

Evaluation of protection against virulent bovine viral diarrhea virus type 2 in calves that had maternal antibodies and were vaccinated with a modified-live vaccine

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Objective—To evaluate the efficacy of an adjuvanted modified-live bovine viral diarrhea virus (BVDV) vaccine against challenge with a virulent type 2 BVDV strain in calves with or without maternal antibodies against the virus.

Design—Challenge study.

Animals—23 crossbred dairy calves.

Procedures—Calves were fed colostrum containing antibodies against BVDV or colostrum without anti-BVDV antibodies within 6 hours of birth and again 8 to 12 hours after the first feeding. Calves were vaccinated with a commercial modified-live virus combination vaccine or a sham vaccine at approximately 5 weeks of age and challenged with virulent type 2 BVDV 3.5 months after vaccination. Clinical signs of BVDV infection, development of viremia, and variation in WBC counts were recorded for 14 days after challenge exposure.

Results—Calves that received colostrum free of anti-BVDV antibodies and were vaccinated with the sham vaccine developed severe disease (4 of the 7 calves died or were euthanatized). Calves that received colostrum free of anti-BVDV antibodies and were vaccinated and calves that received colostrum with anti-BVDV antibodies and were vaccinated developed only mild or no clinical signs of disease.

Conclusions and Clinical Relevance—Results indicated that the modified-live virus vaccine induced a strong protective immune response in young calves, even when plasma concentrations of maternal antibody were high. In addition, all vaccinated calves were protected against viral shedding, whereas control calves vaccinated with the sham vaccine shed virus for an extended period of time. (*J Am Vet Med Assoc* 2006;228:1757–1761)

Bovine viral diarrhea virus is a clinically and economically important disease of cattle and is endemic in cattle populations throughout the world. The virus can be classified into 2 genotypes: type 1 and type 2. These genotypes can be further divided into 2 biotypes: cytopathic and noncytopathic. Bovine viral diarrhea virus can cause acute or persistent infections in adult cattle and young calves.¹

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ABBREVIATIONS

BVDV Bovine viral diarrhea virus
MLV Modified-live virus

rhea virus can cause acute or persistent infections in adult cattle and young calves.¹

Vaccination is one of the most important methods of preventing BVDV infection in a herd, and numerous vaccines are commercially available.² Vaccines may be inactivated or MLV vaccines. The type of vaccine used and immunization schedule should be tailored to the needs of a given herd. Vaccinating pregnant cows and heifers prior to breeding is used to protect calves against persistent infection if exposed to noncytopathic BVDV in utero.³ The high incidence of infection in dairy and beef animals between the time of weaning and 9 months of age has prompted producers to begin vaccinating calves at younger ages.⁴ In a large epidemiologic study,⁴ conventional MLV BVDV vaccines administered to calves younger than 60 days of age were not effective in preventing BVDV infections.

Immunization of calves is complicated by circulating maternal antibodies that may affect the efficacy of the vaccine.⁵⁻⁷ Maternal antibodies are an important part of the passive immunity calves receive from dams after birth. These antibodies protect the calf from pathogens but may also neutralize antigens in the vaccine, preventing the calf from developing an effective immune response.⁸ To prevent maternal antibody interference, producers typically vaccinate calves after antibody concentrations decrease.⁹ Maternally derived BVDV antibody titers should be < 1:32 for successful vaccination of most calves. Depending on the immune status of the dam and the efficiency of colostrum immunoglobulin transfer, this titer may be reached from 70 to 160 days of age.⁶ This leaves a period of time during which calves are vulnerable to infection.

To overcome maternal antibody interference in neonates, traditional management strategies have consisted of administering repeated doses of vaccine or delaying the age of immunization.¹⁰ Another immunologic approach used successfully to overcome maternal antibody interference is addition of adjuvants to vaccines.^{7,11,12} Adjuvants increase the immune response to vaccinal antigens by various mechanisms, including prevention of rapid clearance of antigen, preservation of antigen confirmation, and targeting of antigens to specific types of immune cells.¹³ The objective of the present study was to evaluate the efficacy of an adjuvanted MLV BVDV vaccine for protecting calves

against challenge with virulent type 2 BVDV, with or without circulating maternal antibodies.

