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Porcine reproductive and respiratory syndrome virus and the experimentally infected boar

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Porcine reproductive and respiratory syndrome virus and the experimentally infected boar

by

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ABSTRACT

The general focus of this dissertation is the effect of porcine reproductive and respiratory syndrome (PRRS) virus on the experimentally infected boar and the role of the boar in transmission of PRRS virus (PRRSV) via artificial insemination. The manuscript was written in 3 parts. The effect of PRRSV on semen parameters and seminal shedding of PRRSV in the boar is reported in Paper I. The potential for transmission of PRRSV to gilts through artificial insemination with virus-contaminated semen is reported in Paper II, and in Paper III the use of a killed PRRSV vaccine in boars to reduce seminal shedding of virus is evaluated.

The results of a study in which mature boars were experimentally infected with PRRS virus and monitored for clinical signs of disease, changes in semen parameters, and the presence of PRRSV in the semen are reported in Paper I. Viremia and development of antibody titers were monitored following challenge and reproductive tissues were collected for virus isolation and histopathologic examination. Mild clinical signs of PRRSV infection were noted in 2 of the boars, but changes in semen parameters were not seen. The boars were viremic following challenge and developed indirect fluorescent antibody and serum-virus neutralization titers. Virus was not detected in any of the reproductive tract tissues and no histological lesions were present. Virus was detected in the semen of the 4 infected boars at the time of first collection on either day 3 or day 5 post-challenge and for 13, 25, 27, and 43 days following challenge.

In Paper II, estrus cycles of gilts were hormonally synchronized to allow for artificial insemination of control gilts with semen from a PRRS negative boar. The boar was then infected with PRRSV and semen was collected following infection for artificial insemination of an additional group of gilts. The boar became clinically ill following infection and developed PRRSV antibodies. No lesions were observed in the reproductive tract by histopathologic examination and virus was not isolated from the reproductive tract. All of the gilts remained seronegative for PRRSV antibodies, even though one group of gilts was inseminated with semen containing virus from an experimentally infected boar. There was a marked difference in pregnancy rates one month after insemination, with 4/6 control gilts pregnant and 1/5 gilts receiving virus-contaminated semen pregnant.

The gilts remained clinically normal following insemination and virus was not detected in any of the reproductive tract tissues. Histopathologic examination of reproductive tracts was normal.

The extended seminal shedding of PRRSV discussed in Paper I, led to the evaluation of a killed PRRSV vaccine discussed in Paper III. Four boars were vaccinated intramuscularly with an experimental killed PRRSV vaccine 5 weeks and 2 weeks prior to challenge. The vaccinated boars and 3 control boars were challenged and semen was collected twice weekly for detection of PRRSV. Boars were also evaluated for the development of PRRSV antibodies following infection and the development of a viremia. All 7 boars were viremic at the time of first bleeding on day 4 post-challenge. Vaccination appeared to shorten the length of viremia and seminal shedding of virus in some boars.

INTRODUCTION

Dissertation Organization

This dissertation begins with an abstract and is followed by a review of the literature and a statement of the problem. Three papers present the experimental work performed. The doctoral candidate is the primary investigator and senior author for all 3 papers. The dissertation concludes with a general discussion, additional references, and acknowledgements.

Literature Review

History of PRRS

Porcine reproductive and respiratory syndrome (PRRS) is a relatively newly recognized viral disease of swine. This syndrome was originally called mystery swine disease (MSD) in the United States because the etiologic agent had not been identified.[58] Since its recognition as a disease syndrome of swine, PRRS has been identified by a variety of names. Some of the names this syndrome has been given include: swine infertility and respiratory syndrome (SIRS),[8] abortus blauw,[6] porcine epidemic abortion and respiratory syndrome (PEARS),[151] Seuchenhafter Spätabort der Schweine (infectious late abortion of swine),[88] plague of 1988 - 1989,[7] blue ear disease,[7] blue-eared pig disease,[62] disease '89,[91] pig plague '89,[91] SMEDI-like syndrome,[91] swine reproductive failure syndrome,[91] mystery pig disease (MPD),[115] porcine viral syndrome,[115] new pig disease,[115] and plague of 1988.[115] Porcine reproductive and respiratory syndrome was designated the official name at the First International Symposium on SIRS/PRRS held in St. Paul, Minnesota in 1992.[103] The first cases of PRRS were reported in the United States[58] and Canada[78] in 1987. In November of 1990, the first cases of PRRS in Europe were reported in Germany.[63] During 1991 and 1992 PRRS spread rapidly through Europe with the Netherlands,[154] Spain,[128] Belgium,[149] the United Kingdom,[121] France ,[13] and Denmark[112] reporting cases of PRRS. Prior to the development of a diagnostic method for identifying PRRS infected herds, a diagnosis of PRRS was based on clinical presentation.

Clinical presentation of PRRS was utilized in a survey of members of the American Association of Swine Practitioners in order to try to identify the approximate time of recognized appearance of PRRS and spread of PRRS virus (PRRSV).[166] Criteria used for recognition of PRRSV infected herds included: 1) anorexia, 2) pyrexia, 3) respiratory disease in young pigs, 4) increased stillbirths, 5) increased early farrowings, and 6) increased numbers of mummified fetuses. Results of this survey revealed 1611 herds were considered to be infected in 19 states. Infected herds were also recognized in Chile, Canada, and Switzerland. The first recognized case fitting the criteria was in 1980, with most of the cases being recognized beginning in 1987. Following the development of a serological test for identification of PRRSV antibodies, serological surveys of banked sera were undertaken to identify when PRRSV first encountered the swine population. Swine serum samples collected during the 1980's as part of the Iowa National Animal Health Monitoring System (NAHMS) were evaluated for PRRSV antibodies. Serum samples from 1938 animals representing 91 herds were evaluated.[120] Porcine reproductive and respiratory syndrome virus was found to have encountered naive Iowa swine prior to 1985 and rapidly spread through the state after its introduction to the swine population. In another serological survey of samples collected from 25 Minnesota herds between 1981 and 1991, PRRSV infection was serologically apparent by 1986.[158]

Clinical presentation

The clinical presentation of pigs infected with PRRSV is highly variable, being dependent on a number of factors including age of the pig, health status, management practices, immune status, and reproductive status. In the breeding age pig, general clinical signs associated with PRRSV infection include anorexia, pyrexia, respiratory disease such as coughing and dyspnea, malaise, and in a small number of cases, cyanosis of the extremities. In the breeding age female, clinical signs associated with PRRSV infection also include reproductive failure. As defined by Keffaber, reproductive failure is any failure in reproductive performance from the time of breeding to the time of weaning.[91] Clinical manifestations of reproductive failure in the breeding age female include delayed returns to estrus or lack of cycling, abortions, premature farrowing, and increased numbers of stillborn pigs, mummified fetuses, and pigs born weak which fail to thrive and die shortly after

birth. In the breeding age boar, changes in semen quality following infection have been reported.[49,64,65,156] As many as 25% of boars tested in infected herds had a temporary decline in semen quality.[49,65] It has been speculated that the increased returns to estrus may be due to post-infection infertility of boars.[70]

Infected nursing pigs have respiratory signs including coughing and dyspnea ("thumping"). Diarrhea has been reported to be associated with PRRSV infection in some herds.[27,59] Many of the nursing pigs will die within the first week of life. Nursery pigs are prone to secondary infection with a variety of bacteria including *Streptococcus suis, Salmonella choleraesuis, Hemophilus parasuis, Pasteurella multocida* and *Actinobacillus pleuropneumonia* .[59,93] Infected nursery pigs often become chronic poor-doing pigs. In the older grow/finish pigs clinical disease may present as respiratory disease and poor doing pigs.[28,29]

The clinical presentation of herds infected with PRRSV may be subclinical, acute (epidemic), or chronic (endemic). Subclinically infected herds do not have the typical reproductive and respiratory syndrome associated with PRRSV infection. The only indication of infection is the development of PRRSV antibodies. In the acute form, naive herds infected with PRRSV have severe reproductive failure in the breeding age female and respiratory disease in pigs of various ages.[39,48] Initial presentation is as anorexia, pyrexia, respiratory disease, and malaise in the breeding age pig and finishing pigs. Shortly after this phase, there is an increase in the number of abortions and premature farrowings in those females infected close to the time of parturition and the number of stillborn and weak pigs born increases. Females infected earlier in gestation will have larger numbers of mummies in their litters at the time of parturition. The epidemic phase has been reported to last anywhere from several weeks to several months.[64,81,113] In the endemic form of PRRS, herds become infected and fail to return to the performance level that was present prior to infection. In the breeding age female, reproductive performance is reduced and early infertility problems continue to be a problem for the herd. In younger pigs, the chronic form of PRRS is present as respiratory disease with increased problems of disease due to secondary pathogens. Increased susceptibility to secondary infection results in the development of chronic poor doing pigs that require extra feed, medication, and days to reach market weight.[27,28,93]

Economic impact

The PRRSV has had dramatic economic effects on swine production at both the farm and international levels. Initial outbreaks of PRRS lead to a European Community ban on export of pigs from infected herds in an attempt to prevent the spread of disease.[5] Problems with animal welfare due to lack of adequate facilities for finishing hogs being held on breeding farms and the continuing spread of disease with the movement restrictions eventually led to changes in export restrictions.[62] In the United States (U.S.), pig exports were affected when Mexico and South Korea totally banned the importation of U.S. pigs and Japan restricted importation to pigs from herds in which clinically affected pigs had not been introduced 30 days prior to shipment.[5] Export of live pigs to Korea and Brazil and the export of pig products to South Africa was suspended when PRRS was diagnosed in Britain.[8] Semen importation from Britain and Canada were suspended, respectively, by South Africa and Australia.[8] Imported German pigs are believed to have been the source of PRRSV infection in Spain.[127]

The economic losses to the swine producer are due to pigs lost, nonproductive sow days, establishing larger sow and boar inventories in order to maintain maximum facility usage, increased costs to finish market hogs, increased costs due to veterinary services and drugs, and increased labor costs. A financial model has been developed to evaluate the economic impact of PRRSV infection on a herd during the acute outbreak and assumes that infected herds return to normal following the outbreak.[132] Estimated losses have been reported to be $236/sow in inventory. In herds with "cyclic"[93] or "secondary waves"[50] of infection and herds with chronic problems following infection, determining economic losses due to PRRSV is more difficult.

In determining economic losses, pigs lost include those pigs that had the potential to be born alive but were lost due to abortion, premature farrowing, stillbirth, or mummification, and pigs that were born alive but died prior to being marketed. A study of 4 acutely infected herds in Great Britain showed that up to: 1) 3.3% of sows aborted, 2) 20.6% of sows farrowed prematurely, 3) 26.0% of the pigs born were stillborn, 4) 18.8% of pigs born were mummified, and 5) 88.0% of the neonatal and preweaning age pigs died.[83] In a study of 11 infected herds in Indiana, the number of pigs weaned/litter ranged from 1 - 4 and the nursery death rate approached 50%.[91] In the same study it was found that farms with continuous

farrowing lost a minimum of 10 - 15% of their annual production during the outbreak. In a German study of breeding herds, 200 sows were evaluated for pig losses.[19] During the outbreak the mean total of lost pigs/litter rose from a preoutbreak level of 22.3% to 67.0%. Following the outbreak the mean total of lost pigs/litter returned to a preoutbreak level of 20.3%.

Reduced conception rates of close to 50% reported in some PRRSV infected herds have added to the economic impact of this disease by increasing the number of nonproductive sow days.[91] Following infection, pigs destined for market are often undersized, stunted, and have retarded growth.[92] In a study of 12 finishing herds the average mortality of finishing hogs rose from 2.2% to 4.3% and lung lesions at slaughter increased from 45% to 70%.[29] Losses in the nursery and finishing units have been reported to be more severe in herds with significant enzootic diseases and in herds with lower standards of hygiene and management.[29,93] Secondary infections with *Salmonella choleraesuis, Streptococcus suis, or Hemophilus parasuis* have resulted in morbidity rates of 70% and mortality rates of 15 - 25% .[93] Secondary infections lead to increased vaccine and drug costs and decreased average daily gains.[50,93] Finishing pigs have been reported to take an additional 4 - 6 weeks to reach market weight following herd infection with PRRSV.[144]

Identification of the etiologic agent

Prior to the isolation of PRRSV, a number of organisms were implicated as the cause of PRRS. Attention was focused on the traditional agents associated with the SMEDI (stillborn, mummification, embryonic death, infertility) syndrome, which includes porcine parvovirus, pseudorabies virus, leptospira, porcine enteroviruses, encephalomyocarditis virus, and hog cholera.[34] Determination of the causative agent of PRRS was complicated by the fact that several of these agents as well as mycoplasma, swine influenza virus, a paramyxovirus-type virus, *Chlamydia psittaci,* and *Streptococcus suis* were isolated from suspected PRRS cases.[1,26,30,47,52,53,81,85,124,152] Additional complicating factors arose when researchers in Canada described a proliferative and necrotizing pneumonia (PNP) associated with reproductive disease that was different from the traditional interstitial pneumonia seen with PRRS virus infection.[109] Attention was also focused on the role of the mycotoxin fumonisin in PRRS.[11,12]

Prior to the identification of PRRSV, the infectious nature of PRRS was shown through experimental inoculation of pigs with tissue filtrates from naturally infected pigs. Inoculation of young pigs resulted in typical PRRS interstitial pneumonia and inoculation of pregnant sows resulted in premature farrowings, increased numbers of stillbirths, and large numbers of mummified fetuses.[36,38,43,76,100] Identification of the Lelystad virus, the causative agent of PRRS, occurred in 1991 in the Netherlands.[154] Virus was isolated on porcine alveolar macrophages. Following isolation in macrophages, the virus was shown to produce typical PRRS when inoculated into pigs.[151] The first reported isolation of PRRSV (VR-2332) on a continuous cell line (CL2621) occurred in the United States in 1991.[42] As with the Lelystad virus, VR-2332 was shown to produce typical PRRS when inoculated into pigs.

