particular gene segment entered swine from humans at the time of the North American swine triple reassortment events in or around 1998. These viral genes are thought to have originally entered the human population from birds around 1968. During the past few decades, triple reassortant swine influenza viruses have been isolated from and caused disease in humans, but no or only limited human-to-human transmission has been documented.

HA, NP, NS / Classical swine: Viral genes that seeded the lineage of these gene segments were first isolated from swine in 1930 and are thought to have originally entered the swine population in North America around 1918. Between 1930 and the late 1990s these "classical swine influenza" viruses circulated in North American swine and remained relatively stable. During the past few decades, classical swine influenza viruses have been isolated from and caused disease in humans, but no or only limited human-to-human transmission has been documented in those cases.

NA, M / Eurasian swine: Viral genes that seeded the lineage of these gene segments were originally derived from a wholly avian influenza virus and are thought to have entered the Eurasian swine population in 1979. These genes continue to circulate throughout Eurasia, and prior to April 2009 had not been reported outside the region.

KEY GENETIC UNKNOWNNS

The pH1N1 2009 influenza virus was first detected in Mexico among humans in April 2009. Limited surveillance among species monitored for influenza viruses suggests that this virus might have been circulating undetected somewhere in the world prior to April 2009. Unless and until additional evidence is acquired the true source of the pH1N1 2009 virus will remain unknown.

Identifying the source of the pH1N1 2009 virus may indicate actions that could prevent future pandemics.

There is much we still do not understand about influenza viruses in general and the 2009 pandemic strain in particular. Some key questions remain related to the emergence of this strain as a pandemic virus.

Expanding the geographic coverage of influenza surveillance in all pertinent bird and mammalian species is important to better understand the diversity and ecology of viral populations relevant to human and animal health.

KEY MOLECULAR UNKNOWNNS

Data suggest that other previously unrecognized molecular determinants are responsible for the ability of the 2009 pandemic virus to replicate and transmit in humans. Sequence analysis of the pH1N1 2009 influenza viruses isolated from humans to date has not identified molecular features shown to confer increased transmissibility or virulence in previous studies of other influenza A viruses.

Many of the molecular markers predicted to be associated with adaptation to a human host or to the generation of a pandemic virus, as seen in 1918 H1N1 or highly pathogenic H5N1, are not present in the pH1N1 2009 viruses characterized to date.

GLOSSARY

Antigenic Drift - One of two ways that influenza viruses can change (the other is antigenic shift, see below). Antigenic drift refers to small, gradual changes that occur through point mutations in the two genes that contain the genetic material to produce the main surface proteins, hemagglutinin and neuraminidase. These point mutations occur unpredictably and result in minor changes to these surface proteins. Antigenic drift produces new virus strains that may not be recognized by antibodies to earlier influenza strains. This process works as follows: a person infected with a particular influenza virus strain develops antibodies against that strain. As newer virus strains appear, the antibodies against the older strains might not recognize the "newer" virus, and infection with a new strain can occur. This is one of the main reasons why people can become infected with influenza viruses more than one time and why global surveillance is critical in order to monitor the evolution of human influenza virus strains for selection of which strains should be included in the annual production of influenza vaccine. In most years, one or two of the three virus strains in the influenza vaccine are updated to keep up with the changes in the circulating influenza viruses. For this reason, people who want to be immunized against influenza need to be vaccinated every year.

Antigenic Shift - Antigenic shift is one of two ways that influenza viruses can change (the other is antigenic drift, see above). Antigenic shift refers to an abrupt, major change to produce a novel influenza A virus subtype in humans

(i.e., one that has not circulated previously among people). Antigenic shift can occur either through direct animal (poultry)-to-human transmission or through mixing of human influenza A and animal influenza A virus genes to create a new human influenza A subtype virus through a process called genetic reassortment. Antigenic shift results in a new human influenza A subtype.

Antigens -A substance that elicits a specific (as opposed to nonspecific) immunological response. Foreign antigens typically stimulate a response from the body's adaptive immune system resulting in the production of antibodies and effector T-cells; antigens that produce an immune response can also be called "immunogens."

Epidemic - A disease occurring suddenly in humans in a community, region or country in numbers clearly in excess of normal.