Materials and Methods

Animals—Protocols were reviewed and approved by the institutional animal care and use committee. Twenty-three newborn dairy calves that had not nursed were acquired for the study and were randomly assigned to 1 of 3 groups at enrollment. Calves in group 1 were designated as control calves, calves in group 2 were designated as colostral antibody-negative and vaccinated, and calves in group 3 were designated a colostral antibody-positive and vaccinated. Calves in group 1 ($n = 7$) and group 2 (9) were fed 2 L of pooled colostrum that did not contain antibodies against BVDV within 6 hours of birth and were given a second 2-L feeding of colostrum 8 to 12 hours later. Calves in group 3 ($n = 7$) were given colostrum containing antibodies against BVDV. Dilutions of this colostrum were made with pasteurized whole milk so that the IgG concentration^a administered per feeding was 200 g/2 L. The diluted colostrum was fed within 6 hours of birth and again 8 to 12 hours later. After colostrum treatments, all 23 calves were fed 2 L of a commercially available milk replacer^b twice daily until weaning.

Calves were processed and managed according to routine animal husbandry procedures. Three calves died (1 calf from group 2, at 8 days of age; 2 calves from group 3, at 5 and 65 days of age) prior to challenge with virus. Gross lesions were not observed during necropsy of these calves. The remaining 20 calves were commingled and fed an age-appropriate grain and hay ration ad libitum for the remainder of the study.

Prevaccination serologic assays—Blood was collected from all calves prior to the first administration of colostrum and again at 3 weeks of age (before vaccination). Serum samples were tested for BVDV types 1 (Singer^c) and 2 (A125^c) serum neutralizing antibody titers by use of the constant virus-decreasing serum assay.¹⁴ Twofold serial dilutions (range, 1:2 to 1:8,192) of sera in duplicate were incubated with a constant viral titer (< 500 TCID₅₀) before inoculation of BVDV-free bovine turbinate cells^d in microtiter tissue culture plates.^e Plates were incubated at 37°C with 5% CO₂ for 5 days before being evaluated for virus-induced cytopathic effects. The reciprocal of the last dilution that prevented cytopathic effects was designated the serum neutralizing antibody titer. Geometric mean values were calculated by use of log₂ titers.

Vaccination—Twenty-one calves were vaccinated at approximately 5 weeks of age (day 0). Fourteen calves were vaccinated with a commercially available MLV combination vaccine^f containing bovine rhinotracheitis, BVDV (types 1 and 2), bovine parainfluenza-3, and bovine respiratory syncytial virus. The other 7 calves were sham vaccinated with sterile saline (0.9% NaCl) solution^g and served as controls. All calves were observed daily after vaccination for vaccine-related adverse events.

Hematologic and serologic assays after vaccination—Blood was collected from 5 randomly selected calves (2 calves each from groups 1 and 3 and 1 calf from group 2) 2 and 3 days after vaccination. Biochemical analyses^h were performed on the serum samples, and a CBC was performed on the whole-blood samples. Blood was collected from all calves 28 days after vaccination, and titers of antibody against BVDV types 1 and 2 were determined by use of a serum neutralization assay. One calf in group 3 died before challenge approximately 30 days after vaccination, from causes unrelated to vaccination.

Challenge—All 20 remaining calves received BVDV type 2 (strain 1373)¹⁵ intranasally by use of an atomizerⁱ 104 days after vaccination. The challenge inoculum contained 7.3×10^7 viral particles/mL, and 2.5 mL was atomized into each naris (total volume, 5.0 mL/calf).

Postchallenge observations—Clinical observations were performed daily, from 3 days prior to challenge through

day 14 after challenge. Each calf was visually examined in the pen prior to handling for signs of abnormal respiration, nasal and ocular discharge, diarrhea, and depression. After the visual assessment, calves were restrained for determination of rectal temperature^j and examination for oral cavity ulcers. Calves that died or were euthanatized during the observation period underwent necropsy to determine cause of death.

