Immunologic responses and reproductive outcomes following exposure to wild-type or attenuated porcine reproductive and respiratory syndrome virus in swine under field conditions

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Objective—To compare immunologic responses and reproductive outcomes in sows housed under field conditions following controlled exposure to a wild-type strain of porcine reproductive and respiratory syndrome virus (PRRSV strain WTV) or vaccination with a modified-live virus (MLV) vaccine.

Design—Randomized controlled trial.

Animals—30 PRRSV-naïve 10-week-old female pigs.

Procedure—Humoral and cell-mediated immune responses were monitored while pigs were held in isolation for 84 days after inoculation with the WTV strain (n = 10), inoculation with the WTV strain and 42 days later vaccination with a killed-virus vaccine (10), or vaccination with an MLV vaccine (10). Reproductive outcomes were measured after pigs were released into the farm herd.

Results—Inoculation with the WTV strain, regardless of whether a killed-virus vaccine was subsequently administered, elicited faster and more substantial production of strain-specific neutralizing antibodies, as well as a more rapid generation of interferon-γ secreting cells, than did vaccination with the MLV vaccine. Despite the enhanced immune responses in pigs inoculated with the WTV strain, animals vaccinated with the MLV vaccine produced a mean of 2.45 more pigs than did sows exposed to the WTV strain, mainly because of a lower rate for failure to conceive.

Conclusions and Clinical Relevance—Results suggest that current assays of immunity to PRRSV correlate only imperfectly with degree of clinical protection and that the practice of controlled exposure of sows to a circulating PRRSV strain should be reconsidered in light of negative clinical outcomes. (*J Am Vet Med Assoc* 2006; 228:1082–1088)

RUMINANTS/ SWINE

Infection of pregnant swine with PRRSV can result in abortion[1,2] and fetal death,[2-5] causing substantial economic losses for production facilities.[4,6] In a previous study,[7] sows previously infected with PRRSV were found to be at least partially resistant to reproductive failure following subsequent exposure to the virus, whereas naïve animals were susceptible. Similarly, passive transfer of PRRSV-specific neutralizing antibody to sows during the third trimester of gestation and prior to exposure to PRRSV was shown to prevent abortion and increase fetal survival rate through the weaning stage.[8] Both of these studies, however, were performed under controlled experimental conditions, and it is not clear whether similar results would be obtained under field conditions.

Nevertheless, strategies aimed at enhancing PRRSV-specific immunity in breeding females have been adopted under the assumption that reductions in transmission of the virus, clinical signs, or both can be achieved.[9-12] One technique that has gained popularity, partly because of the overall ineffectiveness of commercially available vaccines,[11] involves controlled exposure of naïve pigs to endemic PRRSV strains. Typically, young sows are injected IM with serum from infected pigs or with virus from cell culture. The underlying assumption is that animals exposed to the virus in this way will not only recover from clinical disease but also develop PRRSV strain-specific immunity prior to becoming pregnant.[12]

Clearly, more information is needed on the relationship between adaptive immunity to PRRSV and protection from clinical disease in swine. The purpose of the study reported here was to compare immunologic responses and reproductive outcomes in sows housed under field conditions following controlled exposure to a wild-type strain of PRRSV or vaccination with an MLV vaccine. The study was designed to not only allow evaluation of the development of humoral and cellular immunity to PRRSV in sows under field conditions, but also allow determination of the effect of these protective responses on the severity of clinical reproductive disease.

Abbreviations

PRRSV	Porcine reproductive and respiratory syndrome virus
MLV	Modified-live virus
IFNγ	Interferon-γ
FFN	Fluorescent focus neutralization

Materials and Methods

Experimental animals and study facility—The study was conducted at a single farm in the Midwestern United

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States. The farm housed 800 breeding sows and approximately 7,800 growing swine during the time of the study and had a long-term (> 5 years) history of active PRRSV infection among swine on the farm. Various control strategies had previously been adopted with limited success, including use of commercial killed and modified-live PRRSV vaccines and controlled exposure of incoming female swine to farm-specific strains of PRRSV. Management practices on the farm were consistent with modern production practices, and all pigs were housed indoors. Animals entering the farm from external sources were quarantined for 75 days in a separate, biosecure isolation building that was divided into 2 rooms.

