

Evaluation of the onset of protection induced by a modified-live virus vaccine in calves challenge inoculated with type 1b bovine viral diarrhea virus

Roberto A. Palomares, DVM, MS; M. Daniel Givens, DVM, PhD; James C. Wright, DVM, PhD; Paul H. Walz, DVM, PhD; Kenny V. Brock, DVM, PhD

Objective—To evaluate onset of protection induced by modified-live virus (MLV) bovine viral diarrhea virus (BVDV) vaccine administered 7, 5, or 3 days before inoculation with type 1b BVDV (strain NY-1).

Animals—40 calves.

Procedures—Calves were assigned to 4 groups: an unvaccinated control group or groups vaccinated with MLV vaccine containing BVDV types 1a and 2 at 7, 5, or 3 days, before inoculation with NY-1 BVDV. Blood samples were collected for leukocyte counts, serum virus neutralization, and virus isolation (VI); nasal swab specimens (NSSs) were obtained for VI, and rectal temperatures were monitored for 14 days after inoculation.

Results—No significant differences in leukocyte counts or rectal temperatures were detected after BVDV inoculation in vaccinated calves. Vaccinated calves had reduced viremia and viral shedding after inoculation, compared with results for unvaccinated calves. On day 5 after inoculation, a higher proportion of calves vaccinated 3 days before inoculation had positive VI from NSSs, compared with NSS VI results for calves vaccinated 5 and 7 days before inoculation. Unvaccinated calves had leukopenia on days 3, 5, and 6 and had higher rectal temperatures on days 7 and 8 after inoculation, compared with temperatures before inoculation. All unvaccinated calves had ≥ 1 positive VI result from NSSs 3 to 11 days after inoculation, and 4 became viremic.

Conclusions and Clinical Relevance—MLV BVDV vaccine prevented fever, viremia, and leukopenia in calves challenge inoculated with NY-1 BVDV. A high proportion of calves vaccinated 3 days before inoculation shed BVDV after inoculation. (*Am J Vet Res* 2012;73:567–574)

Bovine viral diarrhea virus is an important infectious agent that affects cattle worldwide.^{1,2} Infection of pregnant cattle with BVDV may result in abortions, stillbirths, or the birth of calves with congenital defects or that are persistently infected with BVDV.¹ Acute infections with some strains of BVDV may cause immunosuppression and clinical signs of gastrointestinal, reproductive, and respiratory disease.^{3,4} Strategies to prevent and control BVDV include quarantine and other biosecurity measures to control the spread of infection within and between herds, identification and slaughter of persistently infected cattle, and vaccination.^{5–7}

The efficacy of an MLV vaccine to protect against BVDV infection can be indirectly measured by the vac-

ABBREVIATIONS

BVDV	Bovine viral diarrhea virus
GM	Geometric mean
MDBK	Madin-Darby bovine kidney
MLV	Modified-live virus
VI	Virus isolation
VN	Virus neutralization

cine's ability to induce high neutralizing antibody concentrations and a strong cell-mediated immune response. An important factor in the evaluation of BVDV vaccine efficacy is its rapidity in eliciting an adequate immune response to protect cattle against negative outcomes when exposed to the virus within a few days after vaccination. This feature is particularly relevant in certain production systems such as feedlots, where frequent introduction of cattle with unknown BVDV status increases the risk of BVDV infection that could result in severe respiratory disease and a high death rate in affected cattle and substantial economic losses for producers.

A study⁸ to characterize the onset of protection elicited by an MLV BVDV vaccine in calves challenge

Received August 10, 2010.

Accepted March 15, 2011.

From the Departments of Pathobiology (Palomares, Givens, Wright, Walz, Brock) and Clinical Sciences (Palomares, Givens, Walz), College of Veterinary Medicine, Auburn University, Auburn, AL 36849. Presented as an oral presentation at the 89th Annual Meeting of the Conference of Research Workers in Animal Diseases, Chicago, December 2008.

Address correspondence to Dr. Brock (brockkv@auburn.edu).

inoculated with a virulent type 2 strain of BVDV revealed that calves vaccinated 5 or 7 days before experimentally induced infection did not develop clinical signs of BVDV infection, whereas unvaccinated control calves developed severe clinical disease and had a higher death rate. Vaccination of calves 3 days before challenge exposure with the same type 2 strain elicited some protection, as evidenced by a decreased magnitude of viremia and leukopenia after experimental inoculation, compared with that for unvaccinated control calves.⁸ The authors of that study⁸ speculated that such protection may not have been detected if a less virulent BVDV strain had been used for the challenge inoculation.

Studies^{9,10} have identified that type 1b BVDV strains are equally or more prevalent than type 1a BVDV strains and are isolated more frequently from calves that die with gross lesions of pneumonia.¹⁰ Most commercial vaccines marketed in the United States include type 1a, type 2, or both type 1a and type 2 BVDV strains, which might affect their efficacy because cattle are commonly exposed to a different subgenotype, such as type 1b.¹⁰ The objective of the study reported here was to evaluate the onset of protection in cattle vaccinated with an MLV BVDV vaccine 7, 5, or 3 days before experimental inoculation with a type 1b BVDV (strain NY-1). Our hypothesis was that a single dose of a commercial MLV combination viral vaccine (containing BVDV type 1a and 2 strains) would protect cattle from acute BVDV infection when experimentally inoculated with NY-1 BVDV soon after vaccination.

Materials and Methods

Animals—Forty clinically normal beef calves (36 males and 4 females) that were 7 to 9 months old and weighed 191 to 213 kg were used in the study. Calves were seronegative (antibody titer < 1:5) for BVDV as determined via serum VN and had negative results for BVDV via immunohistochemical analysis of ear notch specimens and VI of blood samples. Calves were obtained from a privately owned herd that had an optimal biosecurity program in place and to which new cattle were not introduced (closed herd).

