Title: Evaluation of two procedures to aid in the diagnosis of PRRS in aborted etuses and examination of the dynamics of PRRSV shedding in vaccinated and unvaccinated gilts injected with various doses of PRRSV
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I) Abstract:

PRRS is currently the most economically important viral disease of swine and the most important infectious cause of porcine abortion. Unfortunately, a definitive diagnosis of PRRS abortion is rarely established with currently available laboratory tests on fetal tissues. This study assessed whether fetal serology or PCR on fetal serum and thoracic fluid would augment our ability to diagnose PRRS in aborted fetuses. When compared with virus isolation, neither fetal serology nor PCR yielded false positive results. Fetal serology detected only 26.2% of PRRSV infected fetuses whereas PCR on fetal serum detected 80.8% and PCR on thoracic fluid detected 84.6% of infected fetuses. Although virus isolation still appears to be the gold standard for the diagnosis of PRRS when samples are collected and preserved promptly, PCR proved sensitive and specific and may have an advantage over virus isolation in filed cases where virus isolation has not proven reliable.

This study also addressed the issue of relevant field exposure to PRRSV and the dynamics of PRRSV cycling in breeding age animals. Saliva samples were collected from both vaccinated and unvaccinated gilts for 21 days following exposure to varying doses of PRRSV. A pattern of viral shedding in individual animals was not identified as PRRSV was only isolated from each animal once during the collection period. This study failed to detect an impact of exposure dose on viral shedding in saliva as only low levels of virus were isolated and there were no differences in the number of isolations between the various challenge groups. There was no statistically significant difference in the number of isolations from vaccinated compared with unvaccinated gilts. With the techniques used in this study, virus appears to be shed at low levels in saliva, is not shed continuously at detectable levels, and is shed from day 6 –12 following challenge, regardless of vaccination status.

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II) Introduction:

PRRS is currently the most economically important viral disease of swine and the most important infectious cause of porcine abortion. Unfortunately, a definitive diagnosis of abortion due to PRRSV infection is rarely established with currently available laboratory tests on fetal tissues. Iowa State University Veterinary Diagnostic Laboratory records indicate that a diagnosis of PRRS is established from aborted fetuses in only 3.8% of the cases in which PRRS is eventually documented. As a result, a definitive diagnosis of PRRS-associated reproductive disease often relies on non-fetal samples, some of which do not directly document fetal exposure. Because fetal tissues represent the most common submission in cases of porcine reproductive failure, techniques need to be developed to improve the diagnostic success rate with this sample. This would allow for more precise definition of the real losses associated with PRRS abortion, provide the necessary diagnostic information to address the clinical problem, eliminate the added time and expense of submitting additional samples to diagnose PRRS, and help to eliminate the frustration experienced by diagnosticians, producers, and practitioners over the inability to document fetal infection in cases of PRRS abortion.

Two potential diagnostic tools that have not been routinely employed for the diagnosis of PRRS on fetal tissues are the polymerase chain reaction (PCR) and fetal serology. Fetal serology detects antibodies present in fetal thoracic fluid directed against a specific pathogen. As significant levels of maternal antibody do not cross the porcine placenta, the presence of fetal antibodies directed against an infectious agent provides evidence for in utero exposure. Fetal serology has been widely used to substantiate a number of infectious causes of abortion, including chlamydia, toxoplasma, Cache Valley Fever, and leptospira. Evaluation of a small number of experimentally induced PRRS abortions has demonstrated anti-PRRS antibody in fetal thoracic fluid suggesting that fetal serology has the potential to be a useful diagnostic test in late-term PRRS abortions.

PCR is becoming a more commonly used tool to diagnose a variety of diseases, including PRRS. PCR detects pathogen-specific nucleic acid sequences, and is a highly sensitive and specific technique. PCR has several potential advantages that may make it an extremely useful tool for detecting PRRSV in aborted fetuses. Because PCR does not require viable virus for detection, it may circumvent some of the problems with virus inactivation that have plagued virus isolation from fetal tissues. Unlike fetal serology, PCR does not require both an immunocompetent fetus and sufficient time prior to abortion for seroconversion to detect evidence of viral infection. As such, PCR may prove to be a better method than fetal serology for the detection of PRRS in mid-gestation fetuses.

