2002

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SDSU

SWINE 2001 - 20

Abstract

Porcine reproductive and respiratory syndrome virus (PRRSV) is transmitted through semen via natural mating or artificial insemination. However, the minimal infective dose of PRRSV required to effect transmission through semen is not known. In this study, we induced estrus in PRRSV seronegative gilts and then artificially inseminated these animals with extended, commercial boar semen "seeded" with different concentrations (2, 20, 200, 2,000, 20,000, 200,000 or 2,000,000 $TCID_{50}$/50 ml of semen) of the PRRSV isolate SD 92-23983. Infection of gilts by PRRSV was confirmed by weekly bleedings of these animals to detect seroconversion using the commercial IDEXX ELISA. All pigs (n=7) given ≥ 200,000 doses of PRRSV seroconverted 1 to 3 weeks after insemination. In contrast, only 1/5 and 1/5 pigs seroconverted at dosages of 20,000 and 2,000, respectively. There was no seroconversion (0/14 pigs) at dosages ≤ 200. Pigs, which did not seroconvert 4 to 5 weeks after artificial insemination with semen containing 200, 2,000 and 20,000 infectious doses of PRRSV, were intranasally inoculated with the same amount of virus. After intranasal challenge 4/4 (20,000 doses), 4/4 (2,000 doses) and 0/4 (200 doses) pigs seroconverted within 1 to 3 weeks after inoculation. The results indicate that higher infectious doses of PRRSV/ml are required for transmission via semen compared to intranasal inoculation, and the nested PCR assay can detect viral RNA in semen at concentrations that do not result in transmission of PRRSV by artificial insemination.

Introduction

Porcine reproductive and respiratory syndrome (PRRS) was first recognized in the U.S. in 1987. This disease has emerged as the most economically devastating syndrome in the swine industry. Clinically, PRRS is characterized by respiratory problems in all ages of pigs; poor conception rates in breeding herds; increased pre-and post-weaning death loss, and stillborn pigs born to females infected in late gestation.

Research in our laboratory and by others indicates that PRRS virus (PRRSV) can be transmitted from boars to serologically naïve gilts via semen. Combined data from various studies on boars experimentally infected with PRRSV indicates that these animals shed virus in semen on average for 39 days (range 8 to 92 days) after infection. Gilts artificially inseminated with unextended or undiluted semen collected from experimentally infected boars became clinically ill and seroconverted to PRRSV. In contrast, artificial insemination of gilts with extended semen from boars experimentally infected with PRRSV did not cause seroconversion. The difference in results between these two studies may well relate to the amount of virus present in the semen. Determining the quantity of PRRSV in semen is difficult, because semen contains materials that are toxic to cell cultures.

A polymerase chain reaction assay (PCR) has become the standard test for detection of PRRSV RNA in semen. While some contend that detection of PRRSV RNA in the semen is not indicative of infectious virus, our results indicate good correlation between PCR and a swine bioassay. Thus, producers and swine veterinarians often ask us: Is a positive PCR result for PRRSV in semen an indication that the semen will transmit PRRSV? Currently, there is no data available to provide either an answer to this question or to determine if extension or dilution of semen containing PRRSV is an effective method to reduce the risk of PRRSV transmission via semen from infected boars. Dilution may be effective, because the amount of virus necessary to induce disease through semen transfer is probably several times higher than the concentration of PRRSV necessary to induce disease by intranasal inoculation. In this

study, we determined how much PRRSV was needed to transmit PRRSV via artificial insemination using extended semen.

Materials and Methods

Animals and Housing. Thirty-one, five- to seven-month old PRRSV seronegative gilts were obtained from a commercial breeding herd. On arrival and after estrus synchronization, gilts were bled and serum used to reconfirm the absence of antibodies to PRRSV. Serum was screened for PRRSV antibodies using the commercial IDEXX HerdChek® enzyme-linked-immunosorbent assay (ELISA) (IDEXX Laboratories, Inc. Westbrook, ME).