Thirty 10-week-old female swine were used in the study. All 30 animals came from an outside source without any history of PRRSV infection and were confirmed to be negative for PRRSV infection prior to enrollment in the study by use of a standard serologic test. Twenty gilts were randomly chosen and placed in 1 room of the isolation facility, and the remaining 10 gilts were placed in the other room. After 75 days of seclusion, all pigs were moved to the main herd. At the same time that study animals were introduced to the herd, a contemporaneous cohort of 46 breeding gilts was introduced to the farm according to the farm's routine practices. These animals were raised in the study herd and were naturally exposed to farm strains of PRRSV while growing, although the exact timing of infection was unknown.

Experimental protocol—Nineteen days after being moved to the isolation building, the 20 gilts in the first isolation room were each inoculated IM in the right side of the neck with $10^{3.5}$ $TCID_{50}$ of a wild-type strain of PRRSV (WTV) isolated from a diagnostic sample routinely submitted by the study farm to a commercial diagnostic laboratory. The virus had been isolated on pulmonary alveolar macrophages approximately 18 months prior to the beginning of the study and subsequently grown and titered in MARC145 cells. The open reading frame 5 gene of the WTV strain had been sequenced (GenBank accession No. AY754345) as described[13] and had been found to share 88.6% of nucleotide residues with the corresponding region of the US reference strain (ie, strain VR2332). At the same time, the 10 gilts in the second isolation room were each given a single IM injection of a modified-live PRRSV vaccine.[a] Sixty-one days after being moved to the isolation building, 10 of the 20 sows that had been inoculated with the WTV strain were randomly chosen and given a single dose of a killed PRRSV vaccine.[b]

Evaluation of viral transmission—To confirm that PRRSV was actively circulating in the herd at the time of the study, samples of blood or tissue from 9 herd animals not otherwise involved in the study were collected and submitted for PCR testing for open reading frame 5. Amplification products were submitted for sequencing.

Evaluation of immunologic responses—The day of (ie, day 0) and 14, 28, 42, 56, 70, and 84 days after inoculation with the WTV strain or vaccination with the MLV vaccine, 2 (7 to 10 mL each) blood samples were collected from each animal by means of jugular venipuncture. The first sample was placed in a serum separator tube, and the second was placed in a sterile evacuated tube with heparin. Samples were placed on ice and transported to the laboratory for analysis.

Peripheral blood mononuclear cells were isolated from heparinized blood samples by means of density-gradient centrifugation and analyzed for intensity of the cellular immune response to PRRSV by means of a virus-specific IFNγ test as described.[14] Reference strain VR2332 was used as the source of antigen for stimulation in the INFγ test.

Serum samples were submitted to a commercial laboratory[c] and tested with the diagnostic lab's FFN test[e] to quantify neutralizing antibodies and a commercial ELISA[d] to detect all circulating (neutralizing and non-neutralizing) PRRSV-specific antibodies. All samples were retained until the end of the study and tested simultaneously to eliminate day-to-day variations in testing. Separate FFN tests were conducted on all samples with either the reference strain VR2332 (progenitor of the strain used in the MLV vaccine) or the WTV strain as test antigen.

Evaluation of reproductive outcomes—Once moved out of the isolation building, the sows were allowed to cycle

