PEER REVIEWED

Efficacy of a Modified-live Virus Vaccine Administered to Calves with Maternal Antibodies and Challenged Seven Months Later with a Virulent Bovine Viral Diarrhea Type 2 Virus

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Abstract

A total of 22 commercial dairy calves were used to determine if vaccination with an adjuvanted, modified-live bovine viral diarrhea virus (BVDV) vaccine in the face of maternal antibody will protect calves from a virulent type 2 BVDV challenge seven months post-vaccination, following the loss of maternal antibody. Neonatal calves were obtained prior to consuming colostrum and were randomly divided into three groups. Group 1 calves were fed an antibody-free colostrum supplement within six hours of birth. Calves in Group 2 and Group 3 were fed pooled colostrum obtained from cows vaccinated eight weeks prior to calving with a commercial inactivated combination vaccine containing BVDV1 and BVDV2. Group 2 calves were vaccinated at approximately 4.5 weeks of age with a commercial modified-live virus combination vaccine containing BVDV1 and BVDV2. Calves in Groups 1 and 3 were sham vaccinated. Seven months after vaccination, when calves in Group 3 became seronegative to BVDV2, calves in all three groups were challenged intranasally with virulent BVDV2, strain 1373. Calves that received colostrum and were vaccinated had only mild or no clinical disease. Calves that did or did not receive colostrum and were sham vaccinated developed severe disease, with a mortality rate of 33-50%. In this study young calves vaccinated with an adjuvanted, modified-live virus vaccine in the face of maternal antibody were protected against challenge with a virulent strain of BVDV.

Keywords: bovine, vaccination, BVDV, maternal antibodies

Résumé

On a utilisé un total de 22 veaux laitiers d'une ferme laitière afin de déterminer si la vaccination avec un vaccin à virus vivants modifiés avec adjuvant et des antigènes du virus de la diarrhée viral bovine (BVDV) en présence d'anticorps maternels allait protéger les veaux contre le virus BVDV de type 2 sept mois suivant la vaccination suite à la perte des anticorps maternels. Les veaux néonataux ont été obtenus avant la prise de colostrum et ont été alloués au hasard dans trois groupes expérimentaux. Dans le groupe 1 (n = 6), les veaux recevaient un supplément de colostrum sans anticorps moins de six heures suivant la naissance. Dans le groupe 2 (n = 8) et dans le groupe 3 (n = 8), les veaux recevaient du colostrum mélangé obtenu à partir de vaches vaccinées entre six et huit semaines avant le vêlage avec un vaccin commercial inactivé combinant des antigènes de BVDV 1 et BVDV 2. Les veaux du groupe 2 étaient vaccinés à approximativement 4.5 semaines d'âge avec un vaccin commercial à virus vivants modifiés contenant des antigènes de BVDV 1 et de BVDV 2. Les veaux du groupe 1 et du groupe 3 étaient vaccinés avec un vaccin sans antigènes. Sept mois suivant la vaccination, lorsque les veaux du groupe 3 sont devenus séronégatifs au BVDV 2, les veaux des trois groupes ont été provoqués par voie intranasale avec la souche virulente 1373 du BVDV 2. Les signes cliniques de l'infection au BVDV, le développement de la virémie et la variation dans les décomptes de leucocytes ont été notés pendant 21 jours suivant la provocation. Les veaux qui avaient reçu du colostrum et qui étaient vaccinés montraient très peu ou pas de signes cliniques. Les veaux qui avaient ou qui n'avaient

pas reçu du colostrum et qui avaient été vaccinés avec le vaccin sans antigènes ont été atteints sévèrement par la maladie et avaient un taux de mortalité de l'ordre de 33 à 50%. Dans cette étude, les veaux qui étaient vaccinés avec un vaccin à virus vivants modifiés avec adjuvant en présence d'anticorps maternels étaient protégés contre l'infection avec une souche virulente du BVDV.

