Characterization of protection from systemic infection and disease by use of a modified-live noncytopathic bovine viral diarrhea virus type 1 vaccine in experimentally infected calves

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Objective—To evaluate protection against systemic infection and clinical disease provided by use of a modified-live noncytopathic bovine viral diarrhea virus (BVDV) type 1 vaccine in calves challenged with NY-1 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 type 1 (WRL strain). Calves in both groups were challenged intranasally with NY-1 BVDV on day 21. Calves’ rectal temperatures and clinical signs of disease were recorded daily, total and differential WBC and platelet counts were performed, and serum neutralizing antibody titers against NY-1 BVDV were determined. Histologic examinations and immunohistochemical analyses to detect gross lesions and distribution of viral antigens, respectively, were performed.

Results—After challenge exposure to NY-1 BVDV, 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.


Bovine viral diarrhea virus (BVDV) is a member of the Pestivirus genus, family Flaviviridae, a group that includes hog cholera virus and the causative agent of border disease in sheep. There are 2 biotypes of BVDV (cytopathic and noncytopathic), and 2 genotypes have been defined on the basis of gene sequencing techniques and cross-neutralization assays. Genotype 1 includes the common viral strains used for laboratory reference and in vaccines and has been subdivided into types 1a and 1b. Genotype 2 comprises the BVDV strains associated with acute and peracute infections and a high mortality rate. Variability in the virulence of type 2 BVDV isolates has been demonstrated experimentally and contributes to the variation in clinical severity, which can range from clinically inapparent disease to fatal peracute infections, among cattle with BVDV infection. Antigenic diversity among BVDV isolates has important implications for development of protective immunity.

The range of clinical disease caused by BVDV infection includes infection of the reproductive tract with subsequent reproductive failure (eg, abortion or congenital fetal defects) or persistently infected carrier cattle, mucosal disease, and acute infections characterized by self-limiting diarrhea and immunosuppression. The immunosuppression induced by acute BVDV infection plays an important role in the pathogenesis of the bovine respiratory disease complex and neonatal calf diarrhea. Concurrent experimentally induced infections with bovine respiratory syncytial virus and BVDV result in more severe respiratory tract disease than does infection with either virus alone, and previous studies have revealed that BVDV may contribute to undifferentiated bovine respiratory tract disease, particularly chronic, nonresponsive pneumonia. Infection with BVDV has also been associated with enteritis in neonatal calves. Infection with BVDV plays direct and indirect roles in enteritis in neonatal calves, causing villus atrophy in the duodenum and submucosal inflammation in the intestines as well as potentiating bovine rotavirus infection.

In recent years, concerns have been raised regarding vaccine efficacy at conferring protection against antigenically diverse BVDV isolates. Ideally, vaccination against BVDV results in protection against viremia in the host, thereby preventing infection of target cells in the reproductive and lymphatic systems and subsequent fetal infection and immunosuppression, respectively. The objective of this study was to evaluate the protection afforded by use of a modified-live noncytopathic BVDV type 1 vaccine in calves with systemic infection and clinical disease from challenge exposure with NY-1 BVDV.
Materials and Methods

Calves—Ten 5- to 7-month-old crossbred beef calves that weighed approximately 270 kg were used in the study. All calves had negative results for 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 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 (BT) cells were used for virus isolation and titration assays. All cells were tested for extraneous BVDV by use of a described procedure involving immunoperoxidase, bacteria (including mycoplasmal organisms), and fungi. Cells were grown as monolayers in Dulbecco modified Eagle medium (DMEM) supplemented with equine serum (10%) in a humidified incubator with 5% CO₂. Bovine viral diarrhea virus strain NY-1 (titer, 10^8 TCID₅₀/mL) was used as challenge virus.

Vaccine—The commercially available vaccine containing BVDV type 1 strain WRL (a modified-live, noncytopathic BVDV type 1 strain attenuated by serial passage in swine kidney cells) in combination with modified-live infectious bovine rhinotracheitis, 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 on day 0, and those in group 1 were inoculated with NY-1 BVDV diluted to 10 mL in DMEM. Calves received 1.2-mL aliquots of NY-1 BVDV diluted to 10 mL in DMEM.

Clinical observations—Beginning 2 days before calves were vaccinated (day of vaccination = day 0), rectal temperature and 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 individual unaware of the calf’s treatment group. Histologic changes in tissues (reactive follicles, lymphoid depletion, and lesions) 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, normal; 1, mild; 2, moderate; and 3, severe).

Virus isolation and titration—Virus isolation procedures were performed on fluids from nasal swab specimens,

Virus shedding in nasal secretions—Nasal swab specimens were collected by use of swabs immersed in DMEM that contained gentamicin (100 μg/mL), amphotericin B (0.5 μg/mL), and 2% equine serum.

PBMC preparation—Cells were isolated from blood via centrifugation to harvest the buffy coat layer and washed twice in PBS solution before use for virus isolation.

Necropsy—All calves were euthanatized on day thirty, 9 days after challenge exposure with NY-1 BVDV. 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, and ileum were 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 lesions) 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, 20% to 75% of follicles in node; and 4, > 75% of follicles in node).