Virus isolation—Blood was collected via jugular venipuncture from all calves 15 times during the observation period (from 2 days prior to challenge to 13 days after challenge). White blood cells were isolated according to a described technique.¹⁶ The isolated WBCs were resuspended in 2 mL of medium^k supplemented with equine serum^l and tested for BVDV types 1 and 2 via an isolation assay.¹⁷ Three serial 10-fold dilutions of samples were made, and each diluted sample was added in triplicate to BVDV-free bovine turbinate cell monolayers in microtiter tissue culture plates. Culture plates were incubated for 5 days at 37°C with 5% CO₂ before immunohistochemical staining. Samples were considered to have positive results for BVDV if virus-specific staining was observed in inoculated cells.

Hematologic and serologic analyses—Blood was collected via jugular venipuncture from all calves 13 times during the study period (from 2 days prior to challenge through 11 days after challenge) for hematologic and serologic analyses. Samples were subjected to hematologic analysis by use of a cytometer.^m Blood samples for serologic tests were collected 2 days prior to challenge and again at 7 and 14 days after challenge. Serum neutralizing antibody titers against BVDV types 1 (Singer strain) and 2 (A125 strain) were determined.

Statistical analysis—A general linear model for repeated measuresⁿ was used to evaluate body temperature, WBC counts, clinical score data, and types 1 and 2 BVDV antibody titers. A value of $P < 0.05$ was considered significant.

Results

Adverse vaccine reactions were not observed in any calves. Body temperature was measured rectally in all calves from 3 days prior to challenge to 14 days after challenge, and the mean temperature was determined for each group (Figure 1). Control calves had higher

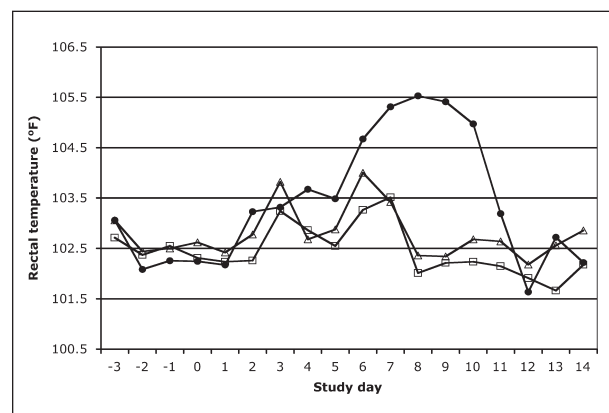


Figure 1—Mean rectal temperatures (°F) in 3 groups of calves before and after challenge (day 0) with virulent type 2 BVDV. Group 1 (black circles; $n = 7$) consisted of seronegative (ie, no anti-BVDV antibodies) control calves that were not vaccinated. Group 2 (white squares; $n = 8$) consisted of seronegative calves that were vaccinated. Group 3 (white triangles; $n = 5$) consisted of calves seropositive for anti-BVDV antibodies that were vaccinated. Notice that there was a significant ($P < 0.05$) difference in mean temperatures between control and treatment groups on days 4 and 7 to 10 after challenge and a significant ($P < 0.05$) difference between group 3 and groups 1 and 2 on day 13. To convert rectal temperatures to degrees Celsius, subtract 32 and multiply by 5/9.

mean rectal temperatures than vaccinates ($P < 0.05$) from 6 to 10 days after challenge. The mean temperature in group 3 was higher than 39.7°C (103.5°F; study cutoff value for pyrexia) on days 3 (39.8°C [103.8°F]) and 6 (40°C [104.0°F]).

Clinical scores—Composite clinical scores were measured in all calves from 3 days prior to challenge to 14 days after challenge, and a mean score was determined for each group. Controls (group 1) had higher ($P < 0.05$) mean clinical scores than vaccinates from 7 to 14 days after challenge. Four of the 7 calves in group 1 died: 1 calf died and 1 calf was euthanatized on day 12 after challenge, 1 calf died on day 14 after challenge, and the fourth calf died on day 21 after challenge. Gross and histologic lesions in those calves were consistent with acute BVDV infection,¹⁸ and type 2 BVDV was isolated from multiple tissues in those calves. Mean clinical scores for group 2 peaked on day 7 after challenge, and those for group 3 peaked on days 4 and 6 after challenge. No calves in groups 2 or 3 died or were euthanatized after BVDV challenge.

