Effect of porcine reproductive and respiratory syndrome virus (PRRSV) exposure dose on fetal infection in vaccinated and nonvaccinated swine

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Summary

Objective: To evaluate the relative susceptibility of vaccinated and nonvaccinated pregnant swine to varied challenge doses of porcine reproductive and respiratory syndrome virus (PRRSV) and the potential for increased challenge doses of PRRSV to overcome vaccine-induced immunity

Method: Fifteen nonpregnant gilts obtained from a PRRS-free herd were vaccinated twice with a modified-live PRRSV vaccine prior to artificial insemination. At 90 days of gestation, these VACC-CHAL gilts and 16 pregnant, nonvaccinated CHAL sows were randomly allotted to one of four experimental groups: a control group that received a sham inoculation, or to groups that received a "low" (10^2 $CCID_{50}$), "middle" (10^4 $CCID_{50}$), or "high" (10^6 $CCID_{50}$) dose of an intramuscular challenge of the NADC-8 PRRSV strain.

Results: The number of infected litters in all dosage groups was significantly higher ($P < .001$) among CHAL females compared to VACC-CHAL females. Dead fetuses and viremia were observed in all litters in the low- and middle-dose groups, and in three of four litters in the high-dose group in the CHAL females; and in no low-dose litters, one of two middle-dose litters, and one of four high-dose litters in the VACC-CHAL females. No fetal death or viremia was identified in control groups. Among infected litters, no significant difference in the percentage of infected fetuses per litter was observed regardless of vaccination status or challenge virus dose. The number of litters with fetal death and infection was significantly lower in the low-dose VACC-CHAL group when compared to the low-dose CHAL group ($P<.01$), but no significant difference was demonstrated between the two medium or two high dose groups.

Implications: Vaccine-induced protective immunity appeared to protect eight of 10 litters from reproductive failure, but may be overcome with increased ($\geq 10^4$ $CCID_{50}$) doses of challenge virus. The lowest PRRSV exposure dose (10^2 $CCID_{50}$) tested in this study caused reproductive failure in naïve, unvaccinated animals. The percentage of infected fetuses per litter observed suggests that multiple fetuses/weakborn pigs should be sampled to ensure that infected animals are represented. Sampling dead or autolyzed fetuses is generally diagnostically unrewarding for PRRSV infection.

Keywords: swine, PRRSV, vaccine, exposure dose, reproduction, breeding herd

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Although the practice of vaccinating breeding stock against porcine reproductive and respiratory syndrome virus (PRRSV) is widespread in the United States swine industry, PRRSV-induced losses continue to occur in some PRRS-vaccinated herds.[1,2] In the field, these losses may be interpreted as vaccine failure or inefficacy. Strain variation in field viruses, suboptimal vaccination procedures, concurrent stress or disease, and nutritional factors have been related to such failures for vaccines in general,[3] and these factors could reasonably be expected to affect the response to PRRS vaccination. One can also encounter variation in the exposure dose of field virus during PRRS epizootics. While vaccination may provide protection against a minimal to modest exposure, high doses of field virus may potentially overcome immunity.[3]

This study was designed to assess the impact of varied PRRSV exposure doses on the susceptibility of sows to infection, clinical disease, and PRRSV-associated reproductive disease, and to determine whether exposure to a higher challenge of PRRSV may be a potential factor in the failure or inefficacy of vaccine-induced protection against PRRSV.

Materials and methods

Animals

Thirty-one breeding females were used in this study. Fifteen 10-month-old nonpregnant gilts ("VACC-CHAL" females) and 16 naturally mated 1.5- to 2-year-old pregnant sows ("CHAL" females) were procured from the same commercial herd, which was deemed free of PRRSV based on clinical and serological history. All animals were found to be serologically negative for PRRSV antibody prior to arrival. On arrival (Study Day 0), they were randomly allotted to study groups, acclimated for 14 days in climate-controlled indoor isolation units at Iowa State University, and then retested for PRRSV antibody by commercial ELISA test (HerdChek® PRRS, IDEXX Laboratories; Westbrook, Maine) (Figure 1).

