Fetal protection in heifers vaccinated with a modified-live virus vaccine containing bovine viral diarrhea virus subtypes 1a and 2a and exposed during gestation to cattle persistently infected with bovine viral diarrhea virus subtype 1b

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Objective—To determine efficacy of a modified-live virus (MLV) vaccine containing bovine viral diarrhea virus (BVDV) 1a and 2a against fetal infection in heifers exposed to cattle persistently infected (PI) with BVDV subtype 1b.

Animals—50 heifers and their fetuses.

Procedures—Susceptible heifers received a placebo vaccine administered IM or a vaccine containing MLV strains of BVDV1a and BVDV2a administered IM or SC. On day 124 (64 to 89 days of gestation), 50 pregnant heifers (20 vaccinated SC, 20 vaccinated IM, and 10 control heifers) were challenge exposed to 8 PI cattle. On days 207 to 209, fetuses were recovered from heifers and used for testing.

Results—2 control heifers aborted following challenge exposure; both fetuses were unavailable for testing. Eleven fetuses (8 control heifers and 1 IM and 2 SC vaccinates) were positive for BVDV via virus isolation (VI) and for BVDV antigen via immunohistochemical analysis in multiple tissues. Two additional fetuses from IM vaccinates were considered exposed to BVDV (one was seropositive for BVDV and the second was positive via VI in fetal tissues). A third fetus in the SC vaccinates was positive for BVDV via VI from serum alone. Vaccination against BVDV provided fetal protection in IM vaccinated (17/20) and SC vaccinated (17/20) heifers, but all control heifers (10/10) were considered infected.

Conclusions and Clinical Relevance—1 dose of a BVDV1a and 2a MLV vaccine administered SC or IM prior to breeding helped protect against fetal infection in pregnant heifers exposed to cattle PI with BVDV1b. (Am J Vet Res 2011;72:367–375)

Abbreviations

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>ACE</td>
<td>Antigen-capture ELISA</td>
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<tr>
<td>BVDV</td>
<td>Bovine viral diarrhea virus</td>
</tr>
<tr>
<td>IHC</td>
<td>Immunohistochemical</td>
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<tr>
<td>MLV</td>
<td>Modified-live virus</td>
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<tr>
<td>PBL</td>
<td>Peripheral blood leukocyte</td>
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<tr>
<td>PI</td>
<td>Persistently infected</td>
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<tr>
<td>UTR</td>
<td>Untranslated region</td>
</tr>
<tr>
<td>VI</td>
<td>Viral isolation</td>
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<tr>
<td>VN</td>
<td>Viral neutralization</td>
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Bovine viral diarrhea viruses are a diverse group of viruses that cause infections and disease in domestic ruminants and wildlife throughout the world.1 Infection with BVDV in cattle causes inapparent to severe disease involving 1 or more organ systems, sometimes with high fatality rates. Organ systems affected by BVDV include the respiratory and digestive tracts, with the reproductive tract and fetus being quite susceptible at critical gestational stages.1 The BVDVs are immunosuppressive and often render an animal more susceptible to other viral and bacterial pathogens.2

Outcomes (abortions, stillbirths, congenital abnormalities, or birth of PI calves) for fetuses infected with BVDV in susceptible and pregnant heifers and cows are dependent on age of fetus at time of exposure.1 Fetuses can become PI when susceptible females are exposed to noncytopathic strains of BVDV at approximately 42 to 125 days of gestation.2 The PI calves are born immuno-
tolerant to the infecting strain and shed virus (often in high numbers) throughout their life in body excretions or secretions, such as nasal secretions. Persistently infected animals are considered to be the principal source of virus for exposure of susceptible cattle and are effective as reservoirs of infection. When PI cattle are placed in pens with susceptible (seronegative) cattle, 70% to 100% of the susceptible cattle are infected, as determined on the basis of results of VI or serologic testing that detects active infection (4-fold increase in antibody titers). Fetuses exposed to BVDV after approximately 125 days of gestation typically react by generating an immune response that eliminates the virus. If the fetus survives the infection, these calves are born with serum antibodies against BVDV. The effect of this congenital infection on the health of these calves remains to be determined, but the impact on other animals is minimal because these congenitally infected calves do not represent a source of infection.

The BVDVs are members of the viral family Flaviviridae, genus Pestivirus. They are classified as cytopathic or noncytopathic biotypes on the basis of the presence or absence of visible cellular pathogenic effects in infected monolayer cultures. Additionally, BVDVs are further classified as genotypes (BVDV 1 and BVDV 2) on the basis of genomic differences. These genotypes also have antigenic differences that are especially evident when VN tests are used. In addition, BVDV 1 and BVDV 2 are further divided into subgenotypes on the basis of viral genomic differences, with 12 BVDV 1 subtypes and 2 BVDV 2 subtypes identified throughout the world. In the United States, BVDV1a, BVDV1b, and BVDV2a are commonly isolated. However, a fourth subtype, BVDV2b, has been isolated from an animal that died of pneumonia in a feedlot. Strains BVDV1a, BVDV1b, and BVDV2a were the BVDV strains detected in diagnostic laboratory accessions and in samples obtained from PI cattle entering a feedlot. With BVDV1b the predominant BVDV subtype (BVDV1b was detected in 77.9% of the cattle PI with BVDV in the feedlot). In a regional survey of beef breeding herds that were tested to detect PI calves in the south central United States in 2006, BVDV1b was the predominant subtype. Commercial vaccines in the United States licensed by the USDA contain primarily BVDV1a and BVDV2a strains.

