ORIGINAL RESEARCH

Preliminary assessment of an inactivated PRRS virus vaccine on the excretion of virus in semen

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Summary: *Seven boars serologically negative to porcine reproductive and respiratory syndrome virus (PRRSV) were inoculated intranasally with PRRSV; four were vaccinated with PRRSV vaccine while the other three served as nonvaccinated controls. Semen samples were collected twice weekly for 32 days to determine the effect of the vaccine on PRRSV shedding in the semen.*

All boars were seropositive by indirect-fluorescent antibody (IFA) assay by day 14 postchallenge (PC). Virus was present in semen at the time of the first collection in each of the seven boars on day 4 PC. The two vaccinated boars that were antibody positive by the Western immunoblot assay shed PRRSV in semen for a shorter time (through days 4 and 7 PC, respectively) compared to the remaining two vaccinated boars (through days 25 and 28 PC, respectively), and the three nonvaccinated boars (through day 32 PC). Neither gross nor microscopic lesions attributable to PRRSV were observed in tissues collected at the termination of the experiment (day 32), and virus isolation attempts from reproductive tissues were negative. Results of this preliminary study suggest that it may be possible to reduce seminal shedding of PRRSV with the use of an inactivated PRRSV vaccine.

The first cases of porcine reproductive and respiratory syndrome (PRRS) were reported in the United States in 1987.[1,2] Clinical signs associated with PRRS virus (PRRSV) infection in the breeding-aged female include delayed return to estrus, reduced conception rates, increased repeat breedings, abortions, early farrowings, and an increased number of pigs born weak or dead. Clinical signs associated with PRRSV infection in the breeding-aged male include lethargy, anorexia, elevated rectal temperatures, and loss of libido.[3-6]

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The role of boar semen in transmission of PRRSV is of concern for producers, veterinarians, boar studs, and regulatory personnel. Currently, the level of concern regarding PRRSV-contaminated semen is such that Australia and South Africa have stopped importing semen from countries in which PRRS has been reported.[7] An epidemiologic study conducted in Britain concluded that there was circumstantial evidence that PRRSV was spread to non-infected herds via purchased semen.[8] Experimentally infected boars have been shown to shed infectious PRRSV in semen for as long as 43 days post-challenge, in the absence of clinical disease,[9] and researchers at South Dakota State University have shown that PRRSV can be transmitted to naive gilts by artificial insemination (AI) using unextended semen from experimentally infected boars.[6]

The purpose of this research was to study the course of PRRSV infection in mature boars and:

- evaluate the effect of an inactivated PRRSV vaccine on serum antibody titers pre- and postchallenge (PC);
- assess the impact of an inactivated PRRSV vaccine on duration of seminal shedding of virus and viremia;
- document the course of clinical signs; and
- evaluate the pathology resulting from PRRSV infection in reproductive and other tissues.

Materials and methods

Animals and housing

Seven boars aged 8–12 months were obtained from two PRRSV-free herds and subsequently confirmed to be serologically negative for PRRSV antibodies by the indirect-fluorescent antibody (IFA) test.[9] The boars were housed in groups of two to three boars per pen in one large room.

Four- to 8-week-old pigs were used in the PRRSV swine bioassay (SB). These animals were also obtained from PRRSV-free herds and verified to be PRRSV antibody negative using the IFA test. Pigs were moved to isolation facilities prior to semen inoculation and were housed in individual isolation units throughout the observation period.

Boar vaccination

Boars were randomly selected for vaccination or no vaccination by herd of origin. Two boars from each herd were vaccinated. Four boars (numbers 31, 32, 5664, and 5665) were vaccinated intramuscularly with 2 mL of an experimental killed PRRSV vaccine 5 weeks and 2 weeks prior to challenge.

Boar inoculation

Boars were inoculated intranasally with 2 mL per naris of $10^{6.5}$ $TCID_{50}$ per mL PRRSV (ATCC VR 2402). This PRRSV strain is a plaque-purified isolate originally derived from a pool of tissues from clinically affected young pigs obtained from a herd undergoing a PRRS outbreak.

Semen collection

Five boars were trained to mount a dummy and semen was collected twice weekly for 6 weeks prior to challenge, on the day of challenge, and then twice weekly for 5 weeks PC using the gloved hand technique.[10] Gloves (Baxter Healthcare, McGaw Park, Illinois) were changed between each boar. Two boars (numbers 5664 and 5666) failed to adapt to the dummy and were collected by electroejaculation[11] beginning on the day of challenge, and then twice weekly for 5 weeks.