Properties and classification

The PRRSV is an nonhemagglutinating enveloped RNA virus with a 45 - 83 nm diameter, a nucleocapsid of 25 - 35 nm and a density of 1.14 mg/ml .[20,23,117,153] The virus is stable for at least 1 month at 4 C and for several months at -70 C and is completely inactivated at 48 hours when held at 37 C and at 45 minutes when held at 56 C.[20] Infectivity of the virus is reduced over 90% at a pH less than 5 or greater than 7.[21] Viral titers of 10^5 - 10^6 $TCID_{50}$/ml have been demonstrated in CL2621 cells.[21] The replicative cycle of PRRS virus is 9 - 12 hours[131] and occurs in the cytoplasm.[21,23,128] The nucleocapsid obtains an envelope by budding through the membrane of the smooth endoplasmic reticulum and is released from the cell by exocytosis.[131]

The PRRSV has properties similar to a group of positive-stranded RNA viruses which include lactate dehydrogenase-elevating virus of mice (LDV), equine arteritis virus (EAV), and Simian hemorrhagic fever virus (SHFV).[125,126] The PRRSV nucleic acid is positive-stranded polyadenylated RNA of approximately 15 kb and consists of 8 open reading frames (ORFs).[45,105] Based on amino acid sequence elements which are found in PRRSV and conserved in EAV and LDV, ORFs 1a and 1b are predicted to encode the RNA polymerase.[105] Open reading frames 2 - 6 have been speculated to encode membrane-associated proteins and ORF 7 to encode the nucleocapsid protein.[105] Comparison of the amino acid sequences of the ORFs of PRRSV, LDV, and EAV indicate that PRRSV is more similar to LDV than to

EAV.[89,105] Equine arteritis virus, LDV, SHFV, and PRRSV all replicate in macrophages.[125] Electron microscopy of cell cultures infected with PRRSV, EAV, or LDV show similar viral and virus-induced structures.[119] No serological cross reaction has been demonstrated between PRRSV and common porcine viruses, many of the RNA enveloped viruses, LDV, or EAV.[23,119,151] Clinically, PRRSV resembles EAV in that infection can be asymptomatic or result in respiratory disease or abortion.[125] Similarities between PRRSV and LDV, EAV, and SHFV suggest that these viruses belong to the recently proposed family Arteriviridae.[45,105]

Antigenic variation

With the development of serological tests for detection of PRRSV antibodies, serological surveys have been performed to assess the prevalence of PRRSV infection. Examination of serological test results has revealed a number of herds serologically positive for PRRSV antibodies with no history of PRRS. The presence of PRRSV antibodies in herds without clinical disease and varying clinical reactions of pigs to challenge with different PRRSV isolates has led to speculation of the existence of virus strains with varying degrees of virulence.[77,111,119] Variants of the related viruses EAV, LDV, and SHFV are known to occur and this was suggestive that PRRSV variants would also occur.[125]

The existence of PRRSV antigenic variation has been confirmed by evaluating the serological responses of sera with PRRSV isolates from around the world. In one study sera collected from German breeding and finishing swine were tested for PRRSV antibodies using the European and U.S. strains of PRRSV.[18] Most of the sow sera were found to be positive with the European virus and negative with the U.S. virus. Of 131 sera collected from finishing pigs 84 were positive with the U.S. isolate and all 131 were positive with the European isolate. The higher the titer with the European virus, the more likely the sample would test positive with the U.S. virus. In another study, 837 sera were collected from 87 herds in 18 states and tested for PRRSV antibodies using the Lelystad and VR-2332 virus isolates.[15] Almost 58% of the samples tested contained PRRSV antibodies and 36.1% of the samples contained antibodies which reacted with both viruses. Lelystad virus antibodies were found in 20.1% of the samples and VR-2332 virus antibodies were found in 43.8% of the samples. Additional studies with monoclonal antibodies have shown European, Canadian, and U.S. isolates of PRRSV are antigenically similar with conserved

epitopes on the 15 kD protein, which suggests cross reaction occurs.[22] Antigenic variation of PRRSV has also been examined by comparing partial nucleotide sequences and amino acid homology between U.S., Canadian, British, and Dutch isolates.[89] Isolates from North America were found to have 87 - 95% sequence homology and only 64 - 67% homology with the European isolates. Amino acid homology between North American and European isolates was 69 - 76%.

Pathogenesis

As previously mentioned, the clinical presentation of pigs infected with PRRSV is highly variable and dependent on a number of factors including age of the pig, health status, management practices, immune status, virulence (strain) of PRRSV, and reproductive status. Pigs are susceptible to PRRSV infection via oronasal, oral, intramuscular, intrauterine, intravenous, and intraperitoneal routes.[44] The sites of replication of PRRSV have not been fully elucidated, although there is a predilection for replication in swine alveolar macrophages (SAMs).

The incubation period has been estimated to be 3 - 5 days with anorexia and fever being the first clinical signs noticed.[42,146] Viremia can be detected as early as 1 day after exposure[35] and has been reported to last for as long as 8 weeks.[62,97,139] Lungs of affected pigs may appear normal on gross examination, provided infection is not complicated by the presence of secondary pathogens.[40,41,59] Experimentally infected pigs killed 2 days following exposure to PRRSV were found to have interstitial pneumonia.[130] Vascular lesions including swollen endothelial cells and thrombi have been suggested to play a role in the transient blue discoloration of extremities that has been reported to occur in some infected pigs.[68] It could also be speculated that the occasionally reported subcutaneous[74] and periorbital[128] edema may also be due to vascular lesions.

Very little is known about the pathogenesis of PRRSV infection in the boar and the effect of virus on the boar reproductive system. Seminal shedding of PRRSV received attention following an epidemiological study which implicated semen as the source of virus in naive herds which had used semen from infected boars and subsequently seroconverted.[137] Ohlinger[116] reported that viral antigen was not detected in semen smears and attempts to isolate virus from semen of naturally infected boars using cell culture techniques has not been successful.[116,137] Seroconversion was also not shown when seronegative pigs were inoculated with

semen samples suspected of containing PRRSV.[116] Researchers at South Dakota State University were able to demonstrate the presence of PRRSV in semen when semen from 2 experimentally infected boars was used to artificially inseminate 2 seronegative gilts.[157] Both gilts had clinical signs of PRRS and seroconverted following insemination. The source of virus in seminal shedding of PRRSV is unknown. The presence of viremia in boars has been suggested as a source of PRRSV contamination of semen.[116] Ohlinger[116] reported that attempts to isolate virus from the reproductive tracts of infected boars were unsuccessful, however, Wensvoort[104] has reported isolation from the genital tract of a boar euthanized 2 weeks after exposure but not from boars euthanized at later dates.

The effect of PRRSV infection on boar reproductive function is also not known. Decreases in semen quality have been reported in naturally infected boars.[49,156] Alterations in spermatozoa motility and morphology were observed in a study of boars infected with PRRSV at 6 artificial insemination centers.[65] In contrast to the previous reports, evaluation of semen quality following experimental inoculation of 2 boars resulted in no changes in concentration, color, progressive motility, or spermatozoa morphology.[157] However, a decrease in volume between pre- and post-infection levels was noted. Two gilts bred with semen from the 2 experimentally infected boars were not pregnant 7 weeks after breeding, suggesting a possible alteration in reproductive performance of the boars. Post-infection infertility of boars has been implicated as a cause of increased returns to estrus.[70] It is not clear if the early infertility observed is due to the inability of spermatozoa to fertilize eggs or due to viral effects resulting in death of the embryo. Deposition of PRRSV into the uterus following natural breeding did not result in a difference in pregnancy rates between exposed and control gilts (Kelly Lager, National Animal Disease Center, Ames, Iowa, personal communication), however, deposition of virus in the uterus was not performed until after the second of 2 breedings performed on consecutive days.[98] If the infertility is due to an effect of PRRSV on the egg/embryo, it may be there is a small window of opportunity when the virus needs to be present and this window may have been missed.

The pathogenesis of PRRSV infection in the breeding age female has been studied extensively. Two approaches have been used to evaluate PRRSV effects on female reproductive performance. One approach has been to inoculate sows and evaluate the effect on the fetus and the second has been to directly inoculate the

fetus or fetal fluids in order to by-pass the maternal component. It is not known if the reproductive effects seen with PRRSV infection are due to maternal, fetal, or an interaction of maternal and fetal changes. Inflammatory and degenerative changes in placentae have been reported and virus-like structures has been identified in endothelial cells of fetal and maternal placental capillaries indicating the potential for placental passage of virus from dam to offspring.[141] Virus has also been isolated from the placenta of a sow experimentally inoculated intranasally with PRRSV.[35] The potential for placental transfer of PRRSV was realized when virus was isolated from piglets born to sows intranasally exposed to PRRSV and PRRSV antibody was identified in precolostral blood.[35,142]

Studies have been performed to evaluate the effect of PRRSV infection during early, mid, and late gestation. Results indicate there is a fetal age difference in susceptibility to development of PRRSV related disease. In one study, sows were inoculated intranasally with PRRSV between 45 and 50 days of gestation.[35] Sixty-nine of 71 fetuses collected on days 7, 14, or 21 were found to be alive at the time of collection. Virus was not isolated from the fetuses, however, virus was isolated from 2 live pigs born to sows approximately 65 days following exposure to PRRSV. The effect of direct inoculation of fetuses with PRRSV was also evaluated in sows between 40 and 45 days of gestation. Fetuses exposed to PRRSV by intramuscular inoculation and collected 4 or 11 days after exposure were found to be alive and virus was isolated from the fetuses. In related research, Lager et al. evaluated the effect of PRRSV on fetuses when amniotic fluid was inoculated with PRRSV.[97] Virus was isolated from fetuses in early, mid, and late gestation that were collected 7 - 31 days following exposure. The younger fetuses were found to replicate virus until midgestation without gross changes, at which time they began to die. These two studies suggest that fetuses of multiple gestational ages are capable of becoming infected with PRRSV but only fetuses of certain ages are susceptible to PRRSV disease. Theories as to the cause of age dependent susceptibility include: 1) nonpermissive maternal-fetal junction until late gestation, 2) development of a susceptible fetal cell population during midgestation, and 3) a fetal immune component which enhances viral replication.[35,97]

Immunology

The development of immunity to PRRSV has been shown to occur in experimentally infected pigs.[69,128] Eight gilts challenged intranasally with PRRSV at 86 - 93 days of gestation gave birth to an average of 5.8 live pigs, 0.6 stillborn, and 2.1 mummies. The same females which were bred 5 months after exposure and challenged at 93 days of gestation (7 - 8 months after first challenge) gave birth to an average of 10.8 live pigs, 0.5 stillborn, and 0.3 mummies. The return of performance parameters to normal following a PRRS outbreak indicates natural immunity develops following exposure to PRRSV,[19,31,73,113,145] however, some herds have chronic or cyclic problems following initial exposure to PRRSV indicating the virus may continue to circulate in the herd.[140]

Infection with PRRSV often leads to secondary infections, suggesting immunosuppression or alteration of immune system functions.[92,108,140,144,155] Galina et al. reported on the interaction of PRRSV with *Streptococcus suis* (*S. suis*) in the development of secondary streptococcal infections.[67] Specific pathogen free pigs were divided into 4 groups. Group 1 received PRRSV and 4 days later received media. Group 2 received PRRSV followed 4 days later with *S. suis*. Group 3 received media followed by *S. suis* 4 days later and group 4 received *S. suis* and 4 days later received PRRSV. Development of clinical central nervous signs typical of *S. suis* infection were evident only in the group 2 pigs indicating PRRSV affects on the host immune system make the host more susceptible to secondary infection.

As mentioned previously, PRRSV replicates in SAMs and results in the destruction of the macrophage. Lung lavages obtained following infection of pigs have revealed decreases in the proportion of SAMs collected from >95% of the cells collected to approximately 50% by day 7 post-challenge, with a relative increase in the proportion of neutrophils.[165] Changes in cell functions were observed, as evidenced by an increase in inflammatory cytokines and a decrease in nonspecific bactericidal activity. Detection of viral and cellular antigens on SAMs using flow cytometric analysis has revealed a down regulation or loss of cellular antigens and failed to show antigens recognized by antisera.[118] Reports of prolonged viremia in the presence of antibody[87,118,143,159] may be due in part to the alterations in cell surface antigens. Splenic periarteriolar lymphoid sheaths, tonsillar crypts, mesenteric lymph nodes and the thymic cortex have been found to be depleted of lymphocytes and enlargement of splenic red pulp macrophages have been observed

following experimental infection of young pigs.[130] Changes in T-lymphocyte subpopulations[165] and decreases in peripheral blood leukocytes have also been noted in experimentally infected pigs.[35,81]

Experimental studies have been performed to evaluate the interaction of PRRSV with the host immune system. Three groups of pigs ages 1, 4, and 10 weeks were evaluated for their ability to respond to foreign antigen with antibody production or cell mediated immunity following challenge with PRRSV.[108] Antigens evaluated for humoral immune system responses were *Brucella abortus, Escherichia coli* pili antigens, and killed pseudorabies virus. Cell mediated immunity responses were evaluated by priming and challenging with dinitrofluorobenzene and measuring the delayed type hypersensitivity (DTH). Results indicated there was no evidence for suppression of the immune response, but rather an antibody and DTH enhanced alteration of the immune system. Additional research has evaluated antibody dependent enhancement (ADE) of PRRSV infections. *In vitro* studies utilizing various dilutions of PRRSV antibody mixed with PRRSV have shown that infectious virus titers in macrophages are enhanced 10 - 100 times by the presence of antibody.[33] In another study, weaned pigs were passively immunized with heat inactivated sera obtained from experimentally infected pigs.[161,162] Blood collected from the weaned pigs over an 80 day period was evaluated for levels of PRRSV antibody using the immunoperoxidase monolayer assay (IPMA) and serum virus neutralization (SVN) test and evaluated *in vitro* for ADE activity. Mean ADE activity was first noted on day 20, peaked by day 40 and gradually declined until it became undetectable between days 59 and 63. The ADE activity was found to develop when SVN antibody levels were less than 1:3 and declined at approximately the same rate as IPMA titers. The authors suggest that a 3 - 4 week window of increased susceptibility may occur in pigs with low levels of passive maternal antibody. The phenomenon of ADE has also been evaluated *in vivo*. Fetuses of sows between 40 and 45 days of gestation were inoculated with uninfected cell culture medium, PRRSV, or PRRSV and antibody.[35] The enhanced replication of PRRSV in fetuses receiving antibody and virus compared to fetuses receiving virus only may explain why immunocompetant late term fetuses appear to be more susceptible to PRRSV.

