Experiences with tonsil biopsy as an antemortem diagnostic test for detecting porcine reproductive and respiratory syndrome virus infection in breeding swine

Mike D. Bierk, DVM; Scott A. Dee, DVM, PhD; Kurt D. Rossow, DVM, PhD; James E. Collins, DVM, PhD; Maria Isabelle Guedes, DVM; Thomas W. Molitor, MS, PhD

Summary

Objective: To summarize results obtained with antemortem tonsil biopsy, and compare them to those obtained during traditional postmortem sampling, for detecting porcine reproductive and respiratory syndrome virus (PRRSV) infection in breeding swine.

Methods: Tonsil biopsies were collected from 42 sows in a 1500-sow breeding herd known to be chronically infected with PRRSV. Animals were euthanized after the biopsy, and tissues were collected using routine necropsy procedures. Two sows that originated from a PRRSV-negative farm were inoculated by the intranasal (IN) route with a total dose of $10^{2.4}$ $TCID_{50}$ of PRRSV, and a third sow was inoculated IN with virus-free cell culture medium. Serum was collected at 2- to 3-day intervals throughout the study, and tonsil biopsies were taken from each animal on day 14 post infection. The animals were euthanized and necropsied. All samples were examined histologically and tested for PRRSV by Taqman polymerase chain reaction and virus isolation.

Results: Tonsillar tissue was identified histologically in the antemortem biopsies from 21 of the breeding sows and two inoculated sows, and in all postmortem biopsies.

Implications: Tonsil biopsy is an inaccurate procedure for antemortem collection of tonsil tissue from breeding swine. Biopsies should be submitted for histopathological confirmation of tonsillar tissue. Tonsil may not be a representative target tissue for detecting PRRSV in pigs of this age, as the virus may reside in other anatomical sites.

Keywords: tonsil biopsy, PRRS eradication, persistent infection, Test and Removal

Received: April 20, 2000
Accepted: June 21, 2000

Porcine reproductive and respiratory syndrome virus (PRRSV) is an enveloped RNA virus classified in the order Nidovirales, family Arteriviridae, and genus *Arterivirus*.[1] Previous studies have demonstrated that PRRSV can induce prolonged viremia in the presence of circulating antibodies, replicate in macrophages, and produce persistent infections.[2] The PRRS virus also resides in lymphoid tissue, and has been isolated for up to 157 days post infection from tonsillar scrapings.[2] Polymerase chain reaction (PCR) has identified PRRSV nucleic acid in tonsil biopsies from experimentally infected 4-week-old pigs up to 252 days post infection.[2,3] Ribonucleic acid viruses such as PRRSV do not revert to inactive states post infection, but continue to replicate, producing a detectable serologic response.[1–3] Persistently infected pigs eventually clear the PRRS virus, and antibody decay occurs over time.[2–4]

Diagnostic tests for PRRSV infections include isolation of viable virus (VI), detection of viral antigen by immunohistochemistry (IHC), identification of viral nucleic acid (PCR, in situ hybridization), and detection of circulating IgG antibodies (IDEXX ELISA test).[5] The IDEXX ELISA tests (IDEXX Laboratories, Westbrook, Maine) may be performed on tissue, serum, or semen, and are available in diagnostic laboratories throughout North America. ELISA test results are expressed as sample to positive (s:p) ratios, with ratios >0.4 considered positive.

The feasibility of eradicating PRRSV in individual herds in the USA is currently being evaluated. One moderately successful approach is Test and Removal,[6,7] a method that has succeeded in eliminating Aujeszsky's Disease virus and *Actinobacillus pleuropneumoniae* from infected farms.[8,9] The protocol for PRRSV consists of blood-testing the entire breeding herd in a single day, identifying previously exposed and acutely infected animals using antibody and nucleic acid tests, and immediately removing these animals from the herd. The IDEXX ELISA (IDEXX Laboratories, Westbrook, Maine) has been used previously in breeding swine to detect PRRSV serum antibodies, in combination with the Taqman™ PCR (Perkin-Elmer Applied Biosystems, Foster City, California) to detect specific PRRSV nucleic acid sequences in sera.[10] Under field conditions, use of this protocol has resulted in the removal of approximately 8%–11% of the breeding herd; however, 96%–97% of these animals were ELISA-positive and PCR-negative on the day of sampling.[6,7]

