



Knowledge

Swine influenza virus: understanding this evolving organism

SUMMARY

Swine influenza virus (SIV) is a constantly changing organism that makes SIV disease management and control difficult. Its role in causing respiratory problems, coupled with being a significant contributor to the porcine respiratory disease complex (PRDC), poses serious

problems for swine veterinarians and producers.

For over 70 years, classical H1N1 virus was the only recognized prevalent influenza virus in swine. Today, the classical H1N1 virus has drifted and shifted its structure to form new variants, with genes derived from human, swine and avian influenza viruses. These changes have challenged the

way SIV is diagnosed and controlled. Understanding the swine influenza virus within a herd or geographic area is needed to make science-based decisions about implementing a control program on swine operations, whether it includes a commercial vaccine such as PneumoSTAR® SIV or a custom vaccine alternative from Novartis Animal Health US, Inc.

Swine influenza virus (SIV) is an inherently changing RNA virus that causes respiratory disease in swine. It is a major contributor to the porcine respiratory disease complex (PRDC), which infects pigs with viral and bacterial pathogens and causes economic losses to pig producers.

SIV has evolved from a seasonal disease caused by a stable genotype (H1N1) to a year-round, endemic respiratory disease caused by multiple SIV genotypes undergoing continuous change.¹ Monitoring pig herds for SIV with isolation and further genetic characterization will help veterinarians determine the most appropriate vaccine options for their clients' herds.

SIV may cause respiratory disease with intermittent clinical signs, including:

- Elevated temperatures
- Clear nasal discharge
- Coughing
- Animals going off feed
- Spontaneous abortions due to high fevers

If left uncontrolled, long-term respiratory problems result in poor performance of market and breeding animals.

History of influenza

Influenza virus has plagued humanity for centuries. Flu-like human disease syndromes were first described in 412 B.C. The Spanish flu pandemic, which also affected swine and avian populations, hit in 1918 and killed an estimated 25 to 50 million people. In 1930, the classical influenza virus, H1N1, was isolated in pigs. For many

years classical H1N1 was thought to be the only strain of influenza in U.S. pig populations.

In 1998, a new swine flu subtype called H3N2 appeared with genes derived from human, swine and avian influenza viruses. It created a swine flu epidemic because pigs were only immune to the H1N1 strain. After a reassortment of H3N2 and classical H1N1, another subtype of SIV, H1N2, appeared. The new strain also caused disease problems in the immunologically naive swine population.

As is typical of RNA viruses, SIV evolves and changes. SIV continues to drift and reassort into a complex collection of viruses, which undergo continuous change. The virus changes over time because of the imperfect replication by RNA polymerase and the propensity for antigenic shift and reassortment of viral RNA segments.

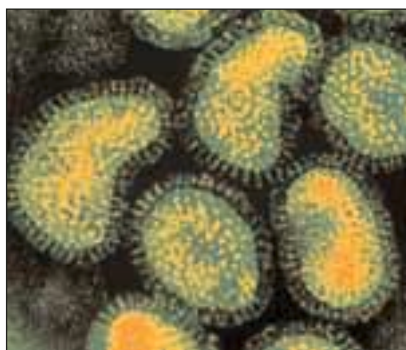
These genetic and antigenic changes are some of the most important factors affecting the epidemiology of swine influenza throughout the world.²

SIV structure

Influenza viruses are enveloped, segmented, single-stranded, negative-sense RNA viruses belonging to the Orthomyxoviridae family. Influenza viruses contain eight RNA genes that code for eight proteins – internal and external structural proteins, RNA polymerase, and non-structural proteins.¹

Nucleoprotein and matrix are used to classify influenza viruses as Types A, B and C. Type A viruses cause disease in animals, birds and humans. In Type A viruses, hemagglutinin (HA) and neuraminidase (NA) genes encode for surface glycoproteins that project from the viral envelope.¹

These external proteins are significant for infection and immunity. The HA is responsible for attachment of the virus to the host receptor and infection of the host cell.³ This protein also raises an antibody response in the host.



Each influenza virus is made up of a core of RNA-genetic material (yellow) surrounded by a spiked protein envelope (green). The tiny external spikes stick to host cells when the virus invades the body. For a detailed representation of influenza virus and the HA gene, please go to the University of Cape Town Department of Medical Microbiology Web site – <http://web.uct.ac.za/depts/mmi/jmoodie/influen2.html>.

SIV: constantly changing

Swine influenza virus is constantly changing, making disease management and control difficult. Due to genetic changes discussed below, there is a platform of influenza viruses capable of infecting immunologically naive swine herds.

