

Monitoring for porcine reproductive and respiratory syndrome virus (PRRSV) in the boar stud

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Why should you monitor for PRRSV in the boar stud?

Soon after the discovery of PRRSV, some field studies described possible transmission of PRRSV to sows via semen.^{1,2} This was later substantiated by several experimental studies which demonstrated seroconversion of sows and (or) the presence of PRRSV in tissues of sows inseminated with PRRSV-contaminated semen.²⁻⁴ Even though one study using extended semen demonstrated that PRRSV transmission via semen may not always occur,⁵ predicting when this might or might not happen is difficult. A recent study demonstrated a dose-dependent effect, ie, when given extended semen that had been inoculated with low concentrations of PRRSV, sows did not show evidence of seroconversion.⁶ However, the actual titer of PRRSV in semen is not known and may differ between boars and within the same boar over time. Even though semen may not always transmit PRRSV, transmission by pig-to-pig contact and aerosols have been described. Therefore, PRRSV-infected boars may still transmit the virus by these routes as well as in semen.

Since many production units are currently utilizing PRRSV test-and-removal programs⁷ and stocking with PRRSV-negative pigs to obtain a PRRSV-free status, it is essential that the boar stud supply PRRSV-free semen and boars to these facilities. With significantly high economic costs from this disease, it is essential to control

the transfer of PRRSV via semen and through direct boar contact.

What clinical signs are observed in PRRSV-infected boars?

Typically, few clinical signs are observed in boars infected with PRRSV.^{1,8-10} Signs that do occur are usually mild and transient. Depression and anorexia has been noted for 3 days post challenge,⁵ as well as fever during the first 2 weeks after inoculation.^{2,9,11} Mild sneezing and coughing of 1 day's duration have also been reported.⁹ There are also some field and experimental reports of semen quality changes after infection with PRRSV, but these are not always observed. Semen volume may be decreased after PRRSV inoculation,^{2,12} while motility, morphology, concentration, and color may be within normal limits.^{2,5} Other studies have demonstrated decreased sperm motility in PRRSV-infected boars, with abnormal sperm morphology consisting of proximal and distal cytoplasmic droplets.⁸

What tests should be used to detect PRRSV in the boar stud?

The polymerase chain reaction (PCR) has been used extensively to detect PRRSV in boar semen.¹¹⁻²⁰ This technique amplifies the PRRSV genome exponentially so that even very small amounts of virus can be identified. Traditional diagnostic techniques such as virus isolation (VI) are not typically performed on boar semen, since

the semen may be toxic to the cells on which the virus is grown. A "swine bioassay" has also been utilized to detect PRRSV in semen, whereby 13 to 15 ml of semen is injected intraperitoneally (IP) into 4- to 8-week-old, PRRSV-naïve (seronegative) pigs. If the semen contains PRRSV, these piglets will become seropositive, indicating that the semen injected IP contains replicating, infectious PRRSV.⁹ Although PCR testing detects PRRSV genome, and not necessarily replicating virus, a comparison of PCR and the "swine bioassay" revealed there was a 94% correlation between the two techniques.¹⁴ Therefore, if the PCR test is positive for PRRSV, there is a significant likelihood that it represents the presence of infectious PRRSV.

In time course studies, boars are viremic and shed PRRSV in semen approximately 1 to 2 weeks prior to the development of PRRSV antibodies.¹¹ Infected, seronegative boars in the acute stages of infection may be detected by VI and (or) PCR testing of serum samples and PCR testing of semen. By 1 to 2 weeks after infection, most boars have seroconverted to PRRSV. Therefore, in the seronegative boar stud it should be fairly easy to detect infected boars. In some seronegative studs, weekly semen testing by PCR and monthly serologic testing of serum is a useful method of routine monitoring for PRRSV.²¹ Others have maintained seronegative boar studs by using two quarantine periods, biosecurity protocols, and serological monitoring.¹⁹

Can persistently infected boars be present in the boar stud and, if so, how do you detect them?