Very little is known about the role of colostrum and passive maternal antibody in PRRSV infections. Antibody has been detected in the colostrum of experimentally infected sows.[151] Passive maternal antibody has been reported by Goyal[71] to persist until 4 - 5 weeks of age and has been reported by Van Alstine[148] to persist 6 - 8 weeks and occasionally up to 16 weeks in pigs nursing immune dams. Yoon et al. have suggested there may be an increased period of susceptibility to PRRSV when low levels of passive maternal antibody are present in the pig.[161,162] As discussed by Molitor, passive immunity can be protective against challenge.[107] Pigs from nonimmune dams were passively given PRRSV antibodies and then challenged. Pigs receiving antibody were not protected following challenge, while challenged pigs born to immune dams were protected. These findings suggest that antibody alone is insufficient to protect from disease and that cell mediated immunity may play an important role in protecting pigs from PRRS.

Epidemiology

Seroprevalence in the United States

Sera collected as part of the Iowa NAHMS project revealed that PRRSV spread rapidly following introduction into the swine population of the state.[120] Seroprevalence studies conducted to assess the spread of PRRSV in the United States revealed variable infection rates. In a survey of sera collected during the second quarter of 1992 from cull breeding swine in 11 states, 7.3% of the samples were positive by indirect fluorescent antibody testing (IFA).[10] Samples collected from 17 states in 1990 as part of the NAHMS project found 36% of herds tested seropositive by IFA.[16,110] The seroprevalence of positive herds ranged from 0% - 82% with a mean seroprevalence of herds within states of 33%. Increased prevalence was associated with the pig dense areas of the United States.

Host species

Limited information is available regarding species susceptible to PRRSV infection. Virus isolation performed on tissues and sera from trapped wild mice and rats obtained from an endemically infected herd were negative for PRRSV.[82] Experimental inoculation of mice and rats intranasally, intraperitoneally, and orally yielded negative results on virus isolation of tissues and sera and gross and microscopic examinations were normal. Experimental inoculation of various species of birds indicates that some birds are susceptible to PRRSV infection.[168] Muscovey

ducks, guinea fowl, Cornish cross chickens, and mallard ducks were inoculated orally via the drinking water with PRRSV. Feces were then collected for virus isolation. Muscovey ducks were found to be resistant to infection, while guinea fowl and chickens were somewhat susceptible to infection. Mallard ducks were found to be highly susceptible and shed virus in their feces through day 24 post-challenge.[168] Clinical signs were not evident in any of the birds and seroconversion was not demonstrated (Jeff Zimmerman, Veterinary Diagnostic Laboratory, Iowa State University, Ames, Iowa, personal communication). Human susceptibility to PRRSV has not been reported and seroconversion has not been demonstrated in laboratory personnel working with the virus.[60]

Transmission

The original source of PRRSV is currently unknown, however, based on serological surveys it is known that PRRSV spreads rapidly through a naive swine population.[120] The routes of transmission of PRRSV from infected to susceptible swine are just beginning to be understood. Transmission of PRRSV to uninfected swine can occur by direct contact with infected swine and has been associated with the purchase and movement of infected swine to uninfected herds.[31,32,55,62,70,91,101,135,136,146,149] German pigs imported into Spain have been incriminated as the source of PRRSV infection in Spain.[127-129] Yoon et al. recently reported on viral shedding of PRRSV in nasal secretions and feces in experimentally infected pigs and found shedding to parallel viremia.[159] In contrast to the report by Yoon et al., virus has not been demonstrated in the feces of experimentally infected pigs using SAMs as the indicator system (Robert Wills, Veterinary Diagnostic Laboratory, Iowa State University, Ames, Iowa, personal communication) and brings into question the role of feces in the transmission of PRRSV. As discussed by Goyal, virus has been found in urine and may be a source of transmission to naive pigs .[71] Transmission has also been shown to occur when weaned pigs are exposed to older pigs.[93] If young pigs are weaned into an isolation facility, pigs do not seroconvert until coming into contact with older pigs.

Seroconversion has been shown to occur when uninfected pigs are comingled with infected pigs several weeks after the pigs were infected.[143,159,167] Seronegative finishing pigs seroconverted when comingled with experimentally infected sows 14 weeks following inoculation.[167] Transmission could not however be shown when seronegative pigs were placed in contact with challenged pigs 26 weeks after

inoculation .[143] The extended period of transmissibility of PRRSV from infected pigs raises questions as to where the virus localizes in the body and if pigs become latent carriers of the virus. Immunosuppression studies using corticosteroids to reactivate virus in experimentally infected pigs were unsuccessful in pigs receiving prednisolone-acetate at 12 weeks post-inoculation.[143] In a related study, three experimentally infected pigs treated with 2 mg/kg dexamethasone intramuscularly for 5 days beginning 110 days after challenge did not become viremic, IFA titers did not change, and virus was not isolated from nasal swabs (Swenson et al., unpublished observations).

Airborne transmission of PRRSV has been speculated to happen and has been estimated to take place at distances of up to 20 kilometers,[2,9,31,46,61,62,64,78,91,95,96,101,135,136,138] however, no evidence has been presented showing airborne transmission happens. In fact, research in progress has been unable to show airborne transmission between infected and uninfected pigs housed in isolation units with a common air source (Robert Wills, Veterinary Diagnostic Laboratory, Iowa State University, Ames, Iowa, personal communication). The role of fomites and vectors in the transmission of PRRSV is unknown, however, under certain conditions it appears PRRSV can survive in the environment for extended periods of time. Pigs placed in facilities used for PRRSV studies seroconverted after being placed in non-disinfected rooms 3 weeks and 4 weeks after the rooms had been emptied.[104] The role of meat from infected pigs in transmission of PRRSV is also not known.

Information about reproductive transmission of PRRSV is just beginning to become available. As previously mentioned, transplacental transmission of PRRSV has been shown to occur.[35,142] The role of semen in transmission of PRRSV is not clearly understood. Epidemiological investigations in Britain implicated semen collected from subclinically infected boars as the source of infection for 9 herds[136] and an epidemiological study in the United States suggested that fresh semen was the source of infection in another herd.[157] Artificial insemination of 2 gilts with freshly collected unextended semen from experimentally infected boars resulted in the development of clinical signs of PRRS and seroconversion by both gilts.[157] Information about the transmission of PRRSV via embryo transfer is not currently available.

Risk factors

Based on experimental findings the use of fresh unextended semen from infected boars, purchase of infected pigs, and placement of pigs in contaminated facilities increases the risk of PRRSV infection occurring. Additional factors implicated as being associated with increased risk of PRRSV infection include density of pigs in the area, climatic conditions, herd size, lack of quarantine facilities, and indiscriminate movement of vehicles and people on a premises.[9,17,62,95,150]

Diagnosis

A preliminary diagnosis of PRRSV infection is based on clinical presentation. Reproductive disease may be characterized by early infertility problems such as poor conception rates or by late term problems such as abortions, weak piglets, and increased numbers of stillborns and mummies. In the respiratory form, a preliminary diagnosis is based on chronic respiratory disease in nursery and/or finishing pigs. There are no pathognomic gross or histopathologic lesions, however, interstitial pneumonia is suggestive of PRRSV infection.[59] A diagnosis of PRRS may be complicated by the presence of secondary pathogens in the lung.[59] Serological tests and tests for detection of virus or viral antigen have been the primary focus for evaluation of PRRSV infection.

Serology

Four serological tests are currently used for the detection of PRRSV antibodies. These tests are the enzyme-linked immunosorbent assay (ELISA), indirect fluorescent antibody test (IFA), immunoperoxidase monolayer assay (IPMA) and serum-virus neutralization test (SVN). The ELISA has been reported to be a simple, cheap, and rapid test to perform following plate sensitization.[4] Antibody titers are reported to appear within 2 weeks of exposure and sows have been found to remain seropositive for at least 5 months.[3] A disadvantage of the ELISA is background reaction which makes this test unsuitable for use as an individual pig test.[4,123] The IPMA is reported to detect antibody by day 6 post-challenge and antibodies can be detected as long as 12 months following challenge, however, in some cases antibody levels decline to undetectable levels by 4 - 6 months.[118] Interfering background reactions require high serum dilutions thereby making the test unsuitable for use as an individual pig test.[123] The IFA test is widely used in the United States for detection of PRRSV antibodies. Titers are detectable by

day 7 post-challenge and rise rapidly by day 14 - 21.[72] Pigs have been reported to become seronegative on the IFA test by 6 - 9 months following infection.[72] The SVN test has primarily been used as a research tool rather than a diagnostic tool due to reports of the test being less sensitive than the preceding 3 tests and due to the fact that SVN antibodies have been thought to be delayed in development compared to IFA antibodies.[25,66,72,106,111] Yoon et al. recently reported on a modification of the SVN test.[160] Addition of 20% swine serum to the protocol resulted in detection of SVN antibodies at 9 - 11 days following exposure to PRRSV.

Limited information is available regarding duration of antibody in the serum of infected pigs. Much of the information available is based on field cases, which can be misleading since time of infection can not be accurately identified in many cases. Yoon et al. compared the IFA, IPMA, and SVN tests for first appearance of antibody, peak antibody production, and decline in antibody titers.[163] Antibody was detected by all 3 tests within 2 weeks of infection, with the IPMA detecting at 5 - 7 days, the IFA at 7 - 10 days and the SVN at 10 - 15 days post-infection. Peak antibody titers occurred at 4 - 5 weeks with the IFA, 2 months with the SVN, and 5 - 6 weeks with the IPMA. Antibody titers detected by IFA rapidly declined following the peak, while IPMA and SVN antibody titers gradually declined. At the conclusion of the sampling period on day 105 post-challenge there was no difference in detection of positive samples by IPMA and SVN, however the IFA test detected fewer positive samples. Assuming a constant decay rate, regression analysis was used to determine when antibody titers would fall below detectable limits. The seronegative threshold was found to be 144 days, 297 days, and 341 days for the IFA, IPMA, and SVN tests. The short duration of IFA antibodies has led to recommendations to test young pigs in order to obtain an accurate picture of PRRSV infection in a herd. Testing of breeding stock only may underestimate the prevalence of PRRSV infection.

Diagnostic information obtained from serological testing has been complicated by the existence of antigenic variants of PRRSV which may escape detection due to the strain of virus in use at a diagnostic laboratory, lack of standardization of serological tests between diagnostic laboratories, high prevalence of PRRSV infected herds, and high seroprevalence within herds. Diagnosis of PRRSV infection as the cause of reproductive failure or respiratory disease is dependent on showing seroconversion using paired samples or a change in titer in

paired samples. Endpoint titers on the IFA test are usually not performed due to the time consuming nature of the test and the cost of performing such a test. In addition, the limited availability of the SVN test has precluded its use as a routine diagnostic tool. For these reasons, serology is primarily used to determine if a herd has been exposed. For a definitive diagnosis, virus isolation and detection of viral antigen are the tests of choice.

Virus isolation

Cells used for virus isolation include: SAMs,[14,51,52,86,122,151,164] the proprietary cell line CL2621,[14,24,66,79] monkey kidney cells (MA104),[94] and cloned MA104 cells (MARC145).[94] Isolation of PRRSV has been reported from lung,[51,72,84,93,102,118,144,147,148] spleen,[72,93,147,148] lymph nodes,[72,148] thymus,[72,147,148] tonsil,[72,148] serum,[13,37,60,72,84,102,118,123,147,148] plasma,[118] buffy coat,[93] urine,[71] feces,[159] placenta,[35] and nasal swabs .[123,159] There are no reports of virus isolation from the semen of infected boars. This may be due to the presence of components in the semen which are cytotoxic for cell cultures.[133,134] Lung and serum are reported to be the samples of choice for virus isolation.[84] Presence of a long viremia in young pigs has made serum a good sample for virus isolation. Due to the transient viremia in mature pigs, viremia may not be present in sows and gilts at the time of abortion or premature farrowing.[148] In cases of late term abortions and early farrowings samples should be collected from weak born pigs rather than mummies, aborted, or stillborn pigs.[59,87,148,164] Poor virus isolation results from mummies, aborted, and stillborn pigs may be due to inactivation of virus in dead pigs. In a study of the susceptibility of PRRSV to different environmental temperatures, virus isolation from positive tissues was 47%, 14%, and 7% when tissues were held at 25 C for 24, 48, and 72 hours.[147] In contrast, virus isolation rates were $\geq$85% from tissues stored at 4 C and -20 C. Serum was thought to have a protective effect on virus as virus was isolated from all but 1 serum sample held at 25 C for 72 hours. Therefore, it has been recommended that samples for virus isolation be kept at refrigerator temperatures during shipment to diagnostic facilities in order to enhance the ability to isolate virus.

The differences in susceptibility of SAMs from one pig compared to another, labor intensive procedure for collection, and problems of infection with mycoplasmas and other agents has led to the investigation and development of stable cell lines which will grow PRRSV. A problem that has been recognized with

the development of cell lines is that the ability to isolate virus from samples of infected pigs has been complicated by the fact that isolates do not uniformly grow in all cell types. In a comparison of SAMs and CL2621 cells, 98 tissues and 73 sera were evaluated for the presence of virus.[14] Seven tissue isolates were made in SAMs and 4 in CL2621 cells. Eighteen of 73 serum samples were found to contain virus using SAMs, but only 2 isolations were made in CL2621 cells. Out of 82 isolates obtained in CL2621 cells, 25 would not grow in SAMs and 28 of the isolates that grew in SAMs did not produce cytopathic effects. Out of 18 isolates obtained in SAMs, 5 would not grow in CL2621 cells. The difference in isolation abilities of various cell systems raises the question as to whether samples for virus isolation should be inoculated in more than one cell type.

Viral antigen detection

Little information is available on detection of viral antigen in tissues. Benfield[22] has reported on the development of monoclonal antibodies which have been used to identify antigen in lungs of infected pigs and Done[60] has reported on the use of fluorescent antibody testing with spleen and lung. Indirect fluorescent antibody tests for detection of virus in semen has been attempted, but has been found to consistently fail at identifying virus.[157] An immunohistochemistry test has recently been reported to detect PRRSV in lung[75] and has been used for PRRSV detection in tonsil, thymus, spleen, lymph nodes and heart (Pat Halbur, Veterinary Diagnostic Laboratory, Iowa State University, Ames, Iowa, personal communication).