Hematologic analysis—White blood cell counts were obtained in all calves from 2 days prior to challenge through 11 days after challenge, and a mean WBC count was determined for each group (Figure 2). Control calves had a substantial decrease in WBCs beginning on day 2 after challenge, and WBC numbers remained low through day 10 after challenge. Calves in group 2 had the smallest shift (20%) in WBC count, with leukopenia most severe on days 5 and 6 after challenge. Calves in group 3 had biphasic leukopenia (shifts in WBC numbers of approx 25%) at 3 to 5 days after challenge and 7 to 10 days after challenge.

Serum neutralizing antibody titers—Titers of serum neutralizing antibody against BVDV were measured before and after colostrum was fed. All calves had titers $< 1:2$ at the time of enrollment (Table 1). Calves in groups 1 and 2 were fed colostrum free of antibodies against BVDV and had negative results of tests for serum antibodies against BVDV before being vaccinated. Calves in group 3 were fed colostrum containing antibodies against BVDV and had a mean titer of antibodies against type 2 BVDV of 8.7_{log2} (ie,

≥ 256) at the time of vaccination. All 5 of those calves had type 2 serum neutralizing antibody titers $\geq 7_{\log2}$ (≥ 128), with the highest titer at 11_{log2} ($> 2,048$).

Twenty-eight days after vaccination, all calves were tested for serum neutralizing antibodies (Table 1). Group 1 was seronegative, whereas vaccinated calves in groups 2 and 3 had titers against BVDV types 1 and 2. Calves in group 2 developed an immune response and had a mean BVDV type 1 serum neutralizing antibody titer of 7.9_{log2} and a mean type 2 titer of 5.5_{log2}. The mean serum neutralizing antibody titer in group 3 decreased, compared with the mean titer on day 0. Neutralizing antibody titers against type 1 virus decreased 0.8_{log2} to 7.7_{log2}, and titers against type 2 virus decreased 1.7_{log2} to 7.0_{log2}.

On the day of challenge (day 104), calves in group 1 were seronegative (Table 1). Group 2 antibody responses continued to increase, with a mean BVDV type 1 serum neutralizing titer of 9.4_{log2} and a mean type 2 titer of 5.6_{log2}. Titers in group 3 were decreased, compared with titers on day 28. Titers against type 1 virus decreased 1.9_{log2} to 5.8_{log2}, and titers against type 2 virus decreased 2.8_{log2} to 4.2_{log2}. The decreases in titers of both types of antibodies were significant ($P < 0.05$).

At 1 week after challenge (day 111), control calves had begun to develop serum neutralizing antibody titers against BVDV type 2 (Table 1). Serum neutralizing antibody titers against type 2 virus in group 2 increased by 3.0_{log2} to 8.6_{log2} and increased by 0.2_{log2} to 4.4_{log2} in group 3. At 2 weeks after challenge (day 118), all 3 groups had increases ($P < 0.05$) in titers against both type 1 and 2 BVDV, compared with titers on day 111.

Virus isolation—Virus isolation was performed on buffy coat cells from all calves from the day of challenge through 13 days after challenge. Results were positive in control calves beginning on day 3 after challenge and continuing through day 13 after challenge. The highest frequency of virus isolation was on day 7 after challenge, when 6 of 7 calves had positive virus isolation results. Two calves had positive results in 5 of the 7 collections after challenge. One control calf had negative results of virus isolation throughout the study. Virus was not isolated from any calves in groups 2 or 3 at any time points.