Introduction

Bovine viral diarrhea virus (BVDV) is a clinically and economically devastating disease that impacts the cattle industry in the US. There are two genotypes of BVDV, type 1 (BVDV1) and type 2 (BVDV2), and each type can be further subdivided into either cytopathic or noncytopathic (NCP) biotypes, depending on how the virus affects cells in culture.[15] Both genotypes of BVDV can affect cattle at any age; however, the NCP BVDV strains are most clinically relevant in the field and can be manifested as either an acute or persistent infection.[12] Noncytopathic viruses represent 75-90% of all diagnostic laboratory BVDV isolations, and NCP are the only viruses associated with persistent, acute, and peracute infections. Cytopathic viruses only have clinical relevance in mucosal disease, which represents a very small number of clinical cases.

Control of BVDV is a major issue, and vaccination has proven to be an effective means of controlling spread of the disease. Some traditional vaccination programs focus on vaccinating calves after maternal antibody has waned; however, this leaves a window of susceptibility[13] when maternal antibodies are no longer protective, but may interfere with vaccine efficacy. In recent years, research has focused on vaccines that can overcome maternal interference by stimulating an immune response and potentially providing protection from disease.[4,18] Some of these studies focused primarily on the ability of calves to mount an immune response to vaccination in the face of maternal antibody, but did not challenge the calves to show efficacy.[1,7,8,9,11] Additional studies included a challenge; however, results of these studies show a range of protection most likely due to variations in vaccine administration and challenge models.[6,16,19]

The objective of the current study was to determine if vaccination of calves in the face of maternal antibody with an adjuvanted modified-live virus (MLV) vaccine provided protection from challenge with a virulent BVDV type 2 strain seven months after vaccination.

Materials and Methods

Animals

Protocols were reviewed and approved by the Rural Technologies Incorporated Institutional Animal Care and Use Committee. Twenty-two newborn, non-suckled dairy calves were acquired for the study, and were randomly assigned to one of three groups at enrollment using Microsoft Excel®. Randomization was completed prior to calf acquisition, indicating which treatment group the calves were to be assigned based on order of enrollment (i.e., birth order). Calves in Group 1 (n=6) were administered an antibody-free colostrum supplement[a] and sham vaccinated; calves in Group 2 (n=8) were colostral antibody-positive and vaccinated; and calves in Group 3 (n=8) were colostral antibody-positive and sham vaccinated. All calves received a MLV vaccine containing bovine rotavirus and bovine coronavirus. Vaccine was administered after birth and at least one hour prior to colostrum or supplement administration according to manufacturer's recommendations. Calves in Group 1 were administered a commercially available colostrum supplement free of antibody within six hours of birth, followed by two liters of a commercially available milk replacer[b] within three hours of initial treatment. Calves in Groups 2 and 3 were fed approximately two liters of pooled BVDV antibody-positive colostrum within six hours of birth. The colostrum had an IgG concentration[c] of 180 g/2 L, and a specific gravity of >1.050. The colostrum was obtained from commercial dairy cows that had been vaccinated approximately eight weeks prior to calving with a commercially available inactivated combination virus vaccine and bacterin.[d] The vaccine included the type 1 (Singer) and type 2 (5912) strains of BVDV. After colostrum treatments (Groups 2 and 3), all (n=22) calves were fed two to three liters of milk replacer twice daily until weaning. All calves were either vaccinated or sham vaccinated at approximately 4.5 weeks of age (range of four to five weeks).

Calves were processed and managed according to routine animal husbandry procedures. The calves were initially housed in individual calf hutches spaced approximately five feet apart. Following weaning at seven to eight weeks of age, all calves were commingled (34 days following vaccination) and housed together for the remainder of the study. The calves were fed an age-appropriate grain and hay ration *ad libitum* throughout the study period.

