

Evaluation of fetal protection against experimental infection with type 1 and type 2 bovine viral diarrhea virus after vaccination of the dam with a bivalent modified-live virus vaccine

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Objective—To evaluate the efficacy of a modified-live virus (MLV) combination vaccine containing type 1 and type 2 bovine viral diarrhea virus (BVDV) in providing fetal protection against challenge with heterologous type 1 and type 2 BVDV.

Design—Prospective study.

Animals—55 heifers.

Procedure—Heifers were vaccinated with a commercial MLV combination vaccine or given a sham vaccine (sterile water) and bred 47 to 53 days later. Heifers were challenged with type 1 or type 2 BVDV on days 75 to 79 of gestation. Clinical signs of BVDV infection, presence of viremia, and WBC count were assessed for 14 days after challenge. Fetuses were collected on days 152 to 156 of gestation, and virus isolation was attempted from fetal tissues.

Results—Type 1 BVDV was not isolated in any fetuses from vaccinated heifers and was isolated in all fetuses from nonvaccinated heifers challenged with type 1 BVDV. Type 2 BVDV was isolated in 1 fetus from a vaccinated heifer and all fetuses from nonvaccinated heifers challenged with type 2 BVDV.

Conclusions and Clinical Relevance—A commercial MLV combination vaccine containing type 1 and type 2 BVDV given to the dam prior to breeding protected 100% of fetuses against type 1 BVDV infection and 95% of fetuses against type 2 BVDV infection. Use of a bivalent MLV vaccine in combination with a comprehensive BVDV control program should result in decreased incidence of persistent infection in calves and therefore minimize the risk of BVDV infection in the herd. (*J Am Vet Med Assoc* 2004;225:1898–1904)

Bovine viral diarrhea is one of the most economically devastating diseases of cattle worldwide.^{1,2} Bovine viral diarrhea virus (BVDV) infections can cause a broad spectrum of clinical signs ranging from

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none to peracute hemorrhagic disease that is associated with a high mortality rate.^{2,4} Acute BVDV infection causes respiratory, gastrointestinal, and reproductive tract disease and is associated with the development of fever and leukopenia.¹ Cattle with persistent infection are a long-term threat to herd mates because they shed BVDV for life; persistently infected cattle may also develop mucosal disease, leading to death.

The BVDV is an enveloped, single-stranded RNA virus that is classified as a *Pestivirus* in the family *Flaviviridae*. Isolates of BVDV can be classified on the basis of biotype (noncytopathic and cytopathic) and genotype (type 1 and type 2)³; genotypes have recently been reclassified as species.⁶ Many strains of each genotype have been identified. The type 1 genotype can be subclassified as type 1a and type 1b.⁷ More than 95% of field isolates are noncytopathic,³ and both genotypes are common.⁸

Acute infection of pregnant cattle with cytopathic or noncytopathic BVDV can result in a variety of conditions in the fetus; this depends on the virulence of the strain and age of the fetus.⁹ The ability to cross the placenta of susceptible cattle and cause fetal infection is the most important evidence of the success BVDV has achieved in the evasion of the host immune system. In early gestation, BVDV infection can cause early embryonic death and fetal resorption. From days 40 to 125 of gestation, infection with noncytopathic BVDV can result in persistently infected calves that shed noncytopathic BVDV for their entire lives. Persistently infected calves are often born weak, have poor survival, and develop mucosal disease, which has a 100% mortality rate. Some calves survive and appear healthy; however, because they shed large amounts of BVDV, they are long-term threats to the herd.

Vaccination against BVDV is an important component of prevention and control programs.¹⁰ One of the biggest concerns with BVDV vaccines that contain only a single BVDV genotype is their inability to cross-protect against heterologous BVDV genotypes (ie, types 1a, 1b, and 2). The animal health industry has responded to the widening diversity of field strains of BVDV reported by adding both type 1 and type 2 BVDV genotypes to vaccines or performing cross-protection studies with vaccines containing a type 1 strain of BVDV. Modified-live virus (MLV) vaccines that contain both genotypes provide protection against respiratory challenge with type 1 and type 2

BVDV.^{b-e} Results of challenge studies¹⁰⁻¹² reveal that the best protection rates develop only against homologous genotypes of BVDV. Clearly, some cross-protection is afforded by a single genotype; however, the wide antigenic diversity and geographic distribution of BVDV genotypically variable strains require the use of more than 1 genotype to induce cross-neutralization antibodies and broad cell-mediated immunity that is protective under field conditions. A great deal of research has been directed at providing protection of the developing fetus and preventing early embryonic death, persistent infections, abortions, congenital defects, and weak calves.^{10,13-21} Results of these studies are variable and range from a high rate of fetal protection (80% to 100%)¹³⁻¹⁶ to a low rate of protection (15% to 20%)²⁰ against the same genotype. Use of a type 1 BVDV vaccine to protect against a type 2 BVDV challenge resulted in protection of only 58% of fetuses.¹⁶

The purposes of the study reported here were to evaluate the efficacy of a commercial modified-live virus combination vaccine and establish the minimum protective dose of the type 1 and type 2 BVDV components in providing fetal protection against a heterologous type 1 or type 2 BVDV challenge.