Emphasis has shifted from efficacy studies that involved the use of acute respiratory challenge within weeks after vaccination with measurements of less severe clinical signs to protection against fetal infection via challenge exposure of pregnant females by use of different BVDV strains. More recently, cattle PI with BVDV have been used for challenge exposure of vaccinated and control animals in studies of acute challenge exposure and fetal protection. The use of PI animals for experimental challenge exposure is similar to challenge exposure for natural field conditions in production agriculture. The purpose of the study reported here was to determine the efficacy of administration of 1 dose of an MLV vaccine containing BVDV1a and BVDV2a to help provide fetal protection in vaccinated heifers challenge exposed by use of PI cattle infected with heterologous BVDV1b strains.

### Materials and Methods

**Animals**—Multiple-source heifers (n = 107) were initially selected for use in the study. Heifers were 13 to 17 months old and had negative results for a VN test to detect antibodies against BVDV1a and BVDV2a and also had negative BVDV-PI status as determined by use of IHC analysis of skin samples (formalin-fixed ear notch skin samples). This phase of the study was performed at a private research facility in Kansas and was approved by the Pfizer Animal Health Ethical Review Board.

The reproductive tract of each heifer was scored prior to the start of the study by the attending veterinarian. At day 0, the heifers were allocated into blocks on the basis of their reproductive tract score and assigned to 3 treatment groups, with each block containing an approximate ratio of 1:2:2 for treatments 1, 2, and 3, respectively. Treatment 1 comprised 22 control heifers injected IM with an MLV vaccine containing bovine herpesvirus-1, parainfluenza-3 virus, and bovine respiratory syncytial virus and an inactivated bacterium containing *Campylobacter fetus* subspecies *venerealis; Leptospira interrogans* serovars *Icterohemorrhagiae, Canicola, Pomona,* and *Grippopryphosa* and *Leptospira borgpetersenii* serovar Hardjo. Treatment 2 comprised 43 heifers vaccinated IM, and treatment 3 comprised 42 heifers vaccinated SC, with a vaccine prepared similarly except that it also included minimum immunizing doses of BVDV1a and BVDV2a. The USDA requires that a release dose for a live BVDV vaccine be 0.7 log10 TCID50 above the minimum immunizing dose within the expiration period. To reduce the possibility of accidental BVDV exposure of the control heifers, heifers in the vaccinated groups (treatments 2 and 3) were housed together in a pasture for 3 weeks after vaccination, whereas the control heifers were placed in a similar but separate pasture. Following this 3-week period, all heifers were commingled for the duration of the study. Heifers were observed at least once weekly for general health following vaccination. Blood samples were obtained on days 0, 21, and 70; serum was harvested and stored at –20°C until assayed by use of a VN test to detect antibodies against BVDV1a and BVDV2a.

Approved products were used to synchronize estrus of the heifers prior to breeding. On days 35 and 36, heifers were bred by artificial insemination; additional breedings were performed via natural service with bulls (negative for PI BVDV status as determined by use of IHC analysis) for the following 7 weeks. On day 105, heifers were examined via transrectal ultrasonography to detect pregnancy. Heifers confirmed to be pregnant were selected for use in the challenge exposure phase of the study in an attempt to maintain the blocks as complete as possible.

**Challenge exposure of heifers**—On day 123, 8 BVDV-PI cattle (3 steers and 3 heifers; body weight, 433 to 525 kg) considered positive for BVDV on the basis of results of IHC analysis and the ACE performed on ear notch samples, PCR assay performed on serum samples, and VI performed on serum and nasal swab specimens in a 96-well infectivity assay were selected for use in the study. All PI cattle were confirmed posi...
Blood samples were collected on days 144, 198, and 198 were used in a VN test to detect antibodies against BVDV1b. All tests used to determine PI status of the cattle and subtyping of the BVDV strains of PI cattle were completed 2 to 3 months before initiation of the study. Serum samples were obtained from the PI cattle on day 123 and used for BVDV1a and BVDV2a serologic tests; on days 123 and 138, whole blood was collected from PI cattle for use in VI from PBLs and nasal swab specimens were collected for use in VI and viral titration.

