Porcine reproductive and respiratory syndrome continues to have a devastating economic impact on the swine industry in the United States. In the past few years, swine producers and veterinarians have attempted to ameliorate the clinical effects of PRRS through acclimatization, defined as deliberate exposure of pigs at an early age to an endemic PRRSv strain to induce development of strain-specific protective immunity. A previous study has suggested that use of acclimatization in conjunction with biosecurity measures may prevent PRRS outbreaks associated with endemic PRRSv strains. Because the most important economic losses associated with PRRS in breeding herds occur late in gestation, when infection can cause stillbirths and abortions, researchers have recommended that gilts be acclimatized at an early age so that they will have sufficient time to recover and develop immunity to the endemic PRRSv strain before being bred.

One strategy used by swine producers to acclimatize pigs is IM inoculation with an endemic PRRSv strain. However, this procedure is labor-intensive, time-consuming, and costly, and alternative strategies that involve inoculating only a portion of the pigs on any farm would be helpful if the economic benefits of reduced labor outweighed any increased costs. One such alternative strategy is to inoculate only a proportion of the pigs in the herd and allow the remainder to become acclimatized by contact with inoculated animals or their secretions. However, the degree of protective immunity developed by animals acclimatized through this type of contact exposure is unknown. If sufficient

**Evaluation of contact exposure as a method for acclimatizing growing pigs to porcine reproductive and respiratory syndrome virus**

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**Objective**—To determine whether 6.5-week-old gilts that have not previously been exposed to porcine reproductive and respiratory syndrome (PRRS) virus can be acclimatized to an endemic strain of the virus by commingling with age-matched gilts inoculated with the endemic PRRS virus strain and whether 10.5-week-old gilts can be acclimatized by commingling with age-matched inoculated or contact-exposed animals.

**Design**—Randomized controlled longitudinal study.

**Animals**—80 gilts seronegative for PRRS on a farm in the Midwestern United States with a history of PRRS.

**Procedures**—20 gilts were inoculated with the endemic PRRS virus strain at 6.5 weeks of age (group 1) and were commingled with 20 gilts that were not inoculated (group 2). Four weeks later, the remaining 40 gilts (group 3) were commingled with gilts in groups 1 and 2. Presence of viral RNA in the tonsils, seroconversion rate, serum neutralizing antibody titers, interferon-γ-mediated cellular immunity, and reproductive outcomes were analyzed.

**Results**—Acclimatization of PRRS virus-naïve pigs was achieved by means of contact exposure at both 6.5 and 10.5 weeks of age. No differences were observed among the 3 groups with respect to development of anti-PRRS virus-specific immune responses or reproductive outcomes.

**Conclusions and Clinical Relevance**—Results suggested that contact exposure of 6.5- to 10.5-week-old pigs that had not previously been exposed to PRRS virus to pigs inoculated with endemic PRRS virus may be an efficient acclimatization strategy for controlling outbreaks on commercial farms on which PRRS is endemic. (J Am Vet Med Assoc 2008;232: xxx–xxx)

**Abbreviations**

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Definition</th>
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<tr>
<td>PRRS</td>
<td>Porcine reproductive and respiratory syndrome</td>
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<tr>
<td>PRRSv</td>
<td>Porcine reproductive and respiratory syndrome virus</td>
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<tr>
<td>FFN</td>
<td>Fluorescent focus neutralizing antibody</td>
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<td>ELIspot assay</td>
<td>Enzyme-linked immunosorbent spot assay</td>
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immunity develops, this method could provide a time- and labor-saving alternative strategy for acclimatizing swine; if sufficient immunity does not develop, use of this strategy could predispose herds to economically damaging outbreaks of PRRS.