After acclimation, VACC-CHAL gilts were vaccinated with 2 cc of a modified-live PRRSV vaccine (RespPRRS Repro™, Noble Laboratories Inc.; Sioux Center,

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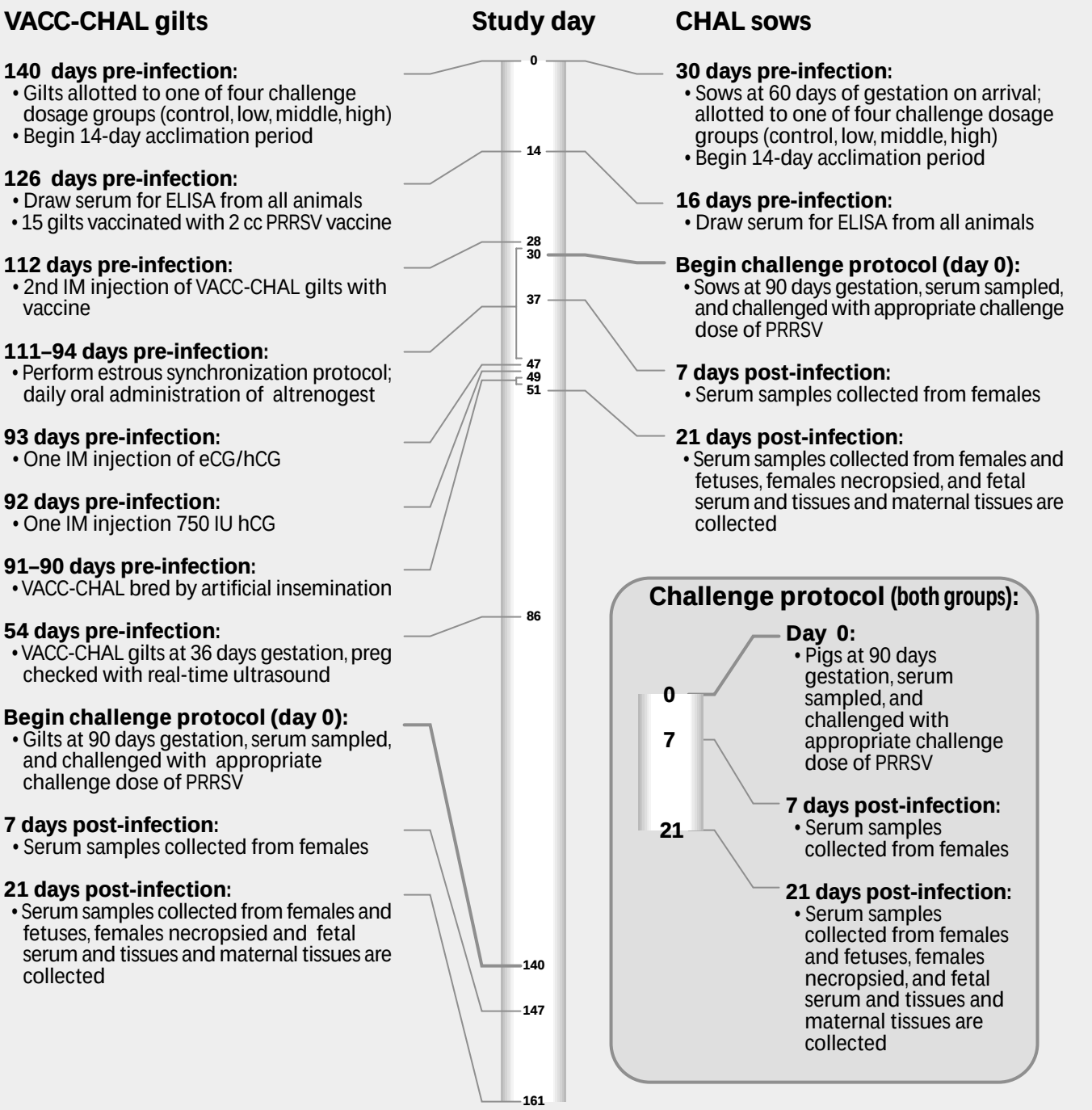
Figure 1: Study timeline



Iowa) via intramuscular (IM) injection 126 and 112 days before challenge. On study day 29, estrous synchronization was initiated. Gilts were given 6 cc altrenogest oral solution (Regu-Mate®, Hoechst-Roussel Agri-Vet Co.; Somerville, New Jersey) in a small amount of feed, providing 13.2 mg altrenogest per head once daily for 28 consecutive days. On day 93 pre-infection (study day 47) each gilt received one IM dose (5 mL) of PG600® (Intervet Inc.; Millsboro, Delaware) to provide 400 IU of pregnant mare serum gonadotropin and 200 IU of chorionic gonadotropin per dose. Thirty hours later, gilts were given 750 USP units of human chorionic gonadotropin (hCG) (Follutein®, Solvay Animal Health, Inc.; Mendota Heights, Minnesota) by IM injection. Gilts were mated twice by artificial insemination at 24 and 36 hours after the hCG injections (91 and 90 days prior to infection) with semen from a PRRSV-negative boar. On day 36 of gestation (54 days prior to infection), 14 of the VACC-CHAL gilts were verified as pregnant by real-time ultrasonography.