Seminal fluid was collected in 400-mL beakers containing a disposable plastic collection bag. To remove the gel fraction, ejaculate was directed onto a sterile gauze covering the mouth of the beaker. For each collection, the sperm-rich fraction and a sperm-poor fraction were collected separately. Following semen collection, the gauze containing the gel fraction was discarded and each fraction of semen was stored at -80°C in 4- to 5-mL aliquots.

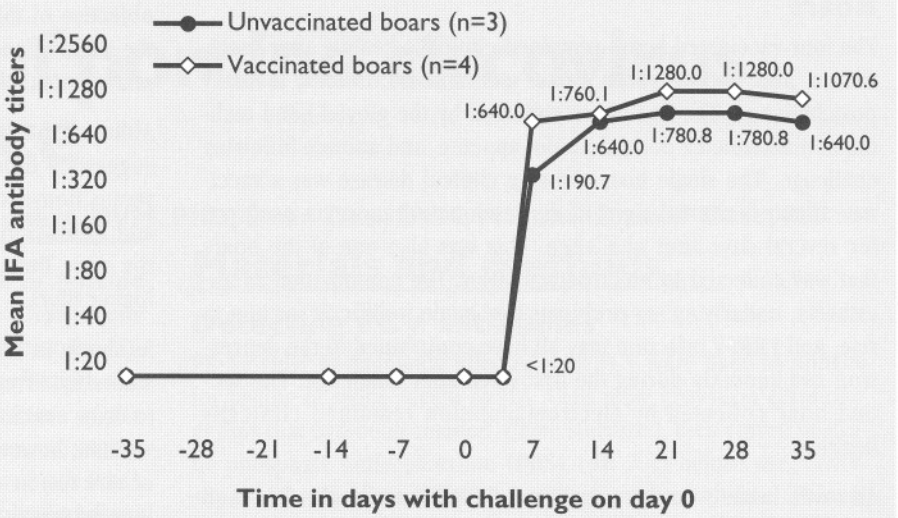
Blood collection

Blood was collected from each boar at approximately 45 day intervals for 3 months prior to vaccination. All seven boars were bled at the time the four boars were vaccinated: 5 weeks and 2 weeks prior to challenge and on the day of challenge (day 0). Following challenge, samples were collected on days 4, 7, 10, 14, and then weekly through day 32 PC, at which time the boars were euthanized. Serum for virus isolation, Western immunoblot assay, and IFA testing was stored at -80° C. Prior to serological testing and virus isolation, serum samples were randomized and animal or date identifiers were removed. Virus isolation from serum was performed as previously described.[12]

Western immunoblot assay

PRRS viral antigens were prepared by infecting confluent MA104 cell monolayers with PRRS virus at a concentration of 10^4 $TCID_{50}$, incubating for 4 days at 37°C, and disrupting in lysis buffer containing protease inhibitors. Control antigen was prepared in the same manner using uninfected MA104 cells. A modified Laemmli procedure was used to separate proteins on a discontinuous slab gel consisting of a 5% stacking gel and 14.0% resolving gel, cross-linked with bis-acrylamide at a ratio of 30.0:0.8. Electrophoresis was carried out on a vertical mini-gel apparatus as directed by the manufacturer. Immediately after SDS-PAGE, viral or cellular proteins and molecular weight markers separated in gels were electrophoretically transferred to a 0.45-mm nitrocellulose membrane using a mini-trans-blot electrophoretic transfer cell by following the recommended procedure of the manufacturer. The membranes were blocked overnight at 4°C with 1% gelatin dissolved in TBS and then reacted with 1:5 diluted serum samples. Antigen-antibody reactions were visualized with optimally diluted goat anti-swine IgG labeled with peroxidase and TMB peroxidase substrate. Appearance of virus-specific reactivity was assessed by comparing the antibody responses to viral and cellular antigens. This procedure was performed five times on each of the prechallenge serum samples.

Figure 1



Mean IFA antibody titers against PRRS virus in vaccinated and unvaccinated boars.