Experimental design and sample size calculation—The study was designed as a randomized, controlled trial. Sample size was calculated^a on the basis of the expected means (7,000, 5,000, and 3,000 cells/ μ L) of the total leukocyte count for each vaccinated group, the SDs (1,200 cells/ μ L) of the means, and a statistical power of 99%.⁸ The calculated sample size was 6 calves/group, but we chose to enroll 10 calves/group to ensure that an adequate number of calves were maintained in each group throughout the study period. Calves were stratified by sex and assigned by use of a random number generator^b to 1 of 4 treatment groups: calves vaccinated 7 days before BVDV challenge inoculation, calves vaccinated 5 days before BVDV challenge inoculation, calves vaccinated 3 days before BVDV challenge inoculation, and control calves not vaccinated before BVDV challenge inoculation.

All 40 calves were housed together at the farm of origin prior to vaccination. As treatment groups were

vaccinated (7, 5, or 3 days before BVDV challenge inoculation), they were separated and transported to the Auburn University BVDV isolation farm. At the BVDV isolation farm, vaccinated calves were housed together in the same pasture and allowed to eat and drink from the same feeders and water troughs. The unvaccinated control calves were transported to the BVDV isolation farm on the day of BVDV challenge inoculation (day 0) to prevent any exposure of nonvaccinated calves to BVDV before inoculation. All calves were inoculated with BVDV on the same day and housed together from that time to the completion of the study. All calf protocols were reviewed and approved by the Institutional Animal Care and Use Committee of Auburn University.

Vaccine—The vaccine used was a USDA-licensed stock material approved for commercial sale.^c The vaccine was an MLV combination vaccine containing type 1a (Singer strain) and type 2 (296 strain) BVDV, bovine herpes virus type 1, parainfluenza virus type 3, and bovine respiratory syncytial virus. A single 2-mL dose of the vaccine was administered IM in accordance with the manufacturer's recommendations.

BVDV challenge inoculation—Calves were experimentally inoculated with a noncytopathic type 1b BVDV isolate (strain NY-1). The NY-1 BVDV strain was originally isolated by researchers at Cornell University from dairy cattle in the United States.¹¹ It has been in continuous propagation since 1957 and is available as a challenge BVDV strain from the USDA National Veterinary Services Laboratories.¹² The stock strain of NY-1 BVDV used in the present study was biologically cloned via successive passages by use of limiting dilutions with subsequent minimal propagation to produce an adequate amount of stock virus for characterization and animal challenge exposure studies. The inoculum used consisted of cell culture supernatants containing 1.36×10^6 cell culture infectious dose 50%/mL of inoculum. The inoculation was performed by intranasal aerosolization of 5 mL of inoculum by use of an aerosolizer^d and a vacuum pump.

Sample and data collection—From each calf, blood samples were collected on days 0, 3, 5, 6, 8, 11, and 14 after BVDV challenge inoculation in blood tubes containing an anticoagulant for determination of leukocyte count. Blood samples were collected on days 0, 3, 5, 6, 7, 8, 9, 11, and 14 after BVDV challenge inoculation in blood tubes without an anticoagulant to obtain serum for VN and VI. Nasal swab specimens for VI were also obtained and body temperature measured by use of a rectal thermometer on days 0, 3, 5, 6, 7, 8, 9, 11, and 14 after BVDV challenge inoculation.

One swab was used to scrape the nasal mucosa of each calf and was then placed in a tube containing 3 mL of Dulbecco modified Eagle medium supplemented with 10% equine serum, L-alanyl, and L-glutamine. Tubes containing nasal swabs were transported on ice until processing. For processing, tubes were mixed by use of a vortex; swabs were removed and discarded, and the remaining medium was stored at -80°C .

For each calf, immediately prior to and for 14 days after BVDV challenge inoculation, clinical signs of disease and clinical scores evaluating signs of depression, appetite, conjunctivitis, diarrhea, nasal discharge, coughing, and dyspnea were assessed and recorded daily. Clinical signs were assessed on a scale from 0 to 3, with 0 representing a lack of clinical signs and 3 representing severe clinical signs. Investigators who assessed clinical signs, collected samples, measured temperatures, and assessed the outcomes were unaware of the group assignment for each calf.¹³

Serum VN—Virus neutralization was performed at serial 2-fold dilutions (1:2 through 1:256) on heat-inactivated serum by use of 100 cell culture infectious dose 50%/mL of type 1 BVDV (strain NY-1) and type 2 BVDV (strain 1373). Samples were incubated for 1 hour at 37°C before the addition of MDBK cells (1.5×10^5 cells/mL). Cultures were incubated for 72 hours in 5% CO₂ at 37°C. The results were expressed as the relative concentration of antibody necessary to inhibit viral infection as determined by an immunoperoxidase staining technique that used a BVDV-specific polyclonal antibody (B-224).

VI—Serum samples and nasal swab specimens were stored frozen at -80°C. For VI, 250 µL of each sample was added to individual 25-cm² tissue culture flasks containing a monolayer of MDBK cells. For cell culture, Dulbecco modified Eagle medium supplemented with 10% equine serum, L-alanyl, and L-glutamine was used. After 3 days of incubation in 5% CO₂ at 37°C, flasks were frozen and thawed; 50 µL of the cell suspension from each flask was then transferred into 3 wells of a 96-well plate seeded with MDBK cells (first plate). The inoculated 96-well plate was incubated for 3 days, and the culture medium from each well was transferred to the corresponding wells of a new 96-well plate seeded with MDBK cells (second plate). Both 96-well plates (first and second) were tested for BVDV antigen by use of an immunoperoxidase staining technique.

