

Pfizer Animal Health

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What's New With Swine Flu?

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Key Points

- The mechanisms of influenza virus evolution—antigenic drift and antigenic shift—rest within the fundamental properties of the virus itself.
- The respiratory tract of swine possesses both avian and human receptors for influenza viruses.
- Genetic reassortments can occur within swine cells co-infected with two or more influenza viruses.
- Currently four clusters of H1 and four clusters of H3 swine influenza viruses (SIVs) have been identified in the US swine population.¹
- The genetic diversity of SIVs sets the stage for the emergence of additional viruses that potentially could make diagnosis and successful vaccination problematic.

The objectives of this bulletin are to highlight key stages in the evolution of SIV in the US and to provide historical context to current conditions within the country.²⁻⁷ This information may be useful to producers and veterinarians as they develop strategies for managing future SIV challenges from continuously evolving H1N1, H1N2, and H3N2 viruses that may frustrate diagnostic approaches and limit the success of existing vaccination programs.

Basic Information About Influenza Viruses

Swine influenza (swine flu or flu) is caused by influenza type A viruses (Figure 1) of the family *Orthomyxoviridae*. Influenza viruses are divided into three groups, namely type A, type B, or type C, but only type A viruses infect pigs. At the center or core of the influenza virus is ribonucleic acid (RNA), which contains the eight genes (PB2, PB1, PA, HA, NP, NA, M1+M2, NS1+NS2) the virus needs to survive and replicate within host cells.⁸

Covering the core of the influenza virus is the matrix protein membrane, which in turn is covered with a lipid envelope. Protruding through the lipid bilayer to the outer surface of the virus are two large spike-

like proteins called hemagglutinin, or HA protein, and neuraminidase, or NA protein. Two of the eight genes contained within the influenza A RNA encode for these two important envelope proteins. Influenza A viruses like SIV use the HA and NA proteins for attaching to and entering the respiratory tract cells they infect.⁹ HA and NA are also the primary viral proteins that the body recognizes as foreign and attacks by producing neutralizing antibodies. Antibody against HA prevents infection by neutralizing the infectivity of the virus, whereas antibody against NA restricts spread of the virus within the host's respiratory tract.¹⁰ Protection against influenza virus is mediated primarily by antibodies to HA, which during the early stages of infection is responsible for the attachment and penetration of the virus into cells. While both HA and NA elicit the production of antibodies, antibody to HA



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has been shown to neutralize virus, whereas antibody to NA has failed to protect.¹¹ This is a key point to remember when designing an appropriate vaccination strategy.

