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1-1-2000

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Wills, R. W.; Doster, Alan R.; Galeota, J.; Sur, J.-H.; and Osorio, Fernando A., "Duration of PRRS Virus Infections and Proportion of Persistently Infected Pigs" (2000). *Nebraska Swine Reports*. 119.

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Duration of PRRS Virus Infections and Proportion of Persistently Infected Pigs

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Summary and Implications

The objective of this study was to more fully characterize persistent PRRSV infections in swine. Twenty-eight 35-day-old segregated-early-weaned pigs were inoculated intranasally with PRRSV. Serum and tonsil biopsy samples were collected on days 0, 7, 14, 28, and then about monthly thereafter until day 251 post inoculation (PI). Virus was isolated from serum and tonsil biopsy samples through days 28 and 56 PI, respectively. Viral RNA was detected in serum and tonsil biopsy samples by RT-PCR through day 251 PI, although no positive serum samples were detected on days 84-196 PI. Greater proportions of day 28 and 56 PI serum samples and tonsil biopsies were found to be PRRSV RNA positive by RT-PCR than positive by virus isolation. Although 20 of 28 tonsil biopsies collected on day 84 PI were positive by RT-PCR, only one of 28 tonsil biopsies collected one month later (day 119 PI) was positive. Three pigs returned to seronegative status on or after day 196 PI. Neither virus nor viral RNA was detected in these ani-

mals beyond day 119 PI. Conversely, five pigs that were persistently infected through day 225 or 251 PI remained seropositive throughout the study although one pig had an ELISA S/P ratio of 0.41, nearly at the cutoff point of 0.40. The results confirm RT-PCR is more sensitive than virus isolation in identifying PRRSV-infected pigs. The abrupt drop in the proportion of pigs with RT-PCR positive tonsil samples from day 84 to day 119 PI indicates most pigs clear the virus within three to four months, but some may remain persistently infected for several months.

Introduction

Porcine reproductive and respiratory syndrome virus (PRRSV) causes a potentially devastating disease in swine herds. Understanding the transmission of PRRSV is paramount to developing successful prevention programs. Research has documented transmission between pigs in direct contact and allowed investigation of how long pigs remain infectious. Early transmission studies demonstrated pigs were persistently infected and capable of transmitting virus for at least two to three months after initial inoculation. Field observations of herds infected for long periods of time and transmission via purchase of clinically normal, but PRRSV-infected, animals highlights the importance of characterizing the

persistence of PRRSV infection.

The proportion of persistently infected animals also directly affects the dynamics of virus transmission within a herd. Since a persistently infected animal is a potential source of infection, the ability to estimate the proportion of persistently infected animals is of critical importance in developing prevention and control programs. Producers often are faced with the decision of whether to introduce previously infected animals into their herds. Currently, it is not clear if pigs that have returned to seronegative status following initial seroconversion are capable of still harboring PRRSV.

The objective of this study was to more fully characterize persistent PRRSV infections in swine. In particular, the study assessed what proportion of inoculated animals become persistently infected with PRRSV. The serological status of persistently infected pigs also was investigated.

Materials and Methods

Thirty-five segregated early-weaned pigs were obtained from a herd known to be free from PRRSV infection. At 35 days of age, the pigs were randomly assigned to one of five isolation rooms. The pigs in four of the rooms were designated as principals and were inoculated intranasally with PRRSV. The pigs in the fifth group were designated negative controls and

**Table 1. Detection of PRRSV from serum and tonsil samples.**

DPI*	Virus Isolation		RT-PCR	
	Serum†	Tonsil	Serum‡	Tonsil
7	28/28	26/28	27/28	27/28
14	20/28	23/28	28/28	28/28
28	2/28	9/28	7/28	27/28
56	0/28	4/28	1/28	21/28
84	0/28	0/28	0/28	20/28
119	NT	0/28	0/28	1/28
147	NT	0/28	0/28	2/28
168	NT	0/28	0/28	0/28
196	NT	0/28	0/28	0/28
225	NT	0/28	1/28	1/28
251#	NT	0/28	1/28	2/28

*Days post inoculation.

†Number of serum or tonsil biopsy samples from which PRRSV was isolated/number tested.

‡Number of serum or tonsil biopsy samples positive for PRRSV RNA /number tested by RT-PCR.

#Necropsy samples.

NTNot Tested.

Table 2. RT-PCR results of pigs in which viral RNA was detected on day 119 PI or beyond

Pig	Days Postinoculation								
	28	56	84	119	147	168	196	225	251
1	T							T	
2	T	T	T		T				
16	T	T	T		T				
24	S,T		T						T
26	S,T	T	T						T
29	T		T						S
34	T		T	T					
40	T	T						S	

S Viral RNA detected in serum sample by RT-PCR

T Viral RNA detected in tonsil sample by RT-PCR

were not inoculated with virus.

Samples were collected on days 0, 7, 14, 28, and then about monthly thereafter until day 251 post inoculation (PI). After collecting blood samples, the pigs were anesthetized so that tonsil biopsies could be taken. A dermatology biopsy punch was used to harvest approximately 4mm x 8mm section of tissue from each palatine tonsil.