Pre-vaccination Serology Assays

Blood was collected from all calves for BVDV serology prior to administration of colostrum or colostrum supplement, at five days of age, and again at 21 days of age. Serum samples were tested for BVDV1 (Singer[e]) and BVDV2 (1373[e]) serum neutralizing antibody titers by use of the constant virus-decreasing serum assay.[2] Two-fold serial dilutions (range 1:2 to 1:256) of sera in duplicate were incubated with a constant viral titer (<500 $TCID_{50}$) before inoculation of BVDV-free bovine turbinate cells[f] in microtiter tissue culture plates.[g] Plates were incubated at 98.6°F (37°C) with

5% CO_2 for five days before being evaluated for virus-induced cytopathic effect (CPE) for BVDV1 and for immunohistochemical staining[17] for BVDV2. The reciprocal of the last dilution that prevented CPE formation or virus-specific staining was designated the serum neutralizing antibody titer. Geometric mean values were calculated by use of $\log_2$ titers.

Vaccination

Twenty-two calves were either vaccinated or sham vaccinated at approximately 4.5 weeks of age (day 0). Eight calves were subcutaneously vaccinated with a commercially available, adjuvanted MLV combination vaccine[h] containing infectious bovine rhinotracheitis virus, BVDV1, BVDV2, bovine parainfluenza-3 virus, and bovine respiratory syncytial virus, according to manufacturer's recommendations. The MLV vaccine contains the same BVDV strains (Singer and 5912) as the inactivated vaccine administered to the cows from which the colostrum was obtained. The remaining 14 calves (Groups 1 and 3) were sham vaccinated with sterile saline solution[i] and served as controls. All calves were observed daily after vaccination for vaccine-related adverse events.

Serologic Assays after Vaccination

Blood was collected from all calves on a monthly basis following vaccination, and BVDV1 and BVDV2 antibody titers were determined using a serum neutralization assay until all calves in Group 3 had titers of ≤2 for both BVDV1 and BVDV2. All calves in Group 3 became seronegative at approximately eight months of age.

Challenge

Twenty-two calves (Group 1 [n=6], Group 2 [n=8], and Group 3 [n=8]) were challenged intranasally with BVDV2 (strain 1373)[3] using an atomizer[j] 215 days after vaccination. The challenge inoculum contained 9.1 X 10^5 virus/mL, and 2 mL were atomized into each naris (total volume, 4 mL/calf).

Post-challenge Observations

Personnel caring for the calves and making clinical observations were masked (blinded) regarding treatment assignment. Clinical observations were performed daily from three days prior to challenge through day 21 after challenge. Each calf was visually examined and scored in the pen prior to handling for signs of abnormal respiration, nasal and ocular discharge, diarrhea, anorexia, and depression, using a scale of 0 to 5. The absence of a clinical sign was scored as 0, and the most severe clinical sign was scored as 5. Briefly, an abnormal respiration score was given if an animal was coughing, had labored breathing, or both; nasal and ocular discharge scores ranged from no discharge, moderate to severe serous discharge, mild to moderate to severe mucopurulent discharge; diarrhea scores ranged from no diarrhea, moderate to severe runny feces, watery/explosive feces, to bloody feces; anorexia scores ranged from no anorexia, mild to severe anorexia, excessive salivation, to exhibiting both signs (anorexic and excessive salivation); and depression scores ranged from no depression, mild to moderate to severe depression, to moribund. After the visual assessment, calves were restrained for determination of body temperature[k] and examined for oral cavity ulcers. These two assessments were also given a score of 0 to 5, with normal rectal temperature (100.4-102.9°F; 38.0-39.4°C) and no oral cavity ulcers scored as 0 and pyrexic (≥105.8°F; ≥41.0°C) or hypothermic (<99.5°F; 37.5°C) rectal temperature and presence of five or more oral cavity ulcers scored as 5. Any calf that displayed a total clinical score greater than 20, including all eight parameters, was euthanized. Calves that died or were euthanized during the observation period were given an additional score of 4. On the day they died, each calf was weighed, then a necropsy was performed to determine cause of death.

Body Weights

Calves were weighed three times during the observation period (-1, 14, and 21 days following challenge) using a portable livestock scale[l] that was validated before and after each weighing period using certified check weights. In addition, calves that died during the observation period were weighed prior to necropsy.