On day 123, rectal temperature and blood samples (whole blood and serum) were obtained from the 50 heifers in the 3 treatment groups (10 in treatment group 1 and 20 each in treatment groups 2 and 3). On day 124 (range of gestational age, 64 to 89 days), all 50 heifers were exposed to the 8 BVDV1b-PI cattle via commingling in an outdoor pen (approx 0.2 ha) for 14 days. Heifers were observed daily for general health and clinical signs indicative of BVDV infection. Rectal temperatures were recorded on days 130, 133, and 138. Blood samples were collected on days 144, 198, and 207 to 209 (following euthanasia); serum samples from days 123, 144, and 198 were used in a VN test to detect antibodies against BVDV1a and BVDV2a.

Serum samples from days 123 and 207 to 209 were tested with a VN test to detect antibodies against BVDV1b. Additional blood samples were collected from the heifers on days 130, 133, and 138; these samples and the whole blood samples obtained on day 123 were used for VI of BVDV from PBLs.

Collection of fetuses—On day 198, heifers were transrectally palpated to confirm pregnancy; 2 heifers in the control group were found to be not pregnant and were assumed to have aborted. The remaining 48 pregnant heifers were transported on day 204 to Oklahoma State University. Fetuses were collected over a 3-day period (days 207 through 209). Fetus collection was randomized such that the numbers of heifers in each treatment group were proportional on each of those 3 days. The heifers were sedated with xylazine hydrochloride and euthanized by penetrating captive bolt; a blood sample was obtained from each heifer, and the fetuses were aseptically removed. The Oklahoma State University Institutional Animal Care and Use Committee approved this phase of the study.

Samples collected from fetuses—Multiple sets of samples were collected from each fetus. Fresh and formalin-fixed ear notch samples were collected for the ACE and IHC analysis, respectively, in accordance with the Oklahoma Animal Disease Diagnostic Laboratory protocol. Fresh samples were placed in 2 mL of PBS solution for the ACE, and formalin-fixed samples were placed in 7 mL of neutral-buffered 10% formalin for IHC analysis. Blood was collected from each fetus via cardiac venipuncture; serum was harvested and stored at –70°C. Duplicate samples from the thymus, kidneys, liver, lungs, and spleen were collected: 1 set of samples was stored at –70°C for VI, and the second set of samples was fixed in formalin for histologic examination and IHC analysis. Samples of the placenta and uterine tissues from the placentomes were collected and placed in formalin for BVDV detection via IHC analysis.

Testing of samples—The VN test was performed by personnel at a company laboratory. Samples were tested for BVDV1a and BVDV2a by use of a 96-well assay with cytopathic virus strains 5960 (BVDV1a) and 129C (BVDV2a). Virus isolation was performed as described elsewhere on PBLs and nasal swab specimens collected from heifers and PI cattle. Similarly, VI was performed on samples of fetal serum, thymus, kidneys, liver, lungs, and spleen. Prior to analysis, fetal tissues were disrupted by grinding approximately 1 g of each respective tissue in medium containing antimicrobials and an antmycotic. Samples were centrifuged, and supernatants were tested in the same manner as for PBL samples. Serum, PBLs, and fetal tissue samples were reported as positive or negative for the presence of BVDV. Results for nasal swab specimens were expressed as the 50% endpoint dilution that had presence of BVDV as calculated by the method of Spearman-Karber. Testing for serum antibodies against BVDV1b was performed in the research laboratory of the investigators at Oklahoma State University.

