RESEARCHREPORT

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SWINE HEALTH

Title: PRRS Eradication pilot study. I. Performance of diagnostic assays in identifying PRRS virus carrier animals - **NPB #98-239**

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ABSTRACT

It has been recognized for several years that PRRS virus produces a persistent infection in swine and carrier animals may transmit virus for months after they were originally 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. Nor do we have adequate information on the ability of current diagnostic assays to identify carriers. Therefore, the objectives of this study were to 1) characterize the proportion of PRRS virus carriers in a population over time and 2) evaluate the ability of diagnostic assays to identify persistently infected animals.

Three week-old pigs (n = 180) were obtained from a herd free of PRRS virus and randomly assigned to one of 2 treatments: inoculation 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 euthanized. Thirty pigs from each group were euthanised on day 7 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 samples (lung, lung lavage, tonsil, tracheobronchial lymph nodes) were collected post mortem. All samples were appropriately processed, coded with random numbers, and stored at -80°C until tested.

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The carrier status of individual 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 tissues; and 3) if 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. At day 105 PI, infectious virus was still present in 90% (10/11) of inoculated pigs. No statistically significant difference was seen in the inoculated group between carriers and non-carriers in ELISA S/P values. RT-PCR on oropharyngeal scrapings detected 80.9% of carrier animals, while RT-PCR on tonsil homogenate detected 66.1% of carriers. No false positive RT-PCR reactions were observed in samples collected from control pigs, i.e., diagnostic specificity was 100%.

INTRODUCTION

Porcine reproductive and respiratory syndrome virus (PRRS virus) is currently the most economically important viral disease of swine in the United States. The clinical importance of PRRS virus is reflected in the rate of submissions to the Iowa State University Veterinary Diagnostic Laboratory (ISU-VDL). The ISU-VDL annually receives 5,000 to 6,000 diagnostic samples for PRRS virus evaluation (e.g., virus isolation or tissue immunoassays) and approximately 120,000 serum samples for PRRS serology (ELISA and IFA). PRRS is still the most serious problem producers confront.

It is generally thought that molecular diagnostics, such as PCR, offer an improvement in diagnostic sensitivity and diagnostic specificity. Many diagnostic laboratories are implementing PCR-based PRRS virus diagnostic test with the expectation of faster turn around and increased diagnostic accuracy of clinical cases. However, few studies have documented the performance of the PRRS RT-PCR assay. Because of the importance of PRRS virus to swine producers and considering the number of cases for PRRS virus diagnostics, we must understand the performance of the PCR-based assay as compared to other diagnostic tests.

OBJECTIVES

The objective of this study was to evaluate the diagnostic performance of PCR-based assays (RT-PCR) for the detection of PRRS virus. In particular, our goal was to evaluate the ability of PCR to detect carrier animals. Carriers are defined as "individuals that harbor an infectious agent in the absence of discernable clinical disease and serve as a potential source of infection." Detection and elimination of carriers from endemically infected herds is the biggest problem we face in the prevention and control of PRRS virus.

Our specific aims were to:

1. Estimate the diagnostic sensitivity and diagnostic specificity of PCR-based assays in acute PRRS virus infections;

2. Estimate the diagnostic sensitivity and diagnostic specificity of PCR-based assays in chronic PRRS virus infections;

3. Determine the diagnostic sample most likely to provide the correct diagnosis from animals in acute or chronic infections.

PROCEDURES

Experimental Design This experiment was designed as a longitudinal study. Biological samples were collected from pigs over time and assayed for the presence of PRRS virus. Three-week-old commercial feeder pigs (n = 180) were obtained from a herd known to be free of PRRS virus. Animals were randomly assigned to two treatment groups: PRRS virus-inoculated (n = 90) and uninoculated control (n = 90). Animals in the PRRS virus-inoculated group were exposed intranasally to 2 mls (1ml/naris) of North American PRRS virus prototype ATCC VR-2332 at a concentration of 10^3 fluorescent foci units/ml. Serum samples were collected on days post inoculation (dpi) –5, 0, 7, 14, 21, and every 14 days thereafter until euthanasia. Sixty animals, 30 inoculated and 30 control, were euthanised at 7 dpi. Twelve animals from each group were euthanised at 63, 77, 91, 98, and 105 dpi with the exception that only 11 inoculated animals were euthanised at 105dpi. Post-mortem samples collected included tracheobronchial lymph nodes, lung, lung lavage, and oropharyngeal lymphoid tissue. Serum, peripheral blood leukocytes, and oropharyngeal scrapings were obtained prior to euthanasia. The PRRS virus carrier status of each individual animal was determined using virus isolation and swine bio-assay.

Diagnostic Testing Virus isolation was performed using standardized techniques with MARC-145 cells. Reverse transcriptase polymerase chain reaction (RT-PCR) was performed on serum from 7 days post inoculation and on oropharyngeal scrapings and tonsil homogenates from dpi 63, 77, 91, 98 and 105. 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. Over time, the proportion of carrier animals decreased, but at 105 dpi, infectious virus was still detected in 90% of animals.

There was no statistically significant difference between infectious carriers and infected animals that had recovered from PRRS virus infection, i.e., we could not differentiate carriers on the basis of antibody S/P values using a commercially available ELISA.

RT-PCR on serum of acutely infected animals (7 dpi) detected 100% of animals infected with PRRS. The diagnostic sensitivity and specificity of PCR on serum from acutely infected animals was 100% and 100%, respectively. RT-PCR on oropharyngeal scrapings had a diagnostic sensitivity of 80.9% and a diagnostic specificity of 100%. PCR performance on tonsil homogenate resulted in a diagnostic sensitivity of 66.1% and a diagnostic specificity of 100%.

DISCUSSION

This experiment has provided significant new information with important implications for 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 imply 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.
   c. These results raise questions about the development of a protective immune response.

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 conclude that the current diagnostic assays cannot efficiently identify carriers.

3. Although this experiment used the latest information in arriving at the best experimental design, retrospectively we recognize that it would have been extremely useful to follow animals out to at least 150 dpi. 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 diagnostic assays to detect carriers.

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PUBLICATIONS AND PRESENTATIONS

Presentations

Horter DC, Pogranichnyy RM, Chang C-C, Yoon K-JH, Zimmerman JJ. Estimating the rate of PRSS virus carriers in an experimental population. November 8, 1999. 80th Ann Meet Conference of Research Workers in Animal Disease, Chicago, Illinois.

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