Different forms of the same virus are often referred to as “strains”. “Strain” is not a defined term for describing PRRS virus but it is commonly used to describe PRRS virus variants.

A genomic sequence is the “DNA fingerprint” of a PRRS virus strain.

PRRS viruses have a chain of 15,000 nucleotide blocks that comprise their genetic strand. It is too costly to sequence the entire strand. Only a small portion, known as Open Reading Frame 5 (ORF 5), is sequenced to differentiate one variant from another. ORF 5 is the genetic blueprint that directs the construction of much of the envelope protein that surrounds the virus. The envelope construction often acts to “trigger” the immune response in recovering pigs. ORF 5 is also known to mutate frequently. The relationship of ORF 5 mutation and immunity qualifies the region as the most useful site to differentiate PRRS virus “strains” and evaluate evolutionary change.

More care must be taken when collecting samples for genomic sequencing than when collecting samples for typical blood tests. Sequencing is 10 to 20 times more expensive than blood testing. Planning is essential to designate the proper animals for sampling, deciding how many pigs to sample, and to determine how frequently to resample.

A collection of PRRS virus sequences can be accumulated from one swine herd over time. The relationship of the sequences is illustrated by a family tree known as a phylogenetic tree or a dendogram. These tree diagrams can be used to indicate whether a new viral “strain” was introduced or the same variants occupied the herd throughout the period.

Tree diagrams quantify with branch lengths, how closely, different PRRS viruses are related. The closer variants are related, the more likely it is that the immunity protecting pigs from one variant will cross protect against the challenge of another. It would seem that the tree diagrams could predict the success of a PRRS vaccine, but currently too little is known about the immune response to PRRS virus infection to use genomic sequencing in this way.
Using “DNA Finger Printing” to Monitor the PRRS Viruses Infecting a Sow Herd

J Roberts

Introduction

A sow herd is not an island. A sow herd is a dynamic population interacting with other populations both inside and outside a pyramid. Many commercial sow herds function on a weekly batch process. On these farms, each week a group of females is weaned, other groups lactate, some groups gestate, and a group is bred. Also each week, a nursery facility must be available to receive weanling pigs. Frequently, an adequate supply of replacement females is required each week to fill breeding groups. Replacement females are often produced at a multiplier farm and multiplier farms depend on a steady supply of seedstock herds. Most sow farms also depend on a steady supply of semen from one or more boar studs. Boar studs replace animals at a fast pace, relying on a pyramid multiplier or seedstock herds to maintain a steady flow of replacement boars. The system is in constant motion and the flow cannot be interrupted without devastating consequences. The system is also unforgiving, as a new PRRS virus “strain” can accompany semen or animal flow, to reaching across an entire production pyramid.

Herd immunity against one PRRS virus “strain” may not protect against a challenge from a different “strain.” We do not have the luxury of managing the PRRS viruses as though they were a single, uniform entity. That is the primary difference between pseudorabies virus control and PRRS virus control programs. The purpose of determining what specific PRRS virus “strains” are present on a sow farm is so future monitoring can identify new viral “strains” should they appear (Murtaugh and Faaberg, 2001). PRRS virus control is most likely to succeed when the PRRS virus “strains” found in a production system are similar (Mahlum, 2000; Roberts, 1999). Therefore, an initial monitor of PRRS virus “strain” variability in a production system provides information about the level of difficulty faced by a management team attempting to control PRRS. Importantly, it is almost impossible to judge the effectiveness of a control program if viruses are not characterized by “DNA finger printing”. When acute “outbreak” symptoms appear in a sow herd, finger printing using genomic sequencing answers the question, “Did the PRRS virus control program fail or was the program destined to fail due to inadequate biosecurity protocols permitting the entry of a new “strain?” These points justify the use of genomic sequencing to monitor the variability of the PRRS viruses in a swine production system.

Terminology

Different forms of the same bacteria or virus are often classed as different “strains.” A functional classification method for PRRS viruses does not exist. Only the very general differentiation of “North American” from “European strains” or “vaccine strains” from “field strains” is recognized (Andreyev et al., 1997; Mengeling et al., 1996). The meaning of the term “strain” when applied to PRRS viruses is uncertain and confusing, but this discussion will consider “strain” to indicate that the viruses have genetic sequences that are different to some degree. On the farm, a different “strain” is often taken to mean a strain to which the pigs are not already immune.

