Control of BVDV infection is achieved by implementing herd health programs focused on limiting exposure to persistently infected carrier calves and optimizing protective immunity through immunization. Antigenic diversity among BVDV isolates affects protective immunity afforded by vaccines. Antigenic heterogeneity among field BVDV isolates has been detected by use of panels of monoclonal antibodies.1 Bovine viral diarrhea virus, a member of the Pestivirus genus, family Flaviviridae, is divided into 2 biotypes, cytopathic and noncytopathic, on the basis of the ability to induce cytopathic effects in host cells in vitro. Both biotypes of BVDV infect cattle, but only the noncytopathic biotype causes persistent infections. Bovine viral diarrhea virus strains are also divided into 2 genotypes by use of gene sequencing techniques and cross-neutralization assays.3,5 Although both genotypes cause disease, the clinically severe, acute BVDV infections are caused by noncytopathic type 2 BVDV.3,4 Variability in the virulence of individual type 2 BVDV isolates has been detected experimentally and accounts, at least in part, for variation among field cases of BVDV infection, which range in severity from clinically inapparent to peracute, fatal infections.6 The genetic diversity among BVDV isolates results in antigenically diverse viruses. Glycoprotein E2 (gp53) is the major antigenic glycoprotein of BVDV and elicits production of high concentrations of neutralizing antibodies in the host following infection or vaccination.7,8 The E2 glycoprotein of BVDV type 1 has 1 immunodominant epitope, whereas that of BVDV type 2 has 3 immunodominant epitopes.8,9 Although type 1 and type 2 strains maintain cross-reactivity to each other with shared epitopes, the strength of the se-

**Objective**—To evaluate protection resulting from use of a modified-live noncytopathic bovine viral diarrhea virus (BVDV) type 1 vaccine against systemic infection and clinical disease in calves challenged with type 2 BVDV.

**Animals**—10 calves, 5 to 7 months of age.

**Procedures**—Calves were allocated (n = 5/group) to be nonvaccinated or vaccinated SC on day 0 with BVDV 1 (WRL strain). Calves in both groups were challenged intranasally with BVDV type 2 isolate 890 on day 21. Rectal temperatures and clinical signs of disease were recorded daily, and total and differential WBC and platelet counts were performed. Histologic examinations and immunohistochemical analyses to detect lesions and distribution of viral antigens, respectively, were performed.

**Results**—After challenge exposure to BVDV type 2, nonvaccinated calves developed high rectal temperatures, increased respiratory rates, viremia, leukopenia, lymphopenia, and infection of the thymus. Vaccinated calves did not develop high rectal temperatures or clinical signs of respiratory tract disease. Vaccinated calves appeared to be protected against systemic replication of virus in that they did not develop leukopenia, lymphopenia, viremia, or infection of target organs, and infectious virus was not detected in peripheral blood mononuclear cells or the thymus.

**Conclusions and Clinical Relevance**—The modified-live BVDV type 1 vaccine protected against systemic infection and disease after experimental challenge exposure with BVDV type 2. The vaccine protected calves against infection and viremia and prevented infection of target lymphoid cells. (Am J Vet Res 2007;65:788–796)
Cross-protective immunity against infection with either type 1 or 2 BVDV is necessary. Cattle vaccinated with a modified-live BVDV vaccine produced antibodies that neutralized 20 strains of BVDV in vitro in 1 study and 10 strains in another study. Both studies used antigenically diverse strains of type 1 and type 2 BVDV in the neutralization assays, with results reflecting favorably on cross-reactivity of the modified-live BVDV vaccine. Experimental challenge-exposure studies have been conducted to confirm that modified-live BVDV type 1 vaccines cross-protect young calves from experimental infection with a virulent strain of BVDV type 2. These studies, which were based on clinical signs, viremia, and hematologic variables, did not evaluate protection against infection of target lymphoid cells of lymphoid organs of vaccinated animals. In a recent report, protection afforded by a noncytopathic BVDV type 1 vaccine in cattle protected against systemic infection and disease after challenge exposure with a noncytopathic BVDV type 1 virus was observed. To our knowledge, cross-protection between genotypes based on inhibition of systemic infection of target lymphoid organs has not been reported.