Leukocyte count—Unclotted blood samples were submitted to the clinical pathology service laboratory at the Auburn University College of Veterinary Medicine for leukocyte analysis. The total leukocyte count for each sample was determined by use of an automatic cell counter.

Statistical analysis—To detect changes in leukocyte counts over time, the mean leukocyte count on day 0 was compared with the mean leukocyte count on days 3, 5, 6, 8, 11, and 14 by the use of repeated-measures analysis for a mixed generalized linear model^c defined by the following equation:

$$y_{ij} = \mu + \tau_i + \beta_j + \epsilon_{ij}$$

where y_{ij} is the response (leukocyte count) of subject j on day i for each treatment group; μ is the mean; τ_i is the effect of day i after BVDV inoculation (day 3, 5, 6, 8, 11, or 14), compared with the effect on day 0; β_j is a variable associated with subject j on day 0 (baseline); and ϵ_{ij} is the error. We assumed that days after inoculation were fixed (so $\sum \tau_i = 1 \cdot \tau_i = 0$) and that the calves used were a random sample of calves from a larger population of calves. Thus, the calves collectively rep-

resented a random effect, so we assumed that the mean of β_j was 0 and that the variance of β_j was σ_{β}^2 . Because the term β_j was common on all days after inoculation of the same calf, the covariance between y_{ij} and $y_{ij'}$ was not 0, but constant across all days and subjects. Changes in rectal temperature over time were analyzed by use of a statistical model similar to the model used for detecting changes in leukocyte counts over time.

An ANOVA was performed to compare means of temperature, leukocyte counts, and antibody titers, respectively, among the treatment groups by the use of a generalized linear model^c with the Scheffe method used to adjust for multiple comparisons. The model was defined as follows:

$$y_{i(y0-y14)} = \mu + \tau_i + \epsilon_i$$

where y_i is the respective response (leukocyte count, rectal temperature, or antibody titers) of subject j to treatment i (control or vaccination 7, 5, or 3 days before BVDV challenge inoculation) at 0, 3, 5, 6, 8, 11, and 14 days after BVDV challenge inoculation; μ is the mean; τ_i is the effect of treatment i (control or vaccination 7, 5, or 3 days before BVDVD challenge inoculation); and ϵ_{ij} is the random effect error. The value for R^2 was used to estimate the goodness of fit of the model. In the present study, R^2 was > 54% for all the continuous variables that were considered.

Virus isolation results from serum samples and nasal swab specimens were analyzed by use of a frequency procedure^f and compared by use of a χ^2 test. A logarithmic base 2 transformation was applied to VN values before calculation and comparison of the GMs. Back-transformed GMs for each viral strain tested were calculated for each group at day 0 and 14 days after BVDV challenge inoculation. For all analyses, values of $P < 0.05$ were considered significant.

Results

Clinical signs of BVDV infection—One calf that was vaccinated 3 days before BVDV inoculation was removed from the study because of an injury not associated with vaccination or inoculation. Clinical disease attributable to BVDV was not observed in any calf during the period between vaccination and BVDV inoculation. Clinical signs of severe BVDV infection (clinical score, 3) were not observed in any calf during the 14 days after BVDV inoculation. Mild coughing, nasal discharge, and loose feces were sporadically observed in a few vaccinated calves (4/10, 2/10, and 2/10 for calves vaccinated 7, 5, and 3 days before BVDV inoculation, respectively) during the 14 days after BVDV inoculation. Of the unvaccinated control calves, 7 of 10 developed mild watery nasal discharge during the course of the study; 3 of those 7 also had loose feces. Of the remaining 3 calves in the unvaccinated control group, 2 had loose feces as the only clinical sign of BVDV infection after inoculation, and 1 did not develop any clinical signs associated with BVDV infection.

Rectal temperature—A significant increase in mean rectal temperature was detected in the unvaccinated control group on days 7 ($P = 0.003$) and 8

Table 1—Mean \pm SEM rectal temperature ($^{\circ}$ C) the day of (day 0) and for 14 days after challenge inoculation with type 1b BVDV (strain NY-1) for calves that were vaccinated with an MLV vaccine containing types 1a and 2 BVDV, bovine herpes virus type 1, parainfluenza virus type 3, and bovine respiratory syncytial virus 7 (n = 10), 5 (10), or 3 (9) days before BVDV inoculation or that were unvaccinated control calves (10).

Day	Calves vaccinated 7 days before inoculation	Calves vaccinated 5 days before inoculation	Calves vaccinated 3 days before inoculation	Unvaccinated control calves
0	39.4 \pm 0.15	39.5 \pm 0.11	39.1 \pm 0.13	38.9 \pm 0.14
3	38.6 \pm 0.28	38.4 \pm 0.18	38.4 \pm 0.13	38.8 \pm 0.17
5	38.2 \pm 0.46	38.2 \pm 0.25	38.5 \pm 0.16	38.6 \pm 0.14
6	38.1 \pm 0.44	38.3 \pm 0.16	38.8 \pm 0.18	38.8 \pm 0.15
7	38.8 \pm 0.10	38.8 \pm 0.13	39.3 \pm 0.13	40.2 \pm 0.29*†
8	38.9 \pm 0.13	38.7 \pm 0.09	38.9 \pm 0.22	40.1 \pm 0.18†§
9	38.8 \pm 0.17	39.0 \pm 0.12	38.9 \pm 0.08	39.0 \pm 0.19
11	39.2 \pm 0.15	39.2 \pm 0.18	39.2 \pm 0.11	39.2 \pm 0.08
14	39.3 \pm 0.07	39.2 \pm 0.09	38.8 \pm 0.12	39.2 \pm 0.13

*Within a column, value is significantly ($P = 0.003$) different from temperature on day 0. †Within a column, value is significantly ($P = 0.001$) different from temperature on day 0. ‡Within a row, value is significantly ($P = 0.015$) different from the value for each of the other treatment groups. §Within a row, value is significantly ($P < 0.001$) different from the value for each of the other treatment groups.

