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PRRSV persistence: Lessons from the big pig experiment

R.M. Molina,1 J. Christopher-Hennings,2 E. Nelson,2 R. Hesse,2 J. Lunney,4 K.-J. Yoon,1 R.R.R. Rowland,1 J.J. Zimmerman1

1Veterinary Diagnostic Laboratory, Iowa State University, Ames, IA; 2Department of Veterinary Science, South Dakota State University, Brookings, SD; 3Department of Diagnostic Medicine and Pathobiology, Kansas State University, Manhattan, KS; 4USDA, ARS, BARC, Beltsville, MD

Introduction

Persistence is unquestionably the single most significant epidemiological attribute of PRRSV infection. PRRSV produce an acute infection characterized by viremia, clinical disease with seroconversion and a chronic, persistent infection in which the virus is present at lower levels in a continuously decreasing percentage of convalescent animals over time (Allende et al. 2000; Rowland et al. 2003). This characteristic of the virus allows it to perpetuate within herds. The duration of persistence is uncertain, but it is recognized as extensive: Wills et al. (1997) isolated virus from one of 4 pigs 157 days post-inoculation; Allende et al. (2000) detected infectious virus by bioassay in 2 of 5 pigs at day 150 post-inoculation; Horter et al. (2002) detected infectious PRRS virus in 51 of 59 (84%) animals between 63 and 105 days post-inoculation, including 10 of 11 (91%) of animals at day 105 post-inoculation; Rowland et al. (2003) isolated virus from tonsil and lymph nodes from pigs infected in utero up to 132 days after farrowing; Batista et al. (2004) detected virus by PCR in tissue pools (tonsil, superficial inguinal and sternal lymph nodes) from 2 of 10 (20%) at 135 days post inoculation (DPI) and from 49 of 50 (98%) animals euthanized 30-100 DPI.

Management strategies directed at prevention and/or control of PRRS requires a qualitative and quantitative understanding of virus circulation at the population level. The purpose of this study was to provide an improved estimate of the percentage of persistently infected within a population over time.

Persistence

Big Pig experiment was PRRS CAP-funded project initiated with three basic objectives: 1) to provide the PRRS research community a set of well-characterized serum and tissue samples from a large population of experimentally infected pigs and age-matched controls; 2) to develop a detailed profile of the immune responses during all phases of infection; and 3) to develop a model for viral persistence at the level of the population. Over the course of the experiment over 20,000 samples (serum and tissue) were distributed to five institutions. Analyses performed in parallel included viral RNA qPCR of serum and lymphoid tissue, virus isolation, serology and bioassays. Several satellite projects were also supported: histopathology, cytokine gene expression in lymphoid tissues, immune cell phenotyping and performance of test on meat juice.

Experimental design

The experiment was designed as a longitudinal study in which pigs were followed for up to 203 days post inoculation (DPI). Pigs were housed in a secure BL-2 facility in the Iowa State University Livestock Infectious Disease Isolation Facility. On day zero, 109 3-week old pigs were intramuscularly inoculated with PRRSV strain VR-2332. Negative control pigs (n = 56) were sham inoculated with cell culture medium by the intramuscular route. Thereafter, at approximately 2 week intervals, serum samples were collected from all animals and a subset of randomly selected animals was euthanized and tissues collected. The presence and amount of virus in tissue and serum was assessed using RT-qPCR, standard virus isolation (VI), and bioassay.

RT-qPCR

Measurement of PRRS viral RNA in serum and tissue samples was performed at South Dakota State University (Dr. J Christopher-Hennings). The protocol has been described previously (Wasilk et al., 2004). Results were reported as the number of RNA templates per ml of serum or gram of tonsil.

No false positive results were observed in negative control pigs.

Detection of virus in serum showed that all inoculated pigs were productively infected with PRRSV, with peak viremic appearing between 7 and 21 DPI. Most pigs cleared the viremia by 42 DPI, with a small percentage (2-7%) of pigs testing positive up to 154 DPI. In contrast, lymphoid tissues (tonsil, submandibular and superficial lymph nodes) were positive through 202 DPI in one or more pigs at each sampling point. No significant difference was detected between gilts and barrows (“gender effect”) in the proportion of qPCR positive animals or duration of persistence by day post infection.

Virus isolation

Recovery of infectious virus in serum and homogenate of lymphoid tissue was performed on MARC-145 cell culture at Kansas State University (Dr. R Hesse). Isolation
of virus was confirmed by staining with FITC-SDOW-17. Pigs were virus isolation-positive on serum and lymphoid tissue at 7 DPI, with the last positive samples detected at 48 and 56 DPI, respectively.

**Bioassay**

Bioassays for the detection of infectious PRRSV were performed as described by Horter et al. (2002). Individual pigs were intramuscularly inoculated with tissue homogenates composed of tonsil, submandibular lymph node, and superficial lymph node from individual pigs. Detection of PRRSV in the serum of bioassays pig by RT-PCR of V1 was used to determine that the pig from which the tissues were derived was persistently infected.

Persistent infection was detected by bioassay up to 175 DPI. Over time post inoculation, the proportion of persistently infected animals peaked around 70 DPI, approached zero between DPI 98 to DPI 119, after which a second spike appeared and peaked around DPI 133.

**Comments**

In agreement with previous reports, viremia as determined on the basis of virus isolation on serum and lymphoid tissues was of short duration. In contrast, RT-qPCR positive tissues were detected in multiple pigs at every sampling throughout the 202 day observation period. These results suggested that virus persisted in a larger proportion of the population and for a longer period of time than previously thought. In contrast to PCR, bioassay positive pigs were detected sporadically in the latter stages of the experiment, with a single positive animal detected at 175 DPI.

The results suggested that RT-PCR was the most sensitive assay for the detection of PRRSV. However, the discrepancy between PCR and bioassay results also strongly suggested that PCR detected non-infectious virus or viral genomic fragments. The mechanism by which this might occur is known. Since PCR results are the basis for the development and implementation of biosecurity protocols for control and elimination of PRRSV, this issue needs to be resolved.

**References**