After a one-week acclimation period, estrus was induced in gilts by two different methods. Nineteen gilts were intramuscularly given 5ml of PG 600®(Intervet Inc., Millsboro, DE) containing 400 IU of Pregnant Mare Serum Gonadotropin (PMSG) and 200 IU Human Chorionic Gonadotropin (HCG). Twelve gilts received altrenogest (Regu-Mate®,Hoechst–Roussel, Somerville, NJ) orally for 15 consecutive days as recommended by the manufacturer.

Estrus induction in gilts was determined twice daily using "teaser boars". Gilts determined to be in estrus were removed to separate isolation rooms for artificial insemination with the SD 92-23983 isolate of PRRSV. No more than four gilts were grouped per isolation room.

Virus. The SD 92-23983 isolate of PRRSV was propagated on MARC-145 cells. The amount of virus in the pool was quantitated by titration on MARC-145 cells in a microtiter assay and the endpoint was determined by visualization of cytopathic effect (CPE). The CPE was confirmed to PRRSV specific by staining with the SR-30 monoclonal antibody conjugated to fluorescein isothiocyanate. The titer of the virus pool was determined using the Kaerber method.

Semen Samples. Semen used for artificial insemination was purchased from a commercial PRRSV naïve boar stud (International Boar Semen, Eldora, IA). Each lot of semen was tested for the presence of PRRS viral RNA using the polymerase chain reaction assay. All lots used in this study were negative for the presence of PRRS viral RNA indicating that the samples were not contaminated with PRRSV.

For insemination, 50 ml of semen was aliquoted into insemination bottles and a predetermined concentration of PRRSV at 2,000,000, 200,000, 20,000, 2,000, 200, 20 or 2 tissue culture infectious doses ($TCID_{50}$/50 ml) was added to the semen sample. Gilts (n = 3 to 7 per group) were inseminated using commercial insemination pipettes and 50 ml of PRRSV "seeded" semen. After insemination, the vulva and surrounding skin of the gilt was washed with Betadine (The Purdue Frederick Company, Norwalk, CT) to remove virus on the external surfaces of the inoculated animal.

Clinical Signs of PRRSV infection. Gilts were observed daily for signs of PRRSV infection, including decreased appetite, lethargy, nasal discharge, coughing and sneezing.

Serum. Blood samples were collected weekly for 4 weeks unless PRRSV antibodies were detected prior to this time. Antibodies were demonstrated using the commercial PRRSV HerdChek® ELISA. Serum samples with a S/P (sample to positive ratio) of ≥ 0.40 were considered positive. Evidence of seroconversion (converting from antibody negative to antibody positive) was considered evidence of transmission of PRRSV via semen.

Polymerase chain reaction (PCR). A nested reverse transcriptase-polymerase chain reaction (nRT-PCR) procedure was used to detect PRRS viral RNA in semen samples.

Results

Clinical signs. Each of the 4 gilts artificially inseminated with 2,000,000 doses of PRRSV was off feed for 24 hours at 3-days after inoculation. There were no clinical signs observed in gilts given doses of 200,000 or lower.

Virus transmission via artificial insemination with extended semen seeded with various concentrations of PRRSV. Transmission of PRRSV through extended semen was most effective at dosages of 2,000,000 and 200,000 with seroconversion demonstrated in 7/7 animals. Only 1/5 animals at 20,000 and 1/5 at 2,000 seroconverted, whereas there was no seroconversion in

animals given doses ≤ 200. Generally, antibody to PRRSV was not detected until at least 2 to 3 weeks after insemination (Table1).

Four of 5, 4/5 and 0/5 gilts artificially inseminated with 20,000; 2,000; and 200 doses of PRRSV, respectively did not seroconvert indicating a lack of PRRSV transmission. In order to determine if these gilts were still susceptible to PRRSV, these animals were intranasally inoculated with the same dosage. The results indicated that the animals given the 20,000 and 2,000 doses seroconverted, but no viral transmission occurred at 200 doses by either route of inoculation. (Table 2).