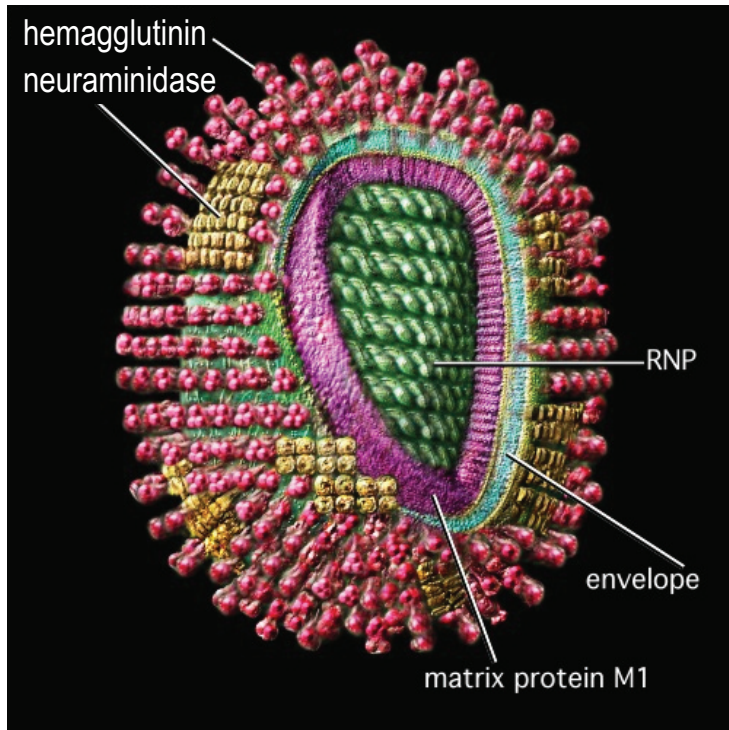


Figure 1—Schematic model of an influenza virus particle (photo: copyright Russell Kightley; rkm.com.au).

Subtyping of influenza type A viruses is on the basis of the HA and NA proteins. Currently, 16 hemagglutinins (H1–H16) and 9 neuraminidases (N1–N9) have been identified in humans, mammals, and birds.¹²⁻¹⁴ The HA and NA proteins of the influenza virus can gradually change through subtle, accumulated mutations in RNA or replication errors. This process is called antigenic drift.¹⁵ If the change in protein occurs at an antibody-binding site, then previously stimulated antibodies may be unable to neutralize the mutated SIV, and the virus can evade the body's immune system. Changes in the HA and NA molecules may be significant enough to cause influenza outbreaks since antibodies to a previously circulating viral strain do not necessarily confer immunity against the same viral subtype that has undergone antigenic drift.¹⁶ In human medicine, this is the reason that the vaccine composition is reviewed annually to determine if the vaccine strains should be changed to provide protection against newly emerging influenza viruses. The same principle applies in veterinary medicine: vaccination of pigs against a particular influenza virus strain may not protect against influenza viruses that may have undergone mutation.^{4,14} Larger and more unpredictable genetic changes called antigenic shifts can also occur in influenza A viruses when animals become infected with more than one

virus at a time.¹⁵ Pigs have receptors in their trachea and lower airways for attachment of both mammalian and avian viruses and for this reason pigs are sometimes referred to as mixing vessels because of their potential role in the emergence of new influenza strains.^{6,17-19}

When two or more influenza viruses of different lineages co-infect a single cell, the segmented nature of their RNA genomes allows genetic reassortment to occur.^{20,21} Genetic reassortment involves the scrambling (reassorting) of internal genes of one virus with those of another so that what emerges from the co-infected cell is a new influenza virus subtype (Figure 2). Potential for this kind of influenza virus mixing is greatest in parts of the world like southern China where large populations of people, birds, and pigs exist in close contact with one another.²² Severity of clinical disease appears to depend upon the infecting SIV strain, the immune status of the animals affected, presence of secondary pathogens, and/or environmental stress factors such as chilling and poor ventilation.

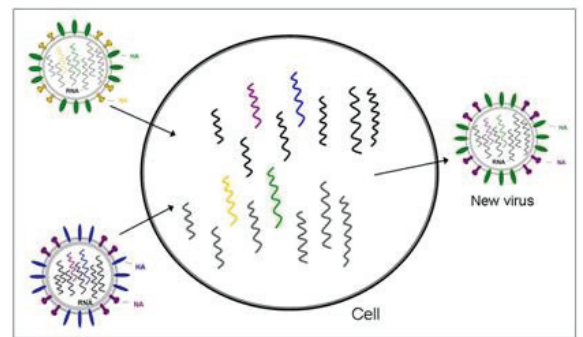


Figure 2—Genetic reassortment of an influenza virus. New influenza virus strains may be formed within an infected pig cell from the co-mingling of internal genetic material from two or more influenza viruses of different subtypes. (Illustration used with the permission of Christine H. Herrmann, PhD, Department of Molecular Virology and Microbiology, Baylor College of Medicine)

While influenza A viruses regularly change by antigenic drift, antigenic shift happens only occasionally. However, antigenic shifts have been responsible for at least three major worldwide disease events during the twentieth century (the Spanish Flu of 1918, the Asian Flu of 1957, and the Hong Kong Flu of 1968) as well as three pandemic threats (Swine Flu Virus Epidemic of 1976 in Fort Dix, New Jersey, Russian Flu of 1977, and the Avian Flu of 1997 and 1999).^{23,24}