Materials and Methods

Challenge viruses—Bovine turbinate cells (passage 20)^f were maintained in proprietary medium solution containing Eagle minimum essential medium^g (MEM) supplemented with 10% equine serum^h and penicillin (100 U/mL), streptomycin (100 µg/mL), and amphotericin D (0.25 µg/mL) at 37°C in 5% CO₂. The cells were tested for contamination with BVDV by use of nested reverse-transcriptase polymerase chain reaction²² at the Animal Disease Research and Diagnostic Laboratory, South Dakota State University (ADRDL-SDSU) and were found to be negative for BVDV.

Type 1 strain BJ¹⁵ BVDVⁱ that originated from a persistently infected Brahman heifer from a farm in Ohio was used in the first challenge study. Type 2 strain PA131¹⁶ BVDVⁱ isolated from the serum of a persistently infected Holstein heifer from a dairy herd in Pennsylvania, was used in the second challenge study. Both challenge viruses were known to induce a high degree of fetal infection and only mild to moderate clinical signs in the dam. Each virus isolate was cultured in bovine turbinate cells that were negative for BVDV, titrated, aliquoted into cryostat tubes, and stored at -80°C. Immediately prior to each challenge, 5 mL of stock virus was rapidly thawed at 37°C and diluted to 1 × 10⁵ TCID₅₀/mL with MEM. The virus suspension was dispensed into 5-mL snap-cap tubes (2.5 mL/tube), placed on ice, and transported to the site where the challenge was to be performed. The titer of the type 1 BVDV challenge was 3.41 × 10⁵ TCID₅₀/mL, and the titer of the type 2 BVDV challenge was 7.34 × 10⁴ TCID₅₀/mL.

Cattle—All procedures involving cattle were approved by the Animal Care and Use Committee at Rural Technologies Incorporated. Angus cross heifers, 14 to 17 months old, were used. Heifers were housed at a research facility near Brookings, SD, and fed an age-appropriate diet throughout the duration of the study. All heifers had serum neutralizing antibody²³ titers of < 1:2 for type 1 and type 2 BVDV and were negative for viral antigen (determined by use of a commercial ELISA^j) prior to the start of the study. Heifers were also tested for T-cell reactivity against type 1 and type 2 BVDV.^{24,k} Heifers with the highest reactivity were excluded

from the study. Fifty-five heifers were included in the study, blocked for randomization on the basis of their T-cell reactivity, and assigned to treatment groups.

Vaccination of heifers—A commercial MLV combination vaccine^l that contained type 1 (Singer) BVDV and type 2 (296) BVDV, bovine herpesvirus 1, bovine parainfluenza 3 virus, and bovine respiratory syncytial virus was used. The vaccine was rehydrated and then diluted, as determined by use of the regulatory guidelines for MLV BVDV vaccines,²⁵ with sterile Dulbecco phosphate-buffered saline solution.^m Heifers were vaccinated with 2 mL of vaccine administered SC (vaccinated heifers) or injected with 2 mL of sterile water (sham vaccine; nonvaccinated heifers) on day 0 of the study and were observed daily for 7 days after vaccination for vaccine-related adverse events. From day 8 after vaccination through to the end of the study, heifers were observed at least 3 times/wk for non-vaccine-related adverse events. Vaccinated and nonvaccinated heifers in each group were housed in separate locations until 1 week prior to challenge, at which time heifers within a group were housed together until the end of the study.

Breeding—Estrous cycles of heifers were synchronized by use of megestrol acetateⁿ supplied in a premix^o and prostaglandin F-2α.^p Heifers were each fed 0.5 mg of megestrol acetate/d for 14 days. Nineteen days after the cessation of megestrol acetate treatment, heifers were treated with 25 mg of prostaglandin F-2α IM and an estrus-detecting device^q was applied to the tailhead. Heifers were observed for signs of estrus (heat) from 2 to 4 days after injection of prostaglandin F-2α and bred via artificial insemination 10 to 12 hours after having been identified to be in standing heat (study days 44 to 53). Prior to insemination, semen was tested for BVDV by use of nested reverse-transcriptase polymerase chain reaction²² at the ADRDL-SDSU, and results were negative.

Pregnancy diagnosis—Pregnancy was diagnosed via rectal palpation and ultrasonography between days 46 to 49 of gestation. Heifers were examined to ensure they were still pregnant immediately prior to challenge (days 75 to 78 of gestation), approximately 30 days after challenge, and approximately 75 days after challenge. No heifers aborted during the study.