Table 2—Geometric mean \pm SEM antibody titer against type 1b (strain NY-1) BVDV the day of (day = 0) and 14 days after challenge inoculation with type 1b BVDV (strain NY-1) for the same calves as in Table 1.

Day	Calves vaccinated 7 days before inoculation	Calves vaccinated 5 days before inoculation	Calves vaccinated 3 days before inoculation	Unvaccinated control calves
0	1.78 \pm 1.21	2.0 \pm 1.18	1.52 \pm 1.05	2.46 \pm 1.27
14	12.69 \pm 1.25*	10.07 \pm 1.38*	11.75 \pm 1.46*	73.51 \pm 1.35*†

*Within a column, value differs significantly ($P < 0.001$) from the value on day 0. †Within a row, value differs significantly ($P < 0.001$) from the value for each of the other treatment groups.

($P = 0.001$) after BVDV inoculation, compared with the mean rectal temperature for the group on day 0 (Table 1). The mean rectal temperature for the unvaccinated control group was also significantly higher on days 7 ($P = 0.015$) and 8 ($P < 0.001$), compared with the respective mean rectal temperatures on those days for the vaccinated groups. In contrast, there was no increase detected in the mean rectal temperatures for calves in the vaccinated groups, compared with the respective mean rectal temperatures for each group on day 0. There was also no difference in the mean rectal temperatures among any of the vaccinated groups on any specific day during the observation period.

Serum VN—Most calves were seronegative for BVDV type 1 and 2 on the day of vaccination (antibody titer $< 1:5$). However, 5 of 40 calves had low antibody titers (1:8 to 1:32) against BVDV type 1b and 2 at the beginning of the study, which were attributed to maternally derived antibodies and not to prior exposure to BVDV. Seropositive calves were evenly distributed among the 4 treatment groups and did not affect the outcomes of interest.

Serum VN antibody titers against NY-1 BVDV were significantly ($P < 0.001$) increased on day 14 after BVDV inoculation in all the vaccinated groups, compared with the NY-1 BVDV antibody titers on day 0. At the end of the study period, the GM antibody titer values were 12.69, 10.07, and 11.75 for calves that were vaccinated 7, 5, and 3 days before BVDV inoculation, respectively (Table 2). No differences were found among the vaccinated groups. Interestingly, unvaccinated calves developed very high VN antibody titers (GM, 73.51) against NY-1 BVDV 14 days after the challenge inoculation,

and this value was significantly ($P < 0.001$) greater than those detected in the vaccinated groups 14 days after challenge inoculation. Eight of 10 unvaccinated calves had a ≥ 4 -fold increase in antibody titers against NY-1 BVDV, which was indicative of an active infection. The 4 unvaccinated calves that became viremic had antibody titers ≥ 64 . In contrast, there was no increase in the GM antibody titers against type 2 BVDV from day 0 to 14 after BVDV inoculation for any of the vaccinated groups or the control group.

VI—Six days after BVDV inoculation, BVDV was detected in the serum of 6 calves (4 from the unvaccinated control group and 1 each from the groups vaccinated 7 and 3 days before BVDV inoculation). The number of calves with viremia in the unvaccinated control group was significantly ($P = 0.04$) higher than the number of calves (n = 0) with viremia in the group vaccinated 5 days before BVDV inoculation but was not significantly different from the number of calves with viremia in the other 2 vaccinated groups (Figure 1). Viremia was not detected in any of the study calves beginning 9 days after BVDV inoculation and for the remaining 5 days of the observation period.

All calves in the unvaccinated group had positive results for BVDV on ≥ 1 nasal swab specimen by day 9 after BVDV inoculation (Figure 2). On days 7, 8, and 9 after BVDV inoculation, the proportion of calves with positive results for BVDV on nasal swab specimens was significantly higher for the unvaccinated group (Figure 1), compared with the proportion of calves with positive results for BVDV on nasal swab specimens in the groups vaccinated 7 ($P = 0.003$), 5 ($P = 0.003$), or 3 ($P = 0.04$) days before challenge inoculation. In contrast,