CHAL sows were naturally mated to PRRSV-negative boars, and were pregnant when they were placed in the isolation facilities. They received no vaccine.

Virus challenge

The NADC-8 PRRSV strain was prepared as previously described.[4] Briefly, the virus was isolated from serum of a weakborn pig on MARC-145 cells. The cell culture was

frozen and thawed and the virus was serially passed two more times. The third passage of virus was titrated and diluted with serum-free minimal essential medium to prepare the low (10^2 $CCID_{50}$), medium (10^4 $CCID_{50}$), and high (10^6 $CCID_{50}$) challenge virus inoculum (2 mL volume). A virus-free control sham inoculum was prepared in a similar fashion from uninoculated MARC-145 cells. Heterogeneity between challenge and vaccine virus was based on temporal and geographical differences when viruses were isolated[5] and genetic differences between the challenge virus and VR-2332 PRRSV strain,[5] the parental strain of vaccine virus that has a 99.7% nucleotide homology with ORFs 2–7 sequence of the vaccine virus.[6]

At 90 days of gestation (0 days post-infection [DPI]), the 14 VACC-CHAL gilts and 16 CHAL sows received one of four challenge exposures to PRRSV injected IM in the caudal thigh:

- a sham inoculation ("control" group);
- 10^2 $CCID_{50}$ of PRRSV ("low-dose" group);
- 10^4 $CCID_{50}$ of PRRSV ("middle-dose" group); or
- 10^6 $CCID_{50}$ of PRRSV ("high-dose " group).

Sampling

Animals were monitored daily for clinical signs and pyrexia. Blood samples were collected via jugular venipuncture from all females on the day of challenge (0 DPI), 7 DPI, and 21 DPI. The serum was separated within 2 hours and frozen at -70°C. All sera were evaluated for PRRSV antibodies by the ELISA test and for PRRSV by virus isolation at the completion of the trial. All animals were euthanized at 21 DPI, and the following maternal tissues were collected: cerebrum, cerebellum, pituitary, tonsil, lung, liver, kidney, spleen, uterus, ovary, and oviduct. Sow lungs were lavaged to collect porcine alveolar macrophages as previously described.[7] At necropsy, fetuses were sequentially numbered beginning at the tip of one uterine horn. Fetuses in spontaneously aborted litters were numbered at random. Thoracic fluid was taken from dead fetuses and serum samples from live fetuses. The serum was separated and the serum and thoracic fluid were stored at -70°C. The following tissues were collected from all fetuses: brain, lung, cardiac muscle, aorta, liver, spleen, tonsil, placenta, umbilical cord, and mediastinal lymph nodes. Maternal and fetal tissues were examined for gross and microscopic lesions.

All fluids (fetal sera and thoracic fluid and sow/gilt sera and lung lavage fluid) were used for isolation of PRRSV as previously described.[8,9] Briefly, cultured cells of the MARC-145 cell line were propagated in Eagle's minimal essential medium supplemented with 10% fetal calf serum and gentamycin sulfate (0.05 mg per mL). The appropriate sample (0.2 mL) was added to the nutrient medium (1 mL) of a confluent monolayer of MARC-145 cells and incubated at 37°C in a humid atmosphere of 5% CO_2. Cell cultures were examined daily for 7 days for cytopathic effect. Culture medium (0.2 mL) from the inoculated wells was used to inoculate a second passage when primary isolation was unsuccessful. Lack of cytopathic effect in these cultures was interpreted as a negative test.

Statistical analysis

The numbers of infected litters and infected pigs per litter were compared between study groups using χ^2 analysis. Results were considered significant at $P < .05$.

