Porcine reproductive and respiratory syndrome diagnostics in the breeding herd: Back to the basics

Laura Batista, DVM, PhD

Due to the negative economic impact that porcine reproductive and respiratory syndrome (PRRS) has on the swine industry,[1,2] diagnostic techniques for this disease have gradually developed. Therefore, it is critical that veterinarians understand the advantages and disadvantages of the currently available technology associated with diagnostic assays for PRRS virus (PRRSV). Veterinarians have changed their strategies from individual management and diagnosis to use of preventive medicine, focusing on reduction of disease transmission, prevention of spread, implementation of effective biosecurity protocols, and application of control programs, eradication procedures, or both, that reduce the economic impact of PRRS. Adequate use and interpretation of the available diagnostic tests for PRRSV can provide very useful information. In addition, it is important to have a clear goal for the strategy to be implemented in each production system. These two premises will allow veterinarians to make an informed decision for implementation of a successful PRRSV control or eradication strategy.

Frequently, we encounter colleagues and producers frustrated by the lack of helpful information derived from the analysis of results that a diagnostic strategy provided. Added to this frustration are the costs incurred in PRRSV diagnosis. PRRS virus diagnosis, control, eradication, or all three present different scenarios. For example, is the virus present in semen, in a load of animals, in a production system? Is the recent outbreak due to lateral introduction of PRRSV? Is the herd stable so that an eradication protocol can be established? Different scenarios require different diagnostic strategies. This article will deal with the most common situations veterinarians encounter in the field while trying to control or eradicate PRRSV in the breeding herd.

Monitoring replacement animals

To assure that purchased replacement animals are PRRSV-naive, reverse transcriptase-polymerase chain reaction (RT-PCR) and enzyme-linked immunosorbent assay (ELISA) should be used in combination. In North America, HerdChek PRRS 2XR (Idexx Laboratories, Westbrook, Maine) is the test of choice.[3] Conversely, in Europe and some Latin American countries, the CIVTEST PRRS (Laboratorios Hipra SA, Girona, Spain) is also available.[4] The main difference between these two ELISAs is that the Idexx ELISA uses nucleocapsid proteins as antigen, and the CIVTEST includes the entire virus.[5] However, this difference is not important, as antibodies against PRRSV that are used diagnostically to detect infected animals are directed against the nucleocapsid (N) protein encoded by open reading frame 7. These are non-neutralizing antibodies.[6] The possibility of a false-positive result due to the gap of 10 to 14 days existing between the animal's initial contact with PRRSV and the production of non-neutralizing antibodies is reduced with the use of reverse transcriptase-nested PCR (RT-nested PCR).[7] Use of RT-nested PCR covers the possibility of a recent infection in the origin barn, contamination during transport, or both. It is important to remember that when RT-nested PCR is used in combination with real-time RT-PCR, sensitivity is higher than with RT-PCR alone.[8] On the other hand, with use of RT-nested PCR, there is a risk of laboratory contamination of a naive sample, a risk that can be decreased by using real-time RT-PCR.[9]

Semen monitoring

Determining the quantity of PRRSV in semen is difficult, because semen contains materials that are toxic to cell cultures.[10] In order to avoid introduction of PRRSV into the herd through semen, real-time RT-PCR is the best option to assure that semen is free of PRRSV. This assay also reduces processing time, therefore allowing delivery of semen the same day it was collected and processed.[11]

Monitoring breeding herd stability

Breeding herd stability assures that PRRSV is not circulating in gilts, sows, and boars housed in the breeding herd.[12] In general, the purpose of this screening is to assess the effectiveness of implemented control measures (eg, gilt acclimatization,[13] mass vaccination,[14] and planned exposure,[15] or herd closure following an outbreak[16]). Three screening techniques should be used to ensure stability of the breeding herd. First and most important, to ensure that there is no active circulation of PRRSV, at least one piglet from each weaned litter must be sampled at 2-week intervals at least three or four times. Groups of five serum samples from these piglets can be pooled every sampled week and submitted for RT-nested PCR or real-time RT-PCR. If all pools are negative, the second step is to introduce PRRSV-naive sentinel animals into the breeding herd. As a general practice, 5- to 6-month-old, PRRSV-naive barrows or rejected gilts are used for this purpose. The sentinels should have nose-to-nose contact with all gilts, sows, and boars in the herd. Blood samples should be collected biweekly from all sentinels, and serum should be tested by PRRSV ELISA. The last serum sample should also be tested by RT-nested