Virus isolation on MARC 145 cells was conducted on serum and tonsillar biopsy samples collected from the principals and a randomly selected negative control pig for a given collection day. Virus isolation was conducted on serum collected on days 7-84 PI and on tonsil homogenates prepared from biopsies or necropsy tissues collected on days 7-251 PI. Serum samples and an aliquot of each tonsil homogenate from days 7-251 PI also were analyzed by Reverse Transcriptase - Polymerase

Chain Reaction (RT-PCR). RT-PCR was done using a Gene Amp EZ r Tth RNA PCR Kit (Perkin Elmer, Branchburg, NJ). Serum antibody levels were assessed by ELISA (IDEXX Laboratories, Westbrook, ME) on all serum samples.

Results and Discussion

Virus isolation and RT-PCR results are summarized in Table 1. The results confirm RT-PCR is more sensitive than virus isolation in identifying PRRSV-infected pigs. Positive RT-PCR results do not necessarily indicate the presence of viable virus, only the presence of viral RNA. At the same time, it appears likely that in order for the viral RNA to be detected out to day 251 PI, replicating virus also must be present for extended periods of time. This was supported by concurrent research in

which bio-assay pigs seroconverted after inoculation with tissue samples collected from pigs inoculated five months previously. Additional research is needed to better determine the duration of time during which pigs remain contagious to susceptible pigs.

As seen in Table 2, the detection of viral RNA by RT-PCR from day 119 to 251 PI was sporadic in that although viral RNA was detected in eight animals during this time, RNA was not detected from the same pig during consecutive months. Furthermore, viral RNA was not detected from both serum and tonsil samples collected on the same day from a pig. The sporadic detection of viral RNA in serum after months of negative samples has been reported previously. Although it is possible that the observation of sporadic positive samples is due to false positive reactions, samples from the seven negative control pigs were consistently negative with one exception. That single exception occurred on day 7, which logically is a time at which a large percentage of samples are positive creating an increased chance of cross contamination. The fact that neither this pig nor other control pigs in direct contact with it developed a serological response enhances the certainty that this was a false positive reaction. Other than that, all of the negative controls remained negative. No other false positive reactions were apparent throughout the rest of the experiment.

Of particular interest is the abrupt drop in the proportion of pigs with RT-PCR positive tonsil samples from day 84 to day 119 PI. These results corroborate earlier work in which PRRS virus was isolated from tonsil scrapings from three of four pigs collected on day 84 PI, but at most, one out of four pigs on subsequent samples. The results demonstrate that although pigs can remain persistently infected for several months, this is a fairly infrequent event, with most pigs clearing the virus between three and four months. Our experiment does not rule out — in fact, it may suggest — that very low levels of replication may continue

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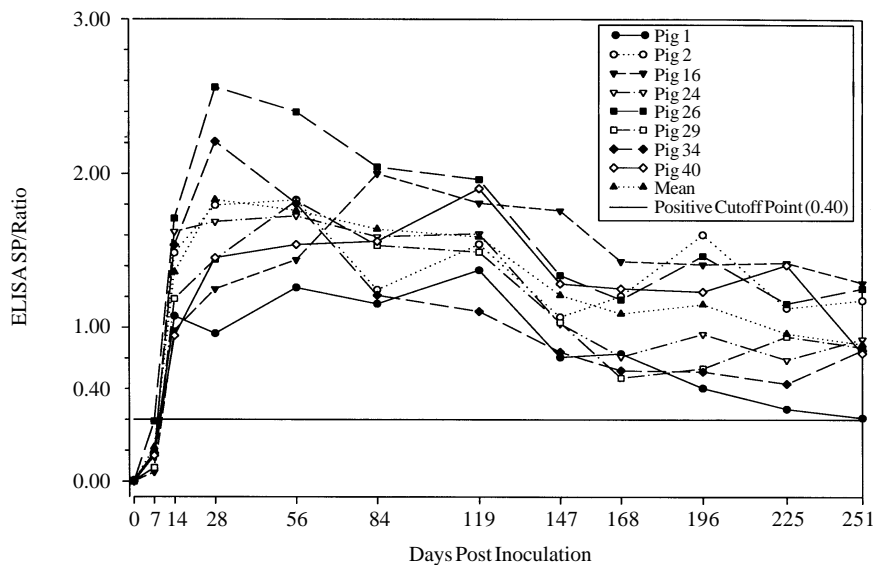


Figure 1. ELISA S/P ratios of persistently infected pigs.

allowing sporadic detection of viral RNA.

All inoculated animals sero-converted. Three pigs returned to seronegative status (ELISA S/P ratios less than 0.40) on or after day 196 PI. Virus was not isolated nor viral RNA detected in these animals beyond day 119 PI. As shown in Figure 1, the eight

pigs that were persistently infected for 119 days or more according to RT-PCR results did not return to seronegative status by the end of the trial although the S/P of Pig 1 was 0.41 on day 251 PI. This pig was positive up to day 28 PI by virus isolation and days 28 and 225 PI by RT-PCR of the aliquot of the virus isolation preparation.

With the exception of Pig 1 the results suggest that an animal that has returned to seronegative status is unlikely to harbor the virus. The results of Pig 1 suggest that viral RNA may still be detected in pigs with an S/P less than or at least near the cutoff point. This finding has significant impact on the use of the ELISA test in the identification of PRRSV infected animals.

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Acknowledgements

The authors thank Blaine Clowser, Jennifer Friesen, Eric Mousel, Andrea McCormic and Rossana Allende for their technical assistance.

This research was partially funded by the Nebraska Pork Producers Association and the National Pork Producers Council in association with the National Pork Board. This research was also supported by USDA National Research Initiative Competitive Grant Program Project Number 96-3520-3678.