The objective of the study reported here was to evaluate cross-protective immunity against systemic infection and clinical disease afforded by use of a modified-live noncytopathic BVDV type 1 vaccine in calves challenged with type 2 BVDV.

**Materials and Methods**

**Calves**—Ten crossbred beef calves 5 to 7 months old and weighing approximately 270 kg were used in the study. All calves had negative results of tests for BVDV, including virus isolation and assays for serum neutralizing antibodies. Calves were housed in biosecurity level 2 isolation rooms and fed a pelleted complete feed at a rate of 2% to 2.5% of their body weight daily. The project was reviewed and approved by the University of Nebraska Institutional Animal Care and Use Committee.

**Cell cultures and viruses**—Bovine turbinate cells were used for virus isolation and titration assays. All cells were tested for extraneous BVDV by use of a described procedure. Bacteria (including mycoplasmal organisms), and fungi. Cells were grown as monolayers in DMEM supplemented with equine serum (10%) in a humidified incubator with 5% CO₂. Bovine viral diarrhea virus strain 890, obtained from the APHIS, Center for Veterinary Biologics, Ames, Iowa (titer, 10⁷ TCID₅₀/mL), was used as challenge virus.

**Vaccine**—The commercially available vaccine contained BVDV type 1 strain WRL (a modified-live noncytopathic BVDV 1 strain attenuated by serial passage in swine kidney cells in combination with modified-live infectious bovine rhinotracheitis virus, bovine parainfluenza 3 virus, and bovine respiratory syncytial virus.

**Inoculation**—Five calves were allotted to each of 2 groups. Calves in group 2 were vaccinated SC on day 0 with BVDV 1. Calves in groups 1 and 2 were inoculated on day 21 via intranasal aerosolization of 10 mL of BVDV cell culture fluid (1.2-mL aliquots of BVDV type 2 isolate 890 diluted to 10 mL in DMEM).

**Clinical observations**—Beginning 2 days before calves were vaccinated (day of vaccination = day 0), rectal temperature and any clinical signs of infection were recorded daily for each calf. In addition, nasal swab specimens were collected from each calf for use in BVDV isolation.

Clinical signs of disease were assigned numeric values on the basis of a scoring system by an individual unaware of treatments. Respiratory rate and dyspnea were scored by use of a 3-point scale (0, normal; 1, rapid, shallow breathing; and 2, dyspnea). Nasal secretions were scored by use of a 3-point scale (0, normal; 1, excessive serous secretion; and 2, excessive mucopurulent secretion). Coughing was recorded as a binary variable (0, not detected and 1, detected). Lethargy was determined by observing whether calves were responsive each time the observer entered an isolation room and scored by use of a 4-point scale (0, responsive; 1, mild signs of depression and inactivity; 2, severe signs of depression and inactivity; and 3, recumbent and unresponsive). Fecal characteristics were scored by use of a 3-point scale (0, normal; 1, unformed feces; and 2, watery feces).

**Hematologic evaluation**—Blood samples were collected daily on days –2 to 11 and days 19 to 30 after vaccination. Blood was collected from the external jugular vein into sterile tubes containing EDTA and was used for determination of total and differential WBC values on the basis of a scoring system by an individual unaware of treatments. Respiratory rate and dyspnea were scored by use of a 3-point scale (0, normal; 1, rapid, shallow breathing; and 2, dyspnea). Nasal secretions were scored by use of a 3-point scale (0, normal; 1, excessive serous secretion; and 2, excessive mucopurulent secretion). Coughing was recorded as a binary variable (0, not detected and 1, detected). Lethargy was determined by observing whether calves were responsive each time the observer entered an isolation room and scored by use of a 4-point scale (0, responsive; 1, mild signs of depression and inactivity; 2, severe signs of depression and inactivity; and 3, recumbent and unresponsive). Fecal characteristics were scored by use of a 3-point scale (0, normal; 1, unformed feces; and 2, watery feces).