Statistical analysis—Data were analyzed by use of a statistical program. Logarithmically transformed serum antibody titers for each heifer (ie, dam) were analyzed with a generalized linear repeated-measures mixed model with fixed effects of treatment, time, and the treatment by time interaction and random effects of block (reproductive tract score) and animal within treatment and block, which was the animal term. Least squares means (back-transformed for titers) were calculated for each treatment and time point. Positive or negative status for viremia for each heifer was analyzed with a generalized linear repeated-measures mixed model with fixed effects of treatment and random effects of block. The Fisher exact test was used to compare rates among treatment groups with regard to positive results for fetuses (VI, serum antibodies against BVDV, IHC analysis for fetal skin, and IHC analysis for fetal tissue) and BVDV fetal infection (fetus was PI, had serum antibodies against BVDV, or was aborted). When a significant (P ≤ 0.05) treatment effect was detected, comparisons were made among treatment groups by use of pairwise t tests.
rum dilution for antibody titer < 1:2 for both BVDV1a and BVDV2a) on day 0, and the control heifers remained seronegative for prechallenge collections on days 0, 21, 70, and 123 (Table 1). Heifers in treatment groups 2 and 3 (who were vaccinated with a BVDV vaccine on day 0) began developing antibodies by day 21, and the geometric least squares mean VN antibody titers against BVDV1a and BVDV2a were significantly higher than those for the control group at days 70 and 123. On day 21, the geometric mean antibody titer for the IM and SC vaccinates against BVDV1a was 8.4 (range, < 2 to 76) and 8.0 (range, < 2 to 256), respectively, whereas the geometric mean antibody titer for the IM and SC vaccinates against BVDV2a was 1.8 (range, < 2 to 16) and 2.5 (range, < 2 to 54), respectively. The geometric mean least squares mean antibody titers on day 70 against BVDV1a were 356.3 (range, < 2 to 1,448) and 241.3 (range, < 2 to 1,218) for the IM and SC vaccinates, respectively, whereas mean antibody titers against BVDV2a were 161.6 (range, 13 to 1,448) and 177.9 (range, 2 to 861) for the IM and SC vaccinates, respectively. On day 123, the mean titer against BVDV1a was 434.2 (range, < 2 to 1,722) and 297.1 (range, 5 to 1,448) for the IM and SC vaccinates, respectively, whereas the mean antibody titers against BVDV2a were 189.4 (range, 19 to 3,444) and 150.1 (range, < 2 to 1,024) for the IM and SC vaccinates, respectively. Group geometric least squares mean antibody titers against BVDV1a and BVDV2a did not differ significantly between the IM and SC vaccinates on days 70 and 123. Also on day 123, 19 of 20 IM vaccinates had antibody titers against BVDV1b of ≥ 16 (1 heifer had a titer of < 4), and 19 of 20 SC vaccinates had antibody titers against BVDV1b of ≥ 8 (1 heifer had a titer of < 4). The mean antibody titer against BVDV1b on day 123 was 55.6 (range, < 4 to 512) in the IM vaccinates and 42.4 (range, < 4 to 512) in the SC vaccinates. All of the placebo-vaccinated (control) heifers were seronegative for BVDV1b (titer < 4), and values for all other days are geometric least squares means. †Heifers were vaccinated with a multivalent MLV vaccine without BVDV (control group [n = 20]) on day 0, bred and confirmed to be pregnant, exposed to 8 cattle PI with BVDV on days 124 through 138, and euthanized for collection of fetuses on days 144 through 198 (Table 1). On day 144, the least squares mean antibody titer for the control heifers against BVDV1a was 270.0 (range, 23 to 1,722) and against BVDV2a was 32.6 (range, 7 to 128). On day 144, the mean antibody titer against BVDV1a was 6,444.0 (range, 1,448 to 27,554) for IM vaccinates and 5,699.7 (range, 362 to 46,341) for SC vaccinates. The mean antibody titer against BVDV2a was 2,689.5 (range, 512 to 16,384) for IM vaccinates and 2,562.4 (range, 10 to 23,170) for SC vaccinates. On day 198, the mean antibody titer for the 8 remaining pregnant control heifers was 3,061.2 (range, 1,218 to 4,096) against BVDV1a and 210.8 (range, 91 to 304) against BVDV2a. On day 198, mean antibody titers against BVDV1a were 3,053.8 (range, 724 to 8,192) for IM vaccinates and 3,019.0 (range, 4,48 to 8,899) for SC vaccinates, and mean antibody titers against BVDV2a were 698.0 (range, 213 to 1,722) for IM vaccinates and 863.4 (range, 152 to 4,096) for SC vaccinates.

Serologic testing and VI after challenge exposure—The 50 heifers responded to exposure to PI cattle by developing elevated VN antibody titers against BVDV1a and BVDV2a in sera obtained on days 144 and 198 (Table 1). On day 144, the least squares mean antibody titer for the control heifers against BVDV1a was 270.0 (range, 23 to 1,722) and against BVDV2a was 32.6 (range, 7 to 128). On day 144, the mean antibody titer against BVDV1a was 6,444.0 (range, 1,448 to 27,554) for IM vaccinates and 5,699.7 (range, 362 to 46,341) for SC vaccinates. The mean antibody titer against BVDV2a was 2,689.5 (range, 512 to 16,384) for IM vaccinates and 2,562.4 (range, 10 to 23,170) for SC vaccinates. On day 198, the mean antibody titer for the 8 remaining pregnant control heifers was 3,061.2 (range, 1,218 to 4,096) against BVDV1a and 210.8 (range, 91 to 304) against BVDV2a. On day 198, mean antibody titers against BVDV1a were 3,053.8 (range, 724 to 8,192) for IM vaccinates and 3,019.0 (range, 4,48 to 8,899) for SC vaccinates, and mean antibody titers against BVDV2a were 698.0 (range, 213 to 1,722) for IM vaccinates and 863.4 (range, 152 to 4,096) for SC vaccinates.

Serum samples obtained from the 48 pregnant heifers on days 207 to 209 (ie, after euthanasia for collection of fetuses) had antibody titers against BVDV1b that ranged from 128 to 8,192, with geometric least squares means of 2,816.1 for control heifers, 987.1 for IM vaccinates, and 1,455.5 for SC vaccinates (Table 1). The mean antibody titer against BVDV1b in the control heifers at the time of euthanasia was significantly higher than the mean titers for the vaccinated heifers.

Eight of 10 control heifers had positive results for BVDV (isolated from PBLs on at least 1 collection day).