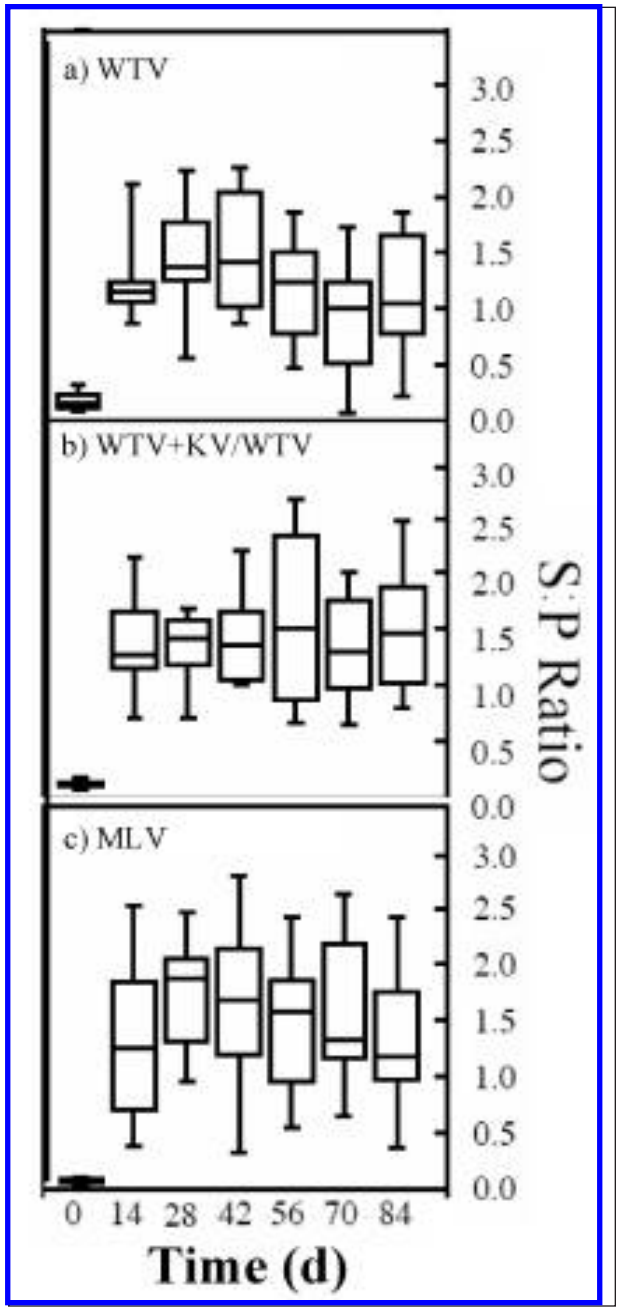

Figure 1—Box plots of antibody titers among pigs inoculated with a wild-type strain of PRRSV (strain WTV; A), inoculated with strain WTV and vaccinated with a killed-virus vaccine 42 days later (B), or vaccinated with an MLV vaccine (C). Antibody titers were measured with an ELISA, and values represent the sample-to-positive control sample (S:P) ratio (an S:P ratio > 0.4 was considered a positive result). For each day, the line through each box represents the median, the box represents the interquartile (25th to 75th percentile) range, and the whiskers represent the full range.

through the herd according to typical practices on the farm and were intermingled with animals not included in the study, including the contemporaneous cohort of 46 breeding sows. Farm records and routine veterinary health assessments, including results of diagnostic testing performed on swine not included in the study, indicated that PRRSV was actively circulating in the herd. Sows in the study population therefore had a high probability of exposure or reexposure to the virus.

Animals were allowed to enter estrus naturally (ie, without chemical synchronization). When animals came into estrus, farm workers, who were unaware of the sows' experimental status, artificially inseminated them by means of standard methods.

At the end of the study period, reproductive outcomes were obtained from the production management records maintained by the farm. Information on dates that sows were bred, date of first parturition, total number of pigs born in the first litter, number of pigs born dead in the first litter, date of removal of the sow from the herd, and reason for removal from the herd was obtained for each animal in the study. Reproductive outcomes of interest were whether the sow gave birth and, if so, whether pregnancy resulted from the first insemination; fetal survival rate (ie, proportion of pigs born alive in the litter); and total number of pigs (alive or stillborn) per litter.

Statistical analysis—Associations between treatment (inoculation with the WTV strain, inoculation with the WTV and vaccination with the killed-virus vaccine, or vaccination with the MLV vaccine) and reproductive outcomes were tested with multivariate linear or logistic regression models. Associations between the intensity of the cellular or humoral immune response and reproductive outcomes independent of treatment were tested with similar multivariate regression models. The effect of treatment on development of the immune response over time was analyzed with a multiple linear regression model that incorporated a repeated-measures design. First-order interaction terms between time (days after inoculation or vaccination) and treatment were included in the model to test the effects of treatment on the rate of immune response development. All analyses were performed with standard software.[c,f] Values of $P < 0.05$ were considered significant.

