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## PRRS VIRUS CARRIER ANIMALS

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**Introduction.** Porcine reproductive and respiratory syndrome virus (PRRS virus) is currently one of the most economically important viral disease of swine in the world. Probably the only infectious agents more costly to producers than PRRS virus are foot-and-mouth disease and classical swine fever viruses.

In terms of prevention and control, the root of the "PRRS problem" is chronic carrier animals. Carriers are individuals that harbor the virus in the absence of apparent clinical disease and serve as a potential source of infection. In this sense, carrier animals are the means by which PRRS virus is maintained in endemically infected herds and the most frequent means of introducing virus into negative herds. It has been recognized for several years that animals infected with PRRS virus become persistently infected. However, PRRS virus infection has not been well characterized with respect to the duration of the carrier state and the proportion of carrier animals within a group over time post infection.

Identification of carrier animals is critical to the control of PRRS. However, we have little information on the ability of current diagnostic assays to identify carriers. It is generally thought that molecular diagnostics, such as PCR, offer an improvement in diagnostic sensitivity and diagnostic specificity. Many diagnostic laboratories are implementing PRRS virus PCRs with the expectation of faster turn around and increased diagnostic accuracy. However, few studies have actually documented the diagnostic performance of PCR-based assays. Because of the importance of PRRS virus to swine producers and considering the number of cases for PRRS virus diagnostics, it is important to understand the performance of the PCR-based assay as compared to other diagnostic tests.

**Objectives** The purpose of the work discussed in this paper was to -

1. Characterize the proportion of PRRS virus carriers in a population over time, and
2. Evaluate the ability of diagnostic assays to identify carrier animals.

**Experimental Design** We used 3-week-old PRRS virus-negative pigs (n = 180). Pigs were randomly assigned to one of 2 treatments: inoculated with PRRS virus (n = 90) or uninoculated control (n = 90). Pigs were exposed intranasally to the North American prototype isolate ATCC VR-2332. Serum samples were collected from all pigs for virus isolation (VI) and/or serological evaluation on days -5, 0, 7, 14, 21 PI, and every 14 days thereafter until animals were euthanised. Thirty pigs from each group were euthanised on day 7 post inoculation (PI) and 12 animals from each group on days 63, 77, 91, 98, and 105 PI. Blood samples and oropharyngeal scrapings were collected ante mortem and tissue

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samples (lung, lung lavage, tonsil, tracheobronchial lymph nodes) were collected post mortem. All samples were processed, randomly numbered, and stored at -80°C until tested.

The carrier status of inoculated pigs was determined as follows:

1. Virus isolation (VI) was attempted on oropharyngeal scrapings;
2. If oropharyngeal scrapings were VI negative, VI was performed on tonsil tissue; and
3. If tonsil tissues were VI negative, swine bioassay was conducted using tonsil homogenate.

Pigs were considered to be carriers if PRRS virus was detected by either VI or swine bioassay; otherwise pigs were considered to be recovered animals (i.e., cleared or non-carrier). Uninoculated pigs served as negative control through the study.

**Diagnostic Assays** Virus isolation was performed using standard techniques with MARC-145 cells. Reverse transcriptase polymerase chain reaction (RT-PCR) was performed on serum, oropharyngeal scrapings, and tonsil homogenates. Serum antibodies against PRRS virus were monitored using a standardized commercial ELISA kit (HerdChek® PRRS ELISA, IDEXX Laboratories, Inc. Westbrook, Maine USA).

**Results** In this experimental population, 100% of pigs still harbored infectious PRRS virus 60 days after inoculation. At day 105 PI, infectious virus was still present in 90% (10/11) of inoculated pigs.

The serum antibody response was consistent with previous reports in the literature. All animals were ELISA positive (S/P  $\geq$  0.4) by day 21 with the exception of one animal that seroconverted by day 35. No statistically significant difference was observed in ELISA S/P values between carriers and non-carriers in the inoculated group. That is, we could not differentiate carriers and non-carrier on the basis of ELISA S/P values.

RT-PCR on oropharyngeal scrapings detected 80.9% of carrier animals on average, while RT-PCR on tonsil homogenate detected 66.1% of carriers. No false positive RT-PCR reactions were observed in oropharyngeal scraping or tonsil samples collected from control pigs, i.e., diagnostic specificity was 100%.

**Conclusions** The results of this experiment have important implications for the prevention and control of PRRS.

1. PRRS virus was present in 90% of animals at 105 dpi. This indicates that a much higher proportion of animals are infected with PRRS virus for a much longer period of time than previously suspected.
  - a. These data suggest that persistent infection is the norm for PRRS virus infection.
  - b. The duration of the infection in individual animals helps explain how PRRS virus is able to remain endemic in commercial herds.

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- c. PRRS virus infection stimulates an active immune response – both cell mediated and humoral. Persistent infection exists in spite of this. Therefore, we have to ask, “what is protective immunity?”
  2. Currently, there is a great deal of interest in eradication of PRRS virus from commercial herds using a test-and-removal strategy. Based on this study, serum samples are the ideal sample for detecting PRRS virus in acutely infected animals and oropharyngeal scrapings are the ideal sample to detect animals persistently infected with PRRS virus. However, we do not believe that the current diagnostic assays are adequate to efficiently and economically identify carriers in the field.
  3. Although this experiment used the latest information in arriving at the best experimental design, we recognize that it would have been extremely useful to follow animals out to at least 150 days PI. This work is in progress. Other future work should focus on expanding our understanding of PRRS virus as a persistent infection, including the mechanisms by which PRRS virus evades the immune response, the role of virus quasi-species in persistent infection, and improved diagnostic assays to detect carriers.