Table 1—Geometric least squares mean (range) VN serum antibody titers* against BVDV1a, BVDV2a, and BVDV1b in heifers vaccinated with a multivalent MLV vaccine without BVDV and with a multivalent MLV vaccine that contained BVDV.

<table>
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<tr>
<th>BVDV subtype</th>
<th>Treatment group†</th>
<th>0</th>
<th>21</th>
<th>70</th>
<th>123</th>
<th>144</th>
<th>198</th>
<th>207–209</th>
</tr>
</thead>
<tbody>
<tr>
<td>BVDV1a</td>
<td>Control</td>
<td>1.0</td>
<td>1.0</td>
<td>1.0</td>
<td>1.0</td>
<td>270.0</td>
<td>3,061</td>
<td>NA</td>
</tr>
<tr>
<td></td>
<td>IM</td>
<td>1.0</td>
<td>8.4</td>
<td>0.1</td>
<td>1.0</td>
<td>644.0</td>
<td>3,053</td>
<td>NA</td>
</tr>
<tr>
<td>BVDV2a</td>
<td>Control</td>
<td>1.0</td>
<td>1.0</td>
<td>1.0</td>
<td>1.0</td>
<td>32.6</td>
<td>987.1</td>
<td>NA</td>
</tr>
<tr>
<td></td>
<td>IM</td>
<td>1.0</td>
<td>1.0</td>
<td>1.0</td>
<td>1.0</td>
<td>2,689.5</td>
<td>1,455.5</td>
<td>NA</td>
</tr>
<tr>
<td>BVDV1b</td>
<td>Control</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>1.9</td>
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<td>NA</td>
<td>2,816.1</td>
</tr>
<tr>
<td></td>
<td>IM</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>55.6</td>
<td>NA</td>
<td>NA</td>
<td>987.1</td>
</tr>
<tr>
<td></td>
<td>SC</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>42.4</td>
<td>NA</td>
<td>NA</td>
<td>1,455.5</td>
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</table>

*Reciprocal titers of < 2 and < 4 were analyzed as 1 and 2, respectively, for calculation of geometric mean titers: Values for days 0 and 21 are geometric means, whereas values for all other days are geometric least squares means. †Heifers were vaccinated with a multivalent MLV vaccine without BVDV (control group [n = 10]), vaccinated IM with a multivalent MLV vaccine that contained BVDV (IM group [20]), or vaccinated SC with a multivalent MLV vaccine that contained BVDV (SC group [20]) on day 0, bred and confirmed to be pregnant, exposed to 8 cattle PI with BVDV on days 124 through 138, and euthanized for collection of fetuses on days 207 through 209.

NA = Not available.

Within a column within a BVDV subtype, values with different superscript letters differ significantly (P ≤ 0.05).
...had mild respiratory disease that consisted of moderate signs of disease (rectal temperature \( \geq 41.4^\circ \text{C} \)), consisting of a high fever (rectal temperature \( \geq 41.4^\circ \text{C} \)), anorexia, and signs of depression. Both of these control heifers had clinical signs of respiratory tract disease. The control heifers had severe respiratory disease consisting of a high fever (rectal temperature \( \geq 41.4^\circ \text{C} \)), abnormal nasal discharge, or both. None of the other heifers had a fever or clinical signs of disease during the challenge-exposure period.

**Clinical disease and rectal temperatures**—After introduction of the PI cattle, there were 6 heifers (2 in each of the 3 treatment groups) that were febrile (rectal temperature \( \geq 40.1^\circ \text{C} \)) on at least 1 sample day or that had clinical signs of respiratory tract disease. The 2 control heifers had severe respiratory disease consisting of a high fever (rectal temperature \( \geq 41.4^\circ \text{C} \)), anorexia, and signs of depression. Both of these control heifers aborted before day 198. Although neither fetus was found, a vaginal swab specimen was collected from one of these control heifers and used for VI. Virus isolated from the vaginal swab specimen was BVDV1b (Figure 1). The 2 IM vaccinates and 2 SC vaccinates had mild respiratory disease that consisted of moderate fever (rectal temperature \( \geq 40.1^\circ \text{C} \)), abnormal nasal...

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**Table 2**—Proportion of heifers exposed to PI cattle that had positive results for VI from PBLs.

<table>
<thead>
<tr>
<th>Treatment group*</th>
<th>123</th>
<th>130</th>
<th>133</th>
<th>138</th>
<th>130, 133, and 138†</th>
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<tr>
<td>Control (n = 20)</td>
<td>0/0</td>
<td>4/10</td>
<td>8/10</td>
<td>2/10</td>
<td>8/10†</td>
</tr>
<tr>
<td>IM (n = 20)</td>
<td>0/20</td>
<td>0/20</td>
<td>0/20</td>
<td>1/20</td>
<td>1/20</td>
</tr>
<tr>
<td>SC (n = 20)</td>
<td>0/20</td>
<td>1/20</td>
<td>2/20</td>
<td>3/20</td>
<td></td>
</tr>
</tbody>
</table>

Values reported represent number of heifers with positive results/number of heifers tested.