Results

Viral transmission in the herd—Sequences of open reading frame 5 amplification products obtained by means of PCR testing of blood or tissue samples from 9 herd animals not otherwise involved in the study were similar or identical to the sequence for the same region of the WTV strain used to inoculate study animals. In addition, all 46 replacement breeding sows seroconverted against PRRSV, indicating natural exposure of these animals to the endemic wild-type strain.

Humoral immune responses—Non-neutralizing antibodies were detected 14 days after inoculation with the WTV strain or vaccination with the MLV vaccine (**Figure 1**), whereas production of neutralizing antibodies was protracted (**Figure 2**). Although all animals seroconverted, no significant differences among treatment groups (inoculation with the WTV strain, inoculation with the WTV strain and vaccination with the killed-virus vaccine, or vaccination with the MLV vaccine) were identified in regard to magnitude or rate of development of the initial humoral response.

In contrast, treatment group had a significant effect on production of neutralizing antibodies, depending on the virus strain used in the FFN assay. Maximum FFN titer against the WTV strain and rate of increase in titer were significantly ($P = 0.001$) higher among sows inoculated with the WTV strain than among sows vaccinated with the MLV vaccine (Figure 2). Conversely, maximum FFN titer against the VR2332 strain and rate of increase in titer were significantly ($P = 0.04$ and 0.007, respectively) higher among sows vaccinated with the MLV vaccine than among sows inoculated with the WTV strain. Rate of increase in FFN titer against the WTV strain in sows inoculated with that strain was sig-