Necropsy—All calves were euthanatized on day 30, 9 days after challenge exposure with BVDV type 2. Specimens were obtained from the thymus for use in virus isolation and from the liver, kidneys, and ileum for aerobic bacterial culture. Specimens obtained from the tonsils, thymus, trachea, esophagus, lungs, liver, kidneys, spleen, rumen, abomasum, duodenum, jejunum (approx 2 m proximal to the ileocecal valve), mesenteric lymph nodes, ileum (approx 15 cm proximal...
to the ileocecal valve), cecum, and colon were fixed in neutral-buffered 10% formalin. The following day, tissues were processed, embedded in paraffin, sectioned at a thickness of 4 µm, stained with H&E, and examined by use of light microscopy. The investigator (DJS) who examined the stained tissue sections and scored the lesions was not aware of the calf from which the tissues originated or of the calf’s treatment group. Histologic changes in tissues (reactive follicles, lymphoid depletion, and lesion) were evaluated and scored by use of a 4-point scale (0, none; 1, mild; 2, moderate; and 3, severe). Lymphocytolysis was scored by use of a 5-point scale (0, none or rare; 1, few cells in follicle of nodes or Peyer’s patches; 2, 10% to 25% of follicles in node; 3, 26% to 75% of follicles in node; and 4, > 75% of follicles in node). 11

Virus neutralization test—Serum-neutralizing antibody titers were determined by combining serial 2-fold dilutions (1:2 to 1:256) of heat-inactivated (56°C for 30 minutes) serum with BVDV type 1 or 2 (150 TCID₅₀/50 µL) in 96-well microtiter plates for 1 hour at 37°C with 5% CO₂. After incubation, 1 × 10⁶ bovine turbinate cells were added to each well and incubated for 5 days at 37°C. Cells were examined with a microscope for changes associated with viral cytopathic effects, and titers of virus-neutralizing antibodies were recorded as the reciprocal of the highest serum dilution that inhibited cytopathic effects, as described. 18

Virus isolation and titration—Virus isolation procedures were performed on fluids from nasal swab specimens, PBMCs, and the thymus. Tissue specimens (5 g each) obtained from the thymus during necropsy examination were homogenized in 20 mL of DMEM with 100 µg of gentamicin/mL and 0.25 µg/mL of amphotericin B/mL. Tissue homogenates were stored at –80°C until tested for BVDV. For virus isolation, thymus specimens, PBMC samples, and nasal swab specimens were diluted to determine TCID₅₀ per milliliter in bovine turbinate cell monolayers and stained by use of an indirect immunoperoxidase test with monoclonal antibody 348 directed against glycoprotein E2 (gp 53), as described, 11 and were examined by personnel unaware of the calf or treatment group of origin.

Immunohistochemical analyses—Paraffin-embedded tissues were sectioned at 5 µm and stained for detection of BVDV antigen by use of an avidin-biotin alkaline phosphatase method. Sections were deparaffinized in xylene, rehydrated in a series of graded alcohol solutions, and treated with protease XIV in 0.5M TBSS (pH, 7.6) for 15 minutes at 37°C. Sections were blocked for 30 minutes at 18° to 24°C in TBSS with 4% equine serum. After blocking, primary antibody (anti-BVDV monoclonal antibody 15C5 directed against gp 48 [diluted 1:1,000 in TBSS]) was added, and sections were incubated for 1 hour at 18° to 24°C. Sections were washed twice in TBSS with 1mM EDTA and 0.05% Tween 20 (4 min/wash). Biotinylated horse anti-mouse immunoglobulin diluted 1:200 in TBSS with 2% normal bovine serum and 4% horse serum was added and allowed to incubate for 15 minutes. Af-

Figure 1—Mean ± SEM rectal temperatures for calves (n = 5/group) that were not vaccinated or vaccinated on day 0 with a noncytopathic BVDV type 1 vaccine. All calves were challenge inoculated intranasally on day 21 with BVDV type 2. *Significant (P < 0.05) difference between groups.