Test and Removal is limited by the inability of currently available serological tests to distinguish persistently infected, non-viremic animals from those that have been previously exposed and have cleared the virus, or are undergoing antibody decay. This presents a serious problem to the swine practitioner and producer. Premature removal of non-infected, seropositive animals may have a significant impact on the profitability of a farm. Conversely, if

Department of Clinical & Population Sciences, University of Minnesota, College of Veterinary Medicine, 385 Animal Science/Veterinary Medicine, 1988 Fitch Ave., St. Paul, Minnesota 55108, Email: deexx004@tc.umn.edu

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

Bierk MD, DEE SA, Rossow KD, et al. Experiences with tonsil biopsy as an antemortem diagnostic test for detecting porcine reproductive and respiratory syndrome virus infection in breeding swine. *Swine Health Prod.* 2000;8(6):279–282.

infected animals are allowed to remain in the herd, risk of PRRSV transmission to naïve pen mates exists.[11] Therefore, it would be economically beneficial to the producer if there were an accurate antemortem test to differentiate PRRSV-infected breeding swine from noninfected, seropositive animals.

Tonsil biopsy has been suggested as an antemortem method for detecting PRRSV-infected animals.[2–4, 12] When this method was used in PRRSV-infected field populations of finishing pigs, PRRSV was detected in 0–60% of the animals tested.[12] However, this study did not state whether samples had been verified as tonsil prior to testing for PRRSV. Although tonsil biopsy is being used on commercial farms in an effort to identify PRRSV-positive breeding animals for removal from infected herds (Reicks D, Hollis B, personal communication, 1999), it has not been properly evaluated in adult swine, and may not provide accurate information to the practicing veterinarian. The purpose of this investigation was to compare tonsil biopsy to traditional necropsy technique as accurate methods for detecting PRRSV-infected breeding animals. Prior to initiating the study, the following questions were raised:

- Can tonsillar tissue be consistently recovered by tonsil biopsy in adult breeding swine?
- Is tonsil an appropriate site for detecting PPRSV in adult breeding swine?
- Is tonsil biopsy a safe and efficacious antemortem diagnostic test for identifying PRRSV-infected adult breeding swine?

Materials and methods

Experimental design: The study consisted of a field study and an experimental animal study. In each phase, antemortem sampling (tonsil biopsy) was compared with postmortem sampling methods for detecting PRRSV-infected sows. Animals in the field study phase originated from a known PRRSV-positive farm, while experimental animals were purchased from a known PRRSV-negative herd (negative serology and no detectable clinical signs for 2 years).

Phase 1: Field study

Animal selection: Forty-two sows were selected from a 1500-sow breeding herd known to be chronically infected with PRRSV. A minimum sample size of 30 animals was required to detect one or more PRRSV-infected sows at an estimated prevalence of ≥10% at a 95% confidence interval. Diagnostic tests were used to select animals that had been previously exposed to PRRSV but were not viremic at the time of sampling. In order to identify candidates, blood samples were collected from a random sample of 60 breeding animals, using standard jugular venipuncture techniques. Sera were tested for PRRSV by PCR and for PRRSV antibodies by IDEXX ELISA. Serum PCR-negative sows with detectable ELISA s:p ratios were selected for sampling.

Sampling methods

Antemortem sampling. Prior to euthanasia of animals, tonsil biopsies were collected using a Miltex uterine biopsy forceps. Sows were restrained using a cable snare placed around the maxilla. Visualization of the tonsil region was enhanced using a Frick's speculum placed between the maxilla and mandible, and a flashlight. The cutting plane of the biopsy forceps was pressed against the palatine tonsil, the handles were compressed, and the sample was withdrawn. One half of the biopsy sample was fixed in 10% neutral-buffered formalin, stored at room temperature overnight, and processed routinely for histopathological evaluation. The other half was kept on ice for VI on porcine alveolar macrophages and MARC-145 cells, and for PCR.[10, 13] Between samplings, the biopsy forceps were disinfected with 10% bleach and rinsed with sterile saline.