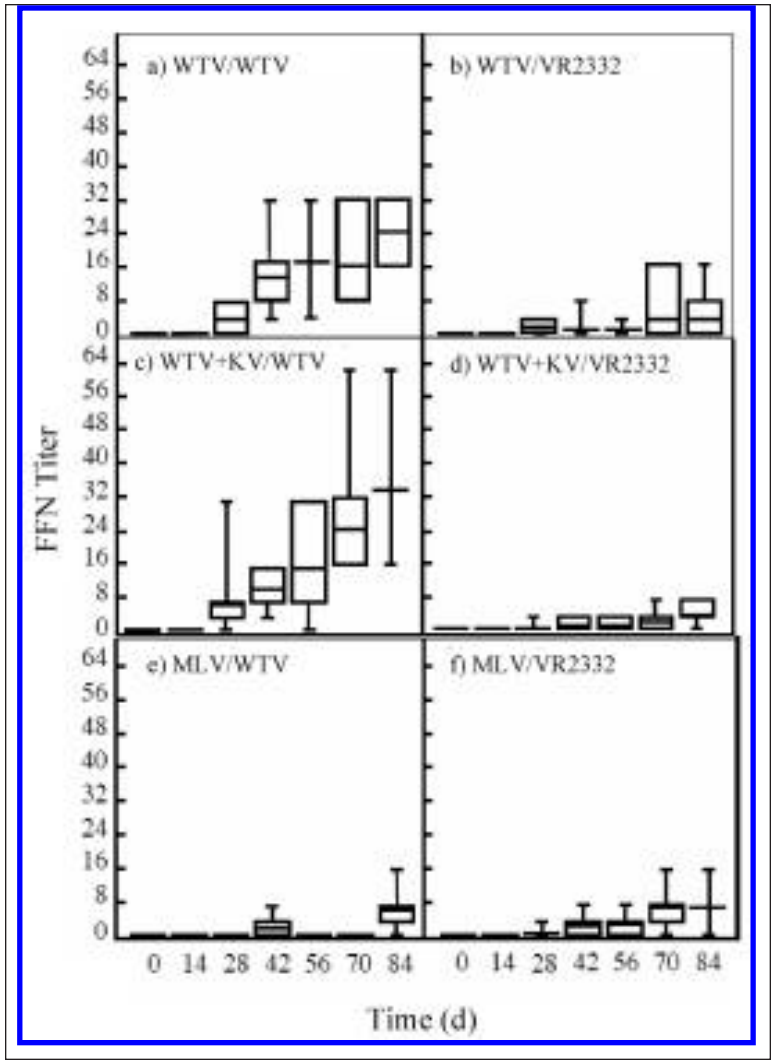

Figure 2—Box plots of PRRSV-specific neutralizing antibody titers among pigs inoculated with PRRSV strain WTV (A and B), inoculated with strain WTV and vaccinated with a killed-virus vaccine 42 days later (C and D), or vaccinated with an MLV vaccine (E and F). An FFN assay was used to measure antibody titers (a titer > 0 was considered a positive result), with strain WTV (A, C, and E) or a reference strain, VR2332 (B, D, and F), used as the test antigen.

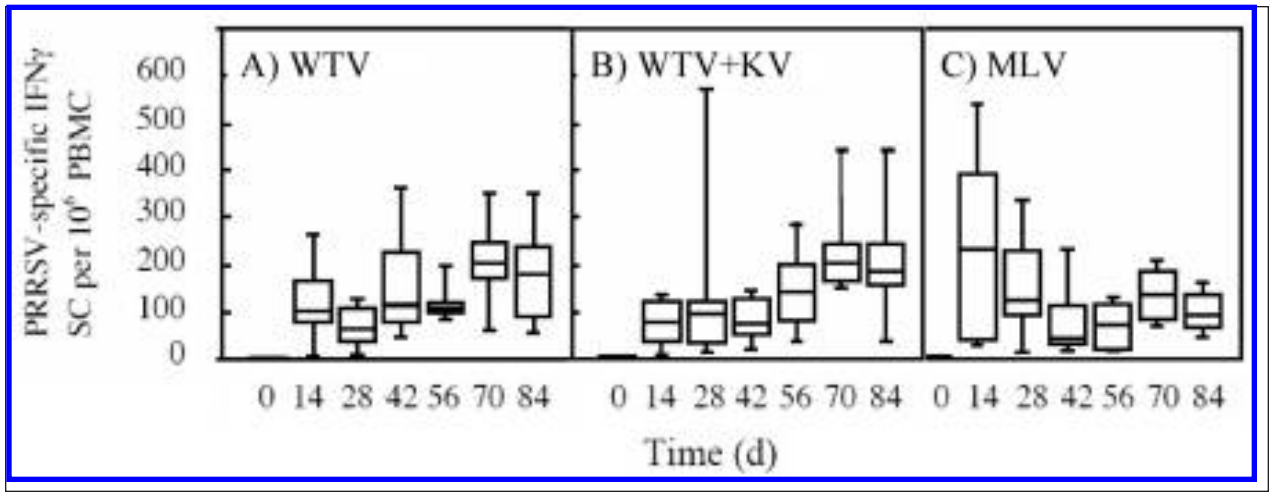

Figure 3—Box plots of cell-mediated immune responses among pigs inoculated with PRRSV strain WTV (A), inoculated with strain WTV and vaccinated with a killed-virus vaccine 42 days later (B), or vaccinated with an MLV vaccine (C). Cell-mediated immune response was measured as number of PRRSV-specific IFN-γ secreting cells per 106 peripheral blood mononuclear cells (PBMC).

Table 1—Reproductive outcomes among sows inoculated with a wild-type strain of the porcine reproductive and respiratory syndrome virus (PRRSV strain WTV; group 1), inoculated with strain WTV and vaccinated with a killed-virus vaccine 42 days later (group 2), or vaccinated with a modified-live virus vaccine (group 3) and among a cohort of untreated sows (control).