Faculté de Médicine Veterinarire, 3200 rue Sicotte, CP 5000, St-Hycinthe, Québec, Canada J2S 7C6;Tel: 450-773-8521, ext 8674; Fax: 450-778-8120; E-mail: laura.batista@umontreal.ca.

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

Batista L. Porcine reproductive and respiratory syndrome diagnostics in the breeding herd: Back to the basics. *J Swine Health Prod.* 2005;13(2):96–98.

PCR or real-time RT-PCR. Finally, tonsils and lymph nodes from culled gilts, sows, or both should be collected, and a homogenate of these tissues should be sent for testing by RT-nested PCR or real-time RT-PCR. If all tests are PRRSV-negative, breeding herd stability has been achieved.[17]

At this point, veterinarians are faced with two options: either continue control through methods including gilt acclimatization, mass vaccination, or both; or proceed to eradicate PRRSV. The final decision will depend on the geographical location of the farm and the probability of lateral introductions (eg, transport,[18] fomites,[19] and nonporcine vectors[20]).

PRRSV sequencing

Precise identification of PRRSV isolates by determination of nucleotide sequence of viral genetic material can be a valuable tool for understanding the success or failure of PRRSV control and elimination procedures, possible sources of introduction into naive herds, and area spread.[21] Also, determination of genetic types of PRRSV isolates allows a fuller understanding of the epidemiology of the disease in a production system. Genomic sequencing of the virus predicts with some accuracy the relatedness between two strains of PRRSV, as well as how closely related they are to vaccine strains.[22] Presently, however, sequence information cannot be used to make inferences about biological properties of PRRSV. Therefore, genomic sequencing will not predict the likelihood of a vaccine successfully preventing the disease, the virulence of a strain, clinical signs of the disease, growth characteristics of the virus, or elicited immune response.[23] Restriction fragment length polymorphism analysis has also been used to discriminate vaccine and field isolates of PRRSV, but the method has not been adopted for genetic studies.[24,25] These two tests can be used only as epidemiological tools. Other inferences should be considered over-conclusions that may produce incorrect decisions.

Dealing with false-positives

Due to the high sensitivity of some serological tests for PRRSV, false-positives are encountered. There is no commercially available serologic test that can differentiate between a pig that has been infected with a field strain of PRRSV and a pig that has been vaccinated and has developed a robust immunity. Also, none of these tests can distinguish whether the animal is viremic, capable of shedding, persistently infected, or has developed neutralizing antibodies against the viral infection.[9] Therefore, serological test results should be interpreted only on a population basis, ie, indicating whether or not the population has been in contact with PRRSV. In order to confirm that the result is a true positive, request an alternative test, preferably one that detects the PRRSV, since serological tests detect antibodies produced against the virus.[9]

Deciding the correct sample size

In general, when testing a population that is expected to be PRRSV-negative, the goal is detection of a recent infection. When our aim is eradication, the goal is identification and elimination of remaining PRRSV-positive animals. In some cases, testing the entire population may be indicated or justified (eg, animals in an isolation unit being introduced into a negative population). However, this is usually cost prohibitive, and the risk of missing a new infection is relatively low even when the entire population is not sampled, particularly when a combination of two or more highly sensitive and specific tests are used (eg, RT-nested PCR, real-time RT-PCR, and Idexx ELISA). In general, it is sensible to use the guideline of 23, 30, and 45 samples with a ≥ 10% prevalence and confidence levels of 90%, 95%, and 99%, respectively.[26]