Figure 2—Mean ± SEM total lymphocyte counts for the same calves as in Figure 1. Notice lymphopenia after challenge inoculation with BVDV in calves that were not vaccinated. See Figure 1 for key.
After washing, substrate was applied to the tissue sections and slides were allowed to incubate for 10 minutes at 20° to 22°C in darkness. Slides were washed in tap water for 2 minutes, counterstained in Mayer hematoxylin, and dehydrated. A coverslip was applied, and each slide was examined for staining as described. Slides for immunohistochemical evaluation were scored by use of a 5-point scale (0, negative; 1, rare or scattered cells; 2, moderate regional staining; 3, widespread staining; and 4, intense staining in multiple regions).

Statistical analysis—An ANOVA for repeated measures in a completely randomized design with an autoregressive error covariance structure and comparison of least squares means were used to detect significant differences between treatment groups for mean values of rectal temperature, WBC counts, lymphocyte counts, neutrophil counts, monocyte counts, and platelet counts. A Fisher exact test, extended for more than 2 outcomes when necessary, was used to detect differences in histologic characters between treatments. A value of \( P < 0.05 \) was considered significant.

**Results**

Clinical observations—Calves in group 1 developed signs of respiratory tract disease (rapid respiratory rate [40 to 60 breaths/min] and dyspnea) with scores of 1 when evaluated on days 28 and 29 (days 7 and 8 after challenge), whereas calves in group 2 received scores of 0 and maintained stable respiratory rates (16 to 18 breaths/min) throughout the observation period. For group 1 calves, calf No. 38 had a fecal characteristic score of 1 on days 26 and 28 to 30, calf No. 39 had a fecal characteristic score of 1 on day 29, and calf No. 28 had a fecal characteristic score of 3 on day 30. In group 1, calf No. 37 received a lethargy score of 1 on days

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**Table 1**—Mean scores for histologic changes in various sections of lymphoid tissues in nonvaccinated (group 1) and vaccinated (group 2) calves (\( n = 5 \)/group) after challenge exposure with BVDV.

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Group</th>
<th>Inflammation</th>
<th>Necrosis</th>
<th>Lymphocytolysis</th>
<th>Lymphoid depletion</th>
<th>Lesion</th>
<th>Immuno-histochemical score</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ileum (proximal)</td>
<td>1</td>
<td>2.4*</td>
<td>1.0</td>
<td>3.0*</td>
<td>3.0†</td>
<td>1.2</td>
<td>3.2*</td>
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<td></td>
<td>2</td>
<td>0.6</td>
<td>0</td>
<td>0</td>
<td>2.8‡</td>
<td>0.6</td>
<td>0.2</td>
</tr>
<tr>
<td>Ileum (distal)</td>
<td>1</td>
<td>2.2</td>
<td>1.6*</td>
<td>3.0</td>
<td>2.8</td>
<td>1.0</td>
<td>2.0</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>0.6</td>
<td>0</td>
<td>0.2</td>
<td>3.2†</td>
<td>1.0</td>
<td>0.2</td>
</tr>
<tr>
<td>Colon (proximal)</td>
<td>1</td>
<td>1.4</td>
<td>0.5</td>
<td>0.2</td>
<td>0.8*</td>
<td>1.0‡</td>
<td>1.0</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>0.6</td>
<td>0</td>
<td>0.6</td>
<td>0.2</td>
<td>0.2</td>
<td>0</td>
</tr>
<tr>
<td>Mesenteric lymph node</td>
<td>1</td>
<td>0.4</td>
<td>0</td>
<td>0.6</td>
<td>0.8</td>
<td>0.8‡</td>
<td>2.8*</td>
</tr>
<tr>
<td></td>
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<td>0</td>
<td>0.9</td>
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<td>0</td>
</tr>
<tr>
<td>Thymus</td>
<td>1</td>
<td>0.2</td>
<td>0.8</td>
<td>1.4*</td>
<td>1.4*</td>
<td>0.8‡</td>
<td>1.8</td>
</tr>
<tr>
<td></td>
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<td>0</td>
</tr>
</tbody>
</table>

*Significant \( P < 0.05 \) difference between groups. †Acute lesions. ‡Chronic lesions.
28 and 29 and calf No. 39 received a lethargy score of 1 on day 28. Calves in group 2 received nasal discharge scores of 1 for calf No. 25 (days 9 to 14, 17, 18, 20, 21, and 23), calf No. 26 (days 14 and 15), and calf No. 27 (days 9 to 14). The remaining scores for group 1 and group 2 calves for all clinical variables were 0 on all days of the study.