Postmortem sampling. Sows were euthanized by intravenous injection of sodium pentobarbital (100 mg/kg). Tissues collected from each sow included lung (apical and middle lobes), heart, kidney, spleen, tonsil, adrenal gland, cornea, brain (sections of brainstem, cerebellum, and cerebrum), lateral retropharyngeal lymph node (LN), sternal LN, medial iliac LN, ovarian LN, superficial inguinal LN, right and left uterine horns, ovaries, and mammary gland. Tissues were processed as described above, and samples were analyzed for PRRSV by PCR and VI. Samples that were PCR-positive, VI-positive, or both, were stained for PRRSV using immunohistochemistry (IHC).[14]

Phase 2: Experimental animal studies

Animal selection, inoculation, and sampling. Three sows were purchased from a PRRSV-negative source and housed in separate rooms in the isolation facility at the University of Minnesota, College of Veterinary Medicine. The PRRSV-negative status of the source farm was verified by ELISA serology and clinical history over a 2-year period. Before the sows were inoculated, sera were collected and tested for PRRSV antibodies by ELISA, and for PRRSV by PCR and VI. Sows A and B were inoculated IN with 5 mL of culture medium containing $10^{2.4}$ $TCID_{50}$ of a PRRSV isolate recovered from a previous study.[15] A negative control sow (sow C) was inoculated by the intranasal route with 5 mL of virus-free cell culture medium. Serum was collected from sows A and B on days 1, 3, 5, 7, 10, 12, and 14 postinoculation, and tested by ELISA, PCR, and VI to ensure that infection had taken place, and that animals were not viremic at the time of antemortem sampling. Sow C was tested on days 1, 7, and 14 postinoculation. Upon receipt of positive ELISA and negative PCR results from sows A and B, and negative ELISA and PCR results from sow C, tonsil biopsies were collected from all animals on day 14 postinoculation. The three sows were then euthanized and necropsied, with tissues processed and tested as described above.

Results

Field study

Antemortem sampling. Prior to euthanasia, all 42 animals were serum PCR-negative, ELISA s:p ratios ranged from 0.35 to 2.25, and PRRSV was not isolated from sera. Biopsy tissue was confirmed to be tonsil by histopathology in 21 (50%) of the 42 sows, and all biopsy samples were PRRSV-negative by PCR and VI. Photomicrographs of tonsil biopsy samples and non-specific oropharyngeal tissue collected during the biopsy procedure are illustrated in Figures 1 and 2.

Postmortem sampling. Tonsil biopsies were confirmed histologically to contain tonsillar tissues in all 42 animals, and all were negative for PRRSV by both PCR and VI. A lateral retropharyngeal LN from one sow was PRRSV-positive by IHC, but not by PCR. The initial VI results were confirmed by a second isolation from the

Figure 1: Photomicrograph of tonsillar tissue collected by antemortem tonsil biopsy procedure. Lymphoid nodule, indicative of tonsil tissue (arrow).