Virus Isolation

Blood was collected via jugular venipuncture from all calves during the observation period (from three days prior to challenge to 21 days after challenge, but only 23 samples/calf were analyzed for virus isolation [one day prior to challenge to 21 days after challenge]). White blood cells (WBC) were isolated according to a previously described technique.[5] The isolated WBCs were re-suspended in 2 mL of media[m] supplemented with equine serum[n] and tested for BVDV using a modification of an isolation assay previously described.[17] Briefly, one 10-fold dilution of each sample was made and each diluted sample was added in quadruplicate to BVDV-free bovine turbinate cell monolayers in microtiter tissue culture plates. Culture plates were incubated for five days at 98.6°F with 5% CO_2. Following incubation, plates were freeze-thawed three times and the samples were passaged onto new cell monolayers and incubated for an additional five days. This process was repeated for a total of three passages before completing immunohistochemical staining for BVDV.[17] Samples were considered positive for BVDV if virus-specific staining was observed in inoculated cells.

Post-challenge Hematology and Serology Analyses

Blood was collected via jugular venipuncture from all calves 25 times during the study period from three days prior to challenge through 21 days after challenge. Samples were subjected to hematologic analysis by use of a cytometer.[o] White blood cell and platelet counts were determined for each animal. Additional blood samples for serologic tests were collected on the day of challenge, 14 days after challenge, and 21 days after challenge. Serum neutralizing antibody titers against BVDV1 (Singer) and BVDV2 (1373) were determined.

Statistical Analysis

An analysis of variance using the PROC GLM procedure of SAS[p] was used to analyze calf bodyweight data. The model included treatment as the main effect to evaluate calf body weight.

Clinical observation, rectal temperature, titer, virus isolation, leukocyte data, and platelet count data were analyzed by ANOVA using the PROC MIXED procedure of SAS for repeated measures. The model included treatment, day, and treatment x day interaction. The random effect of animal and fixed effect of treatment were factors used to evaluate the data to be consistent with previous studies.

Mortality data were analyzed using the z-test for proportions.

The calf was used as the experimental unit. Least square means were calculated and separated using the PDIFF option of SAS. An alpha level of less than 0.05 was used to assess significance between treatments.

Results

Data observed for clinical signs, rectal temperatures, virus isolation, white blood cell count, and platelet count data had a significant time x treatment interaction ($P<0.01$) when single point comparisons between treatment groups were conducted.

Vaccination Reactions

No adverse vaccine reactions were observed in any calves.

Clinical Observations

Body temperature was measured rectally in all calves from three days prior to challenge to 21 days after challenge and the mean was determined for each group. All three groups began to experience elevated mean rectal temperatures on day 5 following challenge that peaked on day 9, and returned to baseline (101.8°F; 38.8°C) on day 12. Calves in Group 2 had significantly lower mean rectal temperatures, compared to calves in Groups 1 and 3 on day 9 (102.9°F vs 104.2°F and 105.4°F; 39.4°C vs 40.1°C and 40.8°C), on day 10 (102.0°F vs 103.1°F and 104.2°F; 38.9°C vs 39.5°C and 40.1°C), and on day 11 (101.8°F vs 102.7°F and 102.7°F; 38.8°C vs 39.3°C and 39.3°C) after challenge.

Clinical observations were recorded for all animals beginning from three days prior to challenge to 21 days after challenge, and the mean of these composite scores was determined for each group (Figure 1). Controls (Groups 1 and 3) had significantly ($P<0.05$) higher mean clinical scores than vaccinates (Group 2) on days 11 through 16 (on average controls were 73.6% higher than the vaccinates), and 18 days (controls were 89.2% higher than the vaccinates) after challenge. In addition, calves in Group 3 had significantly ($P<0.01$) higher mean clinical scores (84.3%) than vaccinates (Group 2) on day 21 after challenge. Mean clinical scores for calves in Groups 1 and 3 peaked on days 11 and 12, respectively; calves in Group 2 peaked on day 11 after challenge. Two of the six calves in Group 1 died (mortality rate of 33%) on day 16 after challenge, while four of the eight calves in Group 3 died or were euthanized (mortality rate of 50%): one calf died on day 11 after challenge, two calves died on day 12 after challenge, and one calf was euthanized on day 22 after challenge. Gross and histologic lesions in those calves were consistent with acute BVDV infection,[10] and BVDV was isolated from multiple tissues in the calves that died. No calves in Group 2 were euthanized or died after BVDV challenge.