Postvaccination rectal temperatures in group 1 and group 2 calves ranged from 38.5°C to 38.8°C and 38.7°C to 39.6°C, respectively. Calves in group 2 had significantly higher mean rectal temperatures on days 2 and 10 after vaccination, compared with calves in group 1. Calves in group 1 had significantly higher mean rectal temperatures on days 24, 28, and 29 (days 3, 7, and 8 after exposure), compared with rectal temperatures on the same days for calves in group 2. All 5 calves in group 1 had pyrexia, with rectal temperatures ranging from 38.8°C to 39.4°C (mean, 39.1°C) on day 24 (day 3 after challenge exposure), 38.8°C to 40.8°C (mean, 39.4°C) on day 28 (day 7 after challenge exposure), and 40.1°C to 41.2°C (mean, 40.4°C) on day 29 (day 8 after challenge exposure), values that were significantly (P = 0.046, P = 0.025, and P < 0.001, respectively) different from the rectal temperatures of calves in group 2 on the same days. Mean rectal temperatures of calves in group 1 decreased substantially on day 9 after challenge and were not significantly higher than the rectal temperatures in calves in group 2 (Figure 1).

Serum neutralizing antibody responses—All calves were seronegative for antibodies against BVDV on day 0, and calves in group 1 remained seronegative throughout the study period. The BVDV type 1 serum antibody titers for calves in group 2 on days 21 and 30, respectively, were as follows: calf No. 25 (8, 32), calf No. 26 (32, 256), calf No. 27 (32, 64), calf No. 35 (16, 64), and calf No. 36 (32, 64). The BVDV type 2 antibody titers were as follows: calf No. 25 (< 4, 4), calf No. 26 (16, 64), calf No. 27 (4, 8), calf No. 35 (< 4, 32), and calf No. 36 (< 4, 4).

Hematologic findings—Beginning on day 24 (day 3 after challenge) and continuing through day 28 (day 7 after challenge), calves in group 1 had significantly decreased mean total leukocyte counts (data not shown) and significantly decreased lymphocyte counts on days 28 and 29 (days 7 and 8 after challenge; Figure 2). Platelet counts in calves of group 1 were significantly (P = 0.02) decreased on day 29 (day 8 after challenge), and neutrophil counts were significantly decreased on days 24 to 27 and 30. On days 3 and 6 to 11 after vaccination, calves in group 2 had significantly (P = 0.02) decreased mean total leukocyte counts. Other significant (P = 0.02) reductions in cell counts developed in calves in group 2 after vaccination; lymphocyte counts were decreased on days 5 to 11, and neutrophils were decreased beginning on day 5 and continuing to day 9, compared with cell counts for calves in group 1.

Virus isolation and viral titers—After challenge exposure, BVDV was isolated from nasal secretions, PBMCs, and the thymus of calves in group 1 but not...
from the nasal secretions, PBMCs, or thymus of calves from group 2. Calves from group 1 shed virus in nasal secretions beginning on day 22 (day 1 after challenge) and continuing through day 30 and were viremic beginning on day 24 (day 3 after challenge) and continuing through day 30, when the calves were euthanatized (Figures 3 and 4). This was in contrast to group 2 calves, which were free of BVDV in nasal secretions as well as in PBMCs throughout the study period. Viral titers in thymus tissues from calves in group 1 ranged from $1 \times 10^1$ TCID$_{50}$/g to $9.6 \times 10^4$ TCID$_{50}$/g of tissue. No virus was detected in thymus tissues from calves in group 2.

