Diagnosis of porcine reproductive and respiratory syndrome

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Porcine reproductive and respiratory syndrome (PRRS), formerly called swine infertility and respiratory syndrome and mystery swine disease, is a recently described viral disease of swine that is found in many parts of the world.[1-5] PRRS is caused by an enveloped RNA virus, approximately 62 nm in diameter, which is sensitive to lipid solvents, heat, and pH levels below 5 or above 7.[6,7]

Three forms of the disease have been described:

1) The epidemic reproductive form of PRRS results in late term reproductive failure.[1,4,5,8,9] On newly infected farms, sows and gilts are often very susceptible to PRRS virus. They become viremic for approximately 2 weeks and if infected in the last trimester of gestation, the virus crosses the placenta to infect the fetuses. Pigs, in utero, develop a prolonged viremia resulting in poor viability and death. Affected sows often farrow mummified, stillborn, weak-born, and normal pigs around days 107–109 of gestation. Weak-born piglets are usually born viremic and remain viremic for 3 or more weeks. Because sows and gilts may be infected early in the last trimester and viremia in adults is transient, they may not be viremic at farrowing.

2) On endemically infected farms where reproduction has returned to pre-PRRS levels, the primary clinical problems occur in the nursery and grower pigs.[2,3] Most piglets will not be born viremic, but may contact the virus when commingled with viremic nursery or grower pigs. Mild to moderate respiratory distress with minimal coughing in nursery and grower pigs is the most common clinical sign in uncomplicated endemic PRRS. Often, the viremic pigs experience atypical manifestations of secondary bacterial diseases that do not respond to usual treatments, become unusually severe, or affect pigs at atypical ages.

Pigs usually become viremic within 3 weeks of moving into the nursery or commingling with older viremic pigs and the viremia can last up to 6 weeks postinfection. An interstitial pneumonia develops within 5 days of infection, but resolves quickly if uncomplicated. Gross lesions of slightly firm, noncollapsing lungs are nonspecific and represent an acute interstitial pneumonia that can be caused by several viruses. Occasionally, eyelid edema and enlarged lymph nodes are observed. Microscopic lesions in the uncomplicated infection are also transient. If uncomplicated, the typical lesion, which may provide a presumptive diagnosis of PRRS, is a proliferative or infiltrative interstitial pneumonia without the bronchiolar necrosis that differentiates it from the lesions of swine influenza. Since viremic pigs are often infected with secondary bacteria, lung lesions are usually complicated and cannot provide a presumptive diagnosis because the milder lung lesions of PRRS are obscured.

3) A subclinical (silent) form of PRRS has been reported in which farms may seroconvert without significant clinical signs.[10]

Many researchers are investigating the epidemiology of PRRS virus infection, characterizing the virus on a molecular basis, and trying to develop diagnostic, therapeutic, and preventive strategies. The volume of scientific information on PRRS is growing rapidly and changing constantly. Currently, a definitive diagnosis of PRRS depends on isolation of PRRS virus from clinically ill animals. Serology can demonstrate previous exposure to the virus.

Differences between laboratories

Many commercial and state diagnostic laboratories can isolate PRRS virus and demonstrate antibodies to PRRS, but interlaboratory variation is higher than desired because tests and reagents have not been standardized among laboratories.

Virus isolation results may vary among laboratories because some use primary porcine alveolar macrophages (PAMs) for virus isolation while others use continuous cell lines, such as CL 2621 or Meat Animal Research Center (MARC) cells. The efficacy of isolation varies among these cell lines, and not all North American strains will grow on each cell type.[11] The reagents and detection systems that are needed to confirm viral growth in cell cultures vary among laboratories and some systems are more sensitive than others. Monoclonal an-

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tibodies to viral subunit proteins show promise for a widely cross-reactive detection system, but are not yet commercially available.[12]

Serology results also may vary among laboratories because different types of tests and different reagents, cell lines, and virus isolates are used for serology. Most laboratories are using an indirect-fluorescent antibody test, while a few laboratories are using a serum neutralization (SN) test. ELISA tests are being developed and are currently in use in research laboratories only. Although most North American isolates appear to be cross-reactive on serology, antibodies to some PRRS virus isolates will not be detected by some serology tests. This probably explains some of the regional differences observed because samples are shipped across the United States for testing. European isolates often will not cross-react with North American isolates. Serology tests based on European isolates will result in many false negative results from North American serum samples.[13]

Factors affecting diagnostic tests for PRRS

Virus isolation

Virus isolation provides a definitive diagnosis, but has some limitations:

- If collected within a few days of farrowing, virus can sometimes be isolated from the serum of sows and gilts that have farrowed affected litters. However, sows and gilts may not be viremic at farrowing because they may be infected early in the last trimester of gestation and viremia is transient in adults (approximately 2 weeks).
- PRRS virus can often be isolated from live, weak-born piglets of affected litters, but the virus is rarely isolated from stillborn fetuses of the same litters. Fetuses often die in utero and may be dead for several days or weeks before farrowing. PRRS virus is probably rapidly inactivated in autolyzed fetal tissues at intrauterine body temperature.
- Ideally, samples should not be collected from pigs found dead because PRRS virus is sensitive to heat and is easily inactivated at room temperature. In one study, over half of the virus in lung samples was inactivated in 24 hours at 25°C. For samples collected from live or euthanatized pigs, chilling or freezing effectively preserves virus viability for shipment to a laboratory.
- Serum is an excellent sample for virus isolation because viremia can endure up to 42 days in nursery pigs and virus in serum will remain viable for the duration of shipment and at temperatures encountered in shipment. When handled properly, the virus can be readily isolated from serum, lung, and lymphoid tissues, such as spleen, tonsil, thymus, and lymph nodes.

Serology

Serology also has some limitations when used for the diagnosis of PRRS:

- IFA antibodies usually appear within 2 weeks of exposure to PRRS virus and may be short-lived, because some pigs become seronegative as soon as 3 months post exposure. ELISA antibodies appear within 3 weeks of exposure and the duration of ELISA antibodies is not known.[14]
- SN antibodies usually do not appear until after clinical signs resolve at 4–5 weeks post exposure.
- Positive serology can occur in young pigs that have not been infected with the virus because passively acquired maternal antibodies usually persist to 6–8 weeks of age, but sometimes persist up to 16 weeks of age.
- Some laboratories offer a serological screening test for PRRS virus at a single serum dilution (usually 1 : 20) that provides a positive or negative result. Other laboratories provide serological titers, which must be interpreted carefully.
- Antibody titers from a single (nonpaired) serum sample are difficult to interpret because some herds may seroconvert to PRRS virus, but have no history of clinical disease associated with PRRS.[10] Pathogenicity among PRRS isolates appears to vary widely.[15] Positive serology tests on these farms implies previous exposure to PRRS virus, but does not confirm current virus infection. Clinical disease in seropositive animals may be due to other pathogens, such as pseudorabies virus or swine influenza virus.
- A change from seronegative to seropositive using a screening test or a four-fold rise in titers between samples collected from the same animal at least 2 weeks apart indicates recent PRRS virus infection. Remember, because sows are often infected during the last trimester, they may be seropositive at farrowing and paired serology will probably not show a four-fold increase at that time.

Antigen detection in tissue

Antigen detection techniques for PRRS, such as tissue-section IFA tests, have been used with limited success by a few laboratories. Several tests are being developed that will greatly aid in the rapid testing for PRRS.

Recommendations for diagnosis

If PRRS virus isolation is desired, tissues should be immediately chilled or frozen after necropsy for shipment to a

laboratory. Sufficient coolant should be packaged with the tissues to prevent thawing and warming during shipment. If shipment is delayed and tissues are received warm, virus isolation results will be unreliable because the virus is probably inactivated.[16]

Reproductive failure cases: Virus isolation

Antemortem diagnosis in piglets

The best diagnostic specimens are from viremic, weak-born, live piglets. Serum can be used for the antemortem diagnosis of PRRS virus in viremic baby pigs.[16] Pigs from *at least three* litters should be sampled. Equal amounts of serum from several pigs in each affected litter can be pooled to reduce the number of submitted samples. Pooling serum samples does not significantly reduce the efficiency of isolation. The more animals that are bled, the greater the chances of detecting the virus.

Postmortem diagnosis in piglets

Lung, spleen, and serum contain high titers of PRRS virus and should be collected from weak-born, recently euthanatized, nonautolyzed piglets for virus isolation.[16] In addition to fresh tissues for isolation, formalin-fixed tissues are recommended for diagnostic histopathology.

Do not use stillborn or dead piglets for PRRS virus isolation because intrauterine temperatures will inactivate the virus and preclude isolation.[16] However, stillborn piglets should be used to rule out other causes of reproductive failure, such as pseudorabies.

Antemortem diagnosis in sows and gilts

Although less reliable than tissues or serum from weak baby pigs, serum from sows and gilts can be used for the antemortem diagnosis of PRRS. For economy, serum from several affected sows can be pooled for a single virus isolation test. For best results:

- collect serum from *at least five* clinically affected sows and gilts within 5 days after farrowing; or
- collect serum from *at least five* prefarrowing sows and gilts (85–100 days gestation). If possible, select those animals that were known to have been lethargic or anorexic.

Boars

Boars are only transiently viremic, but antemortem diagnosis is limited to isolation from serum and serology. PRRS virus is present in semen for unknown lengths of time following infection.[17] Semen is toxic to the cell lines used for virus isolation, so isolation from semen is not currently a useful diagnostic technique. More research is needed before recommendations can be made to ensure a PRRS-free semen supply.

Reproductive failure cases: Serology

Herds known to be seronegative

On farms with reproductive failure that are known to be seronegative to PRRS virus, bleed *at least* 10 sows that have farrowed affected litters. Sows with mummified fetuses are more likely to have been infected long enough to allow seroconversion. Since the herd has been previously documented as serologically negative before the onset of reproductive failure, seropositive results indicate recent exposure to PRRS virus and strongly suggest PRRS as the cause of reproductive disease.