Genetic Drift - See Antigenic Drift

Gradual Adaptation - A process of small genetic changes in the viral genome that allows an influenza virus to adapt over time to become an efficient pathogen in the human host; this process does not involve reassortment events. Researchers believe that the 1918 H1N1 pandemic strain was initially of avian origin and that the virus evolved into a severe pandemic strain in humans through the process of gradual adaptation.

Hemagglutinin (HA) - An important surface structure protein of the influenza

virus that is an essential gene for the spread of the virus throughout the respiratory tract. This protein enables the virus to attach itself to a cell in the respiratory system and penetrate it. It is used to name influenza A subtypes and is referred to as the "H" in the influenza virus subtype (e.g., H5N1). To date, 16 different influenza A hemagglutinin antigens have been identified.

Host - An organism on or in which a parasite (e.g., virus, bacteria) lives.

Morbidity - Disease; morbidity rate is the incidence or prevalence of disease in a specific population during a specified interval of time or a specific point in time.

Mortality - Death; mortality rate is a measure of the number of deaths in a population during a specified interval of time.

Mutation - Any alteration in a gene from its natural state. Specific mutations and evolution in influenza viruses cannot be predicted, making it difficult if not impossible to know if or when a virus such as H5N1 might acquire the properties needed to spread easily among humans.

Neuraminidase (NA) - An important surface structure protein of the influenza virus that is an essential enzyme for the spread of the virus throughout the respiratory tract. This protein enables the virus to escape the host cell and infect new cells. It is used to name influenza A subtypes and is referred to as the "N" in the influenza virus subtype (e.g., H5N1). To date, 9 different influenza A neuraminidase antigens have been identified.

Nucleocapsid - The genome (i.e., the RNA or DNA) and the protein coat (or capsid) of a virus.

Outbreak - Presence of disease in numbers in excess of normal in a specific geographic area or population.

Pandemic -A worldwide outbreak of a disease in humans in numbers clearly in excess of normal. A global influenza pandemic may occur if two conditions are met:

- A new subtype of influenza A virus emerges for which there is little or no immunity in the human population.
- The virus can spread easily from person to person in a sustained manner.

Panzootic - A worldwide outbreak of a disease in animals in numbers clearly in excess of normal.

Pathogenic - Causing disease or capable of doing so.

Reservoir - A person or animal that serves as a host to a pathogenic agent, generally without visible symptoms of the disease or injury.

Seasonal Flu ("Common Flu", "Winter Flu") - Influenza caused by one of the common influenza subtypes known to be circulating in the human population; seasonal influenza peaks in the winter months in the Northern and Southern Hemispheres and tends to be year-round in tropical regions.

Strain - Influenza virus subtypes are further characterized into strains. New strains of influenza viruses replace older strains through the process of antigenic

drift (i.e., small mutations in the genetic material of the virus).

Swine Flu - A respiratory disease in pigs caused by influenza A virus. Outbreaks in swine herds are common; the illness is relatively mild, and most animals recover. Domestic birds can be a source of influenza A in swine, and transmission from humans to swine and from swine to humans has occurred.

Virulence - A pathogen's ability to invade host tissues and the severity of disease produced.

Virulent - Highly lethal; causing severe illness or death.

Virus - Any of various simple submicroscopic parasites of plants, animals, and bacteria that often cause disease and that consist essentially of a core of RNA or DNA surrounded by a protein coat. Unable to replicate without a host cell, viruses are typically not considered living organisms.

Zoonoses - Diseases that transfer from animals to humans.

RESOURCES

Archer B, Cohen C, Naidoo D, et al. Interim report on pandemic H1N1 influenza virus infections in South Africa, April to October 2009: epidemiology and factors associated with fatal cases. *Euro Surveill* 2009;14(42)

Garten RJ, Davis CT, Russell CA, et al. Antigenic and genetic characteristics of swine-origin 2009 A(H1N1) influenza viruses circulating in humans. *Science* 2009 Jul 10;325(5937):197-201

Gibbs AJ, Armstrong JS, Downie JC. From where did the 2009 'swine-origin' influenza A virus (H1N1) emerge? *Virology* 2009 Nov 24;6(1):207