Pathologic findings—Gross and histologic lesions in tissues of the digestive and lymphatic systems were more severe in calves from group 1 than in calves from group 2. Severe (4 calves) or moderate (1 calf) lesions of the digestive tract were evident in calves from group 1, whereas only mild (2 calves) or no (3 calves) lesions of the digestive tract were evident in calves from group 2. In group 1, the ileum of all 5 calves was severely edematous and turgid. Serosal petechiae and hyperemia were evident in the ileum of 3 calves (Nos. 28, 29, and 39). Cavitation was detected in the Peyer’s patches of calf No. 28. Calf No. 36 in group 2 had hyperemia in the duodenum and semisolid feces. Lesions were not detected in the esophagus, abomasum, liver, kidneys, bladder, or bone marrow of any calves. Calves in group 1 had moderate (2 calves) to marked (2 calves) or no (1 calf) thymic atrophy. The calves in group 2 were less affected; 3 calves did not have thymic atrophy, and 2 calves had mild thymic atrophy.

Mean scores for histologic changes in lymphatic tissues of calves from both groups were calculated (Table 1). There was a significant ($P = 0.016$) difference in inflammation in the Peyer’s patches in the proximal portion of the ileum between calves of group 1 and group 2; marked (3 calves), moderate (1 calf), and mild (1 calf) inflammation was in the proximal portion of the ileum of group 1 calves, whereas only mild (3 calves) or no (2 calves) inflammation was detected in the proximal portion of the ileum of group 2 calves. There was also a significant ($P = 0.048$) difference in necrosis in the Peyer’s patches in the distal portion of the ileum between calves of group 1 and group 2; severe (1 calf), moderate (1 calf), mild (2 calves), or no (1 calf) necrosis was detected in group 1 calves, whereas necrosis was not detected in the Peyer’s patches of the distal portion of the ileum of any group 2 calves. There was a significant difference in lymphocytoysis in the Peyer’s patches of the proximal portion of the ileum ($P = 0.018$) and thymus ($P = 0.008$) between calves of group 1 and group 2. Severe lymphocytoysis in the Peyer’s patches of the proximal portion of the ileum was detected in 4 calves and marked lymphocytoysis in 1 calf of group 1, whereas lymphocytoysis was not detected in the Peyer’s patches of the proximal portion of the ileum of group 2 calves. In group 1, histologically acute changes were prominent with moderate to severe lymphocytoysis evident in the ileal Peyer’s patches (Figure 5). In calves of group 2, changes in Peyer’s patches were predominantly chronic in

![Figure 6—Photomicrographs of sections of ileal Peyer’s patches in a vaccinated calf 9 days after challenge inoculation with BVDV. A—Notice chronic histologic changes with marked lymphoid depletion (arrow) and early repopulation of follicles. H&E stain; bar = 50 µm. B—Minimal deposition of BVDV antigen is evident (arrow), and normal follicles are adjacent. Immunohistochemical stain; bar = 50 µm.](image-url)
character and lymphoid follicles were either normal or diminutive. Diminutive follicles had few lymphocytes and consisted of loose stroma, dendritic cells, and some macrophages containing hematoidin pigment (Figure 6). There was no significant quantitative difference in lymphoid depletion in Peyer's patches in the proximal or distal portions of the ileum between group 1 and group 2 calves; marked to severe lymphoid depletion was evident in Peyer's patches of calves of both groups. However, there was significantly more lymphoid depletion in other lymphatic tissues in the proximal portion of the colon ($P = 0.048$) and thymus ($P = 0.048$), compared with calves in group 2. In the calves in group 1, histologically acute changes with marked lymphocytolysis were evident in the thymus (Figure 7). Histologic changes in the thymus of group 2 calves were chronic with marked lymphoid depletion. Evidence of pathogenic bacterial infection was not detected in tissues from any of the calves via bacterial culture or gross or histologic evaluation.