Prevention and control

Since transmission of PRRSV is not completely understood, prevention of PRRSV infection utilizes the same strategies which are used to prevent infection with other swine diseases. Prevention of PRRSV infection is dependent on management strategies which maintain high biosecurity standards such as limiting traffic on the premise, cleaning and disinfecting transport vehicles, limiting access to buildings, and maintaining rodent control programs. Purchase of breeding stock should be done by matching donor and recipient herds with the same PRRS status and stock should preferably be purchased from a limited number of sources. All replacement pigs should be placed in quarantine and tested prior to entry into the herd. The safety of using purchased semen rather than purchased boars for

preventing PRRSV infection is unknown at this time due to the lack of information on seminal shedding of PRRSV and transmission through extended semen.

The control of PRRSV following infection of a herd is based on symptomatic treatment and management practices which reduce stress, minimize exposure to secondary pathogens, and prevent exposure of naive pigs to infected pigs. Recommended symptomatic treatment includes use of electrolytes, antiinflammatory drugs, and antibiotics .[70,99,114] Management practices designed to reduce stress include maintaining adequate environmental temperatures, feeding good quality feed, eliminating drafts, maintaining clean and dry facilities, and delaying iron injections, teeth clipping, and tail docking.[90,99,114,156] Strategies to minimize secondary pathogen exposure and their effects include vaccination for pathogens found on the farm, maintenance of well ventilated draft-free clean facilities, widespread use of antibiotics, and preventing exposure to older pigs. The use of all-in/all-out production strategies, age segregation, and multisite production have been used to stop circulation of PRRSV by preventing naive pigs from coming in contact with infected pigs.[57,93] Management of the breeding herd is another key area for control of PRRSV circulation. Cyclic rebreaks of PRRS which occurred every 3 - 4 months in a herd were found to be associated with the purchase of seronegative gilts.[55]

Vaccines are currently not available, however work is in progress to develop vaccines to protect against PRRS. An experimental inactivated vaccine utilized in a limited number of sows was found to protect against reproductive disease when sows were challenged.[128] Control sows delivered 36 stillborn pigs and no normal pigs while vaccinated sows delivered 23 normal and 2 stillborn pigs. Difficulties may arise in developing PRRSV vaccines with protective antigens due to antigenic variation and in developing vaccination protocols which minimize the potential for the ADE phenomenon.

Limited information is available on eradicating PRRSV from a herd once it is infected. Strategies such as depopulation/repopulation, partial depopulation, test and removal, modified medicated early weaning, and multisite production have been used for other important swine diseases and may be useful in eliminating PRRSV from infected herds.[54,56,57] However, the long range success of these programs for elimination of PRRSV is not known.

Statement of the Problem

Limited information is available about the effects of PRRSV infection on the boar in regards to clinical disease, seroconversion, duration of viremia, effect on semen parameters, and occurrence of seminal shedding of PRRSV and little is known about the role of the boar in seminal transmission of PRRSV. Artificial insemination is becoming increasingly important to the swine industry as a means of improving genetics in a herd while reducing the risk of disease introduction that can occur with the purchase of boars. Epidemiological information suggests that artificial insemination with semen from infected boars may be responsible for transmission of PRRSV.

The purpose of this work was to better define the effects of PRRSV infection on the boar, assess the effect of an experimental PRRSV vaccine in reducing the duration of seminal shedding of PRRSV, and evaluate the ability of extended semen from an experimentally infected boar to transmit PRRSV to naive gilts.

EXCRETION OF PORCINE REPRODUCTIVE AND RESPIRATORY SYNDROME (PRRS) VIRUS IN SEMEN FOLLOWING EXPERIMENTAL INFECTION OF BOARS

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Abstract

Four boars intranasally inoculated with 4 ml ($10^{6.5}$ $TCID_{50}$/ml) porcine reproductive and respiratory syndrome (PRRS) virus (ATCC VR 2402) were monitored for 56 days following exposure. A fifth, unchallenged boar was included as a negative control. Semen samples were collected for 4 weeks prior to infection and for 8 weeks following infection using the gloved-hand technique. Volume, pH, motility, concentration, and sperm morphology were evaluated for each semen sample collected. Blood samples were collected weekly and PRRS virus antibody titers were determined using the indirect fluorescent antibody (IFA) and the serum-virus neutralization (SVN) tests. Virus isolation was performed on serum samples. Boars were euthanized 56 days post-inoculation and tissues were collected for virus isolation and histopathologic examination.

Isolation of virus from semen is technically difficult because of the toxicity of seminal components for cell systems. As an alternative to *in vitro* testing, the presence of PRRS virus in semen was determined by swine bioassay (SB). Four- to eight-week-old SB pigs were inoculated intraperitoneally with 13 - 15 ml of semen. Following semen inoculation, pigs were housed individually and tested for antibodies against PRRS virus at weekly intervals. Positive IFA results on 2 or more consecutive weekly serum samples signaled the presence of infectious PRRS virus in

the semen sample. Isolation of PRRS virus from a subsample of SB pigs provided validation of the test system.

Clinically, 2/4 boars showed mild respiratory signs of 1 day duration following infection. No changes in appetite, behavior, or libido were detected. All boars seroconverted on both the IFA and SVN tests by day 14 post-infection. Virus was isolated from serum between days 7 and 14 post-inoculation. During the monitoring period there was a decrease in semen volume and a corresponding increase in pH, however, this change began 7 - 10 days prior to infection. No differences in sperm morphology, concentration, or motility between the pre- and post-infection samples were observed. Virus was present in semen at the time of the first collection in each of the 4 boars, i.e., 3 days or 5 days after challenge. Virus was detected in nearly all semen samples collected from the 4 infected boars through days 13, 25, 27, and 43, respectively. Neither gross nor microscopic lesions attributable to PRRS virus were observed in tissues collected at the termination of the experiment (day 56), and virus isolation attempts from reproductive tissues were negative.

Introduction

Originally known as mystery swine disease, the first cases of porcine reproductive and respiratory syndrome (PRRS) were reported in the United States in 1987.[1,2] Isolation and identification of a newly recognized viral agent of swine as the cause of PRRS was reported in 1991.[3] Porcine reproductive and respiratory syndrome virus infection is recognized to cause reproductive failure in sows and gilts, including delayed return to estrus, reduced conception rates, increased repeat breedings, abortions, early farrowings, and an increased number of pigs born weak or dead. The effect of PRRS virus infection on boars has not been well characterized. Lethargy, anorexia, increased rectal temperatures, and loss of libido have been reported in boars infected with PRRS virus.[4-7] In field cases, most notably from The Netherlands, decreases in semen quality have been described in infected boars.[5,8,9] An epidemiologic study in Britain revealed circumstantial evidence that PRRS virus was spread to uninfected herds via purchased semen.[10] To date, attempts to isolate PRRS virus from infected boars have been unsuccessful, even when epidemiological evidence has implicated semen from infected artificial insemination (AI) studs as the

source of infection in previously naive herds.[10,11] Currently, concern regarding PRRS virus-contaminated semen is such that Australia and South Africa have stopped importing semen from countries in which PRRS has been reported.[12]

The purpose of this research was to study the course of PRRS virus infection in mature boars and 1) document the course of clinical signs, 2) assess the impact of PRRS virus infection on semen quality, 3) determine if virus was shed in the semen of infected boars and, if so, for what period of time, and 4) evaluate the lesions resulting from PRRS virus infection in reproductive and other tissues. Historically, cytotoxic factors in seminal fluids have made laboratory isolation of viruses from semen on continuous cell lines difficult or impossible to achieve.[13-19] Therefore, a swine bioassay system was developed for the detection of PRRS virus in semen. A similar approach has been used for monitoring the presence of other viruses in animal semen.[17,20-22]

Materials and methods

Animals and housing Five boars, 1 - 1.5 years old, were obtained from a PRRS virus-free herd and subsequently confirmed to be serologically negative for PRRSV antibodies by the indirect fluorescent antibody (IFA) and serum-virus neutralization (SVN) tests. The 4 challenged boars (115, 117, 119, 125) were housed in individual pens in the same room. The control boar (121) was housed in separate facilities to avoid inadvertent PRRS virus infection.

Four- to eight-week-old pigs used in the PRRS virus swine bioassay (SB) were also obtained from PRRS virus-free herds and were verified to be PRRSV antibody negative using the IFA test. Pigs were moved to individual isolation facilities prior to semen inoculation.

PRRS virus The PRRS virus (ATCC VR 2402) was originally derived from a pool of tissues from clinically affected young pigs obtained from a herd undergoing a PRRS outbreak. Inoculation of tissue homogenates into a gnotobiotic pig was followed by virus isolation in swine alveolar macrophages (SAM). The SAM isolate underwent limiting dilution cloning 3 times in SAMs, then adaption and plaque purification in an african green monkey kidney continuous cell line (MA104).

The titer of virus inoculum used in this study was determined by making serial ten-fold dilutions of virus in 96-well microtiter plates[a] using high glucose minimum essential medium[b] supplemented with 30μg/ml neomycin sulfate[c] and 1.2 mg/ml sodium bicarbonate. Virus dilutions were inoculated onto confluent MA104 cells in replicates of 5. Individual wells were observed for cytopathic effect at 5 days post-inoculation. Tissue culture infectious dose 50 titers ($TCID_{50}$/ml) were calculated using the Kärber method.[23]

Boar inoculation Boars were inoculated intranasally with 2 ml/naris of PRRSV at a concentration of $10^{6.5}$ $TCID_{50}$/ml. The virus was administered by use of a syringe fitted with a 16 gauge needle that had been shortened to approximately 5 mm in length. A 35 mm length of rubber tubing[d] (1.6 mm X 0.8 mm) was placed over the cut needle, to facilitate deposition of the virus in the nasal cavity.

Semen collection Semen samples were collected twice weekly prior to challenge, on the day of challenge, and then twice weekly for 8 weeks following challenge from the 4 infected boars. Specifically, 2 boars were collected on days 3, 7, 11, ... , 47, 51, 56 post-challenge (PC) and 2 boars were collected on days 5, 9, 13, ... , 49, 53, 56 PC. The control boar was collected weekly during the same time period.

Semen was collected using the gloved hand technique .[24-29] Polyvinyl chloride gloves[e] were used during semen collection. Gloves were changed between each boar. Seminal fluid was collected in sterilized, prewarmed beakers. To remove the gel fraction, ejaculate was directed onto a sterile gauze covering the mouth of a 400 ml beaker. To avoid cold shock of spermatozoa, the gauze-covered beaker was nested in a 600 ml beaker containing disposable plastic bags[f] filled with warm water. 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, semen was evaluated, and each fraction of semen was stored at -80 C in 4 -5 ml aliquots.

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[a] Corning Glass Works, Corning, NY.
[b] JRH Biosciences, Lenexa, KS.
[c] Sigma, St. Louis, MO.
[d] Fisher Scientific, Eden Prairie, MN.
[e] Baxter Healthcare, McGaw Park, IL.
[f] First Brands Corporation, Danbury, CT.

Semen evaluation Sperm motility was assessed on prewarmed slides within 30 minutes of collection. Sperm concentration was determined by diluting an aliquot of the sperm rich fraction in a 2.9% sodium citrate solution and comparing the optical density to standard spectrophotometric reference values. The pH of the sperm poor fraction was measured with a standard pH meter. Sperm morphology slides were made at the time of collection by mixing 1 drop of semen with 1 drop of eosin-nigrosin stain.[21] Slides were stored at room temperature until they were evaluated at the termination of the experiment. To avoid bias, all slides were assigned randomly ordered numbers and evaluated sequentially using differential interference contrast microscopy at 1,250 X (oil immersion).

Blood collection Blood was collected from each boar at approximately 30 day intervals for 3 months prior to challenge. Following challenge, samples were collected on days 0, 7, 10, 14, and then weekly through day 56 PC, at which time the boars were euthanized. Serum for virus isolation was stored at -80 C and serum for the IFA and SVN tests was stored at -20 C. Prior to serological testing, serum samples were randomized and boar or date identifiers were removed.

Serology *Indirect fluorescent antibody test* Eight chamber slides[g] were inoculated with MA104 cells and allowed to incubate at 37 C for 24 hours. All wells were inoculated with PRRS virus and then incubated at 37 C for an additional 36 - 48 hours. The slides were fixed in an 80% acetone/water solution, dried, and stored at -80 C until needed. Serum IFA titers were determined by making an initial 1:20 dilution of serum samples followed by 2-fold dilutions. The slides were incubated with serum dilutions for 30 minutes at 37 C, then rinsed. Goat anti-swine immunoglobulin fluorescent antibody conjugate[h] was then added and the slides were incubated for an additional 30 minutes at 37 C, after which the slides were rinsed, dried and read by ultraviolet microscopy.

Serum virus neutralization test Serum samples were heat inactivated at 56 C for 30 minutes and 2-fold serially diluted starting at a 1:2 dilution. The diluent used was high glucose minimum essential medium, containing neomycin sulfate and supplemented with 5% fetal bovine serum (FBS)[i]. Challenge virus, diluted in the same diluent was combined with the sera bringing the final virus concentration to

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[g] Nunc, Inc., Naperville, IL.
[h] Kirkegaard and Perry Laboratories, Inc., Gaithersburg, MD.
[i] HyClone Laboratories, Inc., Logan, UT.

50 $TCID_{50}$/0.05 ml. Virus-serum mixtures were incubated at 37 C for 1 hour and 0.05 ml of the mixture was placed on confluent monolayers of MA104 cells in 96-well plates. Cells were propagated in the same medium used to dilute the sera. Cells were observed at 5 days post-inoculation and the neutralization titer of the sera was recorded as the reciprocal of the highest serum dilution in which no cytopathic effect was observed. Serum derived from an experimentally infected pig with a neutralizing antibody titer of 1:16 was used as the positive serum control.