Variable	Group 1	Group 2	Group 3	Control
No. of sows	10	10	10	46
No. of pigs born per litter*	8.80 ± 0.92	11.17± 0.65	9.7 ± 1.29	8.45 ± 1.84
No. of pigs born alive per litter*	8.60 ± 0.80	11.00 ± 0.67	7.9 ± 1.39	7.36 ± 1.60
Proportion of pigs born alive*	0.98 ± 0.01[b]	0.98 ± 0.01[b]	0.76 ± 0.10[a]	0.85 ± 0.18[a,b]
Proportion of sows pregnant following first insemination	0.1[b]	0.5[a]	0.7[a]	0.1[b]
Proportion of sows farrowing	0.5[b]	0.6[b]	1.0[a]	0.5[b]
Mean No. of pigs born alive per sow	4.3[b]	6.6[a]	7.9[a]	3.7[b]

*Data are given as mean ± SEM.
[a,b]In each row, values with different superscript letters were significantly ($P < 0.05$) different.

nificantly ($P < 0.001$) greater than the rate of increase in FFN titer against the VR2332 strain in sows vaccinated with the MLV vaccine.

No significant differences in any of the measures of humoral immunity were found between sows inoculated with the WTV strain and sows inoculated with the WTV strain that subsequently received the killed-virus vaccine, even though the 2 animals that had the highest neutralizing antibody titers (1:64) had received the killed-virus vaccine (Figure 2).

Cell-mediated immune responses—The intensity and rate of development of the cell-mediated immune response to PRRSV, as measured by the proportion of peripheral blood mononuclear cells that were virus-specific IFNγ secreting cells, were highly variable among sows (**Figure 3**). Although treatment group was not significantly associated with maximum cell-mediated immune response, there was an association between treatment group and the rate of increase in the proportion of virus-specific IFNγ secreting cells, with rate of increase significantly ($P = 0.045$) greater in sows inoculated with the WTV strain than in sows vaccinated with the MLV vaccine. No significant difference in cell-mediated immune response was found between sows inoculated with the WTV strain and sows inoculated with this strain that subsequently received the killed-virus vaccine.

Reproductive outcomes—Mean total number of pigs born per litter and mean number of pigs born alive per litter did not vary significantly among treatment groups (**Table 1**). The probability of parturition was significantly ($P = 0.01$) lower for sows inoculated with the WTV strain than for sows vaccinated with the MLV vaccine. In addition, sows inoculated with the WTV strain were significantly ($P = 0.017$) less likely to become pregnant after the first artificial insemination than were pigs vaccinated with the MLV vaccine. However, sows that were inoculated with the WTV strain that did eventually give birth had a significantly ($P = 0.024$) higher proportion of pigs born alive in the litter than did sows vaccinated with the MLV vaccine. Reproductive outcomes were not significantly different between sows inoculated with the WTV strain and sows inoculated with this strain that subsequently received the killed-virus vaccine, except for proportion that became pregnant after the first artificial insemination and mean number of pigs born alive per sow. Mean number of pigs born alive per sow was signifi-

cantly lower for sows inoculated with the WTV strain than for sows vaccinated with the MLV vaccine. Reproductive outcomes for the cohort of 46 replacement breeding sows were not significantly different from values for sows inoculated with the WTV strain.

Association between immune responses and reproductive outcomes—A significant (P = 0.015) positive association was found between maximum FFN titer against the WTV strain and proportion of pigs born alive in the litter. However, maximum FFN titer against the WTV strain was negatively associated (P = 0.046) with the probability that a sow would become pregnant following the first insemination. Other measures of immune response were not significantly associated with any reproductive outcome.

Discussion

In the present study, development of neutralizing antibodies against PRRSV depended on the extent of antigenic similarity between the virus strain used as a reference antigen in the FFN test and the strain to which pigs were first exposed. Specifically, in pigs inoculated with the WTV strain, measured neutralizing antibody titer was higher when the WTV strain was used in the FFN test than when the VR2332 strain was used. In pigs vaccinated with the MLV vaccine, measured neutralizing antibody titer was higher when the VR2332 strain (ie, the progenitor of the vaccine virus strain) was used in the FFN test than when the WTV strain was used. Presumably, this difference was a reflection of increased antibody recognition of epitopes present in the proteins, primarily glycoprotein 5,[15-17] of the autologous virus. The fact that serum antibodies were to any extent capable of impairing the infectivity of a heterologous strain of PRRSV implies that some degree of cross-protective humoral immunity may be conferred by vaccination of pigs.