*Heifers were vaccinated with a multivalent MLV vaccine without BVDV (control group), vaccinated IM with a multivalent MLV vaccine that contained BVDV (IM group), or vaccinated SC with a multivalent MLV vaccine that contained BVDV (SC group) on day 0, bred and confirmed to be pregnant, exposed to 8 cattle PI with BVDV on days 124 through 138, and euthanized for collection of fetuses on days 207 through 209. Heifers that had positive results for VI from PBLs on at least 1 day after challenge exposure (days 130, 133, or 138).

†Within a column, values with different superscript letters differ significantly (P ≤ 0.05).

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**Figure 1**—Dendrogram of BVDV isolates obtained from fetal tissues, a vaginal swab specimen, PI cattle, and reference BVDV strains on the basis of the BVDV 5′UTR sequence. Numbers in the dendrogram represent the percentage of homology between isolates. FC = Fetus from the control group. FIM = Fetus from the IM vaccinated group. FSC = Fetus from the SC vaccinated group. VSS = Vaginal swab specimen obtained from a heifer in the control group after that heifer aborted. Each PI isolate is indicated by a sequential number (ie, P11 through PI8). Study isolates that share 100% homology with a particular PI isolate are grouped together and include the number of that PI isolate (eg, P11, P11, FC1, or FSC1). The sequence of the FIM fetal isolate from the IM vaccinated group was not 100% homologous with any of the PI isolate sequences. 1a = BVDV subtype 1a. 2a = BVDV subtype 2a. 1b = BVDV subtype 1b.

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**ACE and IHC analysis of fetal skin samples**—All 20 fetuses collected from the control heifers had positive results for both the ACE and IHC analysis for BVDV antigen on ear notch skin samples (Table 3). Of the 20 fetuses from the IM vaccinates, only 1 had positive results for the ACE and IHC analysis. The dam of this fetus was seronegative for BVDV1a (titer < 2) and BVDV1b (titer < 4) on day 123, 1 day before exposure to the PI cattle. Only 2 of 20 fetuses in the SC vaccinate group had positive results for the ACE and IHC analysis. The dam of one of those fetuses had serum VN antibody titers of 181 and 16 against BVDV1a and BVDV1b, respectively, on day 123, and the dam of the other fetus was seronegative for BVDV1b (titer < 4) and had a VN antibody titer of 5 against BVDV1a before challenge exposure.

**IHC analysis of fetal tissues**—Fetal thymus, lung, liver, spleen, and kidney tissues; placenta (cotyledon-
ary tissues); and uterine tissue (caruncles) were examined via IHC analysis to detect BVDV antigen. Fetuses from the 8 control heifers had positive results for BVDV antigen in the cotyledonary tissues and thymus, lung, liver, spleen, and kidney tissues but not from the uterine caruncles. One fetus in the IM vaccinate group and 2 fetuses in the SC vaccinate group also had positive results for BVDV antigen in all of the aforementioned tissues, except the uterine caruncles, similar to the fetuses in the control group.

VI—All 8 fetuses of the control group had BVDV in all tissues (Table 3). Examinations were performed on fetal thymus, kidney, liver, spleen, and lung tissues and fetal serum. Five fetuses in the vaccinated groups had at least 1 tissue from which BVDV was isolated. In the IM vaccinate group, BVDV was isolated from 2 fetuses (BVDV was isolated from all tissues of 1 fetus and from all tissues except the spleen and serum of the other fetus). In the SC vaccinate group, BVDV was isolated from 3 fetuses (BVDV was isolated from all tissues of 2 fetuses but only from the serum of the other fetus). The dams of these 5 fetuses had antibody titers against BVDV1b of < 4 to 64 before challenge exposure.

Serologic testing of fetal serum—Serum from the 48 fetuses was tested for BVDV1a, BVDV1b, and BVDV2a by use of the VN test, and 47 of 48 were seronegative (titer < 3) to BVDV1a and BVDV2a (Table 3). One control fetus was seropositive for BVDV1b (titer of 4) but seronegative for BVDV1a and BVDV2a. One fetus from the IM vaccinate group was seropositive (titers of 1,218 against BVDV1a, > 256 against BVDV1b, and 3 against BVDV2a). The dam of this fetus had an antibody titer of 64 against BVDV1b on day 123 before challenge exposure.

Subtyping of BVDV isolates—Virus isolates from the study were sequenced to determine the BVDV subtype via analysis of the 5′-UTR. There were 13 isolates available for sequencing (8 from tissues of the control fetuses, 1 from a fetus of the IM vaccinate group, 3 from fetuses of the SC vaccinate group, and 1 from the vaginal swab specimen obtained from a control heifer after abortion). The virus isolated from the thymus, kidney, liver, and lung tissues of another fetus of the IM vaccinate group could not be detected by use of reverse transcriptase–PCR assay and thus could not be sequenced. All of the isolates, except for 1, were BVDV1b (Figure 1). The isolate from the fetus in the IM vaccinate group yielded a result indicative of multiple sequences for BVDV1 strains, which perhaps represented a mixed infection.