Results

Clinical signs

CHAL sows

Mild fevers (1°–3°C above expected normal values) were observed up to 4 days postexposure. One sow in the low-dose group had mild icterus from 9–14 DPI and aborted at 20 DPI. One sow in the high-dose group aborted at 16 DPI. At postmortem, fetuses from one sow in the middle-dose group were not at the proper phase of gestation and this sow and litter were eliminated from the study. Ten of 11 litters were composed of a mixture of live and dead fetuses; fetuses in one litter in the high-dose group were all alive. Dead fetuses comprised a total of 32% of fetuses in the low-dose group, 30% in the middle-dose group, and 29% in the high-dose group. Autolysis was advanced in approximately 66% of the dead fetuses.

VACC-CHAL gilts

No clinical signs or pyrexia were noted subsequent to inoculation. One gilt in the middle-dose group aborted at 6 DPI; subsequent investigation revealed the cause of abortion to be suppurative endometritis, and this gilt and litter were eliminated from the study. One gilt in the high-dose group aborted at 21 DPI. One gilt in the middle-dose group had no fetuses at postmortem. One litter in each of the middle- and high-dose groups had dead fetuses, representing 33% and 27% of the fetuses in each litter, respectively.

Virus isolation

CHAL sows

Porcine reproductive and respiratory syndrome virus was isolated from serum of nine of 11 sows collected at 7 DPI, and from none of the 11 sows collected at 21 DPI. Virus was isolated from at least one sample in 10 of 11 (90.9%) litters (Table 1) and from 58 of 131 (44.3%) fetuses (Figure 2). Of 58 viremic fetuses, 52 were live at necropsy and six were dead or autolyzed (Figure 2). Viremic fetuses were identified in four low-dose litters (100%), three middle-dose litters (100%), and three

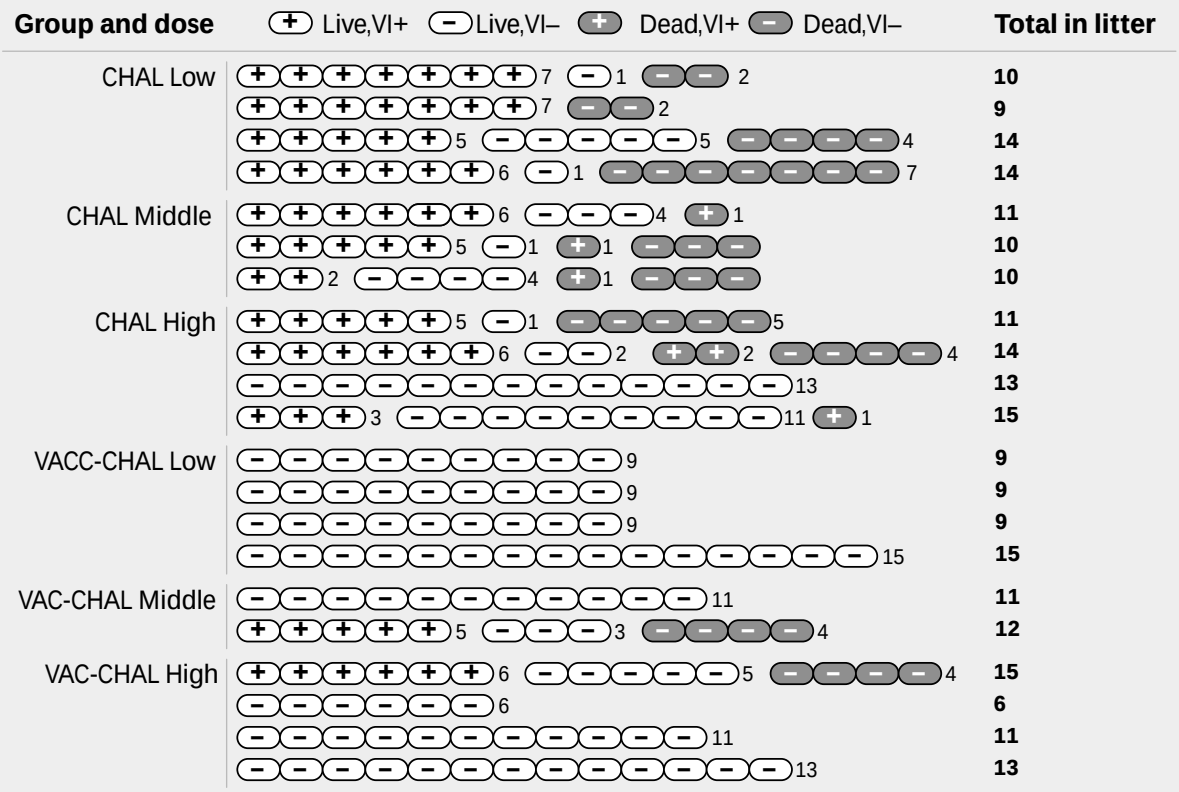
Table 1: Prevalence of PRRSV-infected litters and fetuses within each group