Virus isolation Tissues collected for virus isolation at the time of necropsy included: lung, spleen, kidney, bone marrow from the femur, vas deferens, tail and head of the epididymis, testicle, prostate, seminal vesicles, bulbourethral gland, prepuce, and penis. Tissue homogenates were centrifuged at 2,000 x g for 15 minutes. Tissue supernatant or serum was inoculated onto confluent MA104 cells in 25cm^2 flasks[a]. Inoculated cultures were incubated 18 - 24 hours at 37 C, after which the medium with inoculum was discarded and fresh medium with 4% FBS was replaced on each flask. Cultures were then incubated at 37 C and observed periodically for cytopathic effects. After each passage, cultures were frozen, thawed, and 2 ml of the cell culture medium and cells were inoculated on fresh, confluent MA104 cell cultures in 25 cm^2 flasks from which the growth medium had been discarded and replaced with fresh medium. Two passages were made. Cultures showing cytopathic effect were frozen, thawed, and subinoculated onto MA104 cells in tissue culture tubes[j]. When cytopathic effects were observed in the tissue culture tubes, or after 5 - 7 days, these cells were stained with PRRS virus fluorescent monoclonal antibody[k] and with a polyvalent porcine viral antiserum[l] and anti-porcine immunoglobulin fluorescent antibody conjugate[m].

Histopathology Testicle, epididymis, vas deferens, seminal vesicle, bulbourethral gland, urethra, prepuce, prostate, liver, spleen, and lung were collected for microscopic examination. Tissues were fixed in 10% neutral buffered formalin. Following routine processing, tissues were embedded in paraffin and 5 µm sections were stained with hematoxylin-eosin stain.

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[j] Bellco Glass, Inc., Vineland, NJ.
[k] Provided by D. Benfield, Department of Veterinary Science, South Dakota Center for Livestock Disease Control, South Dakota State University, Brookings, SD.
[l] National Veterinary Services Laboratories, Diagnostic Virology Laboratory, Ames, IA.
[m] National Veterinary Services Laboratories, Diagnostic Virology Laboratory, Ames, IA.

Bioassay of semen samples The presence of PRRS virus in semen was determined by a swine bioassay (SB). Virus uninfected pigs were inoculated with a semen sample, then serologically monitored for evidence of PRRS virus infection. Bioassay pigs were housed individually in isolation facilities to preclude exposure to PRRS virus from other sources. Each pig was inoculated intraperitoneally (IP) with a 13 - 15 ml sample of semen (equal volumes of sperm rich and sperm poor fractions) from a single boar collection using a 20 ml syringe and 20 gauge needle. 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 PRRS virus in the semen inoculum. Otherwise, SB pigs were followed for 5 weeks after inoculation.

Results

Boars The control boar remained clinically healthy throughout the study. Two of the infected boars showed mild sneezing and coughing of 1 day duration. Attitude, appetite, and libido of the 4 challenged boars were not affected following exposure to the PRRS virus. No abnormalities in semen parameters or morphology were observed other than a decrease in volume of the sperm poor fraction and a corresponding increase in pH. The decrease in volume and increase in pH began 7 - 10 days prior to challenging the boars. A corresponding change in the control boar was not seen.

The 5 boars were seronegative for PRRSV antibodies by IFA and SVN on day 0 and at all sampling points prior to challenge. The control boar remained seronegative on both tests throughout the study. One of the 4 challenged boars had a detectable IFA titer on day 7 PC. All 4 boars had detectable IFA titers by day 10 PC and subsequent IFA titers were $\geq$1:320 through day 56 (Fig 1). Detectable SVN antibody titers were evident in 2 of the challenged boars by day 10 PC and in the remaining 2 boars by day 14 PC (Fig 2).

All 4 infected boars had a detectable PRRS viremia between days 7 and 14 PC. At post mortem examination, no gross lesions were observed in the control or 4 challenged boars. No histologic lesions were present. Results of virus isolation were negative for all tissues collected at necropsy.

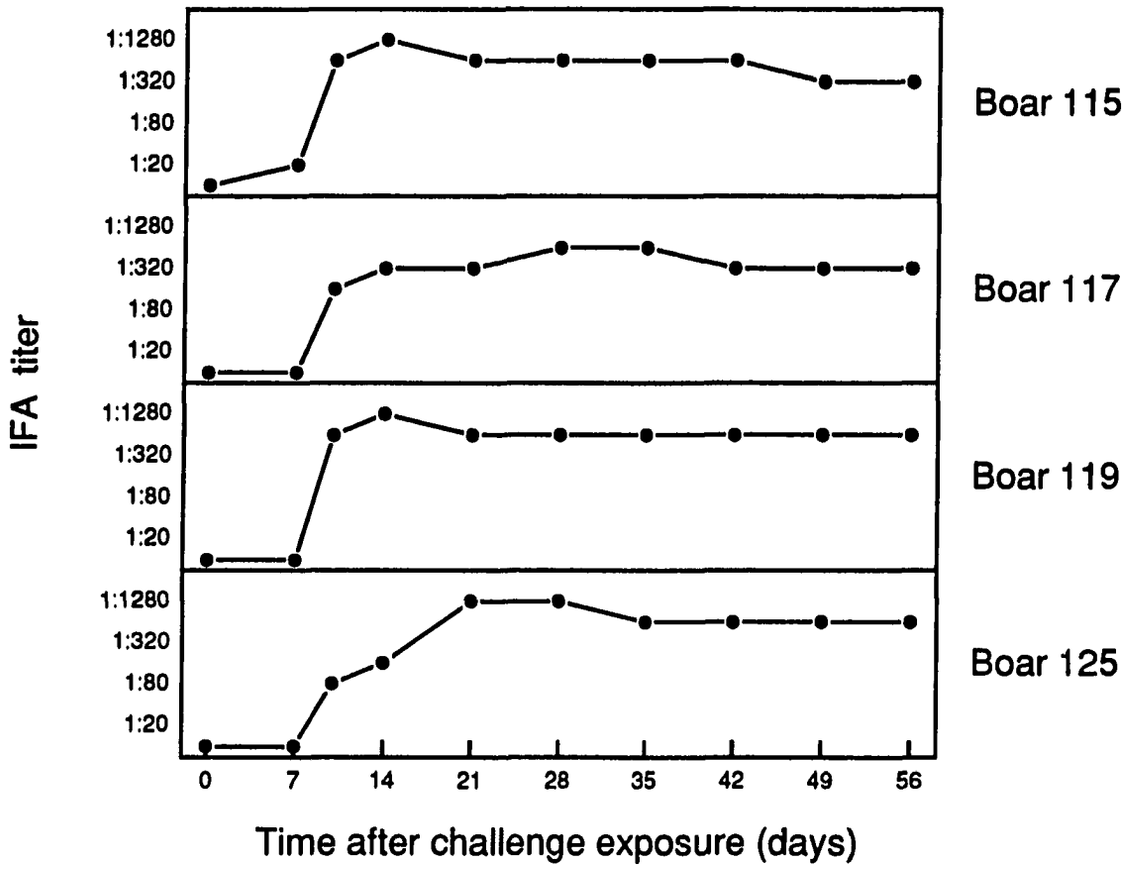

Figure 1. IFA titers following experimental exposure of boars

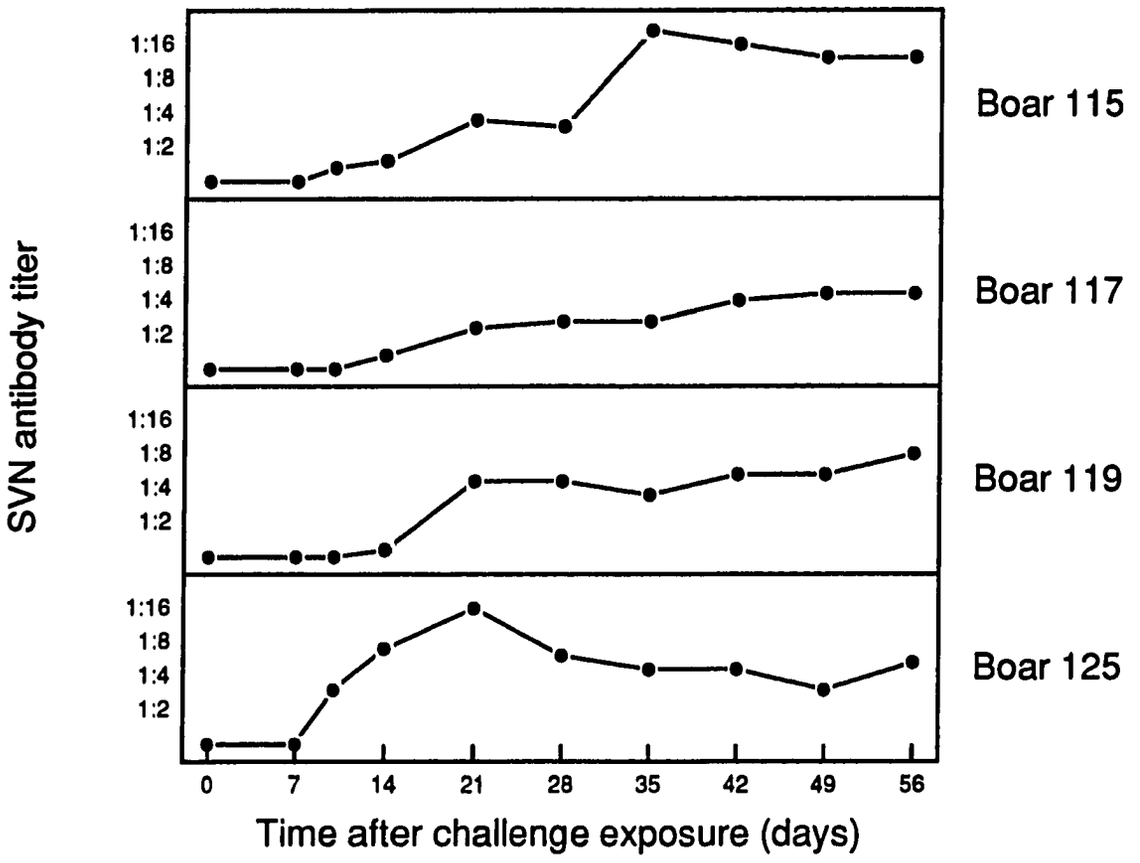

Figure 2. SVN titers following experimental exposure of boars

Bioassay of semen samples Bioassay results for all boars were negative on day 0 and, for the negative control boar throughout the collection period (Table 1). In all challenged boars, PRRS virus was present in semen beginning with the first collection (day 3 or day 5) following challenge. All 4 challenged boars shed virus in their semen through at least day 13 following challenge. Virus was detected in semen from the 4 challenged boars up to 13, 25, 27, and 43 days, respectively, following challenge.

To validate the bioassay protocol, virus isolation was attempted from a subsample of PRRSV antibody-positive SB pigs. Serum samples from 4 of the initial SB pigs, representing each of the 4 challenged boars, were assayed. In each case PRRS virus was isolated, providing evidence that the serological response in SB pigs was caused by infectious PRRS virus.

In general, inoculation with PRRS virus-contaminated semen did not produce clinical disease. Three SB pigs inoculated with PRRS virus-contaminated semen had mild respiratory disease consistent with PRRS virus infection, including nasal discharge and coughing of several days' duration. The remainder of SB pigs remained clinically healthy throughout the study.

Discussion

A number of viral infections are known to result in virus shedding in the semen of swine and other species. Men infected with cytomegalovirus and stallions infected with equine arteritis virus (EAV) may shed virus in semen for an extended period of time without having clinical signs of infection.[18,31,32] Enteroviruses, parapoxviruses, and the viruses of foot-and-mouth disease (FMD), bovine leukosis, infectious bovine rhinotracheitis (IBR), and bovine viral diarrhea (BVD) have been found in semen from infected bulls.[33-36] Foot-and-mouth disease virus, swine vesicular disease virus, porcine parvovirus, picornaviruses, adenoviruses, enteroviruses, Japanese encephalitis virus, pseudorabies virus (PRV), African swine fever virus, and reovirus have been recovered from the semen of infected boars.[19,33,37-43] To this list must be added PRRS virus.

Table 1. Presence of PRRS virus in semen following experimental infection of boars

	Day Post Challenge																										
Boar	0	3	5	7	9	11	13	15	17	19	21	23	25	27	29	31	33	35	37	39	41	43	45	47	49	51	53	56
115	–	+		+		+		+		+		+		+		+		+		–		+		–		–		–
117	–	+		+		+		+		+		+		+		–		–		–		–		–		–		–
119	–		+		+		+		–		–		–		–		–		–		–		–		–		–	
125	–		+		+		–		+		+		+		–		–		–		–		–		–		–	
121[a]	–			–			–				–			–				–			–				–			–

[a]Negative control boar.

Virus isolation from semen on continuous cell lines is difficult. Frequently, cytotoxic factors in semen inhibit the growth of the cells cultured for virus isolation. To circumvent this problem, inoculation of pigs was used to detect PRRS virus in porcine semen. Animal inoculation for the detection of viruses in semen is not a new procedure. This technique has previously been used for detection of IBR virus by inoculation of calves and sheep (Cornell semen test) and for detection of FMD virus by inoculation of cattle, mice, and pigs.[22,44] In addition, sheep have been inoculated with semen from bulls infected with bovine leukosis virus and rabbits have been inoculated with semen from boars infected with PRV.[20-21]

One obvious advantage of the bioassay technique was its ability to screen a large sample volume (13 - 15 ml) as compared to the microliter quantities typically assayed in cell systems. The ability to test large volumes of semen by animal inoculation has been taken advantage of by at least one artificial insemination center which examines semen for the presence of several common pathogenic viruses by inoculating susceptible animals with pooled aliquots of ejaculates in order to achieve surveillance of the entire herd.[17]

The results of this study provided evidence that the swine bioassay procedure detected viable PRRS virus in semen. Clinical signs in 3 bioassay pigs and viremia in 4/4 bioassay pigs indicated that the PRRS virus in semen was infectious, rather than inactivated. Serologically, bioassay pigs responded in a time frame similar to that of the intranasally exposed boars. Fifteen of the 25 SB pigs that seroconverted following IP injection with semen seroconverted by day 7 post-injection and 24/25 pigs seroconverted by day 14.

The seminal shedding of PRRS virus observed in this study is reminiscent of the prolonged seminal shedding reported for EAV, a closely related virus.[45] Stallions infected with EAV can shed virus in semen for a period of weeks to years following natural infection .[46] The continued excretion of PRRS virus after the development of neutralizing antibodies and the disappearance of viremia suggested that the virus continued to replicate within the reproductive tract. A similar situation is seen in stallions infected with EAV where carrier stallions constantly shed virus in semen, but virus is not detected in respiratory secretions, urine, or

blood.[33,34] While virus was not isolated from the reproductive tissues collected from the infected boars at the time of euthanasia, it is possible that virus replication was no longer occurring at the time of euthanasia, as evidenced by the fact that the boars had not shed virus in semen for 2 - 5 weeks prior to that time.