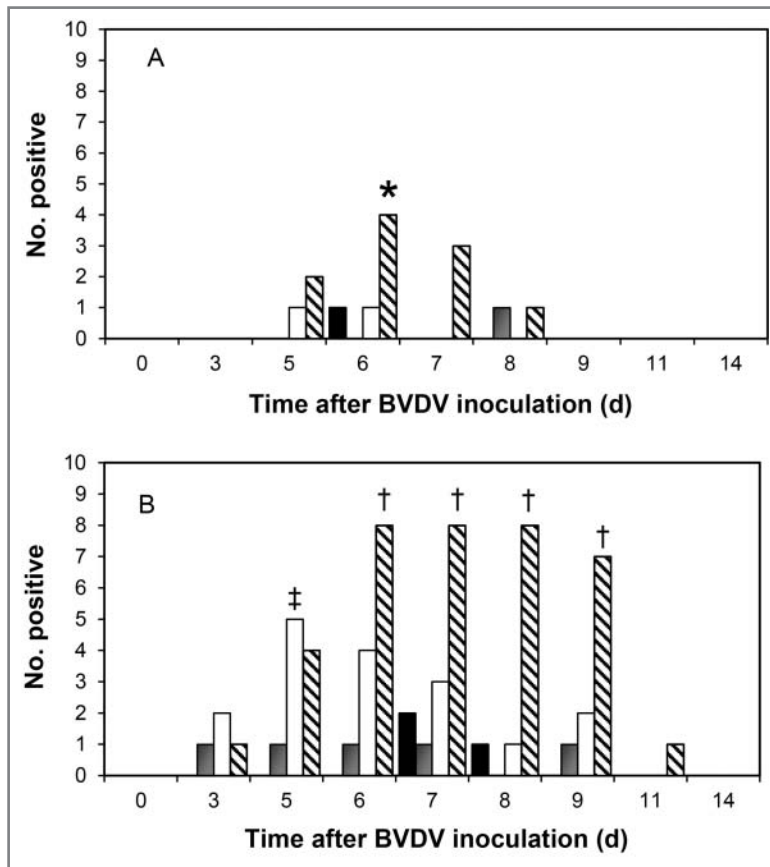


Figure 1—Number of positive results for BVDV via VI from serum samples (A) and nasal swab specimens (B) obtained from calves the day of (day 0) and for 14 days after challenge inoculation with type 1b BVDV (strain NY-1). Calves were vaccinated with an MLV vaccine containing types 1a and 2 BVDV, bovine herpes virus type 1, parainfluenza virus type 3, and bovine respiratory syncytial virus 7 (n = 10; black bars), 5 (10; gray bars), or 3 (9; white bars) days before BVDV inoculation or were unvaccinated control calves (10; diagonal-striped bars). *Value is significantly ($P = 0.04$) higher than that for calves vaccinated 5 days before BVDV inoculation. †Value is significantly ($P < 0.05$) higher than that for each of the other 3 groups. ‡Value is significantly higher than that for calves vaccinated 5 ($P = 0.04$) or 7 ($P = 0.008$) days before BVDV inoculation.

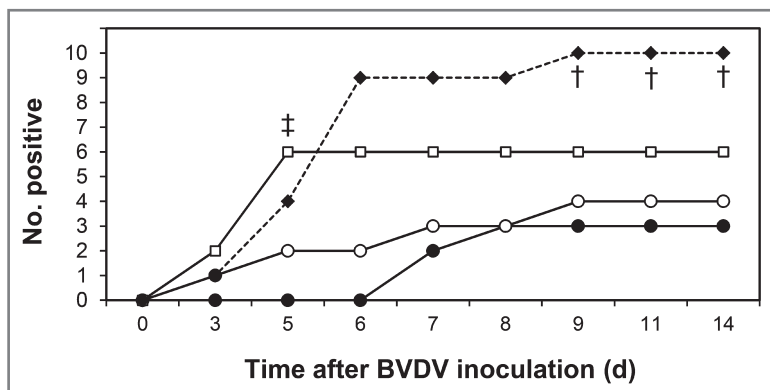


Figure 2—Cumulative number of positive results for BVDV via VI from nasal swab specimens obtained from calves the day of (day 0) and for 14 days after challenge inoculation with type 1b BVDV (strain NY-1). Calves were vaccinated with an MLV vaccine containing types 1a and 2 BVDV, bovine herpes virus type 1, parainfluenza virus type 3, and bovine respiratory syncytial virus 7 (n = 10; solid line with black circles), 5 (10; solid line with white circles), or 3 (9; solid line with white squares) days before BVDV inoculation or were unvaccinated control calves (10; dashed line with black diamonds). †Value is significantly higher than that for calves vaccinated 5 ($P = 0.004$) or 7 ($P = 0.002$) days before BVDV inoculation. ‡Value is significantly higher than that for calves vaccinated 5 ($P = 0.04$) or 7 ($P = 0.002$) days before BVDV inoculation.

on days 7, 8, and 9 after BVDV inoculation, the percentage of calves with positive results for BVDV on nasal swabs did not differ among the vaccinated groups.

Five days after BVDV inoculation, a higher proportion of calves that were vaccinated 3 days before challenge inoculation had positive results for BVDV on nasal swab specimens, compared with the proportion of calves with positive results for BVDV on nasal swab specimens in the groups vaccinated 5 ($P = 0.04$) or 7 ($P = 0.008$) days before challenge inoculations. Of the calves that were vaccinated 3 days before BVDV inoculation, 6 of 9 had positive results for BVDV on ≥ 1 nasal swab specimens between 3 and 9 days after BVDV inoculation (Figure 2). There was no significant difference in the cumulative number of calves that had positive results for BVDV on nasal swab specimens between the group vaccinated 3 days before BVDV inoculation and the unvaccinated control group.

Leukocyte count—Leukopenia was detected in unvaccinated calves on days 3, 5, and 6 after BVDV challenge inoculation, and the mean leukocyte count was significantly ($P < 0.001$) lower on each of those days, compared with that on day 0 (Table 3). This decrease in leukocyte count was associated with the period during which calves in the unvaccinated group became viremic. The mean leukocyte count was significantly ($P < 0.05$) lower for the unvaccinated group on days 3, 5, and 6 after BVDV challenge inoculation, compared with the mean leukocyte counts on those days for the vaccinated groups. Significant variations in the mean leukocyte count were not detected within or among the vaccinated groups throughout the observation period.