SIV Subtypes H1N1 and H3N2

Four clusters of H1 swine influenza viruses have been detected in US swine herds, with three of the clusters appearing to predominate on the basis of gene sequence analysis of contemporary field isolates.^{1,2,4,7,14,25} Similar diversity exists among the H3 SIV subtype. Antigenic and genetic characterization has identified four distinct clusters of H3 viruses, with one

cluster currently appearing to predominate.^{1,3,5,6,26}

Except for the classical swine H1N1 viruses, most of these contemporary H1 and H3 strains are triple reassortant viruses, containing genes of avian, human, and swine influenza virus origin. Because triple reassortant influenza viruses of swine are thought to have an increased affinity for replication in the swine respiratory tract and because multiple lineages of influenza viruses are currently co-circulating in the US swine population, the stage may be set for additional virus variation and the appearance of even more new strains of SIV.^{4,6,7}

H1N1 was first isolated in the US in 1930 and, until recently, this lineage was considered the primary ancestor of the H1N1 SIVs circulating in US swine herds. It is often referred to as “classical” SIV.² The first H3N2 SIV to appear in pigs in the US was detected in 1998, followed the next year by the emergence of three additional genetically and antigenically distinct H3N2 SIVs.^{2,27,28} The H1N1 and H3N2 viruses are found worldwide wherever pigs are raised and are responsible for SIV being one of the most prevalent swine respiratory diseases. Field experience has shown that previous vaccination with an H1N1 vaccine does not guarantee protection against viruses of the H3N2 subtype.²⁷ Similarly, a study has shown that H3N2 vaccination does not fully protect pigs against challenge with antigenic and genetically diverse H3N2 SIVs.^{5,29}

SIV, whether H1N1 or H3N2, causes damage to the respiratory epithelium lining the airways. One of the most noticeable clinical signs is a harsh, barking cough. Acute outbreaks are marked by flu-like signs. Onset is very sudden, usually following a 1- to 3-day incubation period. Morbidity may be as high as 100%, but uncomplicated, mortality is low (usually less than 1%) unless infection occurs in very young pigs or there is a secondary bacterial infection. Most pigs in the herd show signs at the same time. Pigs lose their appetite, become lethargic, huddle and pile. Movement provokes severe paroxysmal coughing that sounds like a pack of dogs barking. Affected pigs run high temperatures (105° F to 107° F) and show labored, open-mouthed, abdominal breathing (thumping). If secondary infection does not occur, clinical signs typically subside within 5 to 7 days in individual animals and within 10 to 14 days in groups.^{25,30}

In SIV-naïve sow herds, abortions during an outbreak may be widespread, ranging from 5% to 10% of the herd. Abortions in these situations are not caused by fetal infection with SIV because the virus does not spread systemically; rather, they are attributed to the high fevers occurring in SIV-infected sows. Disease spread is rapid, typically moving through a breeding-gestation facility within a week. Unlike porcine reproductive and respiratory syndrome (PRRS) virus-induced abortion, abortions associated with SIV infections usually subside in 2 to 3 weeks.^{31,32}

H1N1: A Remarkable Transformation

From when it was first isolated in 1930 until 1998, the classical H1N1 (cH1N1) subtype of SIV was essentially the only SIV lineage circulating in the US swine population.³³ Genetically and antigenically, the cH1N1 SIV and the human influenza virus implicated in the 1918 Spanish Flu pandemic are similar. Work done as late as 2000 to define the antigenic makeup of the cH1N1 SIV strain showed that the virus had remained relatively unchanged since 1930. The virus was generally stable with only a limited number of nucleotide changes leading to amino acid substitutions.^{2,33-35}