Antigenic drift

- Random mutation and single amino acid substitution in HA and NA proteins
- Change is normal and gradual
- Appears to be the reason for change in the H3N2 viruses currently circulating in U.S. swine (see Figure 1)

Antigenic shift

- Reassortment or swapping of virus gene segments between viruses
- Can cause dramatic changes in the virus genome and structure
- H1N2 and H3N2 appears to be caused by antigenic shift

To help control SIV on swine operations, Novartis Animal Health US, Inc. offers two vaccination strategies. Choosing

a vaccination program requires an understanding of SIV and an evaluation of diagnostic results.

Commercial vaccines:

- PneumoSTAR® SIV
 - Provides protection from H1N1 and H3N2 SIV strains with a single 1-mL dose
 - Broad cross-reactivity from strain to strain, regardless of where the strain originated¹¹



Custom SIV vaccines:

- Guard against specific SIV strains found on the farm when commercial vaccines aren't effective against SIV outbreaks
- Produced in USDA-approved facilities

These proteins also help subtype influenza viruses into 16 HA types and nine NA types.

Why and how influenza viruses change

Because influenza viruses contain segmented RNA genes, these segments can sometimes be exchanged when a cell is infected with more than one strain of SIV. As a result, genetic shift or reassortment causes whole RNA segments to rearrange into a completely different SIV strain. Disease can spread quickly because the swine population is immunologically naive to the new SIV strain.

Also, mutations may occur during replication,⁴ thereby changing the nucleotide sequence. Nucleic acids are translated into amino acids,

which become proteins that the immune system recognizes. A nucleic acid change may or may not be sufficient to change the amino acid. If the nucleotide change leads to an amino acid change, this may result in a structural and/or functional change in the protein, which may lead to an antigenic change in the virus. These antigenic sites and the dynamics of the changes are not well-defined for swine influenza viruses.

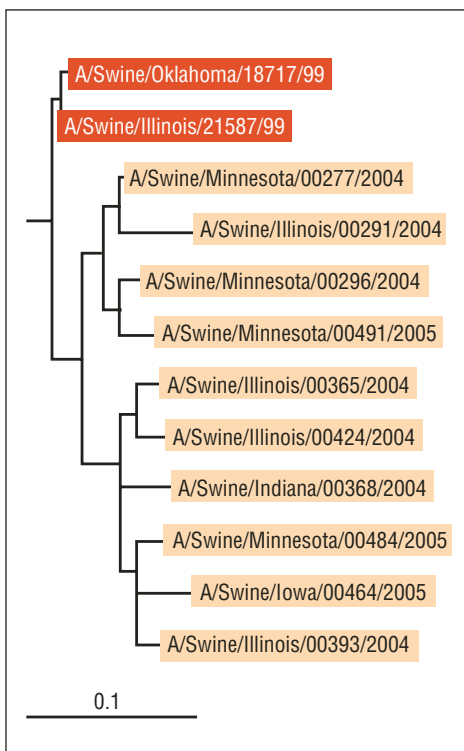
Genetic mutations and amino acid sequence differences may occur, creating a new variant of the virus. This type of change is called genetic drift. If the changes that result from the differences alter the SIV proteins in such a way that the immune system no longer recognizes it, the new variant of the virus may circulate and cause clinical disease.

Implications of changes in influenza viruses

Influenza viruses change in two major ways: genetic drift and genetic shift (or reassortment). The HA and NA proteins are important targets for the immune system (e.g., antigens) and are involved in cellular attachment and release. Therefore, more attention is paid to changes in the HA and NA portions of the genome. When changes occur in the HA or NA genes, the changes are referred to as antigenic drift and antigenic shift.

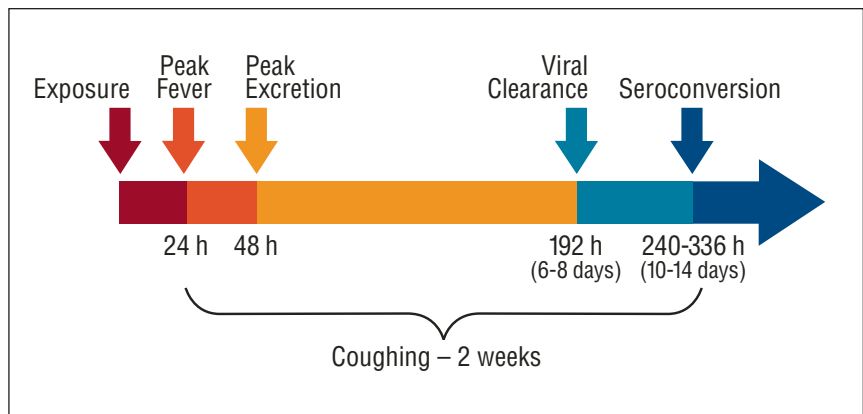
Antigenic drift occurs by random mutation and single amino acid substitution in the HA and NA proteins⁵ during