Sampling for PRRS Virus Isolates

It is best to monitor PRRS virus sequences on a regular basis. Some veterinarians only complete sequencing when sow herd symptoms are visible or nursery mortality begins to increase. However, sampling can find older and different residential PRRS virus “strains” when sow herd disease is not visibly expressed and when nursery mortality is low (Roberts, 2002). Monitoring is more valuable in the long term when a greater range of variants is included.

For most PRRS virus monitoring programs, an effort should be made to collect samples for genomic sequencing every 3 to 4 months. 15 to 25 serum samples are collected from a nursery group with the goal of recovering 2 to 5 isolates. Finding one isolate is a success when prevalence is low. Do not sample pigs randomly. Instead, select lethargic pigs with body temperatures exceeding 103° F (39.4° C). Nursery groups often display PRRS disease symptoms between 6
and 12 weeks of age (Sanford and Desrosiers, 2001).

The recovery of PRRS virus isolates in offspring can be difficult when sow farms contain few infective sows. When it is critical to find at least one isolate, it has been suggested to sample 90 animals to include 6 weekly nursery and finishing pig groups from 8 to 13 weeks of age (Greg Stevenson, personal communication). The serum samples are split, storing one set frozen at minus 80°C and the other set is screened using the PRRS ELISA (HerdChek® PRRS ELISA, IDEXX Laboratories Inc., Westbrook, Maine). The ELISA screen identifies the age group that is experiencing detectable seroconversion and the frozen sera from the group one week younger is used to isolate virus. The seroconverting group and the group that is two weeks younger are the next best candidates.

Sample Collection, Handling, and Storage

Keep samples chilled to maximize the yield of isolates. In the field, hold collected samples in a cooler of ice during sampling and refrigerate samples after collection. Separate the serum before samples are 48 hours old. Avoid warming the serum by limiting the time that samples set at room temperature during clot separation. Send serum samples on ice by overnight delivery to the laboratory. Do not freeze samples or use dry ice, as the freeze-thaw cycle denatures viral RNA (Kurt Rossow, personal communication).

Sequencing of PRRS Viruses

Several steps are necessary to identify the sequence of different nucleotide “links” comprising the “genetic chain” of a PRRS virus variant. First, a small portion of the PRRS viral RNA strand is converted into a massive quantity of DNA particles using an enzymatic reaction known as the polymerase chain reaction (PCR). The specific amplified area of the viral “genetic chain” is known as open reading frame 5 (ORF 5). ORF 5 is most often used to differentiate PRRS virus “strains” as it is more subject to mutational change and often dissimilar when comparisons are made between variants (Dea et al., 2000). The DNA replicates are purified using gel electrophoresis in the second step. The last step is to remove the DNA product from the gel and put it into an automated sequencing device. The sequencer determines the sequential identity of each nucleotide base comprising the DNA. There are four types of nucleotide bases: adenine (A), guanine (G), cytosine (C), and thymine (T). The sequencer reports its findings as a digital color-coded sequential graphic of the A, G, C, and T bases. The digital file is known as a chromatogram (McCarthy, 1998). Sequence chromatograms are the basic elements that define PRRS virus “strains.”

Comparing Two Sequences

When a PRRS variant is isolated from a herd, its genomic sequence must be compared to previous isolates to determine if the new isolate is the same or different. Computer programs are frequently used to compare PRRS virus sequences. The simplest comparison evaluates the nucleotide located at each specific “link” position on two sequences and quantifies the percentage of positions occupied by identical nucleotides (Murtaugh and Faaberg, 2001). For example, two sequences are 98 percent homologous when the order and position of nucleotide bases is 98 percent identical and diverge at only 2 percent of the sites.

Strain relatedness or divergence can be confusing. Many parts of the PRRS virus genome are highly stable and are identical even on different viral “strains.” (Murtaugh and Faaberg, 2001). Therefore, the quantity of divergence may depend on the viral segments included in a comparison. The amount of divergence between two “strains” will be different if comparisons include ORF 5 with another 400 bases rather than the less conserved ORF 5 alone. Divergence estimates do not provide information concerning virulence. The capability of immunity generated against one “strain” to cross protect against another is associated with divergence. However, many other factors are involved and clear predictions of cross protection are not possible (Murtaugh and Faaberg, 2001).

Defining “strains” by the degree of divergence is subjective. Some imprecise guidelines have been suggested for ORF 5 sequences (Collins, 1998). A homology of 99 to 100 percent is evidence that two sequences are identical and ≤94 percent homology indicates that genomic differences are great. This leaves a rather broad undetermined range of 94 to 99 percent. A less confident, but
more concise guideline suggests that similar sequences are ≥97 percent homologous and dissimilar sequences are <97 percent homologous. Despite limitations, homology values often provide reasonable benchmarks to differentiate resident PRRS virus sequences from foreign introductions. Most foreign PRRS viral introductions often have ORF 5 sequence that are >6 percent different from resident PRRS virus variants.