Beginning in 1998, however, clinical disease caused by H3N2 subtypes was recognized in a few states and soon spread throughout the entire country. Described as reassortant viruses, the original H3N2 strain had three genes from a human H3N2 influenza virus that circulated in the human population during 1995, as well as five genes from the cH1N1 SIV. The initial outbreak of influenza attributed to H3N2 occurred during August of 1998 in a North Carolina pig farm with 2,400 breeding sows. Sows were lethargic, had high fevers and went off feed for several days. Morbidity rates approached 100%, 7% of the breeding sows aborted, and 2% of the sows died. Appearing at approximately the same time in Minnesota, Iowa, and Texas were additional H3N2 isolates that had triple reassortants (Figure 3), with genetic material from an avian influenza virus (two genes), from human (three genes) and swine (three genes) viruses as well.²⁷ The emergence of these H3N2 viruses is widely regarded as a remarkable occurrence, as it was the first time reported that the genome of an influenza virus consisted of gene segments from three different viral lineages.²⁷

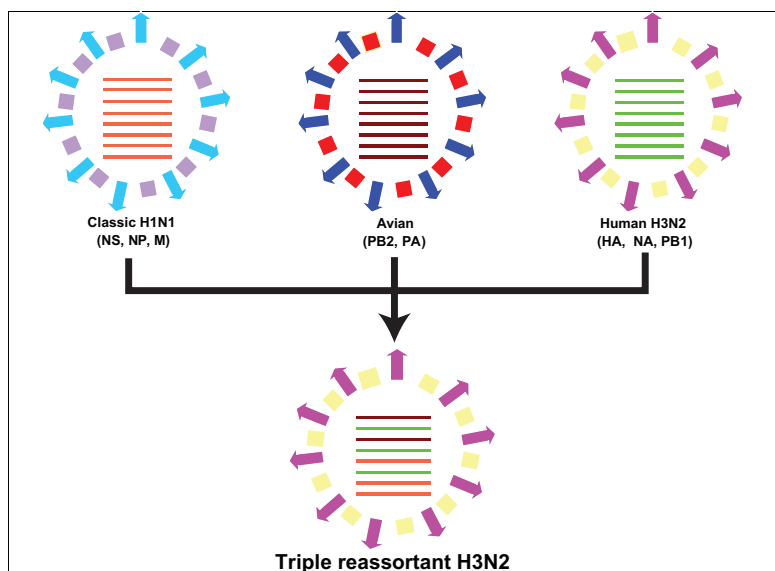


Figure 3—Antigenic shift or reassortment: A new SIV subtype emerged in the US in 1998 as a result of the mixing of genetic material from a human H3N2 influenza A virus, an avian influenza virus, and the classical H1N1 SIV.

Subsequent to the emergence of the H3N2 viruses, genetic changes in both H1 and H3 SIV subtypes were detected with increasing frequency in the US. The dynamics of clinical disease and prevention of outbreaks also changed dramatically. Reassortant H1N1 viruses (reassortants contain genes from swine, avian, and/or human influenza viruses), for example, were reported to infect and cause disease in herds that had been routinely vaccinated with commercial vaccines containing cH1N1 SIV.⁴ Such findings raised concerns among some investigators that vaccines in swine may need to be continually updated as in human medicine.⁴