Hematologic Analysis

White blood cell and platelet counts were obtained for all calves from three days prior to challenge through 21 days after challenge, and mean WBC and platelet counts were determined for each group (Figures 2 and 3). Calves in Group 1 had a 47% ($P\leq0.05$) decrease in WBCs (4.55 K/uL ± 4.0 vs 8.24 K/uL ± 2.6) on day 8, 51% (4.25 K/uL ± 3.1 vs 7.48 K/uL ± 1.7) on day 9, 43% (4.90 K/uL ± 3.1 vs 8.20 K/uL ± 1.7) on day 15, and 30% (6.43 K/uL ± 2.4 vs 9.08 K/uL ± 2.0) on day 16 after challenge when compared to Group 2. Calves in Group 3 had a 65% ($P<0.05$) decrease in WBCs (3.05 K/uL ± 0.5 vs 5.86 K/uL ± 2.1), 56% (3.81 K/uL ± 1.2 vs 8.24 K/uL ± 2.6), and 53% (4.09 K/uL ± 3.2 vs 7.48 K/uL ± 1.7) on days 7, 8, and 9, respectively, and 49% (4.30 K/uL ± 1.4 vs 8.20 K/uL ± 1.7) and 38% (5.22 K/uL ± 2.1 vs 9.08 K/uL ± 2.0) on days 15 and 16 after challenge when compared to Group 2. Calves in Group 2 did not experience leukopenia at any time following challenge. Calves in Group 3 had lower ($P<0.05$) platelet counts when compared to Groups 1 and 2 on days 10 (168 K/uL ± 140.2 vs 311 K/uL ± 145.7 and 450 K/uL ± 135.3), 11 (167 K/uL ± 119.9 vs 262 K/uL ± 138.8 and 397 K/uL ± 94.5), 13 (258 K/uL ± 183.2 vs 331 K/uL ± 206.8 and 471 K/uL ± 142.2), 14 (320 K/uL ± 220.2 vs 380 K/uL ± 213.0 and 575 K/uL ± 134.5), and 15 (402 K/uL ± 205.6 vs 497 K/uL ± 265.3 and 621 K/uL