In addition to addressing improved diagnostics, this study tried to address the issue of relevant field exposure to PRRSV and the dynamics of PRRSV cycling in breeding age animals. Information on how long and at what level reproductive age females shed PRRSV can help provide understanding of when these animals pose the greatest risk to other members of the breeding herd. Secondly, it is important that academicians develop an understanding of relevant field exposures so that valid challenge doses can be used in experimental trials.

III) Objectives:

A) To evaluate whether fetal serology and PCR on fetal thoracic fluid may be useful procedures for the diagnosis of late-term PRRSV abortions.

B) To evaluate PRRSV shedding in groups of vaccinated and unvaccinated gilts challenged with varying doses of PRRSV to develop an understanding of the dynamics of PRRS shedding within a population of breeding-age females.

IV) Procedures:

Pregnant sows and nonpregnant gilts were obtained from a herd that had previously been free of PRRSV infection based on clinical and serological history. All animals were acclimated for two weeks following which serum was reevaluated for PRRSV antibodies by ELISA test.

Nonvaccinated animals: Sixteen pregnant sows were divided randomly into four groups. Low, medium, and high dose groups were inoculated with 10^2, 10^4, and 10^6 TCID of PRRSV (NADC-8) respectively in 2 ml of cell culture medium by intramuscular injection in the posterior thigh at approximately day 90 of gestation. A fourth group served as controls and were given a sham inoculum of uninfected cell culture medium by the same route. Animals were monitored daily for clinical signs and pyrexia. . Saliva was collected from the commisure of the mouth, using sterile, individual plastic pipets, every third day following challenge for 21 days. From the sample collected, 500ul of saliva was diluted in 4.5 mls of cell culture medium and frozen at minus 70 for serial dilution assessment of viral titer. All animals were euthanized on day 21 following inoculation. At necropsy fetuses were sequentially numbered beginning at the tip of one uterine horn; pigs in spontaneously aborted litters were numbered at random. Fetal serum or thoracic fluid from live or dead pigs respectively was evaluated by VI, fetal serology and PCR.

Vaccinated animals: Fifteen nonpregnant gilts were vaccinated intramuscularly with two doses of a commercially available modified live PRRSV vaccine [a] administered two weeks apart. Following vaccination, gilts were dosed orally once daily for 18 consecutive days with 13.2 mg altrenogest oral solution [b] administered in a small amount of feed. On day 19 each gilt received one intramuscular dose (5 ml) of a commercial preparation [c] providing 400 IU of pregnant mare serum gonadotropin and 200 IU of chorionic gonadotropin per dose, followed 30 hours later by 750 IU of human chorionic gonadotropin (HCG) [d] administered intramuscularly. Gilts were bred twice by artificial insemination at 24 and 36 hours following the HCG injections with semen from a PRRS-negative boar. Gilts were evaluated for pregnancy status by real-time ultrasonography on day 36 of gestation.

The fourteen pregnant gilts were divided randomly into three groups of four (low, medium, and high dose groups) and one group of two (controls). Low, medium, and high dose groups were inoculated on day 90 of gestation with 10^2, 10^4, and 10^6 TCID of PRRSV respectively in a manner similar to the nonvaccinated groups. Controls were given a sham inoculum by the same route. Saliva was collected from the commisure of the mouth, using sterile, individual plastic pipets, every third day following challenge for 21 days. All animals were euthanized on day 21 following inoculation and samples were collected as above

Virus isolation, PCR using a nested procedure and fetal serology were performed on all fetal sera and thoracic fluid samples. Virus isoaltion was underatken on all 196 saliva samples collected.