AN ACCELERATED RATE OF CHANGE

Antigenic and genetic studies conducted with SIV field isolates since the dramatic appearance of the H3N2 subtype in 1998 have confirmed that the H1N1 subtype, which had remained essentially stable in US swine for 80 years, was now evolving through antigenic drift and reassortment.² As the timeline in Figure 4 illustrates, four new variant H1 strains emerged in rapid succession in the US during the years 1999 through 2003. The SIV subtype designated H1N2 was first isolated in 1999 from pigs in Indiana that were showing respiratory disease, fever, and inappetance.³⁶ Genetic analyses conducted at the University of Wisconsin revealed that this H1N2 contained four swine, two human, and two avian influenza virus genes.^{36,37} It was determined that this H1N2 most likely arose from the reassortment of a cH1N1 SIV with a triple-reassortant H3N2 SIV isolate. Within two years of the initial isolation in Indiana, H1N2 viruses of the same overall genotype had spread widely within the swine population of the US and were co-circulating with both cH1N1 and triple-reassortant H3N2 viruses.³⁷ Emerging in 2001 was a reassortant H1N1 (rH1N1), which consisted of hemagglutinin and neuraminidase genes from swine cH1N1 viruses plus six internal genes derived from the triple-reassortant H3N2 viruses.^{2,5} Acquisition of the H3N2 internal genes, which have been characterized as almost exclusively avian-like in origin, makes the current second-

generation rH1N1 swine influenza viruses appear to have an increased rate of mutation as the avian genes are prone to errors during replication, and they soon predominate in populations where they become established. Studies conducted with current rH1N1 isolates showed that in general they caused more severe pneumonia than older cH1N1, produced more nasal shedding, and grew to higher titers in the lung, indicating that they have adapted well to swine hosts and may have greater transmission potential while inducing more severe diseases.⁴

The third H1 SIV to emerge was the human-like reassortant H1N2 (hH1N2), which was first isolated in North America in October 2003 in Ontario, Canada, from a group of 6-week-old pigs suffering unexpected, sudden deaths.³⁴ Genetic analyses demonstrated that all eight RNA genes of this isolate were most closely related to the H1N2 viruses that circulated worldwide among the human population during the 2001 to 2002 influenza season. In essence, the 2003 isolation of hH1N2 influenza virus represented an interspecies transmission of a wholly intact human influenza virus to a pig. Additional isolates of hH1N2 influenza viruses in Ontario in 2004 contained genes of mixed swine and human virus lineages. These findings suggested that a human influenza virus lineage could function in cooperation with genes of variable lineages to produce human-swine reassortant influenza viruses that are infectious for and able to replicate in pigs and may reflect adaptation mutations to enhance infection of swine. In early November 2003, the first human-like influenza virus—hH1N2—was isolated in the US in Minnesota from three 4-week-old pigs with respiratory disease.⁷ Genetic analyses of the HA gene revealed high similarity (> 90%) to H1 influenza virus circulating worldwide in the human population during 2002 and 2003. Similar human-like influenza viruses have been isolated from the same production system, suggesting that the virus has adapted to swine.⁷

Approximately two years later (June 2005), H1N1 viruses of human-like HA genotype were isolated from US swine. These viruses were > 98% similar to H1 human influenza viruses circulating in 2004 and < 75% similar to SIV reference strains.⁷ Since then, this human-like H1N1 has spread to pigs outside the immediate geographic region following movement of the infected pigs to other states.⁷

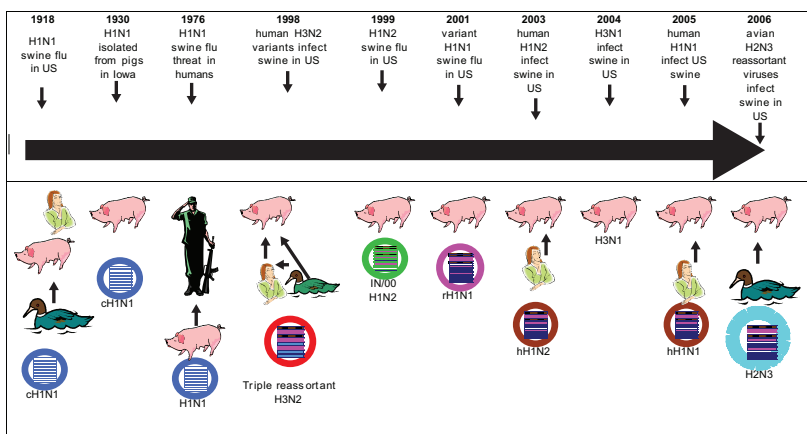


Figure 4—Timeline marking evolution of H1 swine influenza viruses (Illustration courtesy of Marie Gramer, University of Minnesota Veterinary Diagnostic Laboratory).