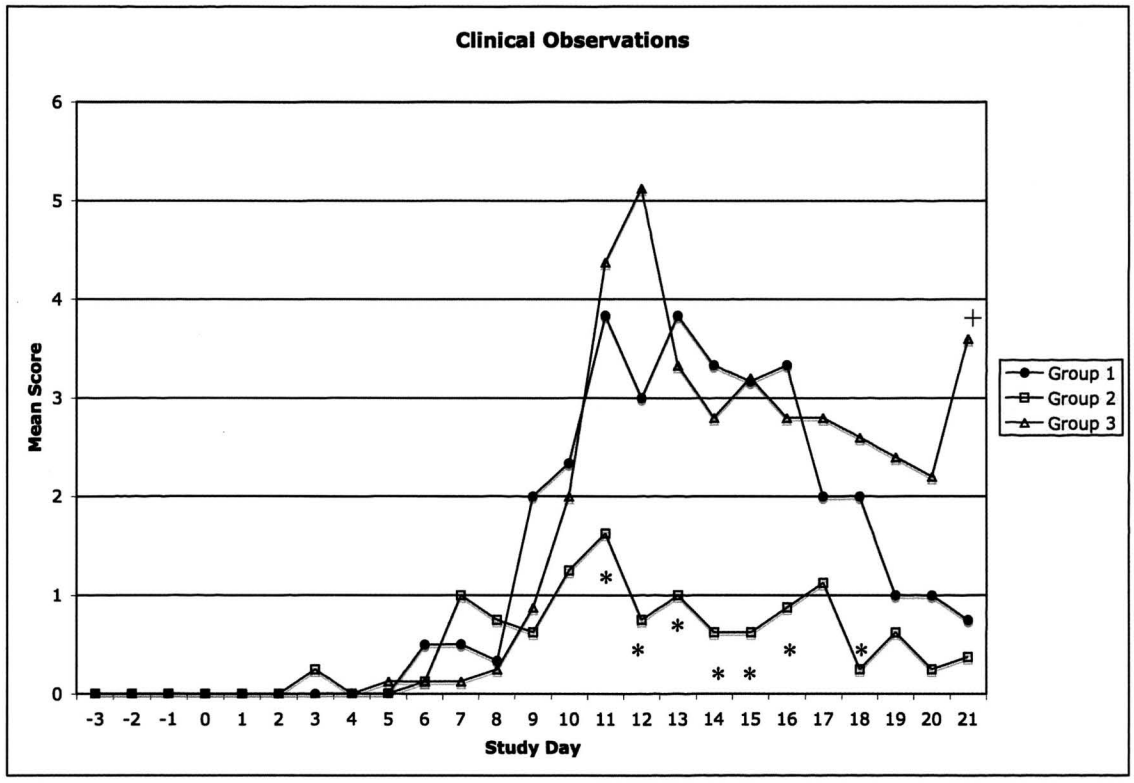

Figure 1. Mean clinical observation scores in three groups of calves before and after challenge (day 0) with virulent BVDV 2. Group 1 (black circles; n=6) consisted of seronegative (i.e., no colostral antibodies) calves that were not vaccinated. Group 2 (white squares; n=8) consisted of calves that were seropositive for anti-BVDV antibodies and were vaccinated. Group 3 (white triangles; n=8) consisted of seropositive calves and were not vaccinated. Notice that there was a significant ($P<0.01$) difference in mean clinical observation scores between calves in Group 2 when compared to calves in Groups 1 and 3 on days 11 to 16 and 18 after challenge (asterisks). In addition, calves in Group 3 had significantly ($P<0.01$) higher mean clinical scores than both Groups 1 and 2 on day 21 after challenge (plus sign).

± 148.0) after challenge. In addition, calves in Group 1 had significantly higher ($P<0.01$) platelet counts when compared to Groups 2 and 3 on day 20 (899 K/uL ± 409.7 vs 585 K/uL ± 128.6 and 566 K/uL ± 304.8) and 21 (867 K/uL ± 366.1 vs 588 K/uL ± 143.0 and 588 K/uL ± 311.3) following challenge. This increase is most likely due to the rate of platelet production exceeding the rate of platelet loss during the disease process. Calves in Group 2 did not experience thrombocytopenia.

Serum Neutralizing Antibody Titers

All calves had serum neutralizing antibody titers against BVDV of <1:2 at the time of enrollment. Calves in Group 1 did not receive any colostrum and tested negative for serum antibodies against BVDV following administration of colostrum supplement. All calves in treatment Groups 2 and 3 were fed colostrum containing antibodies against BVDV, and had mean antibody titers against BVDV1 of $>8.0_{log2}$ (i.e., ≥256) and BVDV2 of $>10.0_{log2}$ (i.e., ≥ 1024) at five days of age. These calves had BVDV antibody titers against BVDV1 of $>8.0_{log2}$ (≥256) and BVDV2 of $>8.0_{log2}$ (≥256) at the time of vaccination.

All calves were tested for serum neutralizing antibodies against BVDV1 and BVDV2 on a monthly basis following vaccination. All calves in Groups 2 and 3 had BVDV2 antibody titers of $>8.0_{log2}$ until day 116, and did not become seronegative ($\leq 2.0_{log2}$) until day 228 of age. Five of the six calves in Group 1 remained seronegative; however, one calf had BVDV1 titers that varied between 3.0_{log2} and 7.0_{log2}, and BVDV2 titers that varied between 0.0_{log2} and 5.0_{log2} in the months prior to challenge. This cyclical pattern was most likely due to a cross-reaction within the assays as the calf did respond similarly to the other calves enrolled in that group after challenge.