Persistence of PRRSV has been demonstrated in tissues from boars for 101 days post exposure, and shedding of PRRSV in semen has continued for 92 days post exposure.¹¹ However, there is significant variability in the duration of shedding between individual boars. This was demonstrated in

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This article has not been peer reviewed. This paper will attempt to answer some common questions and describe monitoring for PRRSV in the boar stud to provide useful information in preventing PRRSV transmission.

This article is available online at <http://www.aasv.org/shap.html>.

Christopher-Hennings J. Monitoring for porcine reproductive and respiratory syndrome virus (PRRSV) in the boar stud. *J Swine Health Prod.* 2001;9(4):186-188.

a recent study where seven boars were given the same PRRSV inoculum and dose at the same time. Individual boars shed PRRSV in semen for a duration of 4, 11 (two boars), 28, 32, 46, and 70 days.¹⁹ In this study, serum, semen, and peripheral blood mononuclear cells (PBMC or “buffy coat”) were collected twice weekly from each boar. After 2 to 3 weeks of negative tests, the boars were euthanized and tissues were evaluated for PRRSV by VI and PCR. In three of seven boars, the tonsil was positive for PRRSV by VI. This would indicate that even though a series of antemortem blood and semen samples are PRRSV negative for 2 to 3 weeks, infectious PRRSV may still be present in tissues, specifically the tonsil. Therefore, using blood and semen samples for testing should not solely be relied on to detect persistently infected boars. If a boar is seropositive for PRRS, there is potential for this boar to be harboring infectious PRRS virus. Therefore, there may be advantages to maintaining a seronegative boar stud to avoid producing persistently infected boars that are difficult to detect.

There is some indication, however, that boars may “clear” PRRSV after infection. This was suggested by a study in which an experimentally inoculated boar was PRRSV negative by VI and PCR testing of serum, semen, PBMCs, and 22 tissues and secretions, 88 days post inoculation.¹⁹ However, the time required for clearance in most boars is unknown, and it may differ among boars, with the possibility that some boars may remain persistently infected. Several factors may also contribute to persistence or clearance, such as concurrent infections and (or) the development of cell-mediated immunity. A recent study with boars revealed that gamma interferon levels, which have the potential to eliminate virus, develop slowly.²² By 318 days post infection, these boars appeared to exhibit a strong, virus-specific immune response measurable by the frequency of T cells capable of producing gamma interferon. Further study is needed to determine whether this response is directly related to viral clearance in the boar, and, if so, how it may be stimulated earlier in the infection.

How can you prevent PRRSV infection in the boar stud?

Effect of vaccination on the boar

One method of control is vaccination of boars with modified-live (MLV) and killed vaccines.^{12,16,23,24} However, the best dos-

age regime and route of vaccination to prevent PRRSV shedding in semen has not been determined and would be an extra-label use of PRRS vaccine in adult boars. Experimental studies have demonstrated that in most boars, a MLV vaccine either eliminated or decreased shedding of wild-type PRRSV in semen.^{12,16,24} Studies have demonstrated that the MLV vaccine can be shed in semen of PRRSV-naïve boars, usually within 1 to 3 weeks after IM vaccination.^{12,16,24} Variability in the duration of vaccine shedding does occur, as one boar was found to shed the vaccine for 39 days after vaccination.¹⁶ Since the vaccine causes seroconversion, it is difficult to determine, by serology alone, which boars are vaccinated and which are infected with wild-type virus. IDEXX HerdCheck® PRRS ELISA S:P values as high as 2.07 or 2.81 have been reported in vaccinated adult boars, so the S:P may be similar whether the boar is vaccinated or has been exposed to wild-type PRRSV.¹⁶ In addition, some semen quality effects may be observed when boars are vaccinated,¹⁶ and vaccination may not protect against possible semen quality effects associated with wild-type PRRSV challenge.^{12,16}

