Interpretation of PRRS tests and Implications for Stud Management

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Introduction

For boar stud managers and their veterinarians, nothing strikes more fear than the thought or reality of a PRRS positive test result. After hearing the horror stories from customers, or maybe having seen an outbreak first hand, there is no close second when it comes to a disease that takes its toll biologically, financially, and mentally. Having a better understanding of what the tests are, what their limitations are, and how we interpret the results won’t alleviate all of the fears, but will help prepare one to logically think through the results and understanding why we are doing the testing in the first place.

Implications of infection sow farms

One of the most frustrating issues with a PRRS virus infection to a sow farm is that the results are unpredictable. Some farms may see little in regard to clinical signs. Others may have weak born piglets, off feed sows in farrowing, late term abortions, retained pigs and increased sow death loss around the time of farrowing, and high preweaning mortalities. There is a sense of helplessness in cases of severe clinical signs that ripples through the staff and production system. The latest estimates from Holtcamp and Kliebenstein in 2011 showed a cost to the US industry of $664 million dollars per year. This was an increase of $104 million dollars from a 2005 estimate\(^8\). Many published estimates and producer data come up with a rule of thumb figure of around $250 per sow loss from a new PRRS virus introduction into a sow herd. Of course this can vary widely. An interesting shift in the 2011 estimate is that about 45% of the losses from a PRRS break were attributed to the sow herd compared to 12% of the losses in the 2005 study. In the field, many farms are vaccinating grow finish pigs in hog dense areas, which may be reducing the losses in the grow finish. Field reports over the last few years are that losses in the sow farms are often more severe in regards to death loss of piglets than what we had typically seen in the past. Most of this is believed to be due to strain differences that have been circulating recently. In a nutshell, PRRS can be really bad for a sow farm, and so the fears are with merit.

Economic impact of a PRRS break in a boar stud

A 2006 paper summarized the impact of a PRRS break on a 200 boar stud\(^1\). While the losses to the boar stud are significant due to depopulation, the reality is that the financial impact downstream is about 8 times greater than it is for the boar stud. This is important to understand, that the primary purpose of testing is to prevent downstream infection of the sow herd.

Summary of losses (Reicks, Boar stud PRRS PCR testing: economics and clinical case reviews, AASV 2006)

<table>
<thead>
<tr>
<th>Number of boars</th>
<th>200</th>
</tr>
</thead>
<tbody>
<tr>
<td>Estimated loss per boar</td>
<td>$812</td>
</tr>
<tr>
<td>Total loss for boar stud</td>
<td>$162,400</td>
</tr>
<tr>
<td>Estimated loss per infected 2500 sow unit</td>
<td>$133,636</td>
</tr>
<tr>
<td>Estimated loss for nursery and finishing pigs</td>
<td>$188,090</td>
</tr>
<tr>
<td>Number of sows served by boar stud</td>
<td>35,000</td>
</tr>
<tr>
<td>Projected number of farms infected</td>
<td>25%</td>
</tr>
<tr>
<td>Estimated TOTAL loss</td>
<td>$1,288,441</td>
</tr>
</tbody>
</table>

**How should boar studs be sampled?**

Boar studs should sample blood or serum rather than semen. It is clear the virus can be detected sooner and more readily in blood than semen in the early stages of infection in a boar\(^2, 3, 4, 5, 6, 7\). The ideal sample is serum because the concentration of virus would be higher than in blood. Boars whose semen will be distributed downstream should be sampled at the time of collection to minimize the risk of distributing semen containing PRRS virus. An alternative is the blood swab method\(^7\). While ejaculating and without restraint, a needle prick is made in an ear vein. Using a polyester tipped swab, the blood is collected and the swab placed into a tube containing saline or PBS (phosphate buffered saline). Most of the universities would need a volume of 0.5 ml to be able to do an individual PCR (polymerase chain reaction) and repeat the test (in the event of a positive or suspect result). Some sensitivity is lost with this technique due to the dilution effect in the saline or PBS and also the dilution effect caused by the red blood cells. Pooling of samples can be done in pools of 3 or 5, understanding that some sensitivity is lost and the chances of missing a positive are greater. This is particularly true during early infection, when the odds are higher that only one positive would exist within the pool.

The blood swab method can be done on the same boars at each collection. In the field, the flinch rate (% of boars responding negatively to the needle prick) has matched the research setting, at around 10%\(^7\). Due to the short time frame needed to do the needle prick, the blood swab method is easier for staff when compared with drawing blood for a serum sample on an unrestrained animal.

Oral fluid sampling is another method that could be applied to sampling of boars for early detection. While the results look promising, more work needs to be done to determine the sensitivity of oral fluids compared to serum during the first week after infection.

**How often should boar studs be tested?**

There is a web based interactive model available to AASV members that can help to determine the likelihood of detecting virus in a boar stud with different sample methods that was developed by Dr. Albert Rovira.\(^9\) For example, a 200 head boar stud sampling 27 boars per week using serum and pools of 5 has a 19% chance of detecting virus in the first week and 60% by the second week after virus introduction. A 400 head boar stud sampling 50 boars per collection day, three times a week, and doing serum PCR in pools of 5 has a 49% chance of detecting virus during the first week and 87% chance by the second week after virus introduction.
The simple answer is that every boar should be blood sampled at every collection and tested individually for all strains of PRRS by PCR. Of course, this is not economically feasible due to the high cost of PCR testing. Most boar studs can afford to do statistical sampling as a means of risk management downstream. I recommend to further manage the risk by selectively sampling boars whose impact of infecting farms downstream is greater. For example:

1. All boars whose semen will be distributed to nucleus or multiplication herds should be sampled each day they are collected.
2. A statistical sample of the population collected each day should be sampled, as determined by the risk downstream farms or the stud are willing to take. The studs I work with do either a 95/10 or 95/5 sample on each collection day. For a large stud the numbers approach 30 or 45 per day, respectively.
3. Any boar that is off feed, feverish, or showing any clinical signs should be sampled immediately. Although clinical signs or fever by themselves are poor indicators of PRRS PCR status, a boar who has been infected with PRRS virus would be more likely to be showing clinical signs or fever than a boar who has not been infected with PRRS virus.