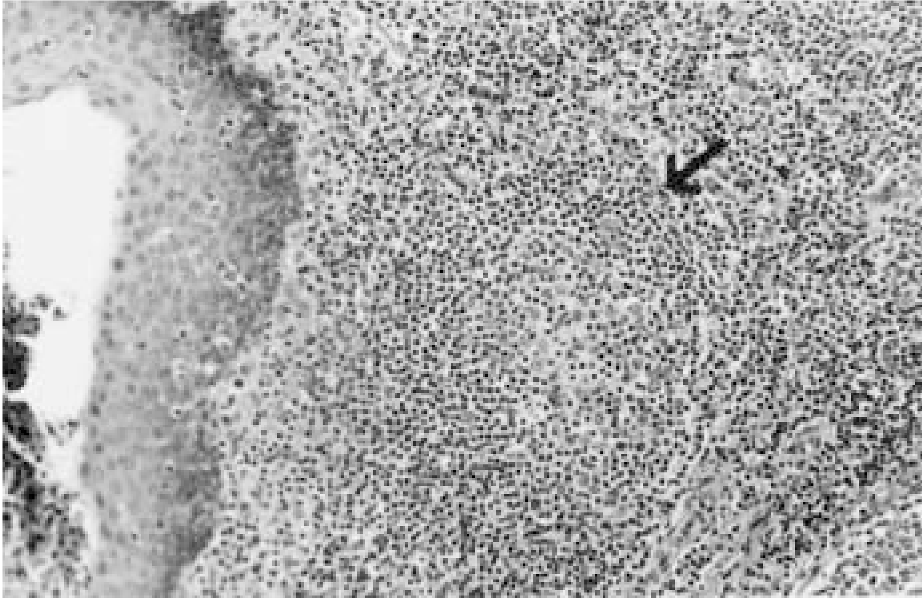
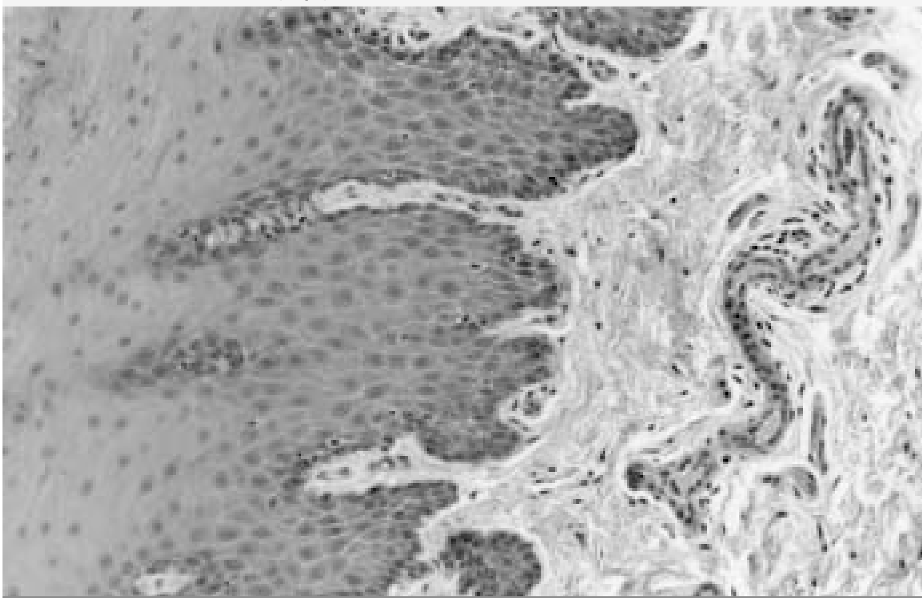


Figure 2: Photomicrograph of non-specific oropharyngeal tissue collected by antemortem tonsil biopsy procedure.



lateral retropharyngeal LN. This animal had an ELISA s:p ratio of 1:20 at necropsy. All other tissues collected from the remaining sows were PRRSV-negative by all described methods.

Experimental animal study

Antemortem sampling. On day 3 after experimental infection, PRRSV was detected in the sera of sows A and B by PCR and VI, and on day 10, ELISA was positive for PRRSV antibody. Sow C remained negative for PRRSV by ELISA, PCR, and VI throughout the 14-day period. Tonsil biopsies were obtained from all three sows prior to necropsy. Histopathological evaluation confirmed the presence of tonsillar tissue in the biopsies collected from sows A and C, but not sow B. The PRRS virus was detected by PCR in the tonsil sample collected from sow A. The PRRS virus was not isolated from any antemortem samples collected after viremia had ceased.

Postmortem sampling. The PRRS virus was detected by PCR in the following tissues collected from sow A: mandibular LN, lateral retropharyngeal LN, medial iliac LN, superficial inguinal LN, ovarian LN, tonsil, heart, uterus, kidney, and umbilical cord, and PRRSV was isolated from all of these tissues except kidney and umbilical cord. The PRRS virus was detected by PCR from the following tissues collected from sow B: uterus, mandibular LN, sternal LN, lateral retropharyngeal LN, and superficial inguinal lymph nodes. All tissues from sow B were negative for PRRSV by VI. All tissue and serum samples from sow C were negative for PRRSV by ELISA, PCR, and VI.