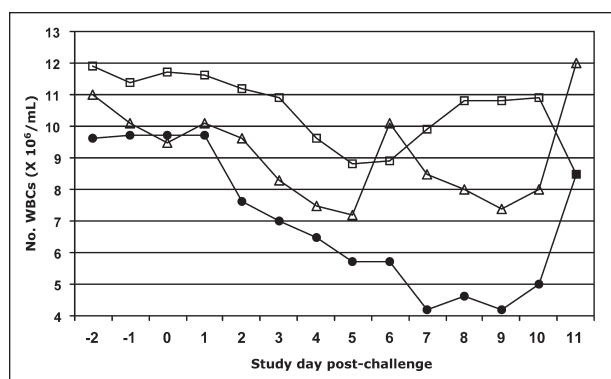


Figure 2—Mean WBC counts in the same calves as in Figure 1. Notice that there was a significant (black circles [$P < 0.05$]) difference in leukocyte numbers between group 1 (black circles) and group 2 (white squares) on days 2 to 5, between group 1 and groups 2 and 3 (white triangles) on days 6 to 8, and between group 1 and group 2 on days 9 to 10 after challenge.

Table 1—Mean (range) values for anti-BVDV serum neutralizing antibody titers (log₂).

Day/BVDV type	Group 1	Group 2	Group 3
Precolostrum/type 1	0.0	0.0	0.2 (0–1)
Precolostrum/type 2	0.0	0.0	0.0
Prevaccination/type 1	0.0	0.0	8.5 (6–11)
Prevaccination/type 2	0.0	0.0	8.7 (7–11)
Day 28/type 1	0.0	7.9 (5–10)	7.7 (3–11)
Day 28/type 2	0.0	5.5 (4–8)	7.0 (6–8)
Day 104			
Postchallenge day 0/type 1	0.0	9.4 (8–11)	5.8 (4–7)
Day 104			
Postchallenge day 0/type 2	0.0	5.6 (4–7)	4.2 (1–6)
Day 111			
Postchallenge day 7/type 1	0.0	9.5 (7–11)	5.6 (3–7)
Day 111			
Postchallenge day 7/type 2	1.3 (0–6)	8.6 (6–11)	4.4 (1–6)
Day 118			
Postchallenge day 14/type 1	5.6 (4–6)	12.6 (12–13)	9.8 (7–13)
Day 118			
Postchallenge day 14/type 2	6.6 (6–7)	12.4 (10–13)	7.0 (2–12)

Discussion

To our knowledge, this is the first study to reveal that a single dose of an MLV multivalent vaccine^f containing BVDV administered at 4 to 5 weeks of age can stimulate a strong protective immune response in calves in the face of high concentrations of maternal antibodies against BVDV. Vaccinated calves had less-severe leukopenia, fewer clinical signs, and lower rectal temperatures after challenge with virulent type 2 BVDV at 4 to 5 months of age. Vaccinated calves were protected against viremia, whereas most control calves were viremic throughout the challenge period. The protective response in vaccinates was not associated with an increase in serum antibody concentrations.

Although a definitive study in which a nonadjuvanted vaccine was compared with the adjuvanted vaccine would be necessary to determine the effects of the adjuvant, it is possible that the adjuvant in the vaccine preparation used in the present study contributed to efficacy. Adjuvanted subunit experimental vaccines have been shown to elicit an immunological response in calves in the presence of passively acquired antibodies.¹¹ However, there are no reports in the literature of the use of adjuvants with live virus vaccines.

The ability of the vaccine used in the present study to protect calves from challenge in the presence of maternal antibody is important because many vaccination programs advocate vaccinating calves at < 4 months of age when maternal antibodies often remain. Maternal antibodies can interfere with vaccination of calves against BVDV at 10 to 14 days of age and may result in susceptibility to BVDV challenge 4 months later.⁵ The rate of decay in serum titer of anti-BVDV antibodies varies; maternal antibodies may persist for 50 to 183 days,^{8,19} and titers as low as 1:32 can inhibit active generation of anti-BVDV antibody in response to vaccination. A large study⁶ of dairy heifers in 2 herds in California revealed that calves should be > 100 days of age to ensure efficacy of MLV BVDV vaccines. In another study,²⁰ calves with a maternal antibody titer > 1:32 seroconverted after vaccination with an MLV BVDV vaccine at 84 days of age, and calves vaccinated at the same age with BVDV MLV vaccine seroconverted when maternal antibody titers were from 1:20 to 1:96.²¹