BVDV challenge—Twenty-eight heifers were assigned to the group to be challenged with type 1 BVDV; 18 heifers were vaccinated, and 10 were nonvaccinated. Twenty-seven heifers were assigned to the group to be challenged with type 2 BVDV; 19 were vaccinated, and 8 were nonvaccinated. Heifers were challenged with BVDV within the first trimester of gestation, on days 76 to 79 of gestation.²⁶ Immediately prior to challenge, a 500-mL biohazard bag was placed over the muzzle of each heifer to induce anoxia. When deep breathing was observed, the bag was removed and virus was administered by use of an atomizer^r that sprayed the challenge virus to the back of the nares on inspiration. Five milliliters was administered to each heifer (2.5 mL/naris). The bag was then placed over the muzzle again until deep breathing was observed.

Clinical assessment—Heifers were observed for 2 days prior to challenge and daily for 14 days after challenge for signs of BVDV infection, including nasal discharge, coughing, oral lesions, ocular discharge, diarrhea, anorexia, and change in attitude. Investigators making clinical observations were unaware of group assignment of heifers. Rectal temperatures were obtained by use of a calibrated digital thermometer.^s

Virus isolation from heifers—Blood samples were collected via jugular venipuncture into evacuated tubes con-

taining EDTA on days -1, 3, 4, 6, 8, 10, and 12 after challenge. Blood samples were processed to obtain the buffy coat. Buffy coats were tested for BVDV by use of a standard virus isolation technique. Briefly, the buffy coat was diluted 1:10 in MEM and added to bovine turbinate cells in microtiter tissue culture plates. Culture plates were incubated for 4 days and passaged twice before immunohistochemical staining for BVDV.²⁷

Sample collection—Blood samples for WBC counts were collected via jugular venipuncture into evacuated tubes containing EDTA 1 day prior to challenge, on the day of challenge, and daily on days 7 to 14 after challenge. Additional samples were collected on days 16, 18, 20, and 24 after challenge in heifers challenged with type 2 BVDV. White blood cell counts were determined by use of a commercial hematology analyzer.⁴

Blood samples for serologic testing were collected via jugular venipuncture into serum separator tubes⁴ on the day of vaccination (study day 0), on days 21 and 28 after vaccination (study days 21 and 28), before breeding (study day 45 or 49), at the time of the first pregnancy check (study day 95 or 98), immediately before challenge (study day 124 or 129), 14 days after challenge (study day 138 or 143), and immediately prior to collection of fetuses (study day 200 or 203).

Serum neutralizing antibody titers for type 1 and type 2 BVDV were measured by use of a standard serum neutralization assay^{23,v}; BVDV strain Singer was used for the type 1 assay, and BVDV strain 125-C was used for the type 2 assay. A constant virus titer (< 500 TCID₅₀) was incubated with 2-fold dilutions (1:2 through endpoint) of sera before inoculation of bovine turbinate cells in microtiter tissue culture plates. Culture plates were incubated for 5 to 7 days and visually assessed for virus-induced cytopathic effect. **Geometric mean titers (GMT)** were calculated by use of log₂ titers.^w

Fetal tissue collection—Fetuses were collected at 152 to 156 days of gestation (75 to 78 days after challenge) to allow sufficient time for viral replication in the fetus to occur. Heifers were slaughtered at an abattoir (by use of captive bolt and exsanguination), and the uterus from each heifer was removed and placed in an individual labeled container. The containers were transported on ice to the ADRDL-SDSU where each fetus was dissected by use of individual sterile instruments. Fetal heart blood, thymus, spleen, and cerebellum were collected, processed, and tested for the presence of BVDV via a virus isolation technique. Briefly, fetal tissues were homogenized diluted 1:10 in MEM supplemented with 2% equine serum and penicillin (100 U/mL), streptomycin (100 µg/mL), and amphotericin D (0.25 µg/mL); plated on bovine turbinate cells; incubated for 4 days; and passaged twice prior to immunohistochemical staining for BVDV.²⁷

Statistical analyses—Analyses of the type 1 and type 2 BVDV challenge studies were performed separately but by use of the same statistical methods.^x Prior to randomization, heifers were blocked on the basis of T-cell reactivity status. Heifers were randomly assigned to treatment groups within reactivity status so that an approximately 2:1 ratio of vaccinated heifers to nonvaccinated heifers was obtained. Treatment group and reactivity status were assumed to be fixed effects. This constituted a 2-way treatment structure with treatment group and reactivity status as the treatment factors in a completely random design structure.

Results of virus isolation from fetuses and buffy coats of heifers were analyzed by use of the Fisher exact test. Analyses of the results of virus isolation from buffy coats were performed only on days when virus was detected in at least 3 heifers.