Figure 1. H3N2 phylogram of a selection of H3N2 viruses currently circulating in U.S. swine



Phylogram courtesy of Marie Gramer, DVM, PhD candidate, University of Minnesota Veterinary Diagnostic Laboratory. The scale is changes per 100 nucleotides and the tree was rooted to a human H3N2 virus strain, which is not shown.

Figure 2. SIV infection timeline



Timeline courtesy of Marie Gramer, DVM, PhD candidate, University of Minnesota Veterinary Diagnostic Laboratory.

viral replication. The change is gradual and part of the normal drift seen with SIV. For the HA gene of influenza viruses, a mutation occurs at the rate of one mutation in every 100 replicated genes. This rate is sufficiently high enough to create several antigenic variants each year.⁶ Antigenic drift appears to be the reason for change in the H3N2 viruses currently circulating in U.S. swine.⁶ Antigenic shift occurs as a result of reassortment or swapping of virus gene segments between viruses and can cause dramatic changes in the virus genome and structure.⁶

Figure 1 is a phylogenetic tree of the hemagglutinin gene sequences from two previously dominant strains of H3N2 swine influenza virus (A/Swine/Illinois/21587/99 and A/Swine/Oklahoma/18717/99, boxed) and 10 currently circulating strains of H3N2 swine influenza virus. Analysis of this phylogram suggests that accumulation of changes in the HA gene of the currently circulating influenza viruses has resulted in continuous drift away from the previously dominant strains.

These changes can have significant impacts on controlling

SIV with vaccination. In some cases, well-vaccinated swine with demonstrable H1N1 swine influenza titers developed clinical influenza when infected with a variant H1N1.⁷ It has become apparent that a platform of influenza viruses exists in U.S. swine that are capable of co-infecting herds, reassorting and undergoing more rapid antigenic drift and antigenic shift.⁷

Controlling and detecting SIV in a pig herd

SIV diagnosis can take many directions depending on the acuteness and severity of the clinical signs or the frequency of outbreaks within a pig flow. See Figure 2.

Preliminary steps in determining if SIV is a factor in PRDC include conducting a necropsy of affected animals and determining if SIV is present. The presence of SIV can be determined through:

- Viral culture
- Histological examination
- Immunohistochemistry techniques
- PCR tests
- Immunoassay tests

Once the presence of SIV as a cofactor in PRDC is identified, the isolate should be subtyped to determine if it is an H1N1, H1N2 or H3N2 virus, and the HA gene sequence should be obtained and compared to reference strains.

Establishing a vaccination program and monitoring success

Once SIV is determined to be a disease that impacts performance, the first step is to incorporate a commercial vaccine into a vaccination program. The success of a vaccination program should be monitored by:

- Reduction of clinical signs
- Incidence of SIV in routine diagnostics
- Incidence of respiratory treatments presumed to be SIV-related

Nasal swab cultures are a good antemortem diagnostic procedure for SIV. Novartis has developed an SIV sampling kit with swabs and transport media to improve the likelihood of isolating SIV from nasal swabs. At necropsy, swabbing the lumens of airways from lungs with pneumonia is also an effective procedure for isolation of SIV. A lung affected with pneumonia is non-collapsing with dark red to purple firm areas of consolidation.

SIV-infected lung



Photo courtesy of Marie Gramer, DVM, PhD candidate, University of Minnesota Veterinary Diagnostic Laboratory.

SIV diagnostic kits

Novartis SIV diagnostic kits are available to help obtain samples in the field. Kits contain:

- Submission form
- Whirpak (various sizes)
- Sealing plastic bags
- Collection swabs
- Specimen transport tubes (carrier media is stable at room temperature until used)
- Materials to sample five, 10 or 15 pigs, depending on need
- Can also be used to obtain Porcine Reproductive and Respiratory Syndrome Virus (PRRSV) isolates

Novartis Diagnostic Laboratories works in close conjunction with many university veterinary diagnostic laboratories across the nation. Important veterinary pathogens that have been isolated at your local laboratory from casework previously submitted for a client can be forwarded to Novartis Diagnostic Laboratories. Assistance can be provided to facilitate transfer of viral and bacterial agents. Call 1-888-466-8325 for more information.