Viral sequences for the isolates from the 8 PI cattle were included in the analysis. On the basis of 100% identity of the isolates, there were 4 PI cattle that matched with the BVDV strains isolated from the fetal tissues or vaginal swab specimen. Nine of the 12 BVDV isolates matched 1 of 2 PI calves. Analysis of all the isolates that could be sequenced indicated that the BVDV strains isolated were all BVDV1b and could be associated with a PI calf.

Summary of test results—Fourteen fetuses were identified as infected with BVDV. Eight control fetuses were identified on the basis of positive results for the ACE and IHC analysis of ear notch samples and VI and IHC analysis of multiple tissues, and 1 control fetus was also seropositive for BVDV1b. Three fetuses in the IM vaccinate group were infected with BVDV (one had positive results for the ACE and IHC analysis of ear notch samples and VI and IHC analysis of multiple tissues, a second fetus had positive results for VI of fetal tissues only, and a third fetus had positive results for serologic testing of fetal serum). Three fetuses in the SC vaccinate group were infected with BVDV (2 had positive results for the ACE and IHC analysis of ear notch samples and VI and IHC analysis of multiple tissues, and the other 1 had positive results for VI of fetal serum). Fetuses were not available for testing from the 2 control heifers that aborted, but a sample of vaginal fluid collected from one of those heifers after abortion had positive results via VI. The proportion of fetuses infected with BVDV, as determined by combining the number of fetuses PI or seropositive (serum antibody titers against BVDV) was significantly greater in the control group (8/8), compared with either of the BVDV-vaccinated groups (3/20 for each of the IM and SC vaccinate groups). Inclusion of the 2 aborted fetuses as evidence of BVDV infection resulted in all 10 control fetuses being affected by challenge exposure.

Discussion

Prevention of PI calves is important for control of BVDV in the cattle industry because PI cattle are believed to be the major source for viral exposure of sus-
ceptible cattle. Testing and biosecurity are important tools for BVDV control, as is a third component, building immunity to BVDV via effective vaccination programs. Effective immunity against BVDV relies on the proper timing of vaccination at the appropriate period of susceptibility, as determined by the pathogenesis of the infection or disease caused by BVDV. The approximate window of susceptibility for establishment of a PI fetus in a naive heifer or cow is day 42 to day 125 of gestation. Thus, vaccination should be performed prior to this period for effective immunity to develop; however, vaccination of naive heifers or cows with MLV products must be performed prior to breeding because these vaccines may have an adverse effect on the ovary or conceptus or on embryonic implantation.161,34

Demonstration of efficacy is important for the assurance of protection afforded by commercial vaccines, which is necessary for licensure of products as well as for the initiation and continued use by producers. Efficacy studies providing evidence of protection against fetal infections and PI calves initially used aerosol administration of laboratory-prepared challenge virus into the nasal cavity of vaccinates and control cattle. Although this artificial challenge may result in exposure with a calculated dose of the virulent virus, this is only a 1-time administration of the virus and there is risk that these strains are less representative of field isolates. Exposure of susceptible cattle in natural conditions is via close contact with a PI animal (calf or adult). The potential for exposure to PI calves in a breeding herd is illustrated by results of a regional study25 that indicated 16.7% of the herds tested contained PI calves, with a within-herd prevalence of 0.3% to 5.2%. More recently, studies3,4,20-28 were conducted that used PI calf as the challenge exposure (acute challenge exposure or challenge exposure to measure protection against fetal exposure) for demonstrating efficacy of vaccines. To our knowledge, the study reported here is the first in which investigators evaluated 1 dose of an MLV vaccine containing BVDV1a and BVDV2a administered before breeding and followed by challenge exposure of pregnant vaccinated heifers to cattle PI with BVDV1b. The commercial vaccine used in the study was licensed by the USDA for IM and SC administration, so the study design included both routes of administration.

The principal goal of the study was to demonstrate efficacy of vaccination in preventing fetal infection; however, there was evidence that the MLV vaccine reduced viremia in the BVDV vaccinates, compared with results for the control heifers. Viremia was identified at least once in 8 of 10 control heifers during the first 2 weeks after exposure to PI cattle. The groups vaccinated with BVDV MLV vaccine had fewer heifers with viremia after the introduction of the PI cattle, with only 1 of 20 IM vaccinates and 3 of 20 SC vaccinates having viremia. Potentially more viremic heifers would have been detected if additional collection dates were included, but increases in the number of samples collected from pregnant heifers would have increased the stress on these animals and their fetuses. In addition, although no clinical disease was observed in most of the heifers, severe respiratory disease was detected in 2 control heifers following exposure to PI cattle. These 2 heifers had a high fever, had signs of extreme depression, and subsequently aborted their fetuses. In contrast, relatively mild disease was observed in only 4 of the 40 heifers that received the BVDV vaccine.