Results of WBC counts were analyzed by use of repeated measures analysis of covariance. The covariates for WBC

counts were the baseline values obtained by calculating the mean of the values determined 1 day prior to challenge and on the day of challenge. Baseline mean was used in the statistical analysis to adjust for baseline difference among heifers. The *P* values for treatment group comparisons were determined on the basis of comparisons of the least square means from the analysis of covariance in which the least square means is adjusted for the covariate.

Results of serologic testing were analyzed by use of a 1-sided comparison and tested for an increased serologic response in the vaccinated heifers, compared with the nonvaccinated heifers, for all days tested except days 203 and 200 (immediately before collection of fetuses in the 2 challenge groups) for the type 1 and type 2 BVDV challenge, respectively, when a 2-sided comparison was performed to test for equality of vaccinated and nonvaccinated heifers. The analysis was performed on log₂ (titer + 1) for any day any heifer had a titer < 2 because these heifers were given a zero (0) value; otherwise, the analysis was performed on log₂ titer. All available data were used in the analyses. A value of *P* < 0.05 was considered significant. All analyses were performed by use of statistical software.^y For the purpose of analysis, it was assumed that the heifer was the experimental unit.

Results

Clinical signs and rectal temperature—No adverse systemic effects or injection site reactions were observed in either challenge group after vaccination. Observations after challenge in all vaccinated and nonvaccinated heifers revealed mild to moderate clinical signs of BVDV infection.

Mean rectal temperature of all vaccinated heifers was within the reference range during the measurement period after challenge, whereas nonvaccinated heifers in the type 1 challenge group had a mean ± SD rectal temperature of 40.4 ± 0.77°C (104.7 ± 1.38°F) on day 8 after challenge. Nonvaccinated heifers in the type 2 challenge group had a mean ± SD rectal temperature of 40.0 ± 0.71°C (104.2 ± 1.28°F) on days 8 and 9 after challenge.

Virus isolation from heifers—In the group of heifers challenged with type 1 BVDV, BVDV was isolated from buffy coats of nonvaccinated heifers on days 3 (2/10 heifers), 4 (2/10), and 6 (7/10) after challenge. On day 8 after challenge, the highest number (9/10) of nonvaccinated heifers were viremic (Table 1). Virus was isolated from the buffy coat of 1 of 18 vaccinated heifers on day 10 after challenge. The proportion of heifers from which virus was isolated from buffy coats was significantly (*P* ≤ 0.001) lower in vaccinated heifers, compared with nonvaccinated heifers, on days 6 and 8 after challenge.

In the group of heifers challenged with type 2 BVDV, BVDV was isolated from buffy coats of nonvaccinated heifers on days 3 (2/8 heifers), 4 (2/8), 10 (4/8), and 12 (4/8) after challenge; all nonvaccinated heifers (8/8) were viremic on days 6 and 8 after challenge (Table 1). Virus was not isolated from the buffy coat of any vaccinated heifer (*n* = 19) on any days on which tests were conducted. The proportion of heifers from which virus was isolated from buffy coats was significantly (*P* ≤ 0.004) lower in vaccinated heifers, compared with nonvaccinated heifers, on days 6, 8, 10, and 12 after challenge.

Table 1—Proportion of vaccinated and nonvaccinated heifers challenged with type 1 or type 2 bovine viral diarrhea virus in which virus was isolated from buffy coats after challenge.

Day after challenge	Type 1 challenge		Type 2 challenge	
	Nonvaccinated	Vaccinated	Nonvaccinated	Vaccinated
1	0/10	0/18	0/8	0/19
3	2/10	0/18	2/8	0/19
4	2/10	0/18	2/8	0/19
6	7/10*	0/18	8/8†	0/19
8	9/10*	0/18	8/8†	0/19
10	0/10	1/18	4/8†	0/19
12	0/10	0/18	4/8†	0/19
Total viremic	9/10	1/18	8/8	0/19
Total PI fetuses	10/10	0/18	8/8	1/19

*Significantly ($P \leq 0.001$) different from proportion of vaccinated heifers from which virus was isolated on the same day after challenge. †Significantly ($P = 0.004$) different from proportion of vaccinated heifers from which virus was isolated on the same day after challenge.
PI = Persistently infected.

WBC counts—In the group of heifers challenged with type 1 BVDV, mean WBC counts in nonvaccinated heifers ranged from 5.20 to 8.88×10^3 cells/ μL and mean WBC counts in vaccinated heifers ranged from 6.77 to 7.79×10^3 cells/ μL on days 7 through 14 after challenge (Figure 1). Mean WBC counts in vaccinated heifers were significantly ($P < 0.01$) higher than those in nonvaccinated heifers on days 7, 8, and 11 after challenge.