Discussion

In the present study, challenge inoculation with NY-1 BVDV did not cause severe clinical signs of BVDV infection in any of the calves. In another study⁸ in which investigators used a similar protocol, experimental challenge exposure with type 2 BVDV (strain 1373) caused severe clinical disease, as evidenced by a high death rate (60%) in unvaccinated calves; those results were consistent with results of other studies¹⁴⁻¹⁶ that involved the use of the same BVDV challenge-exposure strain. One possible reason for the contrast in clinical outcomes between the present study and the previous studies may be variations in the amount of virus used in the challenge-exposure dose or an increased virulence of the type 2 BVDV challenge strain.

Acute BVDV infection in unvaccinated calves was not accompanied by severe

Table 3—Mean \pm SEM leukocyte count on the day of (day = 0) and for 14 days after challenge inoculation with type 1b BVDV (strain NY-1) for the same calves as in Table 1.

Day	Calves vaccinated 7 days before inoculation	Calves vaccinated 5 days before inoculation	Calves vaccinated 3 days before inoculation	Unvaccinated control calves
0	6,780 \pm 801.6	5,738 \pm 341.0	7,106 \pm 583.2	8,646 \pm 790.8
3	7,554 \pm 573.5	7,485 \pm 349.2	6,448 \pm 516.0	4,941 \pm 425.2*†
5	6,900 \pm 651.2	6,877 \pm 451.7	8,161 \pm 877.6	4,978 \pm 271.5*†
6	7,069 \pm 785.8	6,522 \pm 338.4	7,454 \pm 370.3	4,958 \pm 313.1*†
8	7,048 \pm 557.7	7,031 \pm 355.5	7,302 \pm 234.6	7,874 \pm 574.3
11	7,519 \pm 774.5	7,177 \pm 302.6	6,660 \pm 438.0	7,263 \pm 530.4
14	7,869 \pm 580.7	7,798 \pm 576.6	6,680 \pm 350.4	7,163 \pm 498.3

*Within a column, value differs significantly ($P < 0.001$) from the value on day 0. †Within a row, value differs significantly ($P < 0.05$) from the value for each of the other treatment groups.

clinical signs of disease in the present study. This observation is similar to those reported from field investigations. However, the significant increase in rectal temperature detected for the unvaccinated group indicates a deviation from a clinically normal state. Calves that were vaccinated did not have a significant change in rectal temperatures during the course of the study, which is comparable with results of another study¹⁷ that included the use of a type 2 BVDV challenge inoculation. Ridpath et al¹⁸ demonstrated that cattle inoculated with the same NY-1 BVDV strain that was used in the present study developed mild clinical signs of BVDV infection that lasted for a shorter duration, compared with the clinical signs and duration of clinical signs associated with type 2 BVDV strains. Similar to infections caused by the NY-1 strain, acute infections with other BVDV field strains often do not cause severe clinical disease.¹ However, irrespective of the ability of a particular strain of BVDV to cause clinical disease, the deleterious effects BVDV has on the immune and reproductive health of cattle in general, along with the potential for the birth of calves persistently infected with BVDV, create the need for effective control measures to prevent the dissemination of BVDV within and between herds.

The GM antibody titers against the NY-1 BVDV challenge strain used in the present study were significantly increased at day 14 after the challenge inoculation, compared with the GM antibody titers on day 0 for all groups of calves. However, the GM antibody titer for the unvaccinated group on day 14 was significantly higher than that for each of the vaccinated groups. It is possible the lower antibody titers in the vaccinated groups could have been caused by early immune responses (production of type I interferon and neutralizing antibodies and stimulation of cell-mediated immunity in the form of natural killer cells and macrophages) elicited by the vaccine that inhibited antigenic stimulation of the immune system following the challenge inoculation and that prevented viral replication in the respiratory tract. The significant difference in antibody titers between the calves that were unvaccinated and vaccinated in the present study could also be attributed to the fact that unvaccinated calves had a primary humoral immune response that focused on the challenge BVDV strain, but vaccinated calves had a primary humoral response initiated by the heterologous BVDV strains included in the vaccine.

The results of VI from serum samples and nasal swab specimens indicate that vaccination 5 or 7 days before challenge inoculation was effective in protecting calves from developing clinical signs associated with BVDV infection and reducing the amount of viral shedding. This is in accordance with other studies¹⁷ in which BVDV was isolated from nasal swab specimens from all calves in an unvaccinated group but not calves in MLV-vaccinated groups. Fulton et al¹⁹ used 2 doses (vaccination 30 and 17 days before exposure to calves persistently infected with BVDV) of the same MLV vaccine (containing BVDV types 1a and 2) used in the present study and determined that vaccination prevented viremia, whereas BVDV was isolated from nasal secretions and leukocytes from unvaccinated control calves on days 6 and 13 after challenge inoculation. Although BVDV has been isolated from the serum of cattle on day 7 after receiving an MLV vaccine containing Singer or NADL strains of BVDV,²⁰ vaccinated calves in the present study seldom had BVDV isolated from the serum and it was never isolated ≤ 5 days after challenge inoculation.

In the present study, calves in the vaccinated groups did not have a significant decrease in mean leukocyte counts, compared with the leukocyte count for calves in the unvaccinated group, which had marked leukopenia during the observation period. This is consistent with results from other studies^{8,17} in which unvaccinated calves experimentally inoculated with a type 2 BVDV strain all developed leukopenia between 3 and 7 days after challenge inoculation.