Viral infections may cause a reduction in semen quality. Lowered sperm concentration, decreased motility, and increased numbers of abnormal sperm have been reported for stallions infected with EAV, bulls infected with BVD virus, and boars infected with Japanese encephalitis virus.[36,40,48] Diminished semen quality has also been reported with boars infected with PRV or enteroviruses, bulls infected with IBR, and men infected with cytomegalovirus.[21,31,35,49] In this study, alterations in semen quality were not observed in association with PRRS virus infection. Sperm motility, concentration, and morphology remained within normal parameters throughout the experiment. Prior to challenge, a decrease in the volume of the sperm poor fraction and a corresponding increase in pH occurred in the infected boars but not in the control boar. These changes were primarily attributed to the management of the boars. Changing the feeding schedule so as not to conflict with semen collection resulted in boars showing less inclination to dismount the collection dummy early in order to eat. In contrast to these results, a decrease in semen quality in infected boars at artificial insemination centers has been reported.[5,8] Feitsma et al reported that the decrease in semen quality was severe enough to result in a 10% increase in the number of rejected semen samples.[5] In close agreement with the results reported here, Yaeger et al reported no abnormalities in semen color, or sperm concentration, motility, and morphology in 2 experimentally infected boars, although a decrease in volume compared to baseline was seen following exposure to the PRRS virus.[7]

This work showed that infectious PRRS virus may be shed in semen for a considerable period. In an important related development, Yaeger et al recently reported that 2 gilts inseminated with semen from 2 experimentally infected boars showed clinical signs of PRRS, developed antibodies against PRRS virus, and and failed to conceive.[7] Cumulatively, these studies suggest that PRRS virus-contaminated semen may play an important role in the transmission of PRRS virus

and early infertility problems in gilts or sows. Until the biology of PRRS virus infection in boars is better understood, these results pose significant problems for veterinarians and swine producers considering the purchase of herd boars or semen for artificial insemination.

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OUTCOME OF INSEMINATION OF GILTS WITH PORCINE REPRODUCTIVE AND RESPIRATORY SYNDROME (PRRS) VIRUS-CONTAMINATED SEMEN

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Abstract

Six gilts were artificially inseminated (AI) with extended semen from a boar free of porcine reproductive and respiratory syndrome (PRRS) virus infection. Then, one week following PRRS virus exposure, semen was collected from the boar, extended, and used to AI an additional 5 gilts. The boar was inoculated intranasally with 4 ml ($10^{6.5}$ $TCID_{50}$/ml) PRRS virus (ATCC VR 2402). All 11 gilts were bred 3 days in a row using freshly collected and extended semen on each of the 3 days. Gilts were bled on a weekly basis until they were euthanized. Serum samples were tested for the presence of PRRS virus antibodies by the indirect fluorescent antibody (IFA) test and for the presence of PRRS virus using virus isolation on swine alveolar macrophages. Due to the cytotoxic nature of semen for continuous cell lines, a swine bioassay was utilized to confirm the presence of PRRS virus in the semen. A 13 - 15 ml aliquot of undiluted semen was injected intraperitoneally into 4 - 8 week old individually housed seronegative pigs. Bioassay pigs were bled weekly for 5 weeks and serum was evaluated for the presence of PRRS virus antibodies using the IFA test. Two consecutive positive results on the IFA test indicated the presence of PRRS

virus in the semen. The boar was euthanized on day 21 post-challenge. The control gilts were euthanized on day 40 and the gilts exposed to PRRS virus-contaminated semen were euthanized on day 34 following the first insemination. Reproductive tract tissues were collected for virus isolation and histopathologic examination.

No clinical signs of PRRS were noted in the 11 gilts. The boar was depressed and anorexic for several days following challenge, but was physically normal by the time of collection 7 days post-challenge. Sperm motility and morphology were within normal acceptable limits for AI. Virus was detected in undiluted aliquots of semen collected on days 7 and 8 post-challenge, but not in the 3 samples collected prior to challenge or in the semen collected on days 9, 14, or 21 post-challenge. At the time of euthanasia, 4/6 control gilts were pregnant and 1/5 gilts exposed to PRRS virus-contaminated semen was pregnant. None of the gilts seroconverted on the IFA test and virus was not isolated from the serum or reproductive tracts. Virus was not isolated from the reproductive tract of the boar. No histopathologic lesions were noted in the reproductive tracts of the gilts or boar.

Introduction

Porcine reproductive and respiratory syndrome (PRRS) was first reported in the United States in 1987.[1,2] Infection in sows and gilts has been reported to cause reproductive failure characterized by delayed returns to estrus, reduced conception rates, increased repeat breedings, abortions, early farrowings, and an increased number of pigs born dead. Little is known about the role of the boar in female reproductive failure and the transmission of porcine reproductive and respiratory syndrome virus (PRRSV) via semen. An epidemiologic study conducted in Britain concluded that there was circumstantial evidence that PRRSV was spread to non-infected herds via purchased semen. Experimental infection of boars has led to seminal shedding of virus for up to 43 days following infection[3] and insemination of gilts with undiluted semen from experimentally infected boars resulted in 2/2 gilts seroconverting and 0/2 gilts pregnant.[4] 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.[5]

The purpose of this research was to 1) document the course of clinical signs of gilts inseminated with naturally infected semen, 2) determine if there was a

difference in pregnancy rates of gilts receiving normal and virus-contaminated semen, 3) determine if gilts would seroconvert following exposure to PRRSV via the semen, and 4) evaluate the lesions resulting from PRRSV infection in reproductive tissues.

Materials and methods

Animals and housing Eleven gilts and one boar were obtained from PRRS virus-free herds and subsequently confirmed to be serologically negative for PRRSV antibodies by the indirect fluorescent antibody (IFA) test. Gilts were housed in individual isolation facilities. Four- to eight-week-old pigs used in the PRRSV swine bioassay (SB) and as a source of swine alveolar macrophages (SAM) were also obtained from a PRRSV-free herd and verified to be PRRSV antibody negative using the IFA test. Pigs were moved to individual isolation facilities prior to semen inoculation.

PRRS virus The PRRSV (ATCC VR 2402) was originally derived from a pool of tissues from clinically affected young pigs obtained from a herd undergoing a PRRS outbreak. Swine alveolar macrophages were used to isolate virus from the tissues of a gnotobiotic pig inoculated with a pooled tissue homogenate from clinically affected young pigs. The SAM isolate underwent limiting dilution cloning 3 times in SAMs, then adaption and plaque purification in an african green monkey kidney continuous cell line (MA104). The titer of the virus inoculum used in this study was determined by making serial ten-fold dilutions of virus in 96-well microtiter plates[a] using high glucose minimum essential medium[b] supplemented with 30μg/ml neomycin sulfate[c] and 1.2 mg/ml sodium bicarbonate. Virus dilutions were inoculated onto confluent MA104 cells in replicates of 5. Individual wells were observed for cytopathic effect at 5 days post-inoculation. Tissue culture infectious dose 50 ($TCID_{50}$/ml) titers were calculated using the Kärber method.[6]

Boar inoculation The boar was inoculated intranasally with 2 ml/naris of $10^{6.5}$ $TCID_{50}$/ml PRRS virus 7 days after the first insemination of the control gilts. The virus was administered using a syringe which was fitted with a 16 gauge needle

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[a] Corning Glass Works, Corning, NY.
[b] JRH Biosciences, Lenexa, KS.
[c] Sigma, St. Louis, MO.

that had been shortened to approximately 5 mm in length. Rubber tubing[d] 1.6 mm X 0.8 mm was cut into a 35 mm length and placed over the cut needle in order to facilitate deposition of the virus in the nasal cavity.

Estrus synchronization and pregnancy evaluation The gilts were randomly divided into a control group (6 gilts) and a group exposed to virus via semen (5 gilts). The estrus cycles were synchronized so that the virus exposed group came into heat 14 days after the control group and 7 days after the boar was infected with PRRSV. Altrenogest[e] was given orally in the feed at a dose of 11 mg/gilt every 24 hours for 13 days. Dinoprost tromethamine[f] at a dose of 10 mg/gilt was injected intramuscularly (IM) the morning of day 14 and repeated 8 - 10 hours later. A single dose of a product[g] containing 300 IU/gilt human chorionic gonadotropin (HCG) and 600 IU/gilt pregnant mare serum gonadotropin (PMSG) was injected IM on day 15.

Pregnancy status was determined at the time of euthanasia. Statistical significance of pregnancy status between groups of gilts was determined using Fisher's exact test.

Semen collection Fresh semen was collected and extended on each day the gilts were artificially inseminated (AI). Semen was collected into prewarmed thermos bottles lined with a semen collection bag[h] as 2 gel-free fractions, sperm rich and sperm poor, using the gloved hand technique .[7,8,9,10,11,12] Polyvinyl chloride gloves[i] were used during semen collection. To remove the gel fraction, ejaculate was directed onto a sterile gauze covering the mouth of the thermos. Following semen collection, the gauze containing the gel fraction was discarded, semen was evaluated, and a small volume of each fraction of semen was stored at -80 C in 4 - 5 ml aliquots. Semen was collected 8, 7, and 6 days prior to challenge of the boar, at the time of challenge, and 7, 8, 9, 14, and 21 days following challenge.

Semen evaluation Sperm motility was assessed on prewarmed slides within 30 minutes of collection. Sperm concentration was determined by diluting an aliquot of the sperm rich fraction in a 2.9% sodium citrate solution and comparing the optical density to standard spectrophotometric reference values. Sperm

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[d] Fisher Scientific, Eden Prairie, MN.
[e] Hoechst-Roussel, Somerville, NJ.
[f] Upjohn Company, Kalamazoo, MI.
[g] Intervet Inc., Millsboro, DE.
[h] Swine Genetics International, Ltd., Cambridge, IA.
[i] Baxter Healthcare, McGaw Park, IL.

morphology slides were made at the time of collection by mixing 1 drop of semen with 1 drop of eosin-nigrosin stain.[13] Slides were stored at room temperature until they were evaluated at the termination of the experiment. To avoid bias, all slides were assigned randomly ordered numbers and evaluated sequentially using differential interference contrast microscopy at 1,250 X (oil immersion).

Semen extension and artificial insemination Semen extender[j] was prepared according to manufacturer instructions using filtered and heat sterilized water. Fresh extender was prepared at the time of first collection of the boar for AI in control gilts. Fresh semen was collected 3 days in a row for AI. Extender remaining after the first semen extension was refrigerated until use the next day, at which time the required volume of extender was warmed prior to mixing with semen. The same procedure was followed on the third day. Extender remaining after the third day of collection and AI of control gilts was discarded. Fresh extender was prepared at the time of first collection of the boar for AI in exposed gilts. Semen was extended such that each gilt received a total volume of 80 ml (15 ml semen and 65 ml extender) at each insemination and sufficient motile spermatozoa for pregnancy to occur.

Gilts were AI'd using commercially available disposable spiral catheters,[k] semen bottles,[l] and lubricant.[m] Back pressure was applied to induce the immobility response and the spirette was locked into the cervix following lubrication of the spirette. Extended semen was slowly deposited into the uterus. Gilts were AI'd 72, 96, and 120 hours after the HCG/PMSG injection. Extended semen was inseminated into gilts within 2 hours of collection.

Blood collection Blood was collected from each gilt monthly until the time of first insemination, and then weekly until the time of euthanasia. The boar was bled 3 weeks prior to challenge, the day of challenge, and following challenge on days 7, 14, and 21, at which time the boar was euthanized. Serum for virus isolation and IFA was stored at -80 C. Prior to serological testing, serum samples were randomized and pig or date identifiers were removed.

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[j] Swine Genetics International, Ltd., Cambridge, IA.
[k] Swine Genetics International, Ltd., Cambridge, IA.
[l] Swine Genetics International, Ltd., Cambridge, IA.
[m] Swine Genetics International, Ltd., Cambridge, IA.

Serology *Indirect fluorescent antibody test* Eight chamber slides[n] were inoculated with MA104 cells and incubated at 37 C for 24 hours. Virus was inoculated into each well and then incubated at 37 C for an additional 36 - 48 hours. The slides were fixed in an 80% acetone/water solution, dried, and stored at -80 C until needed. Serum IFA titers were determined by making an initial 1:20 dilution in phosphate buffered saline followed by 2-fold dilutions. The slides were incubated with serum dilutions for 30 minutes at 37 C and then rinsed and dried. Goat anti-swine immunoglobulin fluorescent antibody conjugate[o] was then added and the slides were incubated for an additional 30 minutes at 37 C, after which the slides were rinsed, dried and read by ultraviolet microscopy.

Virus isolation Tissues collected from the boar for virus isolation at the time of necropsy included: lung, spleen, bone marrow from the femur, vas deferens, epididymis, testicle, prostate, seminal vesicles, bulbourethral gland, prepuce, and penis. Samples collected from the gilts for virus isolation at the time of necropsy included: ovary, uterus, cervix, placenta, fetuses, and amniotic fluid. Tissue homogenates were centrifuged at 2,000 x g for 15 minutes. Swine alveolar macrophages were grown on 24 well plates[p] in growth medium[q] supplemented with glucose,[r] fetal bovine serum,[s] gentamicin sulfate,[t] penicillin,[u] streptomycin sulfate,[v] amphotericin B,[w] and HEPES.[x] Serum diluted 1:5 in growth medium or tissue homogenates were inoculated onto 18 - 24 hour SAMs in 24 well plates after removal of the growth medium. Inoculated cultures were incubated for 1 hour at 37 C, after which 0.8 ml of growth medium was added to each well. Cultures were then incubated at 37 C and observed periodically for 1 week for cytopathic effects. All

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[n] Nunc, Inc., Naperville, IL.
[o] Kirkegaard and Perry Laboratories, Inc., Gaithersburg, MD.
[p] Costar Corp., Cambridge, MA.
[q] Sigma, St. Louis, MO.
[r] Sigma, St. Louis, MO.
[s] HyClone Laboratories, Inc., Logan, UT.
[t] Schering, Omaha, NE.
[u] Sigma, St. Louis, MO.
[v] Sigma, St. Louis, MO.
[w] Squibb and Sons, Rolling Meadow, IL.
[x] Sigma, St. Louis, MO.

samples were subinoculated onto MA104 cells in 8 chamber slides. Seventy-two hours after inoculation slides were fixed and stained with PRRS fluorescent monoclonal antibody.[y]

Histopathology Tissues collected from the boar at the time of euthanasia included: lung, spleen, vas deferens, epididymis, testicle, prostate, seminal vesicles, bulbourethral gland, prepuce, and penis. Tissues collected from the gilts at the time of necropsy included: ovary, uterus, cervix, placenta, and fetuses. Tissues were fixed in 10% neutral buffered formalin. Following routine processing, tissues were embedded in paraffin and 5 μm sections were stained with hematoxylin-eosin stain.