In the group of heifers challenged with type 2 BVDV, mean WBC counts in nonvaccinated heifers ranged from 4.24 to 7.64×10^3 cells/ μL and mean WBC counts in vaccinated heifers ranged from 6.71 to 7.49×10^3 cells/ μL on days 7 through 14 after challenge (Figure 1). Mean WBC counts in vaccinated heifers were significantly ($P \leq 0.023$) higher than those in nonvaccinated heifers on days 7, 8, 11 to 14, and 24 after challenge.

White blood cell counts in vaccinated and nonvaccinated heifers in both challenge groups were significantly ($P < 0.05$) lower than corresponding baseline values on days 7 and 8 after challenge.

Serology—In the group of heifers challenged with type 1 BVDV, vaccinated heifers developed antibody titers for type 1 and type 2 BVDV by 21 days after vaccination. The antitypes 1 and 2 BVDV antibodies in vaccinated heifers reached a peak prechallenge mean antibody \log_2 titer on day 49 (before breeding) of 11.75 (GMT, $3,444$) and 7.2 (GMT, 147), respectively (Figure 2). Antibody titers decreased slightly prior to challenge, but a strong anamnestic response was observed after challenge; maximum mean antibody \log_2 titers for type 1 and type 2 BVDV of 12.78 (GMT, $7,019$) and 9.54 (GMT, 745), respectively, were detected on day 143 after vaccination (ie, day 14 after challenge).

Nonvaccinated heifers were seronegative for anti-BVDV antibodies until challenge. Mean antibody \log_2

titers for type 1 BVDV in nonvaccinated heifers were 7.12 (GMT, 139) on day 143 after sham vaccination (day 14 after challenge) and 10.7 (GMT, $1,664$) on day 203 after sham vaccination (day of fetus collection). Mean antibody \log_2 titers for type 2 BVDV in nonvac-

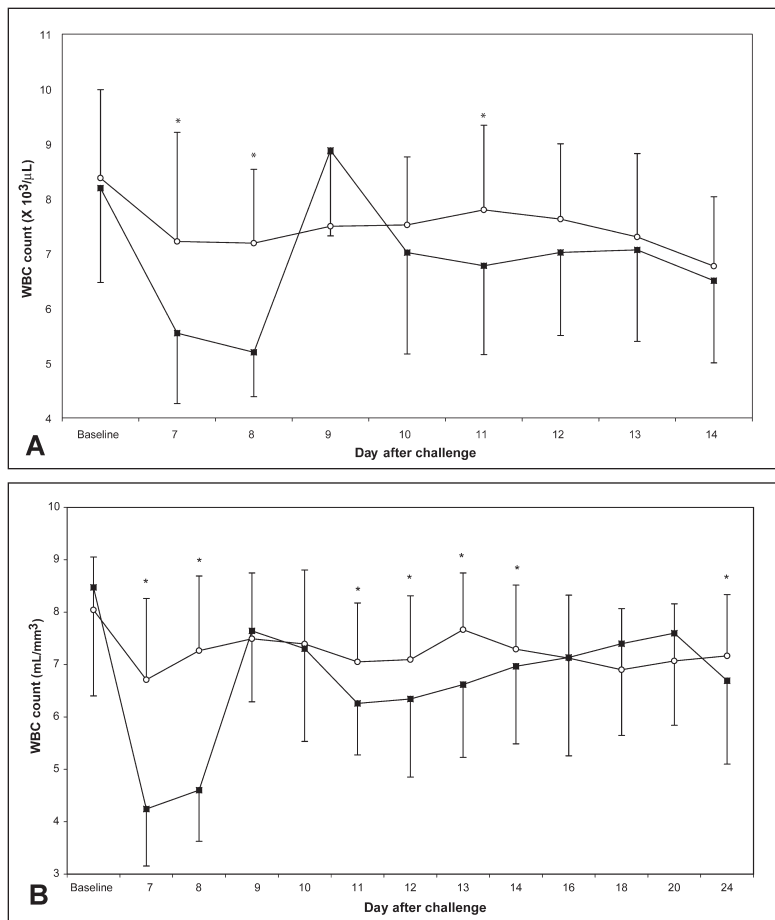


Figure 1—Mean \pm SD WBC counts in vaccinated (circles) and nonvaccinated (squares) heifers 7 to 14 days (A) and 7 to 24 days (B) after challenge with type 1 and type 2 bovine viral diarrhea virus (BVDV), respectively. Baseline values are the mean of the WBC counts on day 1 prior to challenge and day of challenge. *Significantly ($P < 0.05$) different from value for nonvaccinated heifers on the basis of comparisons of least square means. White blood cell counts in vaccinated and nonvaccinated heifers in both challenge groups were significantly ($P < 0.05$) lower than corresponding baseline values on days 7 and 8 after challenge.