The ability of vaccination to reduce the frequency of viremia and viral shedding in nasal secretions in calves exposed to BVDV within days after initial vaccination can reduce the spread of BVDV in commingled cattle, especially in production systems (ie, feedlots) with cattle from multiple sources with unknown BVDV status. Vaccination of high-risk cattle with a single dose of an MLV vaccine 5 or 7 days before exposure may immediately decrease BVDV transmission in cattle at risk of becoming infected because of commingling or exposure to cattle persistently infected with BVDV.⁸ However, it must be emphasized that the present study represents a single vaccination–challenge–exposure study, the results of which cannot be extrapolated to all cattle populations and production systems. The fact that a high proportion (6/9) of calves vaccinated 3 days before challenge inoculation had BVDV isolated from at least 1 nasal swab specimen between 3 and 9 days after BVDV inoculation indicated vaccination does not pre-

vent viral shedding if BVDV infection develops ≤ 3 days after vaccination. However, vaccination, even 3 days before inoculation, resulted in an immune response that decreased the frequency of viremia and viral shedding in nasal secretions, compared with the frequency of viremia and viral shedding in unvaccinated calves. Vaccination also mitigated the severity of the infection as evidenced by no significant variation in mean leukocyte count or rectal temperature in vaccinated calves throughout the observation period. The results of the present study confirm those of another study⁸ in which vaccination 3 days before challenge inoculation provided some but not total protection against BVDV infection by decreasing the degree of viremia and the development of leukopenia after inoculation. However, in that study,⁸ as in the present study, viral shedding in nasal secretions was detected in 4 of 10 calves vaccinated 3 days before challenge inoculation, but the amount of shedding was reduced, compared with that of the unvaccinated control group. Fulton et al³ reported that calves receiving an MLV vaccine containing BVDV types 1a and 2a 3 days before exposure to persistently infected cattle were not completely protected because a substantial proportion of those calves developed viremia.

Modified-live virus vaccines cause a limited infection at the site of injection that emulates a natural infection and triggers a cell-mediated immune response characterized by the proliferation and differentiation of helper and cytotoxic T cells.⁷ Antibody production against viral protein antigens requires interaction between helper T cells and B cells. Several days are required for the proliferation and differentiation of naïve lymphocytes into effector T cells and antibody-producing B cells to effectively control viral infection and replication.²¹ On the basis of the results from the present study, the immune response induced by a BVDV MLV vaccine in calves vaccinated 3 days before challenge inoculation was insufficient to control BVDV replication in the upper portion of the respiratory tract, as evidenced by BVDV shedding in nasal secretions. It is speculated that under natural conditions, BVDV infection within 3 days after MLV vaccination will result in viral shedding for approximately 7 days after exposure. Any virus shed by infected cattle puts other susceptible cattle at risk of becoming infected. However, the reduced frequency of BVDV isolation from the nasal secretions of calves vaccinated 5 and 7 days before challenge inoculation indicates that even 2 additional days of immune stimulation can result in a considerable difference in the physiologic immune response against BVDV infection.

Elements of innate immunity, such as type I interferon, might have played a role in the protection of calves that were vaccinated 5 or 7 days before challenge inoculation by minimizing the clinical signs associated with acute BVDV infection and the shedding of BVDV in nasal secretions.²² The molecular mechanisms by which type I interferon inhibits viral replication include the synthesis of proteins (eg, myxovirus resistance factor, dsRNA-dependent protein kinase, and 2'5'-oligoadenylate synthetase) that interfere with RNA transcription, protection of adjacent cells that have not yet been infected (antiviral

state), and induction of apoptosis of virus-infected cells, all of which impair BVDV viral replication.²³

A single dose of an MLV BVDV vaccine induced protection against type 1b BVDV infection in calves that were challenge inoculated with the virus soon (3 to 7 days) after vaccination as evidenced by a lack of fever and leukopenia as well as a reduced frequency of viremia and viral shedding throughout the observation period. However, a high proportion of calves inoculated with BVDV 3 days after vaccination shed the virus in nasal secretions between days 3 and 9 after BVDV challenge inoculation.

-
- a. PROC POWER, SAS, version 9.1, SAS Institute Inc, Cary, NC.
 - b. Research randomizer, version 3.0, Social Psychology Network, Middletown, Conn. Available at: www.randomizer.org. Accessed Aug 15, 2006.
 - c. Express 5, Boehringer Ingelheim, Ridgefield, Conn.
 - d. Aerosolizer deVilbiss, Somerset, Pa.
 - e. PROC GLM, SAS, version 9.1, SAS Institute Inc, Cary, NC.
 - f. PROC FREQ, SAS, version 9.1, SAS Institute Inc, Cary, NC.
-