Herds of unknown serological status.

On farms where the serological status to PRRS virus is not known, positive IFA serology from single (nonpaired) samples in breeding stock indicates past exposure, probably within 1 year. Because detectable viremia usually lasts 2 weeks or less in older animals, breeding animals may be seropositive, but negative for the virus by isolation.

Single (nonpaired) serological titers, using currently available tests, do not necessarily correlate with the onset and progression of clinical disease.[18] If a four-fold increase in titers can be demonstrated using paired serology, an active PRRS virus infection should be suspected. However, sows are usually infected long before farrowing and serology titers at the time of farrowing are usually stable.

Serology must be combined with positive virus isolation to confirm an active infection in breeding animals. To date, very little is known about the long-term shedding and latency of PRRS virus in seropositive swine. The risk of spread to a seropositive, nonviremic sow or gilt is unknown.

Endemic PRRS cases: Virus isolation

Nursery and grower pigs

PRRS virus can be isolated from the serum of live nursery-age pigs for approximately 4–7 weeks following natural or experimental inoculation.[3,9] This unusually long period of viremia improves the chances of selecting a viremic pig from an endemically infected nursery. Bleed 10 clinically ill pigs that have been in the nursery for at least 2 weeks and 10 pigs that have been in the nursery for 5 weeks. Several samples in each age group can be pooled to reduce laboratory costs.

From recently euthanatized nursery pigs, collect lung, spleen, and serum and quickly chill or freeze them. Additional tissues may be fixed in 10% formalin for diagnostic histopathology. For best results, select clinically ill pigs, preferably with respiratory difficulty, that have been in the nursery or have commingled with older pigs for at least 2 weeks. Because the virus is quickly inactivated in dead pigs at nursery-room temperatures, do not select pigs that have died naturally.[16]

For antemortem and postmortem diagnosis in grower pigs, the samples are the same as for nursery pigs. However, on endemically infected farms, grower pigs may be at the end of the viremic stage and isolation would then be less reliable.[3] Clinically affected pigs with respiratory distress should be selected.

In endemically infected herds, sows and gilts may not be viremic (unless there is concurrent reproductive failure). Also, only a small percentage of baby pigs may be viremic.[2,3] Sampling breeding stock and baby pigs on endemically infected farms without reproductive failure is not very efficient for confirming PRRS by virus isolation.

Endemic PRRS cases: Nursery-pig serology

A definitive diagnosis of PRRS requires virus isolation. Nursery-pig serology can be an adjunct to diagnosis.

The goal of nursery-pig serology is to detect an increasing seroprevalence over time. This may be confounded by passively acquired maternal antibody that usually lasts for 6–8 weeks but can last up to 16 weeks of age. Eartag and bleed 10–20 pigs at 3–4 weeks of age and bleed the same pigs at 7–8 weeks of age. A greater percentage of seropositive pigs at the second bleeding would indicate that virus is actively spreading among pigs on the farm.[19]

Endemic PRRS cases: Herd serology

It may be desirable to know whether a swine herd is seronegative for PRRS virus. The most reliable way to determine whether a herd is seronegative for any given disease is to bleed and test every animal. Obviously, this is impractical. In reality, practical herd serology depends on the prevalence of disease on the farm. The serostatus of a herd is predicted by testing a representative group of animals in the herd. The number and ages of animals to be tested is determined by the assumed seroprevalence in each age group. Seroprevalence to PRRS virus in the breeding herd can vary widely depending on how long the herd has been infected.

Herds with a recent history of reproductive failure have often been infected less than 1 year and tend to have a high seroprevalence (approximately 50%) in breeding animals.[20] To detect seropositive animals with 95% confidence from a group of 30–10,000 animals (assume 50% seroprevalence in the breeding animals) you would need to bleed *at least seven* sows or gilts. We recommend taking 10–20 samples.

In endemically infected herds, which have probably been infected more than 1 year, the seroprevalence is usually low in the breeding herd (20% or less) and high in the finishing hogs (50% or greater).[3,20-22] In a breeding herd of 100 animals, to detect seropositive animals with 95% confidence (assume 10% seroprevalence in breeders and 50% seroprevalence in finishers) you would need to bleed *at least 25* breeders and seven finishing pigs.[3] Because passive antibody is usually gone at 6–8 weeks of age and is always gone by 16 weeks of age, seropositive finishing pigs that are more than 16 weeks old were probably infected with the virus within the last 4 months.[19]

Remember, herd serology only demonstrates previous exposure to the virus and does not provide a definitive diagnosis of PRRS virus as a cause for the clinical problems on the farm.

Because of the industry-wide interest in PRRS and the many researchers investigating this disease, our knowledge of PRRS will grow rapidly. These recommendations for diagnosis may become quickly outdated as more is learned about PRRS.

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