V) Results:

A) Dynamics of PRRSV shedding in vaccinated and unvaccinated gilts:

This segment of the study was intended to assess the duration and magnitude of PRRSV shedding in saliva following IM challenge of vaccinated and unvaccinated, pregnant gilts with 100 (low dose), 10,000 (med dose), and 1,000,000 (Hi dose) TCID PRRSV NADC-8 at 80 days gestation. Saliva was collected from the commisure of the mouth, using sterile, individual plastic pipets, every third day following challenge for 21 days PRRS in 12 vaccinated, 12 unvaccinated and 4 unchallenged controls for a total of 196 saliva samples. PRRSV was only isolated from 7 of these samples. PRRSV was isolated from unvaccinated sows 18 (Hi dose, day 6), 12 (Low dose, day 9), 20 (Low dose, day 9), 14 (Med dose, day 12) 15 (Med dose, day 12); and from vaccinated sows 25 (Hi dose, day 6) and 26 (Med dose, day 9). Titers were only positive at our lowest dilution (1:10). A pattern of viral shedding was not identified as PRRSV was only isolated from each animal once during the collection period. Detectable virus was shed in saliva from day 6 through day 12. Even though there was less than half the number of VI positive saliva samples in the vaccinated group, this was not statistically significant. This study failed to detect an impact of exposure dose on viral shedding in saliva as only low levels of virus was identified and there were no differences in the number of isolations between the various challenge groups. With the techniques used in this study, virus appears to be shed at low levels in saliva, is not shed continuously at detectable levels, and is shed from day 6 –12 following challenge, regardless of vaccination status.

B) The use of fetal serology for the diagnosis of PRRS in aborted fetuses

Serum and/or fetal thoracic fluid was obtained from 140 fetuses. These fluids were analyzed for antibodies to PRRS via Indirect Fluorescent Antibody (IFA) techniques. 61 (43.6%) of the fetal serum samples were positive for PRRS with virus isolation (VI). Using antigen slides prepared with NADC-8 and a fluorescine tagged anti-porcine IgG antibody, 16 of these samples were positive for antiPRRS antibodies. IFA titers were detected only in fluids of fetuses that were VI positive. Of the VI positive fluids, 26.2% were positive on fetal serology. However, only 13.7% of fetal fluids from known infected litters had positive IgG titers. Of the ten litters with infected pigs, fetal serology was only positive in fetuses from 5 of these litters. Thus, if the entire litter is assessed with fetal serology, this technique will only detect PRRS infection in 50% of the litters.

Possibly due to repeated freezing and thawing of serum, none of the samples were positive by IFA techniques with anti-IgM conjugates.

C) Evaluation of PCR on fetal serum and thoracic fluid for the diagnosis of PRRS in aborted fetuses:

Serum and fetal thoracic fluid were evaluated from 57 fetuses with PCR and compared to results from virus isolation (VI). There was 100% agreement between VI and PCR on the 44 VI negative samples (serum and thoracic fluid). Of the 13 VI positive samples from 3 litters, PCR was positive on 11 of 13 (84.6%) thoracic fluid samples. In replicate runs, PCR was positive on 21 of 26 (80.8%) of the VI positive fetal serum samples. Compared to virus isolation, PCR detected 80.8% of positive samples in serum and 84.6% of positive samples in thoracic fluid. If more than one infected fetus per litter was assessed, PCR would not have failed to detect an infected litter. Under idea conditions, PCR was slightly less sensitive but did offer the potential advantage of more rapid turnaround time (24 hours) compared to VI (5-14 days). It should be noted that these findings were under experimental conditions with prompt tissue preservation. Our experience with virus isolation on field cases of PRRS is that virus isolation is minimally productive, probably due to the effects of autolysis on the virus. PCR appears to be a rapid, sensitive and specific method for the diagnosis of

PRRSV. Additional studies are pending to assess the impact of autolysis on PCR to determine whether this test offers an advantage over PCR in the types of specimens routinely submitted to a diagnostic lab.

VI) Conclusions:

Under ideal conditions, virus isolation still appears to be the most sensitive method for the diagnosis of fetal PRRSV infection. PCR is 80-85% as sensitive as VI. Fetal serology appears to be only approximately 25% as sensitive as VI. However, under field conditions, virus isolation has been very disappointing. Future studies will attempt to determine if PCR is less affected by autolysis. If PCR proves to be more sensitive than VI in autolyzed tissues, it would likely be the test of choice for the diagnosis of field cases of PRRS abortion.