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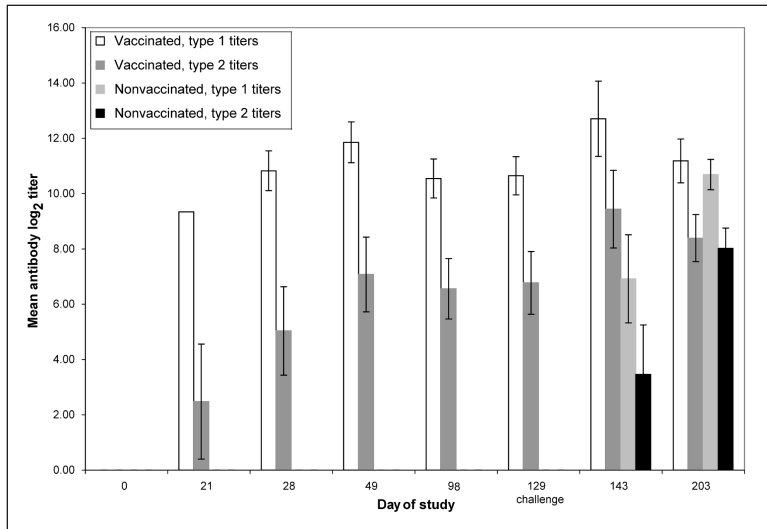


Figure 2—Mean \pm SD antibody \log_2 titers for type 1 and type 2 BVDV in vaccinated and nonvaccinated heifers before and after challenge with type 1 BVDV. Mean antibody \log_2 titers for type 1 and type 2 BVDV were significantly ($P \leq 0.001$) higher in vaccinated heifers than in nonvaccinated heifers at all time points except day 203 (74 days after challenge) on the basis of comparisons of least square means. Day 0 = Day of vaccination. Day 49 = Prior to breeding. Day 98 = First pregnancy examination. Day 203 = Day of collection of fetuses.

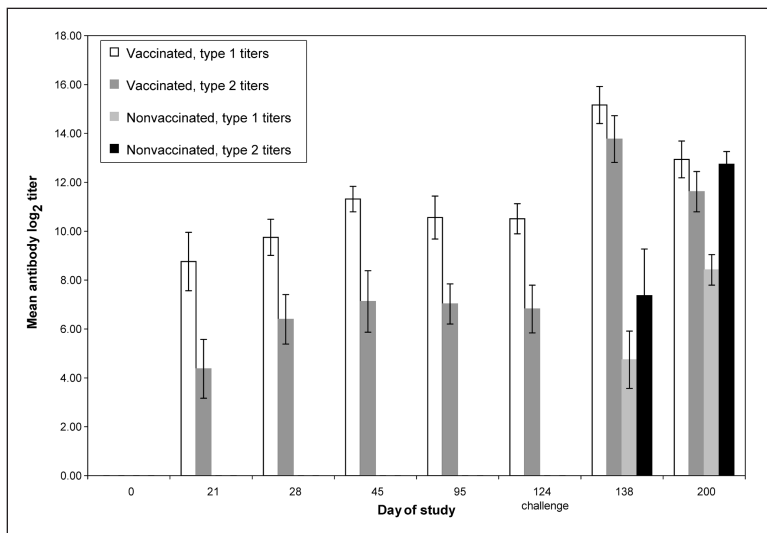


Figure 3—Mean \pm SD antibody \log_2 titers for type 1 and type 2 BVDV in vaccinated and nonvaccinated heifers before and after challenge with type 2 BVDV. Mean antibody \log_2 titers for type 1 and type 2 BVDV were significantly ($P \leq 0.001$) higher in vaccinated heifers than in nonvaccinated heifers at all time points except day 200 (76 days after challenge) on the basis of comparisons of least square means. Day 0 = Day of vaccination. Day 45 = Prior to breeding. Day 95 = First pregnancy examination. Day 200 = Day of collection of fetuses.

inated heifers were 3.35 (GMT, 13) and 8.05 (GMT, 265) on days 143 and 203, respectively.

Mean antibody \log_2 titers for type 1 and type 2 BVDV were significantly ($P \leq 0.001$) higher in vaccinated heifers, compared with nonvaccinated heifers at all time points except day 203 (Figure 2).

In the group of heifers challenged with type 2 BVDV, vaccinated heifers developed antibody titers for type 1 and type 2 BVDV by 21 days after vaccination; peak mean antibody \log_2 titers of 11.33 (GMT, 2,572) and 7.23 (GMT, 150), respectively, were detected on

day 45 after vaccination (before breeding). By challenge (day 124 after vaccination), mean antibody \log_2 titers in vaccinated heifers decreased slightly, but a strong anamnestic response was detected after type 2 BVDV challenge; maximum mean antibody \log_2 titers for type 1 and type 2 BVDV of 15.18 (GMT, 37,231) and 12.80 (GMT, 14,289), respectively, were detected on day 138 after vaccination (day 14 after challenge; Figure 3).