Prevalence of the various H1 viruses in US swine herds has been monitored by investigators at the University of Minnesota Veterinary Diagnostic Laboratory (M. Gramer, personal communication). From 2003 through 2008, 233 H1 SIVs from field cases throughout the US were submitted during March and April (representative of the winter flu season) to the laboratory for genetic analysis. The first approximate 900 nucleotides of the hemagglutinin genes were sequenced and the first approximate 600 nucleotides aligned and compared to reference strains. The data indicated that the cH1N1 SIV genotype had largely been replaced by H1N2 and H1N1 viruses belonging to three genetic clusters: rH1N1 viruses similar to the viruses isolated in Minnesota in 2001, H1N2-like viruses similar to the H1N2 virus isolated in 1999, and human-like H1 viruses like those isolated in 2003 (Figure 5).^{1,38}

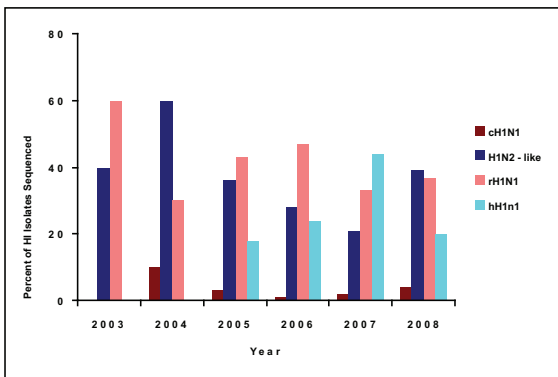


Figure 5—H1 gene sequences for 233 H1 swine influenza isolates obtained during March and April from 2003 through 2008.

Two additional SIV subtypes—H3N1 and H2N3—appeared briefly in US swine herds as a result of reassortants between cH1N1 and the H3N2 viruses introduced in 1998.³⁹⁻⁴¹ H3N1 isolates were obtained in 2004 from swine herds in southern Michigan, central Indiana, and Minnesota. Genetic analysis showed that the H3N1 SIVs had a hemagglutinin gene similar to that of contemporary Cluster III H3N2 SIVs, a neuraminidase gene from a human H1N1 isolate, and the remaining genes from contemporary circulating Cluster III H3N2 SIVs. H3N1 caused lung lesions, and nasal shedding was observed.^{39,40} H2N3 SIV was isolated in September 2006 from several 5- to 6-week old pigs with bronchopneumonia in a multi-sourced commercial nursery in Missouri. The hemagglutinin gene most closely matched that of H2 viruses isolated from North American mallard ducks; the neuraminidase gene was most closely related to the H4N3 avian influenza virus isolated from blue-winged teal; and, with one exception, the remaining internal genes were derived from contemporary triple-reassortant SIVs currently circulating in US swine herds.⁴¹ Although

neither virus has been shown to be efficiently transmitted in the field, their isolation underscores the situation in which swine serve as a mixing vessel for human, swine, and avian influenza viruses.⁴⁰ These data demonstrate that the introduction of H3N2 viruses with genes of human and avian origin into the US swine population has dramatically changed the rate at which H1 swine influenza viruses evolve. Within one year of the first isolation of H3N2, reassortants between the triple-reassortant H3N2 and cH1N1 resulted in an H1N2 with the swine hemagglutinin gene and the remaining genes from the H3N2. Further reassortment produced rH1N1 viruses with the hemagglutinin and neuraminidase from the cH1N1 and internal genes from the triple-reassortant H3N2. Notably, the hemagglutinin genes of these new H1 viruses are genetically more closely related to the reassortant viruses than to cH1N1 isolates, forming genetic clusters distinct from the classic hemagglutinin gene sequences.^{1,4,7} Additionally, the data show that viruses of human origin have spilled over into the US swine population and are continuing to do so. The ongoing isolation of human-like H1N1 and H1N2 influenza viruses suggests that the viruses have adapted to swine and are capable of causing respiratory disease.^{7,14,37}