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 (150 TCID₅₀/50 μL) in 96-well microtiter plates for 1 hour at 37°C with 5% CO₂. After incubation, 1 × 10⁵ BT 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 BVDV cytopathic effects, as described.

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 of amphotericin B/mL. Tissue homogenates were stored at –80°C until tested for BVDV. For virus isolation, thymus specimens and PBMC samples were diluted to determine TCID₅₀ per milliliter in BT cell monolayers. Specimens were also stained by use of an indirect immunoperoxidase test with monoclonal antibody 348 directed against gp 5₃, as described, 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 tris-buffered saline solution (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 applied, and slides were incubated for 30 minutes at 18° to 24°C. Slides were washed as described, and a conjugated avidin-alkaline phosphatase complex was added and allowed to incubate for 15 minutes. 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 analyses—An ANOVA for repeated measures in a completely randomized design with an autoregressive error covariance structure and a 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 [60 breaths/min] and dyspnea) with a score of 2 when evaluated on days 29 and 30 (days 8 and 9 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. All 5 calves in group 1 received lethargy scores of 1 on day 29, and 2 of the group 1 calves (Nos. 22 and 24) had the same lethargy score (1) on day 30. The remaining calves in group 1 calves for all clinical variables were 0 on all days of the study. Calves in group 2 received clinical scores of 0 for all clinical variables on all days of the study.

Postvaccination rectal temperatures in both groups of calves ranged between 38.3° and 38.8°C before exposure to BVDV. Calves in group 2 had a significantly higher mean rectal temperature only on day 4 after vaccination, compared with calves in group 1.
Calves in group 1 had significantly higher mean rectal temperatures on days 25, 26, 29, and 30 (days 4, 5, 8, and 9 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 40.4°C to 41.6°C (mean, 41.1°C) on day 29 (day 8 after challenge exposure), values that were significantly (P < 0.001) different from the rectal temperatures of calves in group 2 on the same day. Mean rectal temperatures of calves in group 1 decreased substantially on day 9 after challenge but were still significantly (P < 0.001) higher than the rectal temperatures of 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. Calves in group 2 had BVDV serum antibody titers ranging from 8 to 32 on day 21 and from 16 to 128 on day 30.

Hematologic findings—Beginning on day 24 (day 3 after challenge) and continuing through day 29 (day 8 after challenge), all calves in group 1 had significantly (P = 0.001) decreased mean total leukocyte counts (Figure 2) and significantly (P < 0.001) decreased lymphocyte counts (Figure 3). Monocyte counts in calves of group 1 were significantly (P < 0.05) decreased on days 24 to 28. On days 6 to 9 and day 11 after vaccination, calves in group 2 had significantly (P = 0.02) decreased mean total leukocyte counts. Other significantly (P < 0.05) reductions in cell counts developed in calves of group 2 after vaccination as follows: lymphocyte counts were decreased on days 5 to 11; monocyte counts were decreased on days 2, 4, and 6 to 9; neutrophils were decreased on days 1 and 7 to 9; and platelets were decreased on days 4 to 7, 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 nasal secretions, PBMCs, or thymus of calves from group 2. Calves from group 1 shed virus in nasal secretions (Figure 4) and were viremic (Figure 5) beginning on day 24 (day 3 after challenge) and continuing through day 29 (day 8 after challenge).

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*Significant (P < 0.05) difference between groups. †Acute lesions. ‡Chronic lesions.
continuing through day 30, when the calves were euthanized. This was in contrast to group 2 calves, which were free of BVDV in nasal secretions and PBMCs throughout the study period. Viral titers in thymus tissues from calves in group 1 ranged from $3.65 \times 10^3$ to $7.12 \times 10^5$ TCID<sub>50</sub>/g of tissue. No virus was detected in thymus tissues from calves in group 2.

**Necropsy**—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. Lesions of the digestive tract were evident only in calves from group 1. Lesions were not detected in the esophagus, abomasum, liver, kidneys, bladder, or bone marrow of any calves. Evidence of pathogenic bacterial infection was not detected in tissues from any of the calves via bacterial culture or gross or histologic evaluation.

Calves in group 1 had moderate (3 calves) to marked (2 calves) thymic atrophy. Calves in group 2 were less affected; 2 calves did not have thymic atrophy, and 3 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 ($P = 0.008$) difference in lymphocytolysis in the thymus between calves of group 1 and group 2: moderate lymphocytolysis was detected in 4 calves and mild lymphocytolysis in 1 calf of group 1, whereas lymphocytolysis was not detected in the thymuses of group 2 calves. In group 1, the ileum of 1 calf was severely edematous and turgid and cavitation was detected in the Peyer's patches. Moderate (2 calves) or mild (2 calves) ileal thickening and turgidity were detected in the other calves of this group. Four calves in group 2 had hyperemia in the ileum and soft feces. There was significantly ($P = 0.024$) more lymphocytolysis in Peyer's patches in calves from group 1, compared with calves from group 2. In calves of group 1, histologically acute changes were prominent with moderate to severe lymphocytolysis evident in the ileal Peyer's patches (Figure 6). In calves of group 2, changes in Peyer's patches were predominantly chronic in character and lymphoid follicles were either normal or diminutive. Diminutive follicles had few lymphocytes and consisted of loose stroma, dendritic cells, and

![Figure 6](image6.png)

**Figure 6**—Photomicrographs of ileal Peyer’s patches in a nonvaccinated calf 9 days after challenge-inoculation with BVDV. A—Notice acute histologic changes with marked lymphocytolysis and edema, and fibrin in surrounding submucosa. H&E stain; bar = 100 µm. B—Marked deposition of BVDV antigen is evident. Immunohistochemical stain; bar = 50 µm.