On the day of challenge (approximately 243 days of age; day 215 post-vaccination [PV]), calves in Group 1 had a mean BVDV1 serum neutralizing antibody titer of $2_{log2} \pm 2.5$ and a mean BVDV2 titer of $0.5_{log2} \pm 1.2$. Those in Group 2 had a mean BVDV1 titer of $1.1_{log2} \pm 0.8$ and a

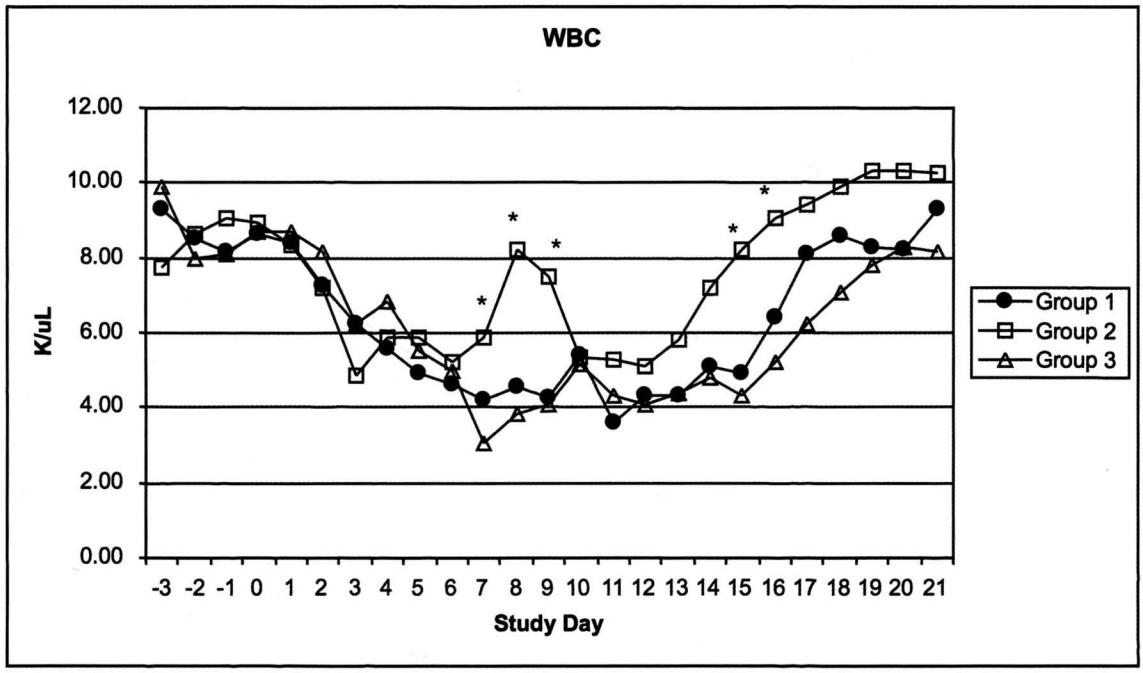

Figure 2. Mean white blood cell (WBC) counts in the same calves as in Figure 1. Notice that there was a significant ($P \leq 0.05$) difference in leukocyte numbers between Group 2 (white squares) and Group 3 (white triangles) on day 7, and between Group 2 and both Groups 1 (black circles) and 3 on days 8 to 9 and 15 to 16 after challenge.