The present study was designed to test the hypothesis that contact exposure would be a successful acclimatization strategy on commercial pig farms. Specifically, the purpose of the study reported here was to determine whether 6.5-week-old gilts that had not previously been exposed to PRRSV could be acclimatized to an endemic strain of the virus by commingling with age-matched gilts inoculated with an endemic PRRSV strain and whether 10.5-week-old gilts that had not previously been exposed to PRRSV could be acclimatized by commingling with age-matched inoculated or contact-exposed animals. Outcome measures that were examined included measures of specific immunity and reproductive performance. The study was conducted on a single farm in the Midwestern United States that had previously acclimatized pigs by inoculating approximately 25% of each replacement gilt group at 6.5 weeks of age to provide sufficient time for a protective immune response to develop before the pigs entered the breeding herd. However, because the age at which gilts are introduced into the breeding herd varies considerably among farms, we also wanted to determine whether gilts could be acclimatized by exposure at 10.5 weeks of age.

Materials and Methods

Animals—Eighty 2.5-week-old replacement gilts purchased from a 2,600-head gilt multiplier farm in Missouri and seronegative for exposure to PRRSV were used in the study. At the time that study animals were purchased, the source farm had never experienced an outbreak of PRRS or had any evidence of PRRSV infection. Sera from 30 randomly selected animals on the farm had been tested on a monthly basis with a commercial ELISA® at an accredited veterinary diagnostic laboratory, and all animals were consistently seronegative. Study gilts were ear tagged to facilitate identification and tracking and were randomly assigned to treatment groups at the time of purchase for the present study.

Experimental farm—Gilts from the source farm were transported to a 2,600-sow, farrow-to-wean confinement facility in Illinois. Shortly after the farm had been established in 1997, an outbreak of PRRS occurred, and the farm had experienced sporadic losses attributable to PRRS. Strict biosecurity measures were subsequently implemented, and an isolation unit was created to quarantine newly introduced animals.

For the 3 years prior to the present study, the farm’s routine protocol was to inoculate a portion of each group of incoming seronegative gilts IM with an endemic PRRSV strain isolated from the farm and to keep each group in the isolation unit for the next 4 weeks before moving it to the grower-finisher unit with the older replacement gilts. At the time of the study, the farm had not experienced any outbreaks of clinical PRRS, despite the presence of wild-type virus on the farm.

Experimental design—On arrival at the study farm, gilts were housed in an isolated nursery that had been disinfected and that had a shower-in entry separate from the rest of the farm. Gilts were randomly assigned to 1 of 3 treatment groups. Group 1 consisted of 20 gilts that were inoculated IM with an endemic PRRSV strain at 6.5 weeks of age and were maintained in an isolation unit. Group 2 consisted of 20 gilts that were contact exposed at 6.5 weeks of age by commingling with group 1 gilts in the same isolation unit, along with 60 other PRRSV-seronegative gilts from the same source farm that were not otherwise used in the study. There were 4 pens in the isolation unit with 25 pigs/pen. Five pigs in each pen were inoculated with the endemic PRRSV strain. Thus, there was a 1:4 ratio between inoculated (group 1) and PRRSV-naïve (group 2 and nonstudy) pigs at the time of acclimatization (6.5 weeks of age). After 1 month, pigs in groups 1 and 2 and the nonstudy pigs were moved to a grower unit.

Group 3 consisted of 40 gilts that were retained in a separate building at the source farm until 10.5 weeks of age. At this time, they were transported to the study farm and commingled with group 1 and 2 gilts and with the 60 nonstudy gilts in the grower unit. There were 6 pens in the grower unit, and group 3 gilts were randomly added to these 6 pens. Because group 3 gilts were introduced into an environment with a mix of inoculated (group 1) and contact-exposed (group 2) pigs, the ratio of infected to noninfected pigs at the time group 3 pigs were introduced could not be ascertained, but would have ranged from 3:7 to 2:5.

Contact-exposed gilts in groups 2 and 3 were commingled with inoculated gilts in group 1, allowing direct contact with inoculated animals. Movement of personnel on the farm was regulated such that the grower unit and the nursery unit were downstream from other areas.