Bioassay of semen samples The presence of PRRSV in semen was determined by a swine bioassay (SB). Uninfected pigs were inoculated with a semen sample, then serologically monitored for evidence of PRRSV infection. Bioassay pigs were housed individually in isolation facilities to preclude exposure to PRRSV from other sources. Each pig was inoculated intraperitoneally (IP) with a 13 - 15 ml sample of semen (equal volumes of sperm rich and sperm poor fractions) from a single boar collection using a 20 ml syringe and 20 gauge needle. 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 after inoculation.

Results

Boar The boar was depressed and anorexic for 3 days post-challenge (PC). By the time of semen collection on day 7 PC his behavior and appetite were back to normal and he was willing to mount the dummy. The boar did not have any other clinical signs through day 21 PC, at which time he was euthanized. Spermatozoa motility and morphology remained within normal limits throughout the study. The boar was seronegative for PRRSV antibodies by IFA at the time of challenge. The IFA titer was 1:2560 on day 7 PC and rose to 1:5120 by day 21 PC. Virus was not isolated from the tissues and lesions were not detected by gross or histopathological examination.

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[y] D. Benfield, Department of Veterinary Science, South Dakota Center for Livestock Disease Control, South Dakota State University, Brookings, SD.

Bioassay of semen samples All of the bioassay pigs remained clinically healthy following inoculation with semen. Pigs inoculated with semen collected from the boar prior to challenge, on the day of challenge, and on days 9, 14, and 21 PC remained seronegative indicating virus was not present in the semen. Pigs inoculated with semen samples collected on days 7 and 8 PC seroconverted, indicating the presence of virus in the semen.

Gilts All of the gilts remained clinically healthy throughout the study and had a strong standing response on at least 1 of the 3 days they were bred. Four of the 6 control gilts were pregnant at the time of euthanasia 40 days after first insemination. The 4 pregnant control gilts had 5, 9, 11, and 11 fetuses within the uterus. One of the control gilts appeared to have cycled but was not pregnant and the other control gilt had a small, anestrus reproductive tract. One of the 5 exposed gilts was pregnant at the time of euthanasia 34 days after the first insemination. This gilt had 12 fetuses in the left horn and 9 fetuses in the right horn. The fetuses were grossly normal except for 2 which had signs of hemorrhage in the tissues. Three of the gilts which were not pregnant had old corpora lutea and developing follicles indicating they were coming back into estrus. The fourth exposed gilt had a small, anestrus reproductive tract similar in appearance to the control gilt's tract. All 11 gilts remained IFA negative throughout the study and virus was not isolated from the reproductive tracts or serum.

Discussion

The role of extended semen in transmission of PRRSV is not clearly understood. Epidemiologic information suggests that semen from infected boars may have been responsible for the transmission of PRRSV into uninfected herds. Unextended semen from experimentally infected boars has been shown to transmit PRRSV to naive gilts. In this study, transmission, as defined by seroconversion, did not occur even though the same amount of unextended semen used to artificially inseminate gilts was shown to be infectious when inoculated intraperitoneally into 4 - 8 week old pigs.

A number of factors may be responsible for the lack of transmission. One possibility could be inactivation of the virus due to the presence of an inactivating agent in the extender or being held at 25 C until extended semen was utilized. The

time lapse from semen collection until insemination was a relatively short period of time of approximately 2 hours. In a preliminary study to evaluate extender and room temperature effects, a limited number of positive semen samples from a previous study were extended in the same manner as the protocol utilized in this study and then held at 25 C for 1 hour followed by storage at -80 C until IP inoculation. Seroconversion indicated that virus was still infectious following extension and holding at room temperature , suggesting that virus inactivation was unlikely (Swenson, unpublished observations).

Other factors which may be responsible for the lack of transmission are route of exposure and virus dose. Although pigs are susceptible to PRRSV infection by a variety of routes, the minimum infectious dose has not been established for each route. The minimum infectious dose for mice exposed to lactate dehydrogenase-elevating virus (LDV) has been shown to vary considerably depending on the route of exposure.[14] Exposure to LDV through IP or tail cartilage injections revealed a minimum infectious dose of 1, while mucosal exposure via the ocular, vaginal, or oral routes required a minimum infectious dose of $10^{5.3}$. Cattle artificially inseminated with a combination of semen and ephemeral fever virus do not seroconvert, however, these same cows were found to be susceptible to infection when inoculated intravenously with 1/10th the dose of virus used in artificial insemination.[15] This information suggests that a higher minimum infectious dose may be required for intrauterine transmission than for IP transmission.

The cause of the marked difference in pregnancy rates is unknown. The lower pregnancy rates in the virus-exposed gilts could be due to PRRSV effects on the gilt, boar, or egg/embryo that would lead to a change in the ability of spermatozoa to fertilize eggs, altered fertilizability of the egg, or changes in development of the embryo due to direct virus effects on the embryo or indirectly through changes in the uterine environment. Attempts were made to remove some variability in the pregnancy rates by using 1 boar to inseminate the 11 gilts and by using estrus synchronization to shorten the time period of artificial insemination to a 2 week time period and thereby reducing the risk of changes in rates due to factors that may influence boar fertility. The boar was housed individually in a controlled environment in order to reduce the effect of temperature or trauma alterations on boar fertility. Routine motility, morphology, and concentration examination of each

semen collection did not reveal any gross abnormalities and all values were within normal acceptable ranges for use in artificial insemination. Although semen parameters were within acceptable ranges and 1/5 gilts became pregnant, we can not rule out a boar effect as the cause of differences in pregnancy rates.

The effect of PRRSV on the gilt and uterine environment at the time of insemination are unknown. It could be speculated that changes in the uterine environment associated with PRRSV may interfere with fertilization, development, or implantation of the fertilized egg. Cows infected with infectious bovine rhinotracheitis virus[16] and gilts exposed to pseudorabies virus[17] have been shown to develop endometritis. Preimplantation murine embryos exposed to cytomegalovirus were found to develop normally, however, embryos from mice inoculated IP with cytomegalovirus were found to be developmentally retarded.[18] When these embryos were transferred to uninfected mice they developed normally, indicating alterations in the maternal enviroment, rather than the virus were responsible for the observed effects. This information would suggest that PRRSV could cause changes that would make the uterus a hostile enviroment for fertilization, embryo development, or implantation. Although no gross or histopathologic abnormalities in the reproductive tracts were observed, lesions may have occurred and resolved by the time of euthanasia 34 days after insemination. A problem with this hypothesis arises when the work of Lager et al. is considered. In this study, gilts were bred on 2 consecutive days followed by deposition of PRRSV in the uterus after the second insemination.[19] No differences in pregnancy rates were observed between the control gilts and gilts exposed to PRRSV (Lager, personal communication). This would suggest PRRSV infection of the uterus does not result in alterations that influence pregnancy. On the other hand, there may be a small window of opportunity when PRRSV must be present for dam effects to occur and this window was missed by inoculating with PRRSV 1 day after the first insemination.

The effect of PRRSV on the egg or embryo is also not known. It is reported in the literature that intrauterine exposure of cows to bovine viral diarrhea virus interferes with fertilization and development of embryos.[20,21] It is also reported that fertilized porcine eggs exposed to porcine parvovirus are developmentally retarded

compared to control eggs.[22] This would suggest that a direct effect of PRRSV on fertilization or embryo development is theoretically possible.

Although a small number of gilts were used in this study, the information obtained suggests that early infertility associated with PRRSV infection does occur, as has been reported from field studies. The cause of the infertility is unknown and may be due to problems associated with infection of the boar or gilt, or due to a direct effect of virus on the egg/embryo and possibly due to an interaction of 2 or more of these events. One of the problems that arises with a study like this is that evaluation of the gilts occurred 34 days after they were inseminated and exposed to virus. We are therefore unable to ascertain if there was a conception problem and/or a problem with embryo development and implantation. Further studies are needed to assess at what stage alterations occur which interfere with pregnancy.

In this study, transmission of PRRSV via extended virus-contaminated semen did not occur. However, we cannot say that transmission through the use of extended semen from infected boars will not occur. Compared to previous work with PRRSV infected boars, the boar in this study shed PRRSV in the semen for an unusually short period. Previous studies have shown that most experimentally infected boars shed for 3 or more weeks. Virus was detected in the semen of the boar used in this study on days 7 and 8 post-challenge but not on days 9, 14, or 21 suggesting that this boar may have been shedding low levels of PRRSV in his semen. Had we used a boar that showed a more typical shedding pattern, different results may have been observed. The previously reported extended seminal shedding of PRRSV indicates there is a risk that PRRSV transmission via semen can occur. Until additional trials of this type are performed, we can not rule out the possibility of PRRSV transmission via extended semen.

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PRELIMINARY ASSESSMENT OF THE EFFECT OF VACCINATION OF BOARS ON THE EXCRETION OF PRRS VIRUS IN SEMEN

A paper to be submitted to Swine Health and Production

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Abstract

Seven boars infected intranasally with 4 ml ($10^{6.5}$ $TCID_{50}$/ml) porcine reproductive and respiratory syndrome (PRRS) virus (ATCC VR 2402) were monitored for 32 days following exposure. Four of the boars were vaccinated intramuscularly with an experimental PRRS virus vaccine 5 and 2 weeks prior to challenge. Semen samples were collected twice weekly following inoculation using either the gloved-hand technique or electroejaculation. Blood samples were collected weekly and PRRS virus antibody titers were determined using the indirect fluorescent antibody (IFA) test. Virus isolation was performed on serum samples. Boars were euthanized 32 days post-inoculation and tissues were collected for virus isolation and histopathologic examination.

Toxic components of semen make isolation of virus from semen technically difficult in cell systems. As an alternative to *in vitro* testing, the presence of PRRS virus in semen was determined by swine bioassay. Pigs 4 - 8 weeks old were housed individually and inoculated intraperitoneally (IP) with 13 - 15 ml of semen. Blood was collected at weekly intervals following IP inoculation and evaluated for the presence of antibodies against PRRS virus. Positive IFA results on 2 or more consecutive weekly serum samples signaled the presence of infectious PRRS virus in the semen sample.

Clinically, 1/7 boars had mild depression of 2 days' duration following infection. No changes in appetite, behavior, or libido were detected. All boars were seropositive on the IFA test by day 14 post-challenge. Virus was isolated from the serum of all 7 boars at the time of first bleeding on day 4 post-infection and to day 28 in one boar. Virus was present in semen at the time of the first collection in each of the 7 boars 4 days after challenge and was detected in nearly all semen samples collected from the 7 infected boars through days 4, 7, 25, 28, and 32 (3 boars). Neither gross nor microscopic lesions attributable to PRRS virus 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 vaccination for PRRS may reduce seminal shedding of PRRS virus.

Introduction

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 age 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 age male include lethargy, anorexia, elevated rectal temperatures, and loss of libido.[3-6]

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.[8,9] 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,[10] and researchers at South Dakota State University have shown that PRRSV can be transmitted to naive gilts by artificial insemination 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 1) assess the impact of PRRSV vaccination on duration of seminal shedding of virus and viremia, 2) document the course of clinical signs, 3) evaluate the lesions resulting from PRRSV infection in reproductive and other tissues, and 4) evaluate antibody titers following vaccination and challenge. Isolation of viruses from semen on continuous cell lines has historically been difficult or impossible to achieve due to cytotoxic factors in seminal fluids.[11-17] Therefore, a swine bioassay system was developed for the detection of PRRSV in semen.[10] A similar approach has been utilized for monitoring the presence of other viruses in the semen of animals.[15,18-20]

Materials and methods

Animals and housing Seven boars, 8 - 12 months of age, were obtained from PRRSV-free herds and subsequently confirmed to be serologically negative for PRRSV antibodies by the indirect fluorescent antibody (IFA) test. The boars were housed in groups of 2 - 3 boars/pen in one large room.

Four- to eight-week-old pigs were used in the PRRSV swine bioassay (SB). These pigs 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 housed in individual isolation units throughout the observation period.

PRRS virus The PRRSV (ATCC VR 2402) was originally derived from a pool of tissues from clinically affected young pigs obtained from a herd undergoing a clinical PRRS outbreak. Inoculation of tissue homogenates into a gnotobiotic pig was followed by virus isolation in swine alveolar macrophages (SAM). The SAM isolate underwent limiting dilution cloning 3 times in SAMs, then adaption and plaque purification in an african green monkey kidney continuous cell line (MA104).

The titer of the virus inoculum used in this study was determined by making serial ten-fold dilutions of virus in 96-well microtiter plates[a] using high glucose minimum essential medium[b] supplemented with 30μg/ml neomycin sulfate[c] and

[a] Corning Glass Works, Corning, NY.
[b] JRH Biosciences, Lenexa, KS.
[c] Sigma, St. Louis, MO.

1.2 mg/ml sodium bicarbonate. Virus dilutions were inoculated onto confluent MA104 cells in replicates of 5. Individual wells were observed for cytopathic effect at 5 days post-inoculation. Tissue culture infectious dose 50 ($TCID_{50}$/ml) titers were calculated using the Kärber method.[21]

Boar vaccination Four boars (31, 32, 5664, 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/naris of $10^{6.5}$ $TCID_{50}$/ml PRRSV. The virus was administered using a syringe which was fitted with a 16 gauge needle that had been shortened to approximately 5 mm in length. Rubber tubing[d] 1.6 mm X 0.8 mm was cut into a 35 mm length and placed over the cut needle in order to facilitate deposition of the virus in the nasal cavity.

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 post-challenge (PC) using the gloved hand technique.[22-27] Gloves[e] were changed between each boar. Two boars (5664, 5666) that failed to adapt to the dummy were collected by electroejaculation[28] 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.[f] 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 - 5 ml aliquots.