When to take another direction

When a genetic information database of influenza viruses is established and a vaccination program implemented, monitoring should continue as clinically warranted. Respiratory outbreaks should be documented and SIV isolation conducted in an attempt to compare the HA genetic sequence of the current virus strain to the other strains in the database. If the frequency or severity of documented SIV outbreaks is increasing, the HA genetic sequence evaluation may help determine if changes have occurred that could be causing an increase in SIV incidence. To determine more conclusively if the changes are due to antigenic shift or antigenic drift, analysis of all eight gene segments is necessary.

Why characterize SIV

Defining SIV isolates by their antigenic and genetic characteristics helps in following and understanding the evolution of swine influenza within a pig herd. PCR and antigen capture ELISA can determine if a sample is infected with SIV. They can be used on nasal swabs or lung tissues for

detection of swine influenza virus.⁸ Virus isolation is helpful for subtype-specific PCR to determine hemagglutinin (H) subtype; the PCR can also be used directly on nasal swabs or lung tissue, but sensitivity is lower than other methods of detection.⁸

Genotyping or genetic sequence analyses of the HA and NA genes of circulating influenza viruses can reveal more than just the subtype. When studied carefully, they can reveal mutations in antigenic regions of the molecule that may have contributed to the virus' ability to evade the immune system and spread.¹

Subtyping and sequencing SIV isolates from disease outbreaks may provide useful information to the swine practitioner and producer, who may be seeking to alter vaccine strategies on the basis of the results.¹ Creating a phylogenetic tree of HA genes from the different strains found on an operation shows the relationship between strains and may help determine if a new strain is the result of a presumed antigenic drift or antigenic shift. These extra diagnostic steps are very helpful in determining if a current vaccine is effective in an operation's swine population.

Preventing SIV

Vaccination programs for controlling SIV are commonplace in swine operations. However, because of the ever-changing nature of SIV, problems arise in vaccinated pig herds. Commercial vaccines that include H1N1 and H3N2 offer protection for the most common influenza strains when the vaccines were licensed, but changes occur within strains and subtypes over time. These changes may allow for evasion of the immune systems of a pig population.

PneumoSTAR® SIV includes an H1N1 and an H3N2 isolate from the late 1990s. The ImmunSTAR® adjuvant provides protection with one dose. Most protein antigens (especially when administered in small quantity) need to be administered with an adjuvant to assure a high-quality/high-quantity, memory-enhanced antibody response.⁸ ImmunSTAR is the only swine adjuvant formulated to offer protection in a single 1-mL dose.

As more strains are added to a vaccine, the “window of immunogenicity”⁹ needs to be monitored. Too many or too little antigens may induce

tolerance, rather than an active response for the given antigen.⁹ When three strains are included in a vaccine, the “window of immunogenicity” requires either an increase in total antigenic mass or maintenance of the antigenic mass by reducing each antigen.

Within the window of immunogenicity, with or without an adjuvant, larger antigen doses generally result in greater antibody responses up to a point at which suppressive activities become exaggerated. However, production of the highest titer is not always the best result. A moderate titer of high-affinity antibody may be preferable to a high titer of low-affinity antibody. High-affinity antibody generally results from immunization with smaller quantities of antigen than needed for production of the highest titer. For an animal to sustain an antibody response, a continual or intermittent supply of antigen is needed. One way an adjuvant may aid the immune response is by forming a depot of antigens at the injection site, resulting in the sustained release of small quantities of antibodies over a long period of time. This approach gives sustained stimulation while minimizing

its suppressive effects.⁹

When presented with a viral challenge, the immune system must determine which antibodies have the highest affinity to the antigen and expand this population of B lymphocytes or B cells. Vaccination with strains closer to the infecting strain should make this process easier for the body.

As SIV continues to evolve, it is important to use available diagnostic tools to find a best-fit vaccine. In some cases, a commercial vaccine will provide enough protection to cover a herd against SIV. If the SIV strain is sufficiently different from the strains in the commercial vaccine, a custom vaccine with the specific strain can be developed to create a better immune response and protective immunity. In an experimental study funded in part by the Minnesota Pork Board, more complete protection was observed with a homologous virus vaccine than with a commercial vaccine.¹⁰ According to the study, it may be necessary to review and update the SIV strains in each vaccine with continuous monitoring of the field isolates for their genetic and serologic diversity.¹⁰

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