At 24 weeks of age, all of the gilts were moved to a finisher unit. After the second estrus was detected in the finisher unit, gilts were moved to gestation crates and bred by artificial insemination. The semen used for artificial insemination was obtained from boar studs maintained on isolated stud farms known to be free from PRRS for at least 5 years prior to the present study. The boar studs were regularly tested for PRRS and monitored for clinical signs of PRRS on a daily basis. When semen was collected from the boar studs, a blood sample was collected from the ear vein of every third boar. Serum was obtained, and serum samples were pooled in groups of 5 and sent to the University of Illinois Veterinary Diagnostic Laboratory for testing by means of a reverse-transcription, multiplex PCR assay for North American and European strains of PRRSV; results were negative. Although the semen itself was not tested, the boar-testing procedure and farm history indicated that the semen most likely did not contain PRRSV.7,8

At 112 days of gestation, gilts were moved to crates in a farrowing room. All-in–all-out procedures were used for the farrowing room, and the farrowing room was disinfected after each group of sows was transferred out. After farrowing, sows were moved to a separate barn and were subsequently removed from the herd as part of the farm’s depopulation-repopulation efforts.
Inoculation with PRRSv—Gilts in group 1 were acclimatized by means of IM inoculation on the right side of the neck with 10^{1.3} TCID_{50} of an endemic PRRSv strain (open reading frame 5 sequence GenBank accession No. AV754343), as described. The dose used was selected on the basis of past experience with acclimatization with the same virus strain, which had been isolated from pooled serum samples from nursery pigs with clinical signs of PRRS and had initially been cultured on porcine alveolar macrophages at the University of Minnesota.

For preparation of the inoculum, an aliquot of the endemic PRRSv strain was cultured in MARC 145 cells at the University of Illinois, and aliquots of titrated cell culture supernatant from passage 2 were shipped on dry ice in appropriately labeled cryovials to the farm, where cryovials were stored in a liquid nitrogen tank. To avoid any confusion, the vials used to store aliquots of inoculum were of a different make and model from any other vials stored in that liquid nitrogen tank. The inoculum was thawed and mixed with saline solution immediately prior to inoculation. The herd veterinarian administered all inoculations throughout the study.

Sample collection—For all pigs, 2 blood samples were collected by means of external jugular venipuncture at the time of introduction to the study farm (ie, 6.5 weeks of age for groups 1 and 2 and 10.5 weeks of age for group 3) and at 19.5, 36.5 (ie, immediately prior to artificial insemination), and 48.5 (ie, day 85 of gestation) weeks of age. The first sample was collected in a 10-mL evacuated serum separator tube; the second sample was collected in a 10-mL evacuated tube containing sodium heparin. Samples were stored at 4°C until analyzed.

In addition, tonsillar biopsy specimens were obtained from all pigs at 12.5, 19.5, and 36.5 weeks of age, except that tonsillar biopsy specimens were obtained from only 20 of the 40 group 3 pigs at 12.5 weeks of age. Lingual tonsils were sprayed with lidocaine (2%) solution. Under focused illumination (torch light), biopsy specimens were collected with sterile disposable 6-mm punch biopsy instruments and individually sterilized 310-mm curved Kocher hemostat forceps, as described. Tissue samples were placed in 2-mL microcentrifuge tubes containing 1 mL of stabilizing buffer. Samples were stored at 4°C until analyzed.

Immunologic outcomes—Development of PRRSv strain-specific immunologic responses was evaluated by testing for anti-PRRSv antibodies and PRRSv-neutralizing antibodies in serum samples, measuring the number of mononuclear cells secreting PRRSv-specific interferon-γ in blood samples, and testing for the presence of viral RNA in tonsillar biopsy specimens. A commercial ELISA kit was used to test for anti-PRRSv antibodies. All samples were tested by the University of Illinois Veterinary Diagnostic Laboratory. Pigs were considered seropositive if the sample-to-positive ratio was ≥ 0.4.