Gorman OT, Bean WJ, Kawaoka Y, et al. Evolution of influenza A virus nucleoprotein genes: implications for the origins of H1N1 human and classical swine viruses. *J Virol* 1991 Jul;65(7):3704-14

Hay AJ, Gregory V, Douglas AR, et al. The evolution of human influenza viruses. *Philos Trans R Soc Lond B Biol Sci* 2001 Dec 29;356(1416):1861-70

Karasin AI, Schutten MM, Cooper LA, et al. Genetic characterization of H3N2 influenza viruses isolated from pigs in North America, 1977-1999: evidence for wholly human and reassortant virus genotypes. *Virus Research* 2000;68(1):71-85

Kawaoka Y, Krauss S, Webster RG. Avian-to-human transmission of the PB1 gene of influenza A viruses in the 1957 and 1968 pandemics. *J Virol* 1989 Nov 1;63(11):4603-8

Kilbourne ED, Smith C, Brett I, et al. The total influenza vaccine failure of 1947 revisited: major intrasubtypic antigenic change can explain failure of vaccine in a post-World War II epidemic. *Proc Natl Acad Sci U S A* 2002 Aug 6;99(16):10748-52

Ma W, Lager KM, Vincent AL, et al. The Role of Swine in the Generation of Novel Influenza Viruses. *Zoonoses Public Health* 2009 May 20

Myers KP, Olsen CW, Gray GC. Cases of swine influenza in humans: a review of the literature. *Clin Infect Dis* 2007 Apr 15;44(8):1084-8

Olsen CW. The emergence of novel swine influenza viruses in North America. *Virus Research* 2002;85(2):199-210

Ortiz JR, Sotomayor V, Uez OC, et al. Strategy to enhance influenza surveillance worldwide. *Emerg Infect Dis* 2009 Aug; 15(8):1271-8

Reid AH, Taubenberger JK. The origin of the 1918 pandemic influenza virus: a continuing enigma. *J Gen Virol* 2003 Sep;84(Pt 9):2285-92

Sheerar MG, Easterday BC, Hinshaw VS. Antigenic Conservation of H1N1 Swine Influenza Viruses. J Gen Virol 1989 Dec 1;70(12):3297-303

Shinde V, Bridges CB, Uyeki TM, et al. Triple-reassortant swine influenza A (H1) in humans in the United States, 2005-2009. N Engl J Med 2009 Jun 18;360(25):2616-25

Shope RE. Swine Influenza: III. Filtration Experiments and Etiology. J Exp Med 1931 August 1;54(3):373-85

Vincent AL, Lager KM, Ma W, et al. Evaluation of hemagglutinin subtype 1 swine influenza viruses from the United States. Veterinary Microbiology 2006;118(3-4):212-22

Webby RJ, Swenson SL, Krauss SL, et al. Evolution of Swine H3N2 Influenza Viruses in the United States. J Virol 2000 Sep 15;74(18):8243-51

Webster RG, Bean WJ, Gorman OT, et al. Evolution and ecology of influenza A viruses. Microbiol Mol Biol Rev 1992 Mar 1;56(1):152-79

Zhou NN, Senne DA, Landgraf JS, et al. Genetic Reassortment of Avian, Swine, and Human Influenza A Viruses in American Pigs. J Virol 1999 Oct 1;73(10):8851-6

Zimmer SM, Burke DS. Historical perspective--Emergence of influenza A (H1N1) viruses. N Engl J Med 2009 Jul 16;361(3):279-85

All glossary definitions are found at one or more of the following sources (or are adapted from those sources):

CDC: Avian Influenza (Bird Flu). Accessed July 1, 2009
[Web page: <http://www.cdc.gov/flu/avian>]

Flu.gov. Glossary
[Web page: <http://www.pandemicflu.gov/glossary/index.html>]

Ryan KJ, Ray CG. Sherris medical microbiology: an introduction to infectious disease, 4th ed. 2004 New York: McGraw-Hill

USDA. Avian Influenza (Bird Flu): Avian Influenza Glossary of Terms
[Web page:
http://www.usda.gov/wps/portal/usda/usdahome?navid=AI_GLOSSARY&parentnav=AVIAN_INFLUENZA&navtype=RT]