H3N2 SIV: Continuing Antigenic Change

During the period between 1998 and 2007, the H3N2 viruses also have continued to circulate and evolve, adding another layer of complexity to the current epidemiological picture in the US swine population. Sequence analyses of the triple-reassortant H3N2 viruses have shown that their hemagglutinin genes belong to one of three genetically distinct influenza virus clusters:⁶

- Cluster I includes the prototypes A/Swine/Texas/4199-2/98 (Sw/TX/98), a triple-reassortant H3N2, and A/Swine/North Carolina/35922/98 (Sw/NC/98), a double-reassortant H3N2. Viruses in Cluster I appear to be most closely related to the H3N2 influenza viruses isolated from humans in 1995.^{27,36} A/Sw/TX/98 virus is the H3N2 reference strain used in serologic assays in most diagnostic laboratories in the US.
- Cluster II contains A/Swine/Colorado/23619/99 (Sw/CO/99), whose hemagglutinin gene is more closely related to the A/Sydney/5/97 human-like H3N2 influenza viruses isolated from humans during 1997 and 1998 than the H3N2 viruses isolated from swine in 1998 (A/Sw/TX/98).^{3,6}
- Cluster III includes prototype viruses A/Swine/Oklahoma/18089/99 (Sw/OK/99) and A/Swine/Illinois/21587/99 (Sw/IL/99), viruses whose hemagglutinin gene is most similar to that of human influenza viruses circulating in the US during 1996—A/Wuhan/359/95-like viruses. The H3N2 viruses that predominate across the US are most similar in hemagglutinin genotype to the Cluster

III-like viruses.^{1-3,6}

Genetic analyses of contemporary H3N2 influenza viruses circulating in the US swine population show that a growing number of isolates are diverging from the prototype Cluster III classification, so much so that these H3N2 viruses are now being classified as Cluster IV viruses (referred to by some investigators as Cluster III variant).^{14,42} This suggests that continuous antigenic drift or shift in Cluster III since 1998 may have resulted in virus variants.^{26,42}

Prevalence of the H3N2 clusters in US swine herds has also been monitored by investigators at the University of Minnesota Veterinary Diagnostic Laboratory.¹ During the months of March and April from 2003 through 2008, a total of 151 H3 isolates were submitted for genetic analysis. Results showed that Cluster I (Sw/TX/98) and Cluster II (Sw/CO/99) were not identified at all from 2006 through 2008, and Cluster III (Sw/IL/99) was seldom identified. This is significant because it means that contemporary triple-reassortant H3N2 SIVs may not be cross-reacting in diagnostic hemagglutination-inhibition tests using antigens prepared from H3N2 strains representing the major genetic clusters that have circulated in US swine herds since 1998. Such a situation not only poses a serious diagnostic problem, it also raises concerns about the effectiveness of SIV vaccines prepared from H3N2 parent strains isolated in 1998. Replacing Cluster I, Cluster II and Cluster III H3N2 SIVs are Cluster IV strains, which were identified by gene sequence analysis in 131 of the 151 H3 isolates in the University of Minnesota study (Figure 6). Both antigenic and genetic differences were noted within the Cluster III and Cluster IV viruses.¹