**Immunohistochemical findings**—In calves of group 1, high concentrations of BVDV antigen were detected in lymphatic tissues, particularly in areas of the Peyer's patch germinal centers, mesenteric lymph nodes, and thymic cortex. The distribution and intensity of staining were correlated with lesions in the tissues, with the most intense staining evident in Peyer's patches, followed by the mesenteric lymph nodes, lymphatic tissue in the proximal portion of the colon, and thymus. Bovine viral diarrhea virus antigen was found in dendritic cells and occasionally in nearby intestinal-associated submucosal macrophages adjacent to lymphoid nodules with positive staining. Deposition of BVDV antigen in dendritic cells was significantly ($P = 0.024$) greater in the proximal portion of the ileum and mesenteric lymph nodes ($P = 0.048$) in group 1 calves (Figure 5), compared with group 2 calves (Figure 6), which had no staining in those tissues with the exception of the Peyer's patches of 1 calf in which minimal staining was detected. Marked deposition of BVDV antigen in dendritic cells was evident in the thymus of group 1 calves (Figure 7), whereas BVDV antigen was only rarely evident in the thymus of group 2 calves. In other lymphoid tissues, little (tonsil and retropharyngeal lymph node) or no (prescapular lymph node) antigen was detected in calves of either group.

**Discussion**

A modified-live noncytopathic BVDV type 1 vaccine protected calves against viremia, systemic infection, and clinical disease after challenge exposure to noncytopathic BVDV type 2 isolate 890 in the study reported here. The vaccinated calves were protected against viremia and infection of lymphoid target cells in multiple lymphoid organs. Virus was not recovered from nasal secretions of vaccinated calves, indicating that replication of virus in the upper portion of the respiratory tract was prevented. In contrast, nonvaccinated calves challenged with BVDV type 2 890 developed higher rectal temperatures (days 24, 28, and 29) following exposure, with peak febrile responses (rectal temperature, 41.3°C) and increased respiratory rates developing on days 28 and 29 (days 7

![Image](image1.png)

![Image](image2.png)

**Figure 7**—Photomicrographs of sections of the thymus in a nonvaccinated calf 9 days after challenge inoculation with BVDV. A—Notice acute histologic changes with marked lymphocytolysis (arrows). H&E stain; bar = 50 µm. B—Marked deposition of BVDV antigen is evident in dendritic cells (arrow). Immunohistochemical stain; bar = 25 µm.
and 8 after challenge exposure). Vaccinated calves were protected against systemic replication of challenge virus because decreased leukocyte and lymphocyte counts were not detected in those calves after challenge exposure. Moreover, infectious virus was not recovered from PBMCs or thymic tissues, and immunohistochemical tests of lymphatic organs confirmed that virus was not distributed systemically. In contrast, 3 nonvaccinated calves were viremic by day 3 after challenge and all 5 calves were viremic by day 5, continuing through day 9 after challenge. The nonvaccinated calves were leukopenic on days 24 and 26 through 28 and lymphopenic on days 28 and 29 after challenge exposure.

Virus was also consistently reisolated from thymus tissues of nonvaccinated calves, and marked deposition of antigen in dendritic cells and occasional submucosal macrophages was widely distributed in lymphoid tissues in those calves during immunohistologic examination. In contrast, virus was not reisolated from thymus tissues of vaccinated calves and antigen was not evident in lymphoid tissues in those calves.