When FFN tests were conducted with autologous antigen, maximum neutralizing antibody titer and rate of increase in titer were significantly higher among sows inoculated with the WTV strain than among sows vaccinated with the MLV vaccine. One possible reason for this discrepancy is the relative attenuation of the vaccine virus strain, compared with wild-type strains such as WTV, reflecting reduced replication efficiency in pigs. Accordingly, the ineffectiveness of currently available vaccines in controlling PRRSV infection may result from their inability to elicit an adequately intense or rapid neutralizing antibody response in commercially raised swine.

Reduced replication efficiency of the vaccine virus strain could also explain the apparent difference in cell-mediated immune response found in the present study. Although maximum cell-mediated immune response, measured as the proportion of peripheral blood mononuclear cells that were virus-specific IFNγ secreting cells, was not significantly different among treatment groups, the rate of increase was significantly greater in sows inoculated with the WTV strain than in sows vaccinated with the MLV vaccine. Importantly, the VR2332 strain was used as the antigen in this assay. Because the cellular response to PRRSV is predominantly directed against glycoprotein 5, which is relatively nonconserved, and glycoprotein 6, which is relatively conserved,[18,19] use of this viral strain could have altered the assay results. For instance, unpublished data generated in our laboratory suggest that the measured cell-mediated immune response is nearly twice as great when a PRRSV strain autologous, rather than heterologous, to the strain to which pigs were initially exposed was used in the assay.

In the present study, the cell-mediated immune response developed more rapidly than it did in a previous study.[14] This might be attributable to increased nonspecific activation of the innate immune system in sows raised under farm conditions. Because the present study was conducted in a field environment, these animals were more likely to experience concurrent infection and other environmental stressors than is typical for sows in an experimental setting, and increased activity of the innate immune system (eg, production of interferon-α) could have promoted development of cell-mediated immunity to PRRSV.[20,21] However, additional research is needed to determine whether these factors actually do affect immune responsiveness of pigs.

Results of previous studies[7,22,23] involving pigs raised in controlled settings have suggested that exposure of pigs to a particular PRRSV strain provided protective immunity during subsequent exposure to that same strain. These findings, in combination with the failure of commercial vaccines to consistently provide protection against strains circulating in the environment,[9,12,24] have led to the widespread adoption of a policy of intentionally exposing naïve animals to herd-specific PRRSV strains as a strategy for controlling farm outbreaks of the associated disease. So far, both successes and failures regarding the effectiveness of this type of deliberate exposure to PRRSV as a preventative measure have been described in anecdotal reports.[12] However, to our knowledge, the present study is the first to rigorously investigate the use of such controlled exposure as a strategy for management of PRRSV infection. To the extent that our results can be generalized, we conclude that this procedure should not be used on commercial farms, unless the incurred losses can be balanced by economic gains in other stages of production. In this regard, although inoculation of sows with the WTV strain prior to their introduction in the herd, compared with vaccination with an MLV vaccine, was associated with a higher proportion of live births per litter, the lower proportion of sows that farrowed resulted in an average reduction of 2.45 live births for each sow introduced into the herd. Reproductive outcomes of sows inoculated with the WTV strain prior to introduction to the herd were statistically indistinguishable from outcomes for sows naturally exposed to the endemic strain. Whether vaccination with the MLV vaccine enhanced productivity relative to no intervention at all could not be determined in the present study because of the unavailability of naïve pigs that could be introduced into the herd after 75 days of isolation. However, given the observed clinical consequences arising from exposure to the WTV strain, it is likely that such animals would have had poor reproductive performance when encountering PRRSV following introduction into the herd.