Serum titers of PRRSv-neutralizing antibodies were measured with an FFN assay at the South Dakota Animal Disease Research and Diagnostic Laboratory. The reference antigen used for the FFN assay was the endemic PRRSv strain used for acclimatization.

An ELIspot assay was used to measure the number of blood mononuclear cells secreting PRRSv-specific interferon-γ, as described.

The endemic PRRSv strain used for acclimatization was used as the reference antigen in the ELIspot assay.

A reverse-transcription PCR assay was used to detect viral RNA in tonsillar biopsy specimens. For samples with positive assay results, gel-purified amplions were sequenced with automated DNA sequencers at the W. M. Keck Center for Comparative and Functional Genomics at the University of Illinois Biotechnology Center. All amplicons were sequenced in both directions to minimize error rates and resolve ambiguous bases.

Reproductive outcomes—Effects of acclimatization on reproductive outcomes were evaluated by collecting information on litter size (ie, total number of piglets born), number of piglets born alive, and number of piglets weaned.

Statistical analysis—Immunologic and reproductive outcome data were compared among groups by means of the Kruskal-Wallis test or χ² test. Standard software was used for all analyses. Values of P < 0.05 were considered significant.

Results

Immunologic outcomes—All 80 gilts were seronegative when introduced to the study farm, with sample-to-positive ratios ranging from 0 to 0.124 (Figure 1). At 19.5 weeks of age, all 80 gilts were seropositive, with sample-to-positive ratios ranging from 0.410 to 3.799. Ratios obtained at 19.5 weeks of age did not differ significantly (P = 0.345) among the 3 treatment groups.

Given that all 80 gilts were seronegative for anti-PRRSv antibodies when introduced to the study farm, PRRSv-neutralizing antibody titers were not measured at this time point. At 19.5 weeks of age, all 80 gilts had neu-
any of the 19 pigs in group 1, 19 pigs in group 2, or 40 pigs in group 3 that were tested. Percentage difference in nucleotide sequences for amplicons obtained from tonsillar biopsy specimens collected at 12.5 and 19.5 weeks of age and the nucleotide sequence for the open reading frame 5 sequence of the endemic PRRSv strain ranged from 0.01% to 0.06%.

Reproductive outcomes—No outbreaks of reproductive disease occurred on the farm during the study period. Litter size, number of piglets born alive, and number of piglets weaned did not differ significantly (P = 0.50, 0.63, and 0.63, respectively) among the 3 treatment groups (Table 1). 

Table 1—Reproductive performance for pigs acclimatized to PRRSv by inoculation at 6.5 weeks of age (group 1; n = 20), contact exposure at 6.5 weeks of age (group 2; 20), or contact exposure at 10.5 weeks of age (group 3; 40). Horizontal bars represent median values.

<table>
<thead>
<tr>
<th>Group</th>
<th>Litter size</th>
<th>No. born alive</th>
<th>No. weaned</th>
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<tbody>
<tr>
<td>Farm*</td>
<td>10.4 ± 3.3</td>
<td>9.9 ± 3.3</td>
<td>9.8 ± 3.2</td>
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<tr>
<td>1</td>
<td>10.3 ± 3.2</td>
<td>10.2 ± 3.0</td>
<td>9.8 ± 3.8</td>
</tr>
<tr>
<td>2</td>
<td>10.3 ± 3.0</td>
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</tr>
<tr>
<td>3</td>
<td>11.1 ± 3.6</td>
<td>10.3 ± 3.5</td>
<td>9.8 ± 3.4</td>
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Data are given as mean ± SD.
*Represents data for all first-parity sows on the farm where the study was conducted for the month during which most of the study sows farrowed.