Results of the study reported here correlate with previous reports of studies\(^{14,16}\) that used a BVDV type 1 vaccine and BVDV type 2 challenge virus. Vaccination of calves with a noncytopathic BVDV type 1 vaccine resulted in diminished disease signs and nasal shedding of virus and prevented development of leukopenia and viremia after experimental infection with BVDV type 2 isolate 890.\(^{16}\) Another study\(^{14}\) reported similar results from use of a cytopathic BVDV type 1 vaccine with the same challenge virus, BVDV type 2 strain 890. Despite similar results obtained with a noncytopathic or cytopathic virus for vaccination,\(^{14,16}\) differences in immune responses after infection with the different BVDV biotypes have been reported.\(^{20,21}\) In 1 study,\(^{20}\) neutralizing antibody responses developed earlier and to higher concentrations in calves after infection with noncytopathic BVDV, compared with the homologous cytopathic BVDV. Lymphocyte reactivity in in vitro lymphocyte proliferative assays was significantly higher in calves exposed to the cytopathic biotype during the first inoculation, compared with calves that were initially inoculated with the noncytopathic biotype.\(^{20}\) Another study\(^{11}\) revealed that calves inoculated with a noncytopathic BVDV were more likely to be viremic and shed virus from the respiratory tract, compared with calves inoculated with cytopathic virus, despite the higher concentrations of neutralizing antibodies. The authors speculated that biotype may play a role in distribution of virus in host tissues during the course of infection.\(^{21}\) Protection against infection of target lymphoid cells of lymphoid organs from vaccinated animals was not evaluated in the preceding studies\(^{14,16,20,21}\) or in another study\(^{15}\) that used BVDV type 1 vaccine and BVDV type 2 challenge.

The vaccine provided cross-protective immunity against systemic infection and disease after challenge exposure in the study reported here. We have previously attributed the protective immunity afforded by this vaccine to replication of the vaccine BVDV in the host.\(^{11}\) Consistent with that report, vaccine virus replicated and was distributed systemically in vaccinated calves as evidenced by decreased leukocyte and lymphocyte counts, which were mild and transient and remained within reference ranges for those cell populations.\(^{22}\) Lymphocyte depletion was extensive in the Peyr’s patches and mild in the mesenteric lymph nodes, prescapular lymph nodes, and tonsils of vaccinated calves. Differences between vaccinated and nonvaccinated calves in the character of lesions in the lymphatic organs were clearly distinguishable. As previously described in detail, lesions in the lymphatic organs of vaccinated calves were chronic in nature, whereas nonvaccinated calves inoculated with NY-1 BVDV developed acute lesions (severe lymphocytolysis) in lymphatic organs after challenge exposure.\(^{11}\) Similar effects of modified-live vaccines on vaccinated animals have been reported, including decreased circulating lymphocytes and neutrophils,\(^{23}\) transient viremia,\(^{23}\) and recovery of BVDV from ovaries.\(^{24}\) Moreover, in the present study, lesions detected in the lymphoid organs of vaccinated calves were chronic and no longer active and the calves were protected against viremia and systemic replication of noncytopathic type 2 BVDV.

The fact that vaccination effectively prevented viremia and infection of lymphoid target cells suggests that postexposure immunosuppression caused by infection with BVDV type 2 may be prevented by vaccinating calves with type 1 BVDV vaccines. Prevention of viremia by vaccination is important because viremia precedes fetal infection and infection of target cells of lymphoid organs. Prevention of viremia then precludes both fetal infection and establishment of persistent infection as well as infection of target cells of lymphoid organs and immunosuppression. Cross-reactivity of BVDV vaccine as described in the present study, which provides cross-protective immunity, is an important property because antigenic variation is a prominent feature of BVDV isolates that commonly circulate in the cattle population.

References

a. Plain Ration, No. 31786HB24, ADM Alliance Nutrition Inc, Quincy, Ill.

b. Bovine turbinate cells, National Veterinary Services Laboratory, USDA, Ames, Iowa.

c. Bovine viral diarrhea virus strain 890, APHIS, Center for Veterinary Biologics, Ames, Iowa.

d. Jencine 4, Schering-Plough Animal Health, Union, NJ.

e. Atomizer No. 163, DeVilbiss Health Care Inc, Somerset, Pa.

f. Monoclonal antibody 348, VMRD Inc, Pullman, Wash.

g. Biotinylated horse anti-mouse IgG (H&L), Vector Laboratories, Burlingame, Calif.

h. Alkaline phosphatase substrate kit 1, Vector Laboratories, Burlingame, Calif.

i. Vector Red, Vector Laboratories, Burlingame, Calif.


6. Kelling CL, Steffen DJ, Topliffe CL, et al. Comparative virulence of isolates of bovine viral diarrhea virus type II in experiment-