All of the heifers responded with increased antibody titers against BVDV1a or BVDV2a (or both) after exposure to PI cattle, which indicated that they were all exposed to BVDV during the challenge-exposure period. The response of the vaccinates with anamnestic antibody concentrations after exposure to PI cattle was similar to that reported in another study.1 Some of the PI cattle appeared to have a greater potential to expose susceptible heifers. There were cattle PI with BVDV that matched multiple fetal viral isolates, yet there were others with no relationship to a fetal isolate. Because all PI cattle were shedding virus at the beginning and end of the exposure period, this variation could have been related to the virulence of the virus from these PI cattle or to differences in socialization patterns of the animals that impacted the amount of exposure of the pregnant heifers. Similar to results of other studies, use of PI cattle was an effective method of exposure because all control heifers aborted or had PI fetuses following challenge exposure.

Criteria used to diagnose the infected fetuses in the study reported here were quite inclusive. They included VI of tissues obtained from several organs of fetuses, IHC analysis of those same fetal tissues and the placenta, IHC analysis of formalin-fixed ear notch samples, ACE of fresh ear notch samples, VI of fetal serum, aborted fetuses, or antibodies against BVDV in fetal serum. If any one of these tests had a positive result, the fetus was considered positive for BVDV infection in this study. To be considered a positive result for VI of fetal tissues, only one of the tissues had to yield infectious virus. These multiple tests for infection status included more criteria than were included in other studies.20,21 For example, investigators in a study21 conducted to determine whether MLV vaccine protected against nasal challenge exposure of BVDV1b in pregnant heifers defined infected fetuses on the basis of results of IHC analysis of fetal tissues, VI of pooled fetal tissues and fluids (allantoic, amniotic, or both), and ACE of fresh skin samples. All 16 control fetuses from 2 trials in that study21 had positive results for IHC analysis and VI of fetal tissues and ACE of skin samples, and 11 of 16 had positive results for VI of fetal fluids. The vaccinated groups in that study all had negative results, except for 1 animal that had positive results for IHC analysis and VI of fetal tissues. In another study,22 a single criterion (VI of fetal tissues) was used to determine BVDV infection. In the study reported here, 3 fetuses were considered infected but had positive results for only one of the aforementioned criteria, and 2 fetuses were aborted. The viruses causing these infections may have varied virulence potential, compared with that in other strains. In the present study, we used additional criteria for determination of fetal infection, including IHC analysis of fetal skin samples and antibody testing of fetal serum. Thus, it is difficult to compare protection against fetal infection among studies because of a lack of uniformity in experimental design and criteria for defining fetal infection.

The mechanisms for protection against the heterologous strain, BVDV1b, by an MLV vaccine containing
BVDV1a and BVDV2a likely involve multiple components of the host immune system. The humoral immune system, as evaluated by neutralizing antibody concentrations, offers some predictive value for potential protection against BVDV. Both MLV and killed vaccines induce antibodies against a wide range of BVDV strains. In those studies, which involved the use of BVDV1b TGAC or Nebraska in the VN test, vaccines containing BVDV1a strains induced antibodies against BVDV1a and lower titers against BVDV1b. These results were similar to results of the present study when the serum samples for day 123, which were obtained 1 day before challenge exposure to PI cattle, were tested for neutralizing antibodies against BVDV1a, BVDV2a, and BVDV1b. Antibody titers against BVDV1b were reduced before the introduction of the PI cattle, compared with titers against BVDV1a and BVDV2a, and there were 2 vaccines that were seronegative (titer < 4) for BVDV1b. Antibody titers against BVDV1b on day 123 (i.e., 1 day before challenge exposure) were an indication of whether the fetuses would become infected after heifers were challenged exposure to PI cattle. To our knowledge, this is the first study in the United States in which titers against BVDV1b were reported for heifers at the time of challenge exposure to BVDV1b. In another study, an MLV vaccine containing BVDV1a protected fetuses after the vaccinated and control fetuses were experimentally challenged exposed to BVDV1b via intranasal deposition of the virus into the nasal cavity. In that study, antibody titers against BVDV1a and BVDV2a, but not BVDV1b, before and after challenge exposure were reported.

We concluded that 1 dose of an MLV vaccine containing BVDV1a and BVDV2a at minimum immunizing doses in addition to other immunogens administered before breeding reduced the risk of fetal infections in pregnant heifers challenged exposure to BVDV1b via PI cattle. Although protection was not total with only 1 dose of vaccine, evidence from this study indicated that the MLV BVDV1a and BVDV2a vaccine helps confer protection against a heterologous BVDV strain, BVDV1b. Efficacy of the vaccine was similar between groups of heifers vaccinated IM or SC. Vaccination in addition to biosecurity and test-and-removal of PI cattle are integral tools for the control of BVDV-PI cattle.

References

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