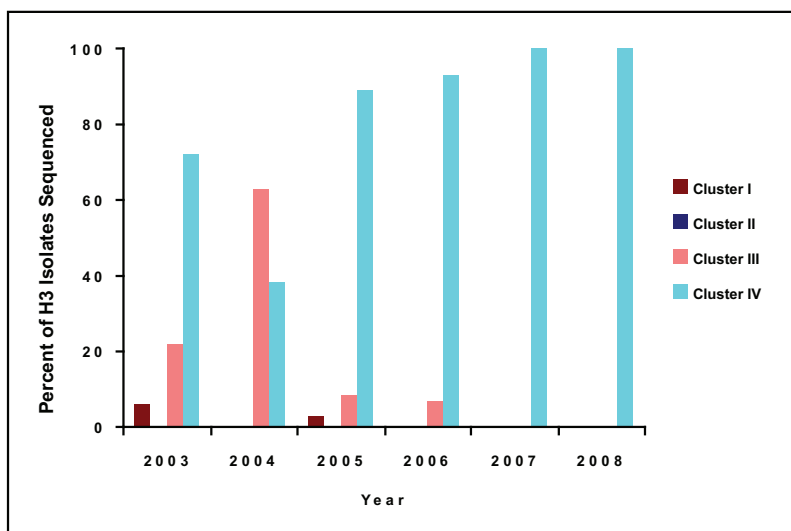


Figure 6—H3 gene sequences for 151 H3 swine influenza isolates obtained during March and April from 2003 through 2008.

A Genetically Diverse Reservoir of Swine Influenza Viruses

Until 1998, swine influenza in the US was relatively stable, with classical H1N1 SIV that emerged in 1930 being the only major virus lineage causing disease. This cH1N1 remained highly conserved both genetically and antigenically for 80 years. As the preceding review has noted, the emergence of H3N2 viruses late in 1998 rapidly changed the epidemiology of disease throughout the country. Between 1999 and 2001, the exchange of genes between cH1N1 and triple-reassortant H3N2 viruses resulted in the new H1N2-like and reassortant H1N1 groups. Between 2003 and 2005, human-like H1N2 and H1N1 viruses appeared. Adding complexity to the situation, the H3N2 viruses also showed a propensity for antigenic changes, with Cluster IV field isolates becoming the predominant H3 genotype in all geographic regions of the US. Clearly, antigenic and genetic variation currently exists in contemporary H1 and H3 SIV isolates. SIVs isolated from US swine in 2007 and submitted to the Minnesota Veterinary Diagnostic Laboratory for antigenic and genetic analyses belong predominantly to three H1 clusters and one H3 cluster:¹

- Reassortant H1N1-like (rH1N1)
- H1N2-like
- Human-like H1 (hH1)
- Cluster IV H3N2 (sometimes referred to as Cluster III variant)

If the H1 and H3 SIVs belonging to these lineages continue to co-circulate, the emergence of additional reassortant viruses with unique combinations of surface and/or internal protein genes is likely for the following reasons:²

- Pigs have cellular receptors to receive avian, human, and swine influenza viruses.
- The population of susceptible swine with no immunity to influenza is continuously renewed.
- Existence of swine herds in close proximity to human and bird populations.

What is less clear are the implications of antigenic and genetic variation for SIV vaccination and control programs. Investigators know that escape from vaccinal immunity can occur when the HA of infecting strains varies appreciably from the HA of the vaccine strain and the changed HA is no longer completely recognized by the pig's immune system. Therefore, outbreaks of swine influenza can occur in herds that have been previously exposed either naturally or by vaccination. The mutability of the virus makes it a hard target to hit. What is unknown is the exact level or type of variation necessary to cause vaccine escape. The question to address through detailed analyses of HA gene sequences and antigenic characteristics is what constitutes a significant change affecting vaccine efficacy and what does not. With the acquisition of

avian genes for replication, current H1 viruses are evolving more rapidly, and therefore have an enhanced ability to evade established herd immunity.^{4,14} As a result, current killed SIV vaccines may not be able to provide the coverage necessary for the changing mix of circulating swine influenza viruses. The challenge for the swine industry is to develop a surveillance system that rapidly identifies the genetic and antigenic characteristics of new and emerging circulating viruses so that efficacious vaccines against potentially devastating influenza viruses might be provided in a timely manner.

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