Pooling

Pooling of samples for virus detection is a common field practice; however, there is limited knowledge concerning what kind of samples and how many samples should be pooled for the best diagnostic results. Pooling has been perceived as a solution to the problem of economic constraints and the need to sample many animals, particularly in negative or low-prevalence populations. Samples are combined or pooled and tested as a single sample. Currently, serum and semen samples are pooled only for RT-PCR diagnosis. Caution should be exercised, since validation of this technique should be performed both for RT-PCR and other diagnostic tests. It is clear that viral titer and number of negative samples play important roles in dilution of a positive sample. Our group is currently doing research in this area and very interesting data will soon be available. Meanwhile, practitioners should contact their diagnostic laboratory of choice and discuss sample pooling in order to avoid generating false-negative results which could prove highly costly, particularly at the end of a control or eradication program.

Alternate tests

There are many other available tests which can be used as alternatives or corroboration diagnostic options for PRRSV. However, some are cumbersome and others have low sensitivity, and they may not be offered on a commercial basis. These tests include serum virus neutralization, indirect fluorescent antibody, fluorescent focal neutralization, immunohistochemistry staining, fluorescent antibody staining, and virus isolation. We must also remember that while positive results on any of these tests indicate either the presence of antibodies against PRRSV or that PRRSV is present in the sample, a negative test does not necessarily indicate that the pig is free of PRRSV or has not previously been in contact with the virus.

Conclusion

Control and eradication of PRRSV depends on correct sample-testing selection, diagnostics assessment, sample handling, and the sensitivity of the test used: all interact to provide a reliable result. A complete herd history, reliable production records, assessment of clinical signs, and a combination of the proper diagnostic tests, adequate interpretation of results, and the practitioner's experience are the best resources a veterinarian can use to successfully manage PRRS and its economic impact on swine production.

References

1. Dee SA, Joo HS, Polson DD, Marsh WE. Evaluation of the effects of nursery depopulation on the profitability of 34 farms. *Vet Rec*. 1997;140:498–500.

*2. Polson DD, Marsh WE, Dial GD. Financial implications of mystery swine disease (MSD). *Proc Mystery Swine Disease Committee Meeting, Livestock Conservation Institute.* Denver, Colorado. 1990:8–28.

*3. Snyder ML, Mermer B, Anderson PR, Wensvoort G, Hill HT. Evaluative data for an immunodiagnostic ELISA for PRRS. *Proc 2nd Int Symp PRRS*. 1995:15.

*4. Sitjà M, Medrano A, Busquets N, Caballero JP, Artigas C. Presencia de glicoproteínas del virus del PRRS en el antígeno de un ELISA Indirecto. Utilidad y Significado [Presence of glycoproteins of PRRS virus in the antigen of an indirect ELISA. Use and significance]. *VII Symposium Veterinario Avedila*. 2003:2–5.

*5. Caballero J, Rebordosa X, Sitjà M, Segundo R, Llopart D, Artigas C. Comparativa de dos ELISAs comerciales en la detección de anticuerpos post-vacunales anti-PRRSV [Comparison of two commercial ELISAs in the detection of post-vaccination antibodies against PRRSV.] *Memorias del 1er Symposium AMVEC Peninsular*. Mérida, México. 2004:189.

6. Albina E, Piriou L, Hutet E. Immune response in pigs infected with porcine reproductive and respiratory syndrome virus (PRRSV). *Vet Immunol Immunopath*. 1998;61:49–66.

7. Nelson EA, Christopher-Hennings J, Benfield DA. Serum immune response to the proteins of porcine reproductive and respiratory syndrome (PRRS) virus. *J Vet Diagn Invest*. 1994;6:410–415.

8. Egli C, Thur B, Liu L, Hofman MA. Quantitative TaqMan RT-PCR for the detection and differentiation of European and North American strains of porcine reproductive and respiratory syndrome virus. *J Virol Methods*. 2001;98:63–75.

9. Christopher-Hennings J, Faaberg KS, Murtaugh MP, Nelson EA, Roof MB, Vaughn EM, Yoon K-J, Zimmerman JJ. Porcine reproductive and respiratory syndrome (PRRS) diagnostics: Interpretation and limitations. *J Swine Health Prod*. 2002;5:213–218.