Discussion

Tonsil tissue was obtained by antemortem biopsy from only 21 of the 42 purchased sows, and from two of the three experimentally infected sows. In contrast, tonsil tissue was obtained by postmortem biopsy from 100% of the animals. Therefore, it appears that histopathological verification is critical to ensure that tonsil biopsy submitted for detection of PRRSV is truly tonsil. It is unknown whether practitioners using tonsil biopsy are confirming their results in this manner, and it is strongly recommended that they do.

In contrast to previous reports,[2–4] PRRSV was not detected in any of the tonsil samples collected. One explanation for this observation could be that our study focused entirely on sows, while previously published work was based on experimental infection of nursery age pigs. The duration of PRRSV viremia in the nursery pig has been reported to range from 3–35 days, in contrast to 9–11 days in adult animals.[16,17] While PRRSV was not detected in tonsils of any of the 42 animals in the field study, it was detected in one sow in a lymphoid region not accessible in the live animal. Therefore, tonsil biopsy may generate false negative results. Finally, only one of the two experimentally infected animals had detectable PRRSV in the tonsil, although the virus was detected in other sites throughout the body in both sows.

In our experience, the protocol for tonsil biopsy was technically challenging and would be difficult to apply to large breeding herd populations. Three people were required to obtain each sample: one to restrain the sow, a second to collect the sample, and another to illuminate the tonsil using a flashlight. Furthermore, collection of the sample resulted in extensive trauma to the oropharyngeal region of the sow, and the entire process frequently took 2–3 minutes to compete. In contrast, two people can collect a blood sample in 30–60 seconds.

Animals from only one farm were used in this experiment, a significant limitation of the study. Whether these results are representative of those from other infected farms remains to be seen. One could speculate that in an acute outbreak, the recovery of PRRSV-infected tonsils might be much higher. The major difference in the results obtained by Kolb[12] and the results from our study may relate to the difference in the stage of the disease in the animals. In the Kolb study, acutely infected finishing pigs were sampled, and PRRSV was detected in 0–60% of the animals sampled by PCR and VI. However, since the tissues were not examined microscopically, it is unknown whether PRRSV was undetected in some samples because there was no virus in the tissues, or because inappropriate samples had been collected. The veterinarian responsible for sample collection in our study had no prior experience with the biopsy procedure, and different results might have been obtained if experienced personnel had been employed. Finally, the percentage of PRRSV-infected tonsil samples might have been higher if more sensitive assays (i.e. nested PCR or swine bioassay) had been used.[18] However, use of nested PCR assays may result in false positive readings because of laboratory contamination (dust, non-specific nucleic acid, etc),[19] and the availability and cost of animals and facilities may limit the practicality of swine bioassay. We selected tests for this study on the basis of their availability to swine practitioners through US diagnostic laboratories, and did not consider research facilities as centers for routine testing.

This is the first report of the use of tonsil biopsy to detect PRRSV in adult breeding swine. This study differs from published reports in a number of respects: sows were studied instead of nursery age pigs, the field study allowed for the evaluation of naturally infected sows, and the experimental animal study used a relatively low total dose of PRRSV ($10^{2.4}$ $TCID_{50}$).

Implications

On the basis of our results, we conclude that:

- Antemortem tonsil biopsy is inaccurate for collection of tonsil in unanesthetized sows when conducted by inexperienced veterinarians.
- Tonsil may not be a representative target tissue for detection of PRRSV in sows, and
- PRRSV may reside in sites other than tonsil in naturally infected sows.

Therefore, it is important that practitioners understand the limitations of tonsil biopsy before using this technique to detect PRRSV-infected sows when the goal is elimination of PRRSV from infected populations.

Acknowledgments

The authors would like to thank the University of Minnesota Agricultural Experiment Station and PIC for providing the necessary financial support for this project.