Nonvaccinated heifers were seronegative for anti-BVDV antibodies until challenge. The mean antibody \log_2 titers for type 1 BVDV in nonvaccinated heifers were 4.89 (GMT, 30) on day 138 and 8.44 (GMT, 347) on day 200 after sham vaccination. The mean antibody \log_2 titers for type 2 BVDV in nonvaccinated heifers were 7.59 (GMT, 193) and 12.75 (GMT, 6,889) on days 138 and 200, respectively.

Mean antibody \log_2 titers for type 1 and type 2 BVDV were significantly ($P \leq 0.001$) higher in vaccinated heifers than in nonvaccinated heifers at all time points except on day 200 (Figure 3).

Virus isolation in fetuses—Bovine viral diarrhea virus was isolated from all 4 fetal tissues tested in all fetuses from nonvaccinated heifers (10/10 fetuses from heifers challenged with type 1 BVDV and 8/8 fetuses from heifers challenged with type 2 BVDV). Virus was not isolated from any tissue of fetuses from vaccinated heifers (0/18) challenged with type 1 BVDV. Virus was isolated from all 4 tissues of 1 fetus from a vaccinated heifer challenged with type 2 BVDV (1/19; Table 1).

Discussion

Examination of the ability of a vaccine to prevent BVDV infection has historically centered on the magnitude of the antibody response induced by the vaccine. The results of our study provide evidence that a single dose of a bivalent BVDV vaccine can induce a level of immunity that prevents fetal infection with BVDV. The BVDV type 1 and type 2 fractions established the minimum protective dose for the purpose of

registration of the combination vaccine with the regulatory authorities. In our study, the observation that antibody titers at the time of challenge for type 1 BVDV were > 10-fold higher than those for type 2 BVDV is consistent with the results of an earlier study.²¹ The ability of the type 2 BVDV challenge to induce a high (approx 25- and 120-fold difference in type 1 and type 2 titers, respectively) anamnestic antibody titer for both type 1 and type 2 BVDV, whereas challenge with type 1 BVDV induced only a low to moderate (approx 5-fold difference) anamnestic antibody titer for type 1 and type 2 BVDV, highlights the antigenic and

immunologic differences between these 2 BVDV genotypes and has been previously observed by the authors for a variety of BVDV strains. These results lend more support to the recommendation that BVDV vaccines contain both genotypes of BVDV.

In our study, the decrease in total WBC counts from baseline values in heifers after BVDV challenge has been reported in other BVDV challenge studies.^{21,28,29} In our study, WBC counts in vaccinated and nonvaccinated heifers in both challenge groups were significantly ($P < 0.05$) lower than corresponding baseline values on days 7 and 8 after challenge. On these days, mean WBC counts in nonvaccinated heifers decreased by 35% to 50% from baseline values, whereas the mean WBC count in vaccinated heifers decreased by only 15%. In nonvaccinated heifers in both challenge groups, a rapid increase in mean WBC count was detected on day 9 after challenge; this may reflect the innate immune system response to infection. In the presence of a mild decrease in mean WBC count from baseline values on days 7 and 8 after challenge, vaccinated heifers prevented infections with BVDV in all but 1 fetus after challenge with BVDV.

The results of our study are similar to those of a previous study²¹ in which the use of a bivalent MLV BVDV vaccine provided protection to $> 90\%$ of fetuses against a heterologous type 1 or type 2 BVDV challenge. Our study differed from the previous study in that different challenge isolates of BVDV and larger, more statistically relevant numbers of animals were used. The challenge isolates used in our study were known to cause persistent infection in calves and mild to moderate clinical signs in the dam. The type 2 BVDV isolate used in the previous study²¹ caused persistent infection in calves, severe clinical illness and death in dams, and abortions.

Virus isolation from fetuses was consistent in that all 4 fetal tissues (cerebellum, heart blood, spleen, and thymus) yielded either positive or negative results. Results of virus isolation are the gold standard for diagnosis of persistent infection in fetuses³⁰; therefore, the results of virus isolation were conclusive in establishing vaccine efficacy in protecting the fetus against heterologous challenge with BVDV.

The proportions of fetuses protected in our study were higher than those reported in studies in which monovalent BVDV MLV vaccines were used. Two MLV vaccines containing a single type 1 BVDV provided protection against a type 1 BVDV challenge in 83%¹⁵ to 92%¹⁴ of fetuses. In 2 studies^{13,18} in which 2 inactivated type 1a BVDV vaccines were used, 100% of fetuses were protected against type 1 challenge; however, challenge with type 2 BVDV was not performed.