References

1. Baker JC. The clinical manifestation of bovine viral diarrhoea infection. *Vet Clin North Am Food Anim Pract* 1995;11:425–445.
2. Houe H. Epidemiological features and economical importance of bovine virus diarrhoea virus (BVDV) infections. *Vet Microbiol* 1999;64:89–107.
3. Fulton RW, Briggs RE, Ridpath JF, et al. Transmission of bovine viral diarrhoea virus 1b to susceptible and vaccinated calves by exposure to persistently infected calves. *Can J Vet Res* 2005;69:161–169.
4. Makoschey B, Janssen MGJ, Vrijenhoek MP, et al. An inactivated bovine virus diarrhoea virus (BVDV) type 1 vaccine affords clinical protection against BVDV type 2. *Vaccine* 2001;19:3261–3268.
5. Kelling CL, Grotelueschen DM, Smith DR, et al. Testing and management strategies for effective beef and dairy herd BVDV biosecurity programs. *Bovine Pract* 2000;34(1):13–21.
6. Brock KV. Strategies for the control and prevention of bovine viral diarrhoea virus. *Vet Clin North Am Food Anim Pract* 2004;20:171–180.
7. Reber AJ, Tanner M, Okinaga T, et al. Evaluation of multiple immune parameters after vaccination with modified live or killed bovine viral diarrhoea virus vaccines. *Comp Immunol Microbiol Infect Dis* 2006;29:61–77.
8. Brock KV, Widel P, Walz P, et al. Onset of protection from experimental infection with type 2 bovine viral diarrhoea virus following vaccination with a modified-live vaccine. *Vet Ther* 2007;8:88–96.
9. Fulton RW, Ridpath JF, Confer AW, et al. Bovine viral diarrhoea virus antigenic diversity: impact on disease and vaccination programmes. *Biologicals* 2003;31:89–95.
10. Fulton RW, Ridpath JF, Ore S, et al. Bovine viral diarrhoea virus (BVDV) subgenotypes in diagnostic laboratory accessions: distribution of BVDV1a, 1b, and 2a subgenotypes. *Vet Microbiol* 2005;111:35–40.
11. Lee KM, Gillespie JH. Propagation of virus diarrhoea virus of cattle in tissue culture. *Am J Vet Res* 1957;18:952–953.
12. Clifford JR. *Standard reference preparations, test reagents, and seed cultures for laboratory test reagents*. Department of Veterinary Services memorandum No. 800.97. Fort Collins, Colo: USDA APHIS Veterinary Services, 2006.
13. O'Connor AM, Sargeant JM, Gardner IA, et al. The REFLECT statement: methods and processes of creating reporting guidelines for randomized controlled trials for livestock and food safety. *J Swine Health Prod* 2010;18:18–26.
14. Liebler-Tenorio EM, Ridpath JE, Neill JD. Distribution of viral antigen and tissue lesions in persistent and acute infection with the homologous strain of noncytopathic bovine viral diarrhoea virus. *J Vet Diagn Invest* 2004;16:388–396.
15. Stoffregen B, Bolin SR, Ridpath JF, et al. Morphologic lesions in type 2 BVDV infections experimentally induced by

- strain BVDV2-1373 recovered from a field case. *Vet Microbiol* 2000;77:157–162.
16. Ridpath JF, Bendfeldt S, Neill JD, et al. Lymphocytopathogenic activity in vitro correlates with high virulence in vivo for BVDV type 2 strains: criteria for a third biotype of BVDV. *Virus Res* 2006;118:62–69.
 17. Kelling CL, Hunsaker BD, Steffen DJ, et al. Characterization of protection against systemic infection and disease from experimental bovine viral diarrhoea virus type 2 infection by use of a modified-live noncytopathic type 1 vaccine in calves. *Am J Vet Res* 2007;68:788–796.
 18. Ridpath JF, Neill JD, Peterhans E. Impact of variation in acute virulence of BVDV1 strains on design of better vaccine efficacy challenge models. *Vaccine* 2007;25:8058–8066.
 19. Fulton RW, Johnson BJ, Briggs RE, et al. Challenge with bovine viral diarrhoea virus by exposure to persistently infected calves: protection by vaccination and negative results of antigen testing in nonvaccinated acutely infected calves. *Can J Vet Res* 2006;70:121–127.
 20. Bolin SR, Ridpath JF. Specificity of neutralizing and precipitating antibodies induced in healthy calves by monovalent modified-live bovine viral diarrhoea virus vaccines. *Am J Vet Res* 1989;50:817–821.
 21. Janeway CA, Travers P, Walport M, et al. Adaptive immunity to infection. In: Janeway CA, Travers P, Walport M, et al, eds. *Immunobiology: the immune system in health and disease*. 6th ed. New York: Taylor and Francis Group, 2005;409–459.
 22. Chase CC, Elmowalid G, Yousif AA. The immune response to bovine viral diarrhoea virus: a constantly changing picture. *Vet Clin North Am Food Anim Pract* 2004;20:95–114.
 23. Lenschow DJ, Lai C, Frias-Staheli N, et al. IFN-stimulated gene 15 functions as a critical antiviral molecule against influenza, herpes, and Sindbis viruses. *Proc Natl Acad Sci U S A* 2007;104:1371–1376.



Correction: Accuracy of isoflurane, halothane, and sevoflurane vaporizers during high oxygen flow and at maximum vaporizer dial setting

In the report “Accuracy of isoflurane, halothane, and sevoflurane vaporizers during high oxygen flow and at maximum vaporizer dial setting” (*Am J Vet Res* 2011;72:751–756), the affiliation paragraph should have read as follows:

From the Department of Clinical Studies, School of Veterinary Medicine, University of Pennsylvania, Philadelphia, PA 19104 (Ambrisko, Klide), and the Division of Anaesthesia and perioperative Intensive Care, Department for Companion Animals and Horses, University of Veterinary Medicine, Veterinaerplatz 1, 1210 Vienna, Austria (Ambrisko). Dr. Ambrisko’s present address is Division of Anaesthesia and perioperative Intensive Care, Department for Companion Animals and Horses, University of Veterinary Medicine, Veterinaerplatz 1, 1210 Vienna, Austria.

Correction: Comparison of use of an infrared anesthetic gas monitor and refractometry for measurement of anesthetic agent concentrations

In the report “Comparison of use of an infrared anesthetic gas monitor and refractometry for measurement of anesthetic agent concentrations” (*Am J Vet Res* 2011;72:1289–1304), the affiliation paragraph should have read as follows:

From the Department of Clinical Studies, School of Veterinary Medicine, University of Pennsylvania, Philadelphia, PA 19104 (Ambrisko, Klide), and the Division of Anaesthesia and perioperative Intensive Care, Department for Companion Animals and Horses, University of Veterinary Medicine, Veterinaerplatz 1, 1210 Vienna, Austria (Ambrisko). Dr. Ambrisko’s present address is Division of Anaesthesia and perioperative Intensive Care, Department for Companion Animals and Horses, University of Veterinary Medicine, Veterinaerplatz 1, 1210 Vienna, Austria.