Although other studies^{22,23} have revealed a priming effect of MLV vaccines in the presence of maternal antibodies, those studies did not include a viral challenge to determine if the immune response was protective. In the first study,²² calves with maternal antibodies against BVDV were vaccinated at 10 days of age. Those calves had higher BVDV cell proliferation levels at weaning than did calves in a nonvaccinated group, but after revaccination, there was no difference in the cellular proliferative responses between revaccinated calves and calves that were being vaccinated for the first time.²² In the other study,²³ a cellular priming effect occurred in calves with maternal anti-BVDV antibody that were vaccinated with an MLV vaccine while the BVDV humoral immune response was blocked. Calves vaccinated with MLV BVDV vaccine at 7 weeks of age developed BVDV-specific CD4⁺ and $\gamma\delta$ -T-cell responses, whereas calves vaccinated with an inactivated vaccine at the same time did not develop BVDV-specific

T-cell responses. On subsequent revaccination with MLV BVDV at 14 weeks of age, the calves developed an anamnestic response regardless of whether they had been initially vaccinated with MLV or inactivated BVDV vaccine. This finding indicates that BVDV immune memory was established after the initial inactivated vaccination despite the fact that no BVDV-specific T cells could be detected.²³

Maternal antibody interference may also depend on the route of BVDV exposure.^{23,24} Calves with BVDV maternal antibodies that were infected intranasally at 2 to 5 weeks of age with type 2 BVDV (isolate 1373) were protected from clinical disease. Those calves developed BVDV-specific CD4⁺, CD8⁺, and $\gamma\delta$ -T-cell responses.²³ Calves in another study²⁴ were monitored until they reached 7 to 9 months of age, at which time the BVDV types 1 and 2 serum antibody titers had completely decayed. Calves were rechallenged with the 1373 isolate and were found to be protected against infection and signs of disease despite the fact that no anti-BVDV serum antibodies were detected prior to challenge.²⁴ After challenge, the titer of anti-BVDV antibodies increased dramatically, indicating function of BVDV-specific immune memory. Interestingly, a similar response was seen in the present study with a parenterally administered vaccine. Antibody titers continued to decrease after vaccination and only increased after challenge with virulent BVDV.

The results of the present study indicated that vaccination with the MLV vaccine studied protected calves from challenge by virulent type 2 BVDV, even in the presence of maternal antibody. This finding has important implications for the management of veal and dairy heifers, which may be given initial vaccinations at a very young age, and for beef calves, in which suckling calves are often vaccinated at the time of branding, when calves are 1 to 3 months of age.

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- a. Midland Bioproducts Corp, Boone, Iowa.
 - b. Land O' Lakes Animal Milk Products Co, Shoreview, Minn.
 - c. National Veterinary Services Laboratory, Ames, Iowa.
 - d. American Type Culture Collection, Manassas, Va.
 - e. Greiner Bio-One, Frickenhausen, Germany.
 - f. Pyramid 5, Fort Dodge Animal Health, Fort Dodge, Iowa.
 - g. Bimeda Animal Health Inc, Riverside, Mo.
 - h. Food Animal Diagnostic Profile, South Dakota State University Animal Disease and Diagnostic Laboratory, Brookings, SD.
 - i. Gelman Sciences, Ann Arbor, Mich.
 - j. GLA Agriculture Electronics, San Luis Obispo, Calif.
 - k. Cellgro, Mediatech Inc, Herndon, Va.
 - l. Atlanta Biologics, Norcross, Ga.
 - m. Abaxis, Union Center, Calif.
 - n. SAS, version 9.1, SAS Institute Inc, Cary, NC.
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New Veterinary Biologic Products

Product name	Species and indications for use	Route of administration	Remarks
Newcastle Disease Vaccine, Subunit (Dow AgroSciences LLC, Indianapolis, IN, US Vet Lic No. 642)	A plant-cell-produced subunit vaccine for the immunization of healthy chickens against Newcastle Disease	SC	USDA licensed 1/27/06