10. Mengeling WL, Lager KM. A brief review of procedures and potential problems associated with the diagnosis of porcine reproductive and respiratory syndrome. *Vet Res.* 2000;31:61–69.

11. Christopher-Hennings J, Nelson EA, Nelson JK, Hines RJ, Swenson SL, Hill HT, Zimmerman JJ, Katz JB, Yaeger MJ, Chase CCL, Benfield DA. Detection of porcine reproductive and respiratory syndrome virus in boar semen by PCR. *J Clin Microbiol*. 1995;33:1730–1734.

*12. Dee SA. A protocol for defining breeding herd stability and classifying farms according to PRRS status to identify potential intervention strategies: A summary of 200 farms. *Proc IPVS.* 1998;2:262.

13. Dee SA. An overview of production systems designed to prepare naïve replacement gilts for impending PRRSV challenge: A global perspective. *J Swine Health Prod.* 1997;5:231–239.

*14. Philips R, Dee SA. Evaluation of mass vaccination and unidirectional flow for elimination of PRRS. *Proc IPVS*. 2002:130.

15. Batista L, Torremorell M, Pijoan C. Experimental exposure to porcine reproductive and respiratory syndrome virus (PRRSV) in gilts during acclimatization. *J Swine Health Prod*. 2002;10:147–150.

16. Torremorell M, Moore C, Christianson WT. Establishment of a herd negative for porcine reproductive and respiratory syndrome (PRRSV) from PRRSV-positive sources. *J Swine Health Prod.* 2002;10:153–160.

*17. Batista L, Pijoan C, Baidoo S. Eradication of porcine reproductive and respiratory syndrome virus (PRRSV) by serum inoculation with the homologous PRRSV strain. *Proc IPVS.* 2004:289.

*18. Dee SA, Torremorell M, Deen J, Pijoan C. An evaluation of the thermo-assisted drying and decontamination (TADD) system for the elimination of porcine reproductive and respiratory syndrome virus from contaminated livestock transport vehicles. *Proc CRWAD*, 2004:110.

19. Otake S, Dee SA, Rossow KD, Deen J, Joo HS, Molitor TW, Pijoan C. Transmission of porcine reproductive and respiratory syndrome virus by fomites (boots and coveralls). *J Swine Health Prod*. 2002;10:59–65.

20. Otake S, Dee SA, Rossow KD, Moon RD, Pijoan C. Mechanical transmission of porcine reproductive and respiratory syndrome virus by mosquitoes, *Aedes vexans* (Meigen). *Can J Vet Res*. 2002;66:191–195.

21. Batista L, Pijoan C, Lwamba H, Johnson CR, Murtaugh MP. Genetic diversity and possible avenues of dissemination of porcine reproductive and respiratory syndrome virus in two geographic regions of Mexico. *J Swine Health Prod.* 2004;12:170–175.

22. Andreyev VG, Wesley RD, Mengeling WL, Worwald AC, Lager KM. Genetic variation and phylogenetic relationships of 22 porcine reproductive and respiratory syndrome virus field strains based on sequence analysis of open reading frame 5. *Arch Virol*. 1997;142:993–1003.

23. Wesley RD, Mengeling WL, Lager KM, Clouser DF, Landgraf JG, Frey M. Differentiation of a porcine reproductive and respiratory syndrome virus vaccine strain from North American field strains by restriction fragment length polymorphism analysis of ORF-5. *J Vet Diagn Invest*. 1998;10:140–144.

*24. Wesley RD, Mengeling WL, Andreyev V, Lager KM. Differentiation of vaccine (Strain RespPRRS) and field strains of porcine reproductive and respiratory syndrome virus by restriction enzyme analysis. *Proc AASP. Nashville, Tennessee.* 1996:141–143.

* 25. Murtaugh MP, Faaberg KS. How to interpret and use PRRSV sequence information. *Proc Allen D. Leman Swine Conf.* 2001:60–66.

*26. Cannon RM, Roe RT. *Livestock Disease Surveys: A Field Manual for Veterinarians*. Canberra: Australian Bureau of Animal Health. 1982.

* Non-refereed references.