Tissue samples

Tissues collected from the boars at the time of necropsy included: lung, spleen, kidney, bone marrow from the femur, vas deferens, epididymis, testicle, prostate, seminal vesicles, bulbourethral gland, prepuce, and penis. Tissues for virus isolation and histopathology were processed as previously described.[1]

PRRSV in semen

The presence of PRRSV in semen was determined by a swine bioassay. Individually housed PRRSV uninfected pigs were inoculated intraperitoneally (IP) with a 13- to 15-mL sample of semen (equal volumes of sperm-rich and sperm-poor fractions) from a single boar collection. Serum samples were collected from SB pigs at the time of IP inoculation and at weekly intervals thereafter. Two or more consecutive IFA-positive results from weekly samples were considered indicative of the presence of infectious PRRSV in the semen inoculum. Otherwise, SB pigs were followed for a total of 5 weeks PC.

Results

Boars

The four vaccinated boars remained clinically healthy after vaccination and six of seven boars remained clinically healthy postchallenge. The five boars collected by the gloved-hand technique maintained a normal libido, appetite, and attitude following challenge. The single boar showing clinical disease was a vaccinated boar—clinical signs of depression and anorexia occurred for several days after challenge. This was also one of the boars that was collected by electroejaculation. The combination of anesthesia, underlying leg problems that made it difficult for him to rise, and PRRSV infection may all have contributed to the depression and anorexia during the first week after challenge. The second boar collected by electroejaculation remained clinically healthy.

All seven boars were seronegative for PRRSV antibodies by IFA at the time of challenge. The four vaccinated boars and two of three nonvaccinated boars had detectable IFA antibody titers by day 10 PC and subsequent IFA antibody titers were >640 through day 32 PC (Figure 1). After challenge, IFA titers rose more rapidly in the vaccinated group of boars and by day 21 PC the mean antibody titer in the vaccinated boars was two-fold higher than the nonvaccinated boars. All serum samples collected from vaccinated and nonvaccinated boars prior to challenge were negative for PRRSV antibodies on the Western immunoblot assay except for samples collected from two of the vaccinated boars on the day of challenge (day 0). The PRRSV antibodies detected in the day-0 serum of vaccinated boars 31 and 32 were specific for the 15 kd, 19 kd, and 26 kd viral proteins. All seven boars were viremic on day 4 PC and a detectable viremia was present for as long as 28 days in one nonvaccinated boar (Table 1).

At postmortem, no gross lesions were observed in the boars and histologic findings were unremarkable. Virus isolation was negative for all tissues collected at the time of necropsy.

PRRSV in semen

In all boars, PRRSV was present in semen beginning with the first collection on day 4 PC (Table 2). Challenged, nonvaccinated boars shed virus in their semen until the time of euthanasia on day 32 PC. The four vaccinated boars shed virus in semen through days 4, 7, 25, and 28 PC, respectively.

Discussion

Previously, it was shown that infectious PRRSV may be shed in the semen of infected boars for a considerable period of time, even in the absence of clinical disease.[9] Yaeger, et al., showed that PRRSV could be transmitted to naive gilts via naturally contaminated semen.[6] Cumulatively, these studies suggest that PRRSV-contaminated semen may play a role in transmitting PRRS virus to gilts or sows. Prolonged seminal shedding and transmission of PRRSV by boars is similar to the seminal shedding and venereal transmission known to occur in stallions infected with equine arteritis virus (EAV), an agent closely related to PRRSV. In stallions, vaccination against EAV has been shown to reduce or eliminate seminal shedding of virus and the development of the carrier state.[13,14] The objective of this work was to make a preliminary assessment of the effect of an inactivated PRRSV vaccine on the duration of viremia, seminal shedding, and serum antibody response in boars.

Although the number of boars used in this study is small, the data suggests a difference in serum antibody response in vaccinated versus nonvaccinated boars following challenge. Both vaccinated and nonvaccinated boars developed IFA titers at approximately the same time; however, the vaccinated boars developed slightly higher titers.

As shown in Table 1, duration of viremia among boars varied considerably. Given the number of animals in the study, it is difficult to draw conclusions as to the effect of vaccination on duration of viremia; however, the data suggest a trend for a decreased period of viremia in vaccinates. The two boars that were viremic the longest were collected by electroejaculation. We can speculate that the longer duration of viremia was in part due to the added stress associated with anesthesia and electroejaculation.