PCR results. The RT-PCR assay detected PRRS viral RNA at virus concentrations of ≥ 100 $TCID_{50}$/50 ml of semen.

Summary and Conclusions

Seminal viral load is likely to be directly related to the transmissibility of PRRSV by natural mating or artificial insemination. The results of this study indicate PRRSV can be transmitted through extended semen at doses ≥ 2,000 $TCID_{50}$/50 ml semen (≥ 40 $TCID_{50}$/ml of semen). However, only 20% of the gilts inseminated at doses of 2,000 and 20,000 seroconverted compared to 100% seroconversion in all animals given doses ≥ 200,000 $TCID_{50}$/50 ml semen (≥ 4,000 $TCID_{50}$/ml of semen). This indicates that higher doses of PRRSV are more likely to result in transmission via artificial insemination than lower doses. Transmission via intranasal inoculation was more efficient at doses of 2,000 and 20,000 compared to artificial insemination because a higher number of gilts seroconverted.

The PCR assay was able to detect PRRS viral RNA at virus concentrations of 100 $TCID_{50}$/50 ml of semen (2 $TCID_{50}$/1 ml of semen). Infection of gilts via artificial insemination required a dose of virus at least 20-fold higher than the amount detectable by the PCR assay. Thus, the PCR assay may detect PRRS viral RNA in semen at concentrations that are below the threshold dose for transmission of infectious virus.

Studies are now in progress to develop a quantitative PCR assay that can determine the amount of viral RNA in semen samples in an effort to determine what concentration of viral RNA correlates with the minimal infective dose to transmit PRRSV via artificial insemination. Such an assay should improve producers "peace of mind" since the quantitative PCR assay could be used to predict when PCR positive semen samples are a high, moderate or low risk for transmission of PRRSV.

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Table 1. Transmission of PRRSV by artificial insemination using extended semen seeded with different concentrations of PRRSV.

Dose ($TCID_{50}$/50 ml of semen)	No. pigs positive/No. pigs inoculated	Time (weeks) after inoculation that pigs seroconverted
2	0/4	No seroconversion
20	0/3	No seroconversion
200	0/7	No seroconversion
2,000	1/5	4
20,000	1/5	3
200,000	3/3	2,2,3
2,000,000	4/4	1,2,3,3

A positive pig was an animal that demonstrated seroconversion as indicated by the IDEXX ELISA test on serum from blood collected at weekly intervals after artificial insemination. The numbers in the last column indicate the time in weeks after inoculation that individual pigs were demonstrated to seroconvert from antibody negative to antibody positive. Seroconversion is indicative of transmission of PRRSV via artificial insemination with semen seeded with different concentrations of PRRSV.

Table 2. Transmission of PRRSV by intranasal inoculation of gilts that did not seroconvert from artificial insemination.

Dose ($TCID_{50}$/50 ml of semen)	**No. pigs positive/No. pigs inoculated**	**Time (weeks) after inoculation that pigs seroconverted**
200	0/4	No seroconversion
2,000	4/4	1
20,000	4/4	1,2,3,3

Gilts, which had not previously seroconverted at these concentrations of virus after artificial insemination (Table 1), were intranasally inoculated with the same dose of virus 4 to 5 weeks later. A positive pig was an animal that demonstrated seroconversion as indicated by the IDEXX ELISA test on serum from blood collected at weekly intervals after artificial insemination. The numbers in the last column indicate the time in weeks after inoculation that individual pigs were demonstrated to seroconvert from antibody negative to antibody positive. Seroconversion is indicative of transmission of PRRSV.

Acknowledgements

Funding for this project was provided by the National Pork Producers Council, the United States Department of Agriculture NRICGP (# 9203683 and 9502254), the South Dakota Agricultural Experiment Station, and the Department of Veterinary Science, South Dakota State University.