	Exposure dose group				
	Control	**Low**	**Middle**	**High**	**Total**
CHAL	0/4*	4/4	3/3	3/4	10/11
	0%†	57% (36–78)	51% (30–64)	43% (27–57)	51% (27–78)
VACC-CHAL	0/2*	0/4	1/2	1/4	1/10
	0%†	0%	42%	40%	41% (40–42)

* Number of virremic litters/numbers in group

† Average percentage of viremic fetuses within infected litters (range of percentage of viremic fetuses within a litter)

Figure 2: Viremic live or dead fetuses per litter



high-dose litters (75%). The number of viremic fetuses in the low-, middle-, and high-dose groups did not differ significantly. Percentages of viremic fetuses within affected litters varied from 26.6%–77.7 %. Porcine reproductive and respiratory syndrome virus was isolated from lung lavage fluid from eight of 11 sows at necropsy (21 DPI). No virus was isolated from control sows or fetuses.

VACC-CHAL gilts

No virus was isolated from gilt sera at 7 or 21 DPI, or from any lung lavage fluids collected at necropsy. No virus was isolated from fetuses from the low-dose group. Virus was isolated from two of 10 litters (one litter in each of the middle- and high-dose groups) and from 11 of 116 (9.5%) fetuses (five of 12 [41.7%] and six of 15 [40%] fetuses per litter from the middle- and high-dose groups, respectively). All viremic fetuses were live; no dead fetuses yielded virus. No virus was isolated from any fetuses in litters without dead fetuses or from control gilts or fetuses.

Microscopic lesions

CHAL sows

Microscopic lesions in exposed sows were limited to mild perivascular lymphoplasmacytic infiltration in the uterine submucosa in approximately 66% of sows. No significant gross or histologic lesions were observed in control sows or in fetuses from exposed or control groups.

VACC-CHAL gilts

Minimal uterine submucosal perivascular lymphoplasmacytic infiltrates were observed in one gilt in the middle-dose group, while no significant lesions were observed in either nonchallenged control group. No significant gross or histologic lesions were observed in fetuses.

Serology

All animals were seronegative for PRRSV antibody by ELISA test (ELISA S:P ratio < 0.4) prior to challenge (CHAL sows) or vaccination (VACC-CHAL gilts). All CHAL sows had seroconverted by 21 DPI (Table 2). Control CHAL sows remained seronegative throughout the study. All VACC-CHAL gilts were seropositive at 0 DPI and had an increase in ELISA S:P ratio between 0 DPI and 21 DPI. Control VACC-CHAL gilts (n=2) had similar positive ELISA S:P ratios at 0 and 21 DPI.

Discussion

The intramuscular challenge exposure route was chosen for this study to assure that each animal received the intended specific challenge dose. Reported comparisons of intramuscular and intranasal dosing of PRRSV have not demonstrated any significant differences in onset or degree of humoral immune response or infection rate

Table 2 ELISA results before and after PRRSV inoculation

Day post-inoculation	CHAL mean	CHAL range	CHAL SE	VACC-CHAL mean	VACC-CHAL range	VACC-CHAL SE	Control VACC-CHAL mean	Control VACC-CHAL range	Control VACC-CHAL SE
0	<0.4	not applicable	not applicable	0.730	0.511-1.094	± 0.059	0.627	0.602-0.653	± 0.0255
21	1.083	0.700-1.627	± 0.081	2.26	1.925-2.578	± 0.067	0.598	0.588-0.608	± 0.014

in young pigs.[10,11]

Although ultrasound examination at day 36 postbreeding indicated pregnancy in one VACC-CHAL gilt in the middle-dose group, she was found not pregnant at necropsy. No maternal clinical signs or aborted fetal tissues were observed and there were no gross or microscopic lesions found at necropsy that would support a diagnosis for the apparent resorption of the fetuses.