All three nonvaccinated boars were shedding virus in semen at the end of the study on day 32 PC. These results are compatible with a previous report of prolonged seminal shedding of PRRSV by boars[9] and a recent study carried out by researchers at South Dakota State University, in which four boars infected with PRRSV were found to shed PRRSV in their semen through at least day 21 PC.[15] In contrast, the duration of seminal shedding of PRRSV in two boars that responded serologically to vaccination, as determined by Western immunoblot assay, was considerably shorter (through days 4 and 7 PC, respectively) than the control boars used in this study (32 days) and shorter than the duration of semen shedding reported in a previous study (through days 13–43 PC). The variable duration of semen shedding and occasional intermittent shedding of PRRSV by boars makes it difficult to accurately assess the effect of vaccination on the duration of seminal shedding. However, when the information on the duration of seminal shedding from all of these studies is considered, it appears that vaccination reduced the length of seminal shedding in two of the vaccinated boars. The effect of vaccination on the two

Table 1

Presence of PRRS virus in serum after experimentally infecting boars

	Day postchallenge							
Boar	0	4	7	10	14	21	28	32
31*	–	+	–	–	–	–	–	–
32*	–	+	–	+	–	–	–	–
5664*†	–	+	+	–	+	+	–	–
5665*	–	+	–	–	–	–	–	–
33	–	+	+	–	+	–	–	–
5666†	–	+	+	+	+	–	+	–
6725	–	+	–	–	–	–	–	–

*Vaccinated boars

†Electroejaculated boars

Table 2

Presence of PRRS virus in semen after experimentally infecting boars

	Day postchallenge									
Boar	0	4	7	11	14	18	21	25	28	32
31*	–	+	+	–	–	–	–	–	–	–
32*	–	+	–	–	–	–	–	–	–	–
5664*†	–	+	+	+	+	+	+	+	–	–
5665*	–	+	+	+	+	–	+	–	+	–
33	–	+	+	+	+	+	+	+	+	+
5666†	–	+	+	+	+	+	+	–	–	+
6725	–	+	+	+	+	–	+	+	+	+

*Vaccinated boars
†Electroejaculated boars

remaining vaccinates is difficult to assess. These boars stopped shedding earlier than the nonvaccinated boars, but within the time period previously observed in nonvaccinated boars. Since the nonvaccinated boars were still shedding virus at the termination of the experiment, it is not possible to compare the mean duration of shedding between vaccinated and nonvaccinated groups.

Seminal shedding of PRRSV presents a risk for producers purchasing boars or semen for AI. Boars were found to shed virus in the semen after they were no longer viremic and had seroconverted by the IFA test. For example, boars 5665 and 6725 were no longer viremic by day 7 PC and had seroconverted by the IFA, but were still shedding virus in the semen on days 28 PC and 32 PC, respectively. This implies that the only way to determine whether a boar is shedding PRRSV in semen is to evaluate the semen for the presence of virus. This poses substantial problems. Virus isolation on cultivated cells is commercially available and relatively inexpensive, but virus isolation from semen is not a sensitive procedure (i.e., false negatives). Swine bioassay and polymerase chain reaction (PCR) are being used in research situations to detect PRRSV in semen, but both tests are expensive and are not readily available for routine use.

At present, we do not have a resolution to the dilemma of seminal shedding of PRRSV. There are no PRRSV vaccines currently licensed for use in breeding swine. The modified live virus (MLV) vaccine recently licensed in the United States is only approved for use in young pigs. Product literature indicates that pigs vaccinated with the MLV vaccine develop a prolonged viremia following vaccination. Until proven otherwise, it may be assumed that breeding pigs would also develop a viremia after vaccination and that boars could shed live vaccine virus in the semen. Although a small number of boars were involved in this study, the results suggest that inactivated vaccines may reduce the length of seminal shedding in boars. Certainly, these results justify further research into the use of inactivated PRRSV vaccines for breeding swine.

Implications

- Boars infected with PRRSV shed virus in the semen for extended periods.
- Inactivated PRRSV vaccines may reduce or prevent seminal shedding.
- Boars may shed PRRSV in the semen in the absence of a detectable viremia or serum antibody response.

Acknowledgments

This study was supported by Bayer Corporation, Agriculture Division — Animal Health, Shawnee Mission, Kansas.

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