**PRRS ELISA testing**

PRRS ELISA testing is an important part of PRRS monitoring for a boar stud. There have been cases where a certain strain of the virus was not detected by the primers in the PCR test. There also is the possibility of the sample being damaged in transport and the RNA being damaged and unrecognizable by the test. A sample that gets too warm would be an example. The PRRS ELISA test also looks at a portion of the virus which does not change much from strain to strain. Rather than looking for the virus, or a portion of the virus, it is looking for antibodies. Antibodies are produced by the immune system in response to an infection. The testing of antibodies is not as sensitive to handling issues and to date, I am not aware of a strain of the virus that was not detected by the PRRS ELISA test. It does take time for the immune system to produce enough antibodies to be detected. In the case of the PRRS ELISA test, generally samples will turn positive around 9 days after exposure to virus but it does vary from animal to animal. Most people use 14 days as a reliable time frame. The purpose in running the ELISA test is to catch a strain of virus missed by PCR, or if there is handling issues. I recommend that boar studs run a portion of their samples once a week with the ELISA test. I also recommend that any boar treated or off feed for any reason get sampled immediately for PCR. Then 1-2 weeks later, the boar should be retested by PCR and also tested by ELISA at that time.

**What happens to the samples at the lab?**

Samples are typically sent to the lab the day of collection so that PCR tests can be ran the same day. For boar studs that are not within driving distance of a lab, samples are normally shipped overnight to the diagnostic lab. There are two basic steps to getting results: Extraction and PCR. For the extraction step, the goal is to isolate, purify, and concentrate the virus. During the PCR step, if there is genetic coding in the sample that matches what the primers are looking for, it is
amplified and gives a positive result. The number of amplification steps that it takes before the sample came back positive is what is reported as the “CT” value. Thus, a lower CT value means a stronger positive. If the Diagnostic Lab tells you that your sample was positive at a CT of 24, that is a strong positive. Generally CT values in the 37 or higher range are suspect results and need to be verified. If there is virus present, every amplification step doubles the amount of virus. So if there is one strand of virus, after 37 amplification steps there should be well over 1 billion nucleic acid segments to detect. You can see why the test is so sensitive.

Mutations of the virus can potentially result in false negative results. In other words, there really is virus in the sample, but the genetic coding of the virus has mutated to the point that none of the primers (readers) in the PCR test recognize the virus, and a negative result is reported. This is becoming less common as primers have been adjusted and added to the various PCR test kits, but is always still a possibility. For this reason, I always recommend the stud to do weekly PRRS ELISA testing in addition to the PCR test. Another possible issue with PCR testing is sample handling. There is a possibility that the virus is damaged in storage, for example, excessive temperatures, and that it is now not recognized by the primers. This is the reason the diagnostic labs ask you to put the sample on ice or refrigeration. Think of it as spoiling, as with spoiled meat that has been left out.

Most of the PCR tests available today are ran in a couple hours. Add a couple hours for the extraction process, and that is the typical turnaround time. Additional delays could be due to having a large number of samples to prepare. The results are normally available late afternoon or early evening. This allows enough time so that the major labs serving boar studs can rerun samples that evening if there are suspicious results. In the meantime the boar stud manager and veterinarian can plan on what to do if the sample really is positive.

Why are there a lot less false positives today than there were 5 years ago? First, the various buffers and master mixes that are used throughout the process are generally prepared through refined automated processes, whereas in the past, many of these materials were prepared in the lab by hand. The second reason is that most of the labs today only have to open the original sample once, everything else utilized a closed sample and there is no manual pipetting between steps. One can imagine that when there were more manual steps in the process, virus could easily “escape” from a positive sample and contaminate the lab. Especially with the amount of amplifications (remember 1 becomes 1 billion)!

What happens after a positive result?

Generally positive results are reported directly to the veterinarian working with the boar stud, and a decision as to what to do is needed. Normally, I recommend the samples to be re-ran individually, and to go back to the original sample so that the entire process is redone. Currently, this is the standard procedure at Iowa State University, South Dakota State University, and University of Minnesota Veterinary Diagnostic Labs11,12,13.
If the sample comes back positive, the boar stud should be closed and no more semen distributed. The semen from that production day should not be on the farm, and should not be sent out. If the semen has already been sent to sow farms, it needs to be removed from the sow farms or distribution areas. There is fair chance that there is live virus in those samples.

Normally an attempt is made to save some of the boars from getting infected. To do this, the entire population needs to be serum sampled the next morning. If not, there is little chance of getting ahead of PRRS virus. There were a number of success stories in testing the whole population right away, removing the positives, and retesting. However, there has been less success in the last 5 years as new strains have evolved and seem to spread much faster than in the past.

**Withholding of semen**

Semen should always be withheld until negative results are obtained. There is little value in testing a stud if semen could have already been used when results are obtained. If it is impossible to do this, it may be more appropriate to test weekly using ELISA and PCR tests, understanding that downstream infection is much more likely to have occurred when positives are found.

**Summary**

PCR has been an incredible tool to reduce the risk of downstream infection from a boar stud PRRS break. Knowing how to utilize this test will help protect the integrity of the boar stud and dramatically reduce the losses downstream.

**References**

1. Reicks D, Boar stud PRRS PCR testing: economics and clinical case reviews, AASV 2006