![Figure 7](image7.png)

**Figure 7**—Photomicrographs 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 and an adjacent normal follicle. H&E stain; bar = 100 µm. B—Minimal deposition of BVDV antigen is evident. Immunohistochemical stain; bar = 50 µm.
and some macrophages containing hematoidin pigment (Figure 7). There was no significant quantitative difference in lymphoid depletion in Peyer's patches between group 1 and group 2 calves; moderate to marked lymphoid depletion was evident in Peyer's patches of calves from both groups. However, there was significantly more lymphoid depletion in other lymphatic tissues (eg, cecal tonsils, mesenteric lymph nodes, and prescapular lymph nodes) of calves in group 1, compared with calves in group 2.

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. Deposition of BVDV antigen was significantly (P = 0.008) greater in Peyer's patches, the cecal tonsil (P = 0.008), and mesenteric lymph nodes (P = 0.048) in group 1 calves (Figure 6), compared with group 2 calves (Figure 7), which had very little or no staining in those tissues. In other lymphoid tissues, very little (tonsil and retropharyngeal lymph node) or no (prescapular lymph node) antigen was detected in calves of either group.

Discussion

In the study reported here, a modified-live noncytopathic BVDV type 1 vaccine protected calves against viremia, systemic infection, and clinical disease after challenge exposure to NY-1 BVDV (noncytopathic, type 1). The host response to vaccination blocked challenge exposure to NY-1 BVDV (noncytopathic, type 1). The protective immunity conferred by the vaccine BVDV in the host because vaccinated calves had decreased leukocyte and lymphocyte counts beginning 5 days after vaccination and continuing through day 11, with cell counts returning to baseline values by day 21 after vaccination. However, the decreases in leukocyte and lymphocyte counts in those calves were mild and transient and remained within reference ranges for those cell populations. Effects of replication of vaccine BVDV systematically and in target tissues were still evident 30 days after administration of vaccine, when the calves underwent necropsy examination. Lymphocyte depletion was extensive in the Peyer'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. Lesions in the lymphatic organs of vaccinated calves were chronic in nature, as evidenced by lymphocyte depletion. Debris from lymphocytolysis had been removed, so there was no or little remaining BVDV antigen, and diminution of lymphoid follicles with mostly stroma, macrophages, and dendritic cells was evident. In contrast, nonvaccinated calves inoculated with NY-1 BVDV developed acute lesions in lymphatic organs after challenge exposure, a finding that was consistent with results of a previous report. Acute lesions included more severe lymphocytolysis in Peyer's patches and the thymus and more severe lymphoid depletion in cecal tonsils and mesenteric and prescapular lymph nodes. The effects of BVDV vaccine virus on leukocyte and lymphocyte populations and on lymphoid target tissues in this study were consistent with findings from studies of other modified-live BVDV vaccine strains. Vaccination of calves with modified-live BVDV causes a decrease in the number of circulating lymphocytes and neutrophils. Transient BVDV vaccine–induced viremia was reported in calves that received the modified-live BVDV vaccine. In another study, BVDV was isolated from ovaries removed from cattle on days 8, 10, and 12 after administration of a modified-live BVDV vaccine and BVDV antigen was detected by use of immunohistochemical analysis on days 10 to 30 after administration. 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 1 BVDV. These findings suggest that the transient suppression of leukocyte numbers was not associated with clinical immunosuppression and that vaccination was effective at inducing immunity. Protection against viremia and infection of target lymphatic organs is important because a potentially important consequence of the immunosuppressive effects of naturally occurring acute BVDV infections is potentiation of the pathogenic effects of other pathogens of the respiratory or gastrointestinal system.

Results from our study revealed that the BVDV vaccine protected calves against BVDV type 1 infection, viremia, and dissemination of virus. The fact that vaccination effectively prevented infection of lymphoid target cells suggests that postexposure immunosup-
pression may be prevented in vaccinated animals. These findings are consistent with those of a previous study in which heifers vaccinated with the same noncytopathic type 1 modified-live BVDV (strain WRL) 21 days before breeding were challenged exposed at days 55 to 100 of gestation by IV administration of BVDV type 1 (strain 7443). In that study,13 vaccination protected against viremia and leukopenia and 92% of calves born to vaccinated heifers were not persistently infected with BVDV. Those findings, coupled with the data from our study, indicate the ability of the vaccine to prevent viremia during the acute stages of infection. This is clinically relevant because production losses in cattle are primarily associated with reproductive failure and the immunosuppression associated with acute infections and viremia, events that predispose calves to respiratory or enteric disease.

References