References–refereed

1. Cavanaugh D. Nidovirales: A new order comprising Coronoviridae and Arteriviridae. *Arch Virol.* 1997;142:629–633.

2. Wills RW, Zimmerman JJ, Yoon KJ, Swenson SL, McGinely MJ, Hill HT, Platt KB, Christopher-Hennings J, Nelson EA. PRRS virus: a persistent infection. *Vet Micro.* 1997;55:231–240.

5. Collins JE, Dee SA, Halbur P, Lautner B, McCaw M, Polson D, Rodibaugh M, Sanford E, Yeske P. Laboratory diagnosis of PRRS virus infection. *Swine Health Prod.* 1996;4:33–35.

6. Dee SA, Molitor TW. Elimination of PRRS virus using test and removal process. *Vet Rec.* 1998;143: 474–476.

7. Dee SA, Molitor TW, Rossow KD. Epidemiological and diagnostic observations following the elimination of PRRS virus from a breeding herd of pigs by the test and removal protocol. *Vet Rec.* 2000;146: 211–213.

8. Kluge JP, Beran GW, Hill HT, Platt KB. Pseudorabies (Aujeszky's Disease). In: Straw BE, D'Allaire S, Mengeling WL, Taylor DJ, eds. *Diseases of Swine.* 8th ed. Ames, Iowa: Iowa State University Press. 1999:233–246.

9. Nielsen R, Thomsen AD, Vesterland SD. Pleuropneumonia caused by *Haemophilus parahaemolyticus*. An attempt to control the disease at two progeny testing stations by serological blood testing followed by removal of the seropositive animals and their litter mates. *Nord Vet Med.* 1976;28:349–352.

11. Dee SA, Joo HS, Henry S, Tokach L, Park BK, Molitor To, Pijoan C. Detecting subpopulations after PRRS virus infection in large breeding herds using multiple serologic tests. *Swine Health Prod.* 1996;4:181–184.

13. Bautista EM, Goyal S, Yoon IJ, Joo HS, Collins J. Comparison of porcine alveolar macrophages and CL 2621 for the detection of PRRSV and anti-PRRS antibody. *J Vet Diagn Invest.* 1993;5:163–165.

14. Halbur PG, Miller LD, Paul PS, Meng XJ, Huffman EL, Andrews JJ. Immunohistochemical identification of PRRS virus antigen in the heart and lymphoid system of 3 week old CDCD pigs. *Vet Pathol.* 1995;32:200–204.

16. Yoon IJ, Joo HS, Christianson WT, Morrison RB, Dial GD. Persistent and contact infection in nursery pigs experimentally infected with PRRS virus. *Swine Health Prod.* 1993;1(4)5–8.

17. Christianson WT, Choi CS, Collins JE , Molitor TW, Morrison RB, Joo HS. Pathogenesis of PRRS virus in mid-gestation sows and fetuses. *Can J Vet Res.* 1993;57:262–268.

19. Gleason SC, Currier R, Quinlisk P. Public health response to a potentially rabid bear cub. *MMWR.* 2000;48:971–973.

References – non-refereed

3. Wills RW, Doster AR, Galeota J, Sur JH, Osorio FA. Duration of PRRS virus infections and proportion of persistently infected pigs. *Proc 3rd Int Symp PRRSV.* 1999;139.

4. Benfield DA, Nelson J, Rowland RR, Lawson SR, Steffen M, Rossow KD, Collins JE. Persistence of PRRS virus. *Proc AASP.* 1999;305–308.

10. Tune KA, DeCesare B, Collins JE, Kapur V. New diagnostic tools for the detection of infectious agents. *Proc AD Leman Conf.* 1997;170–172.

12. Kolb JR. Monitoring the risk of introducing PRRSV using tonsil biopsy, serum, buffy coat and ORF 7 PCR in seropositive replacement animals. *Proc AASP.* 1998;413–418.

15. Bierk MD, Dee SA, Rossow KD, Guedes MI, Molitor TW. A diagnostic investigation of PRRS virus persistence in adult breeding swine. *Proc AASP.* 2000;403–404.

18. Horter D, Pogranichy R, Chang CC, Yoon KJ, Zimmerman JJ. How long does PRRSV persist in pigs after infection? *Proc AASP.* 2000;401–402.