Comparing Many Sequences

Quantifying divergence has limited use, since only two sequences can be compared at once. Analyses of the isolates recovered from multiple sites over time must compare many sequence differences between herds, within herds, and across time (Murtaugh and Faaberg, 2001). Phylogenetic tree diagrams can compare multiple sequences at the same time (Murtaugh and Faaberg, 2001). Phylogenetic trees use all possible combinations of divergence to build valid family tree diagrams depicting the relatedness of the isolated variant sequences.

A phylogenetic tree shows the ancestral family lineage of sequences. Interpretation is based on the length of the branches. Short branches connect similar sequences. Many short branches connect clusters of isolates that are similar. Differing clusters are connected by long branches (Murtaugh and Faaberg, 2001). The range of divergence within clusters gives a sense of definition to a “strain.” For example, it is not unusual to identify a cluster representing a year of isolation with every sequence reporting homology values exceeding 97 percent. Vaccine “strains” can also be entered into the trees to show their relatedness to field variants.

Cluster identification makes it easier to identify associations that exist between PRRS viral “strains” and herd changes over time. The interpretation of a tree diagram requires knowledge of production history, health history, management, and replacement animal flow. The process is aided when isolates can be identified on the tree by herd location and date they were found (Roberts, 2001).

When the same clusters exist in the same herd flow across time, it indicates that management has avoided the entry of “foreign strains.” When a new cluster appears on a tree comparing at least one year of isolates and the finding is associated with high mortality, it suggests the introduction of a foreign variant. Increased “strain” variation due to foreign sow herd entries is associated with greater mortality in the offspring (Roberts, 1999). The prognosis for PRRS virus control is not favorable when many dissimilar viral clusters are identified in a pyramid. It is worse when individual sow herds display several dissimilar clusters in less than two years.

It is possible for two very different “strains” to reside simultaneously in the same sow population (Dee et al., 2001). When a foreign “strain” enters a sow herd, it competes with existing resident “strains.” The foreign “strain” has an advantage when it is unrecognized by herd immunity. In this situation, all animals are susceptible. The foreign variants will eventually express a sweeping clinical manifestation that appears to competitively exclude old resident variants in the short term. Often, the old variants are redetected after a year or two as herd immunity brings the foreign introduction under control (Roberts, 2002).

Standard modified live virus vaccine sequences should always be included in tree diagrams. They serve as reference sequences to enable the identification of vaccine variants that may be isolated. Sometimes, vaccine variants are the only isolates found. Vaccine strains are “foreign strains” when first introduced into a herd. Vaccine strains also mutate like field strains once introduced and continue to spread through susceptible animals (Murtaugh and Faaberg, 2001; Torrison et al., 1996). Once a population establishes immunity to a vaccine strain, it is a “resident strain” that competes with other “resident field strains.” The true vaccine strain may be competitively excluded over time, as vaccine strains are less able to replicate in pigs than field strains (Domingo et al., 1996). Variant viruses evolving from the vaccine strain are more likely to replicate effectively in pigs and survive long term in the herd (Domingo et al., 1996). However, the presence of modified live virus vaccine strains in a tree diagram requires an interpretive decision. It has been proposed that vaccine strains can cause clinical episodes (Botner et al., 1997). If other field isolates are found, a determination of whether vaccine-like strains are associated with clinical disease is not possible. The association can be assumed if the history includes acute disease with detection of vaccine-like strains isolated by
PCR directly from the serum of many clinical pigs in the absence of field strains. This is usually not the situation.

Summary

Proper sample care is more critical when isolating PRRS viruses than the care afforded samples taken for common serologic blood tests. Samples must always be chilled. PRRS virus sequencing is available from several diagnostic laboratories. Sequence interpretation can be done by comparing the divergence of two sequences at a time. However, interpretation is much better using phylogenetic tree diagram to compare many sequences at once. Free software is available to build phylogenetic tree diagrams from sequence collections. Anyone attempting PRRS virus control within a production system will find it worthwhile to use phylogenetic trees. Interpretation of diagrams is difficult for disconnected participants. Usually sound interpretations are based on the collaboration of managers possessing knowledge of production and health history with individuals who have experience applying tree diagrams.

References