Blood collection Blood was collected from each boar at approximately 45 day intervals for 3 months prior to vaccination. All 7 boars were bled at the time the 4 boars were vaccinated 5 weeks and 2 weeks prior to challenge and the day of challenge. Following challenge, samples were collected on days 4, 7, 10, 14, and then

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[d] Fisher Scientific, Eden Prairie, MN.
[e] Baxter Healthcare, McGaw Park, IL.
[f] Swine Genetics International, Ltd., Cambridge, IA.

weekly through day 32 PC, at which time the boars were euthanized. Serum for virus isolation and IFA testing was stored at -80 C. Prior to serological testing and virus isolation, serum samples were randomized and boar or date identifiers were removed.

Serology *Indirect fluorescent antibody test* Eight chamber slides[g] were inoculated with MA104 cells and incubated at 37 C for 24 hours. All wells were inoculated with PRRSV and then incubated at 37 C for an additional 36 - 48 hours. The slides were fixed in an 80% acetone/water solution, dried, and stored at -80 C until needed. Serum IFA titers were determined by making an initial 1:20 dilution of serum samples in phosphate buffered saline followed by 2-fold dilutions. The slides were incubated with serum dilutions for 30 minutes at 37 C and then rinsed and dried. Goat anti-swine immunoglobulin fluorescent antibody conjugate[h] was added and the slides were incubated for an additional 30 minutes at 37 C, after which the slides were rinsed, dried and read by ultraviolet microscopy.

Virus isolation Virus isolation was done on SAMs. Macrophages were collected from young PRRS-free pigs and stored at -80 C until needed. Macrophages were then thawed, diluted in growth medium[i] supplemented with glucose,[j] fetal bovine serum ,[k] gentamicin sulfate ,[l] penicillin,[m] streptomycin sulfate,[n] amphotericin B ,[o] and HEPES,[p] and seeded onto 24 well plates.[q]

Tissues collected from the boars for virus isolation 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. Tissue homogenates were centrifuged at 2,000 x g for 15 minutes and inoculated onto 18 - 24 hour SAMs in 24 well plates after removal of the growth medium. Serum diluted 1:5 in growth medium was inoculated onto 18 - 24 hour SAMs in 24 well plates after removal of the growth medium. Inoculated cultures

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[g] Nunc, Inc., Naperville, IL.
[h] Kirkegaard and Perry Laboratories, Inc., Gaithersburg, MD.
[i] Sigma, St. Louis, MO.
[j] Sigma, St. Louis, MO.
[k] HyClone Laboratories, Inc., Logan, UT.
[l] Schering, Omaha, NE.
[m] Sigma, St. Louis, MO.
[n] Sigma, St. Louis, MO.
[o] Squibb and Sons, Rolling Meadow, IL.
[p] Sigma, St. Louis, MO.
[q] Costar Corp., Cambridge, MA.

were incubated for 1 hour at 37 C, after which 0.8 ml of growth medium was added to each well. Cultures were then incubated at 37 C and observed periodically for 1 week for cytopathic effects. All samples were subinoculated onto MA104 cells in 8 chamber slides. Seventy-two hours after inoculation slides were fixed and stained with PRRS conjugate[r] and read.

Histopathology Testicle, epididymis, vas deferens, seminal vesicle, bulbourethral gland, urethra, prepuce, prostate, kidney, spleen, and lung were collected for microscopic examination. Tissues were fixed in 10% neutral buffered formalin. Following routine processing, tissues were embedded in paraffin and 5 µm sections were stained with hematoxylin-eosin stain.

Bioassay of semen samples The presence of PRRSV in semen was determined by a swine bioassay. Individually housed PRRSV uninfected pigs were inoculated intraperitoneally (IP) with a 13 - 15 ml sample of semen (equal volumes of sperm rich and sperm poor fractions) from a single boar collection using a 20 ml syringe and 20 gauge needle. 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 after inoculation.

Results

Boars The 4 vaccinated boars remained clinically healthy following vaccination and 6/7 boars remained clinically healthy following challenge. The 5 boars collected by the gloved-hand technique maintained a normal libido, appetite, and attitude following challenge. The single boar having clinical disease was a vaccinated boar. Clinical signs of depression and anorexia occurred for several days following challenge. This was also one of the boars that was collected by electroejaculation. The combination of anesthesia, underlying leg problems which made it difficult for him to rise, and PRRSV infection probably all contributed to the depression and anorexia during the first week following challenge. The second boar collected by electroejaculation remained clinically healthy.

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The 7 boars were seronegative for PRRSV antibodies by IFA at the time of challenge. All 4 vaccinated boars and 2/3 nonvaccinated boars had detectable IFA titers by day 10 PC and subsequent IFA titers were ≥1:640 through day 32 (Fig 1). All 7 boars were viremic on day 4 PC and a detectable viremia was present for as long as 28 days in one challenged boar (Table 1).

At post mortem, no gross lesions were observed in the boars and histologic lesions were not observed. Virus isolation was negative for all tissues collected at the time of necropsy.

Bioassay of semen samples 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 vaccinated boars shed virus in their semen through days 4, 7, 25, and 28, respectively, following challenge.

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.[10] 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 an important role in the transmission of PRRSV to gilts or sows. Prolonged seminal shedding and transmission of PRRSV by boars closely parallels 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.[29,30] The objective of this work was to make a preliminary assessment in a small group of boars of the effect of an inactivated PRRSV vaccine on the duration of viremia, seminal shedding, and serum antibody response.

As shown in Table 1, there was considerable variability in the duration of viremia between boars. When the mean duration of viremia is compared between the vaccinated and nonvaccinated boars it appears that vaccination reduced the length of viremia. The two boars that were viremic the longest were collected by electroejaculation. The longer duration of viremia may be in part due to the added

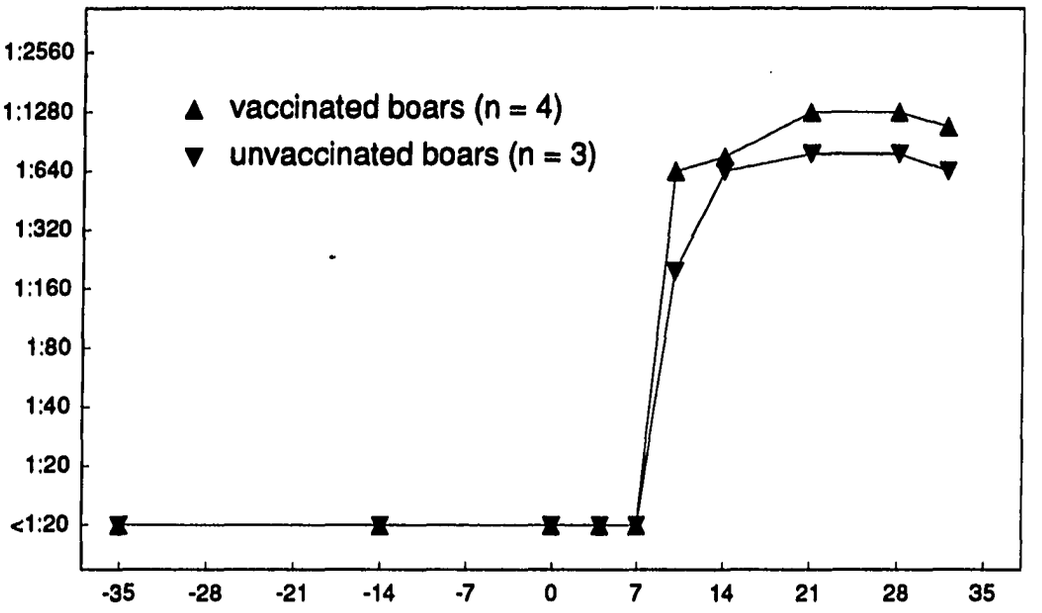

Figure 1. IFA titers following vaccination and challenge of boars

Table 1. Viremia following experimental exposure

	day post challenge							
BOAR	0	4	7	10	14	21	28	32
31[v]	–	+	–	–	–	–	–	–
32[v]	–	+	–	+	–	–	–	–
5664[ve]	–	+	+	–	+	+	–	–
5665[v]	–	+	–	–	–	–	–	–
33	–	+	+	–	+	–	–	–
5666[e]	–	+	+	+	+	–	+	–
6725	–	+	–	–	–	–	–	–

[v] = Vaccinated.
[e] = Electroejaculated.

Table 2. PRRS virus in semen following vaccination and experimental exposure

	day post challenge									
BOAR	0	4	7	11	14	18	21	25	28	32
31[v]	–	+	+	–	–	–	–	–	–	–
32[v]	–	+	–	–	–	–	–	–	–	–
5664[ve]	–	+	+	+	+	+	+	+	–	–
5665[v]	–	+	+	+	+	–	+	–	+	–
33	–	+	+	+	+	+	+	+	+	+
5666[e]	–	+	+	+	+	+	+	–	–	+
6725	–	+	+	+	+	+	+	+	+	+

[v] = Vaccinated.
[e] = Electroejaculated.

stress on the boars that was associated with anesthesia and electroejaculation. Both vaccinated and nonvaccinated boars developed IFA titers at approximately the same time, however, the vaccinated boars developed slightly higher titers (Fig 1). Although the number of boars used in this study is small, the data is suggestive of a difference in response of boars following vaccination and challenge compared to nonvaccinated boars.

One aspect of PRRSV infection that has received little attention as a possible factor in the clinical expression of PRRSV is genetic background. Seminal shedding of PRRSV is shown in Table 2. This study was terminated on day 32 based on a previous boar challenge study in which 3/4 boars were no longer shedding in semen on day 31. The boars used in this study were from 2 different herds. Four boars (5664, 5665, 5666, 6725) were from Herd A, while three boars (31, 32, 33) were from Herd B. Interestingly, the data suggests that the herd of origin may have had an effect on the results.

It is difficult to assess the effect of vaccination on the boars from Herd A due to 2 factors. First, since the 2 nonvaccinated boars were still shedding virus at the termination of the experiment we do not know when the boars would have stopped shedding. Second, the lack of seminal shedding by the 2 vaccinated boars from Herd A at the termination of the experiment may have been normal cessation of shedding or may have been due to vaccination.

In contrast, a difference in seminal shedding between the vaccinated and nonvaccinated boars from Herd B is evident. The two vaccinated boars from Herd B shed for considerably shorter time when compared to the nonvaccinated boar from Herd B and the 4 challenged boars from Herd A. In a previous study, boars from Herd B were housed in the same facilities, collected on a similar twice a week schedule, and challenged with the same dose of virus and by the same route as the boars in this study. In the previous study (Table 3), 4 nonvaccinated boars from Herd B shed virus in their semen through days 13, 25, 27, and 43 following challenge. When the Herd B boars from both studies are compared, it appears that vaccination reduced the length of seminal shedding in this group of boars. Housing and management, challenge dose and route, twice a week semen collection schedule, and age of the animals were comparable in both studies. The major difference between the two groups of boars was the genetic background. The small number of boars used in the two studies make it difficult to draw absolute conclusions, but,

Table 3. Presence of PRRS virus in semen following experimental infection of boars

	Day Post Challenge																												
Boar	0	3	5	7	9	11	13	15	17	19	21	23	25	27	29	31	33	35	37	39	41	43	45	47	49	51	53	56
32[v#]	–	+		–		–	–		–		–		–	–		–	No collections beyond day 32											
31[v#]	–	+		+		–	–		–		–		–	–		–	No collections beyond day 32											
119	–		+		+		+		–		–		–		–		–		–		–		–		–		–	
125	–		+		+		–		+		+		+		–		–		–		–		–		–		–	
117	–	+		+		+		+		+		+		+		–		–		–		–		–		–		–
33[#]	–	+		+		+	+		+		+		+	+		+	No collections beyond day 32											
115	–	+		+		+		+		+		+		+		+		+		–		+		–		–		–

[v] = Vaccinated.

[#] = Experiment terminated day 32.

these results suggest that genetic makeup may be a factor in determining the duration of seminal shedding and that vaccination may reduce the length of seminal shedding in boars.

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GENERAL DISCUSSION

Since the identification of PRRS as a new disease of swine and the identification of the virus, considerable information has become available in regards to the respiratory effects in hogs destined for market and the reproductive effects in the breeding age female. Relatively little information is available as to the effects this virus has on the breeding age boar. The purpose of this work was to document clinical signs, changes in semen parameters, development of PRRSV antibodies, length of viremia, and occurrence of seminal shedding in the experimentally infected breeding age boar. With the identification of seminal shedding of PRRSV, additional studies were developed to assess the role of extended semen in transmission of PRRSV to naive females and to evaluate the efficacy of an experimental PRRSV vaccine to reduce seminal shedding.

This work has shown that mature boars are capable of shedding PRRSV in their semen shortly after experimental infection and can shed for extended periods. Vaccination of boars was shown to reduce the length of seminal shedding and may be an important tool for the control of PRRSV in infected herds. Transmission of PRRSV to naive gilts with PRRSV-contaminated semen was not shown to occur in this work, however, there appeared to be a PRRSV effect on pregnancy.

The information obtained from this work has helped to better define the effects of PRRSV on the mature boar. This work has also raised questions about PRRSV which need additional investigation. One area that needs further investigation is the assessment of minimum infectious dose based on route of exposure. Virus-contaminated semen was found to be infectious when given IP, however, the same dose of semen in extender was not found to be infectious when given intrauterine. This suggests a difference in minimum infectious dose depending on the route of exposure. Although transmission was not shown to occur with the use of extended semen in this study, additional work in this area needs to be performed in order to better assess the risks of transmission associated with the use of AI. The difference in clinical disease and length of seminal shedding of PRRSV between the 3.5 year old boar and the 8 - 18 month old boars suggests there may be an age dependent difference in response to PRRSV infection. An age dependent difference in duration of seminal shedding of EAV has been reported to

occur and is suggestive that an age dependent difference may occur in boars infected with PRRSV. Further work also needs to be done to address the role of PRRSV in the early infertility syndrome that is reported in the literature. This work suggested a difference in pregnancy rates due to PRRSV infection, however, the cause of the difference in pregnancy rates could not be determined. It is currently not known if the early infertility is due to effects of the virus on the semen, egg/embryo, or dam. Additional work in this area will help to better define the role of the boar in early infertility.

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