Under the conditions of this study, the lowest PRRSV challenge-exposure dose resulted in fetal infection and death similar to the higher challenge doses in nonvaccinated naïve animals. No significant difference in the percentage of infected litters or in the percentage of infected fetuses per litter was identified between different challenge doses in the nonvaccinated sows. The infection rate of litters of nonvaccinated sows was significantly higher ($P<.0005$) than that in the vaccinated groups (10 of 11[90.9%] versus two of 10 [20%]). Vaccine-induced immunity appeared to protect eight of 10 litters from fetal infection under the conditions of this study; however, a significant difference ($P<.01$) in infection could be demonstrated only between the low-dose VACC-CHAL and CHAL groups. The comparison between VACC-CHAL and CHAL groups did not demonstrate a significant difference in litter infection rate in the middle- and high-dose groups; the loss of subject females in the middle-dose group had a detrimental effect on the statistical outcome.

Apparent incompleteness of vaccine-induced protective immunity may be challenge-dose dependent, in that the low-challenge dose did not produce any infected litters in the vaccinated gilts while the middle-challenge dose produced infection in one of two litters, while the high-challenge dose produced infection in one of four litters. The percentages of viremic fetuses within these two PRRSV-infected litters were similar to those found in the nonvaccinated infected litters, which would be expected since the maternal immune response should not affect the progress of an intrauterine infection once the virus has crossed the maternal-fetal barrier.

No virus was isolated from the lung lavage fluid of vaccinated and challenged animals, which is consistent with previous experimental reports.[4] Microscopic lesions identified in the maternal tissues and the lack of lesions in the fetuses are consistent with findings of other investigators.[12]

In the 13 PRRSV-infected litters, virus was isolated from 64 of 112 (57%) live fetuses and from six of 44 (13.6%) dead fetuses (Figure 2), which is consistent with previous reports.[9,13,14] This indicates that isolation of virus from dead or autolyzed fetuses is generally unrewarding compared to virus isolation from weakborn or stillborn pigs, probably due to the instability of the virus in decomposing tissues.[4,9,13,14]

Previous studies have demonstrated that PRRSV strain NADC-8 infection will induce protection against reinfection with the homologous virus.[4,9] Immunity against homologous challenge prevented fetal infection for 604 days post initial infection.[4] However, protection against heterologous strains appears to be less complete and inconsistent,[15–18] which is consistent with our findings. Collectively, these observations suggest that clinical protection may be dependent upon the antigenic similarity between the immunizing and challenge viruses. In addition, the present study also suggests that clinical protection induced by field viruses against reinfection by heterologous strains may be challenge dose dependent, although additional studies are required to confirm this hypothesis.

These findings, along with information on strain differences and the protection provided by homologous challenge,[4,9] would suggest that safeguarding the breeding herd depends on manipulating a complex interaction based on the antigenic similarity between the challenge virus and the vaccine or field virus strains from which herd immunity was established, and the challenge dose. In light of these factors, acclimation of breeding stock and biosecurity cannot be solely replaced by vaccination programs.

From a diagnostic standpoint, these findings underscore the need for care in selecting samples for laboratory study. In a typical PRRSV-infected litter, the number of noninfected fetuses may range from 30%–70%. If samples are collected from only a limited number of aborted/weakborn pigs, there is the possibility that only noninfected pigs will be sampled; therefore, sample size can be critical when trying to identify PRRSV infection. Because of the variable distribution of infected pigs in a litter, samples pooled from multiple weakborn pigs submitted for virus isolation are still among the best specimens. Under optimal laboratory conditions, tissue or fluids from dead fetuses rarely provide positive virus isolations. Considering that the typical specimen submitted to the laboratory is a dead fetus from the field, the poor virus isolation rates for PRRSV are not surprising.

Implications

- The use of altrenogest in this study constituted an extra-label use for research purposes only. We do not advocate the use of this product in commercial swine production.
- Vaccine-induced protection may be incomplete at higher exposure doses.
- Earlier studies demonstrated long-term solid immunity induced by natural exposure to field virus against re-exposure to the homologous virus.[4,9] Exposure to heterologous virus as mimicked by this study may provide less reliable protection. Protection of breeding swine is likely dependent on the immunological similarity between immunizing and challenge strains.
- Immunization will not replace biosecurity and herd acclimation/stabilization practices.
- Diagnosis of PRRSV-related reproductive disease cannot be reliably achieved by sampling dead fetuses. Multiple samples from live- and/or weakborn fetuses are required for practical diagnostic attempts.

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