The MLV vaccine, when used according to label instructions, has proven effective in preventing clinical signs of disease, viremia, and leukopenia associated with the respiratory form of PRRSV. Since clinical signs of PRRSV in boars are uncommon and viremia is relatively short, even with wild-type PRRSV virus, the primary reason for boar vaccination is to prevent PRRSV shedding in semen. However, we have demonstrated in boars vaccinated with MLV vaccine that shedding of the vaccine and wild-type virus can occur.^{12,16} Two inactivated vaccines have also been evaluated in boars. One preliminary study indicated the possibility of reduction, but not elimination, of seminal shedding of PRRSV with use of the inactivated vaccine.²³ In another study, boars vaccinated with the inactivated vaccine and challenged with wild-type virus became viremic, and shedding of virus in semen was observed.²⁴ Therefore, there is no assurance that using inactivated vaccines will prevent PRRSV shedding or viremia when boars are exposed to a wild-type virus. Two studies have demonstrated an absence of viremia and shedding of virus when seropositive boars were challenged with homologous virus.^{11,24} However, it would be

difficult to ensure challenge with homologous virus in a field situation. For a vaccine to be useful in boars, it should induce a “sterilizing” immunity that will prevent viremia and PRRSV shedding in semen. However, the optimum vaccine dose, route of administration, and duration of immunity that would protect the reproductive tract and semen of the boar from PRRSV infection is unknown.

Quarantine and Biosecurity

Quarantine periods for herd additions are essential in maintaining a PRRSV-free boar stud. During these periods, serological monitoring, most commonly with the IDEXX HerdCheck® ELISA, can be used to confirm seronegative status. This test is useful in determining PRRSV herd status, but there have been suggestions that a very small percentage of animals, from previously seronegative herds with no known herd introductions or PRRSV exposures, may show a positive reaction on this test.²⁵ Re-testing or using other serological tests may confirm the status of these individual boars. However, even if additional tests demonstrate a negative PRRSV-antibody status, the boar should still be treated as suspect. Other serologic tests may not be as sensitive as the ELISA, interpretation of results can be highly subjective, high background may be a problem with some samples, and antigenic variation of PRRSV isolates may influence the results.²⁶ Therefore, ELISA-seropositive boars should be separated and placed with a sentinel animal, or serum and semen may be submitted for detection of PRRS virus, or both. If seronegative boars are housed near the seropositive boars, they should also be tested for PRRSV antigen to rule out the possibility of recent virus transmission.

In PRRS-seropositive boar studs, the duration of quarantine may have an effect on the number of boars that continue to shed PRRSV in semen. In one field study in a seropositive boar stud, 12 of 131 boars (9.1%) were positive for PRRSV in semen by PCR when quarantine was 25 days or greater, and only seven of 440 boars (1.6%) were positive when quarantine was 45 days or greater.²⁷ Therefore, in seropositive herds, there appears to be some benefit in longer quarantine periods to prevent PRRSV transmission via semen.

Summary

Transmission of PRRSV via semen and boars may occur. Since clinical signs and semen abnormalities may not always be observed, testing to detect PRRSV antibodies or antigen is necessary. Diagnostically, there appear to be advantages in producing a PRRSV-free boar stud rather than trying to determine whether boars already exposed to PRRSV are shedding the virus. During the first 2 weeks after exposure to PRRSV, the virus can be readily detected by PCR or VI testing of serum or semen; by the second week, antibodies to PRRSV are detectable. In contrast, it can be difficult to detect persistently infected boars in a PRRS-seropositive boar stud (previously exposed to PRRSV). Shedding of PRRSV in semen may be intermittent and quite variable in duration between boars; viremia is usually absent; and boars may still harbor infectious virus in tissues even after serum, PBMC, and semen have been tested and found free of PRRSV. Whether the boar stud is PRRSV seropositive or seronegative, excellent biosecurity, as well as quarantine periods and diagnostic testing are needed to prevent the virus from entering or leaving the boar stud.

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