Discussion

Results of the present study suggested that contact exposure of 6.5- to 10.5-week-old pigs that had not previously been exposed to PRRSv to pigs inoculated with an endemic PRRSv strain may be an efficient acclimatization strategy. Pigs in all 3 treatment groups in the present study developed anti-PRRSv and strain-spe-
cific neutralizing antibodies, had endemic viral RNA in tonsillar biopsy specimens, and developed evidence of strain-specific interferon-γ–mediated cellular immunity, indicating that pigs in all 3 groups developed immunity to the endemic PRRSv strain. In addition, no significant differences were found among groups in regard to any of the immunologic or reproductive variables measured.

In the present study, we were able to detect viral RNA in tonsillar biopsy specimens collected at 12.5 and 19.5 weeks of age, and all pigs had seroconverted by 19.5 weeks of age. Thus, clearing of the virus from the tonsils appeared to have begun sometime after 6 weeks after infection in all 3 groups. The lower proportion of group 3 pigs positive for viral RNA at 19.5 weeks (9 weeks after exposure) may have been due to more rapid clearing of the virus in pigs in this group. For instance, it is possible that group 3 pigs were exposed to a lower dose of PRRSv than were pigs in groups 1 and 2, which were inoculated or directly exposed at 6.5 weeks of age, and, hence, were able to clear the virus faster. Alternatively, the immune system in group 3 pigs may have been more mature at the time of exposure and, thus, more adept at clearing the virus.

Between 36.5 weeks and 48.5 weeks, neutralizing antibody titers of pigs in all 3 groups in the present study remained constant or increased. This strongly suggested that there was continued antigenic stimulation associated with the circulating farm strain of PRRSv. At the time the present study was conducted, naive gilts that were brought to the farm were acclimatized to PRRSv and moved to the grower unit monthly, resulting in continuous input of live virus into the grower unit, which was connected via a hallway to the gestation barns. Groups of gilts were moved from the grower to the finisher units and then into the gestation barns on a weekly basis. Thus, despite the on-farm biosecurity protocols, sows in the gestation barn were likely to have been exposed more than once to PRRSv as a result of animal movement from the nearby grower units. This situation is typical of that on operational swine farms. Previous studies\(^1,5,16\) have indicated that neutralizing antibodies and interferon-γ–mediated cellular immunity play a role in protection against PRRS. The lack of a clinical PRRS outbreak in the face of continuous exposure to endemic PRRSv would suggest that acclimatization in this setting was protective or that the endemic PRRSv strain was relatively nonpathogenic.

The present study did not evaluate the relative merits of using versus not using an acclimatization strategy. Ideally, we would have included a nonexposed group of pigs at the same farm to serve as a negative control group. However, current management practices at the study farm precluded use of such a control group. Also, because we did not detect the introduction of novel PRRSv strains onto the farm during the time of the study, we were unable to evaluate whether acclimatization would be beneficial with respect to challenge with novel or divergent PRRSv strains.

In conclusion, our findings suggest that acclimatization of 6.5- to 10.5-week-old pigs by means of contact exposure to pigs inoculated with an endemic PRRSv strain may be a time-saving, labor-saving, and economically efficient strategy to avoid outbreaks associated with endemic PRRSv on commercial swine farms. The approximate 1:4 ratio of inoculated to contact-exposed pigs used in the present study offers some guidance as to the proportion of animals that might need to be inoculated to acclimatize swine herds with similar characteristics. Further research is needed to assess the effectiveness of acclimatization in protecting pigs in the face of a PRRS outbreak. In addition, in areas with large numbers of swine operations, it would be essential to consider the risk of infection for nearby farms.

At the farm where the present study was conducted, the acclimatization procedure was stopped in July 2006. Although acclimatization on the farm was considered successful, PRRSv was still considered a cause of persistent reproductive losses, even in the absence of outbreaks. The farm was completely depopulated, cleaned, and disinfected, and only PRRSv-naïve animals were subsequently introduced. Mean number of piglets weaned per sow since elimination of PRRSv from the farm was approximately 10, representing a slight improvement over the situation during the time that the farm was chronically infected with PRRSv.

References