The vaccine containing 2 genotypes of BVDV used in our study and another study²¹ provided broad protection against infection of fetuses and the dam's respiratory tract by 4 heterologous BVDV strains (2 type 1 and 2 type 2 genotypes). Our study reports the results of challenge with North American strains of type 1 and type 2 BVDV that cause mild to moderate clinical signs in the dam. In the other study,²¹ a European type 1 strain of BVDV that is mildly pathogenic in the dam and a North American type 2 BVDV strain that is high-

ly pathogenic in the dam and abortigenic were used. All 4 strains are known to cause persistent infection in the fetus. This is in contrast to the lower rate of fetal protection provided by an MLV vaccine containing a single type 1 BVDV strain that provided protection against a type 2 BVDV challenge to 58% of fetuses.¹⁶ The lack of strong cross-protection after use of a single type 1 BVDV vaccine was also reported in several outbreaks of type 2 BVDV-associated reproductive disease in cattle vaccinated with a type 1a strain of BVDV.³¹ The results of these studies indicate that vaccines should contain more than a single BVDV genotype to induce cross-protection in the field.

The use of an MLV combination vaccine containing type 1 and type 2 BVDV in cattle prior to breeding has considerable merit for protection of fetuses against BVDV infection. Vaccination with a bivalent BVDV vaccine combined with a comprehensive BVDV control program that includes quarantine and testing of newly introduced cattle, a herd screening and monitoring program, and a biosecurity program should result in decreased incidence of BVDV infection and minimize the risk of persistent BVDV infections.

^aAnnual reports 1991–2002, Animal Disease Research and Diagnostic Laboratory Reports, South Dakota State University, Brookings, SD. Available on request.

^bImmunogenicity of Titanium 5 L5 following BVDV 890 (type 2) challenge, challenge study test results, TR-01, 1998, AgriLaboratories, St Joseph, Mo. Available on request.

^cImmunogenicity of Titanium 5 L5 following BVDV NY-1 (type 1) challenge, challenge study test results, TR-02, 1999, AgriLaboratories, St Joseph, Mo. Available on request.

^dImmunogenicity of Master Guard following type 2 challenge, challenge study test results, TR-06, 1999, AgriLaboratories, St Joseph, Mo. Available on request.

^eBVD vaccination and challenge trials for BVD types 1 and 2 vaccines, technical bulletin, TB99-107, 1999, Boehringer IngelheimVetmedica, St Joseph, Mo. Available on request.

^fAmerican Type Culture Collection, Manassas, Va.

^gMediatech Inc, Herndon, Va

^hHyclone, Logan, Utah.

ⁱCourtesy of Dr. Kenny Brock, Auburn University, Auburn, Ala.

^jBovine viral diarrhea virus antigen-capture ELISA, Syracuse Bioanalytical, East Syracuse, NY.

^kDr. James A. Roth, Department of Veterinary Microbiology and Preventive Medicine, Iowa State University, Ames, Iowa.

^lBreed-Back FP 5, Boehringer Ingelheim Vetmedica Inc, St Joseph, Mo.

^mDulbecco phosphate-buffered saline (0.9% NaCl) solution, JRH Bioscience, Lenexa, Kan.

ⁿMGA, megestrol acetate, Pharmacia Animal Health, Kalamazoo, Mich.

^oSuperMix 0.8, Land O'Lakes, Saint Paul, Minn.

^pLutalyse, prostaglandin F-2 α , Pharmacia Animal Health, Kalamazoo, Mich.

^qKAMAR heatmount detector, KAMAR Inc, Steamboat Springs, Colo.

^rGelman Chromist, Sigma-Aldrich, St Louis, Mo.

^sGLA Agriculture Electronics, San Luis Obispo, Calif.

^tAbaxis HMT, Abaxis, Union Center, Calif.

^uMonoject Corvac tubes, Kendell Medical, Mansfield, Mass.

^vSerum neutralization assay, Benchmark BioLaboratories, Lincoln, Neb.

^wMicrosoft Excel, Microsoft Corp, San Francisco, Calif.

^xMilliken Associates Inc, Manhattan, Kan.

^ySAS system, version 8.2, SAS Inc, Gary, NC.

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Product name	Species and indications for use	Route of administration	Remarks
Feline Leukemia Vaccine, Live canarypox vector (Merial Inc, US Vet Lic No. 298)	For vaccination of healthy cats, 9 weeks or older, as an aid in the prevention of disease caused by feline leukemia virus	Needle-free transdermal delivery system	USDA licensed 9/20/04
Salmonella Newport Bacterial Extract (Epitopix LLC, US Vet Lic No. 365)	For use in healthy cattle 6 months of age or older as an aid in the control of disease and fecal shedding caused by infection with <i>Salmonella</i> Newport	SC	USDA licensed 11/03/04