Intriguingly, measured immune responses to PRRSV were less predictive of the severity of reproductive disease than was the method of exposure in the present study. For instance, although there was a weak positive association between maximum FFN titer against the WTV strain and the proportion of pigs born alive in the litter, no such relationship could be established when humoral immune response was measured at approximately day 85 of gestation. Moreover, measures of the cell-mediated immune response were not significantly associated with any of the measured reproductive outcomes. The lack of a consistent relationship between the intensity of the cell-mediated immune response and the extent of protection from clinical reproductive disease among swine in commercial[25] and experimental[20] settings has been reported previously. The reasons for this inconsistent association are unclear, but could reflect uncontrolled variation in host immunity or in the environmental conditions under which infected swine were housed. Compounding this variation is the frequent use of heterologous virus as a source of antigen to stimulate the IFNγ response in the assay of cell-mediated immunity. Use of a divergent viral strain (eg, strain VR2332) could have reduced the sensitivity of the test. Alternatively, the severity of clinical disease may have been reflective of the viral strain through mechanisms not strongly dependent on host immunity.[26] It is interesting to note that there was a marginally significant negative association between neutralizing antibody titer and the proportion of sows that conceived following the first artificial insemination. Thus, reproductive efficiency, like growth rate,[27] may in some cases be negatively correlated with the intensity of the immune response.

The relationship between immunity to PRRSV and clinical disease in pigs is clearly complex, and this intricacy is augmented by the inherent genomic and antigenic variability of this virus. Unlike the relatively stable pseudorabies virus,[28] an alphaherpesvirus of swine that has been all but eradicated with the help of a highly effective vaccine and for which the intensity of the IFNγ response elicited by the MLV vaccine correlates with protective immunity,[29,30] PRRSV's malleability enables it to escape immunologic surveillance by eliciting strain-specific responses.[7,22,23] Therefore, understanding the interactions among immunity, viral antigenic variation, and clinical disease will be essential for controlling PRRSV and other viruses with similar genetic architecture and dynamics of infection.

a. Ingelvac PRRS MLV, Boehringer Ingelheim Vetmedica Inc, St Joseph, Mo.
b. PRRomiSe killed PRRSV vaccine, Intervet Inc, Millsboro, Del.
c. South Dakota Veterinary Diagnostic Laboratory, Bookings, SD.
d. HerdCHECK PRRS Elisa, Idexx Labs, Westbrook, Me.
e. SAS for Windows Release 8.2, SAS Institute Inc, Cary, NC.
f. Systat, version 10.2, Systat Software Inc, Richmond, Calif.

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Selected abstract for *JAVMA* readers from the *American Journal of Veterinary Research*

Pharmacokinetics of amoxicillin administered in drinking water to recently weaned 3- to 4-week-old pigs with diarrhea experimentally induced by *Escherichia coli* O149:F4

Gerda M. Jensen et al

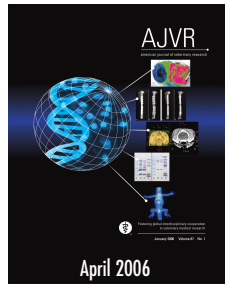
Objective—To measure effects of *Escherichia coli* O149:F4–induced diarrhea on water consumption and pharmacokinetics of amoxicillin after administration in drinking water.

Animals—24 recently weaned 24- to 28-day-old crossbred pigs.

Procedure—10 pigs were inoculated with *E coli* O149:F4; all 10 pigs subsequently developed diarrhea. Pigs were medicated by administration of amoxicillin in the drinking water (0.75 mg/mL) for a 4-hour period on 2 consecutive days. Fourteen age-matched noninfected healthy pigs (control group) were medicated in a similar manner. Blood samples were obtained from both groups daily, and plasma concentrations of amoxicillin were analyzed by use of high-performance liquid chromatography.

Results—Diarrhea reduced the area under the plasma concentration-versus-time curve (AUC) and maximum plasma concentration (C_{max}) of amoxicillin on the first day of medication by 56% and 63%, respectively. The AUC of amoxicillin on the second day of medication for diarrheic pigs did not differ significantly from that of control pigs on the first day of medication.

Conclusions and Clinical Relevance—*E coli*–induced diarrhea reduced the AUC of amoxicillin and time that plasma concentration of amoxicillin was > 0.025 μg/mL and, hence, the likelihood of having a therapeutic effect on the first day of administration in drinking water. On the assumption that plasma concentrations may indirectly reflect concentrations at the site of infection, analysis of our results suggests that higher doses of amoxicillin may be appropriate for administration in drinking water during a 4-hour period on the first day that pigs have diarrhea attributable to *E coli* O149:F4. (*Am J Vet Res* 2006;67:648–653)



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