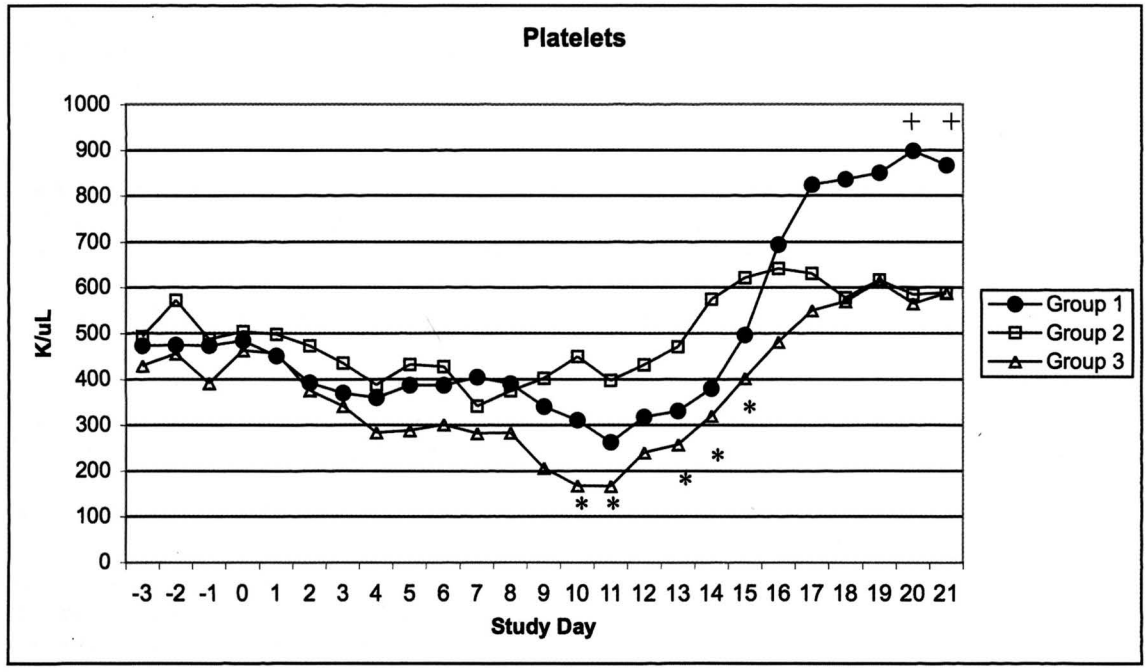

Figure 3. Mean platelet counts in the same calves as in Figures 1 and 2. Notice that there was a significant ($P<0.05$) difference in platelet counts between Group 3 (white triangles) and both Groups 1 (black circles) and 2 (white squares) on days 10, 11, and 13 to 15 after challenge (asterisks). In addition, Group 1 had a significant increase ($P<0.01$) in platelet counts when compared to both Groups 2 and 3 (plus signs) on days 20 and 21 after challenge.

mean BVDV2 titer of $1.6_{log2} \pm 1.5$. Calves in Group 3 had a mean titer of $1.0_{log2} \pm 0.5$ against BVDV1 virus and a mean titer of $0.8_{log2} \pm 0.8$ against BVDV2 virus.

At two weeks after challenge (day 229 PV), all three groups had minimal increases in titers against BVDV1 and dramatic 10-fold increases in titers against BVDV2 when compared with day 215 PV. Serum neutralizing antibody titers against BVDV1 in Group 1 increased by 1.5_{log2} to $3.5_{log2} \pm 3.0$, and BVDV2 titers increased by 10.1_{log2} to $10.6_{log2} \pm 1.1$. Titers against BVDV1 increased in Group 2 by 2.9_{log2} to $4.0_{log2} \pm 2.3$, and by 9.7_{log2} to $11.3_{log2} \pm 1.1$ against BVDV2. Calves in Group 3 had titers against BVDV1 increase by 1.0_{log2} to $2.0_{log2} \pm 2$, and by 10.0_{log2} to $10.8_{log2} \pm 1.3$ against BVDV2. At three weeks after challenge (day 236 PV), all groups had a slight increase in antibody titers against both BVDV1 and 2. Group 1 had an increase of 1.5_{log2} against BVDV1 and an increase of 2.95_{log2} against BVDV2. Group 2 had an increase in BVDV1 titers of 0.9_{log2} and an increase in BVDV2 titers of 1.1_{log2}. BVDV1 titers increased by 2.2_{log2} and BVDV2 titers increased by 1.4_{log2} in Group 3. There were no statistically significant serological differences between groups at any time point.

Body Weights

The average weight of the calves at time of challenge was 548 lb (249 kg); 537 lb (244 kg) for Group 1, 557 lb (253 kg) for Group 2, and 550 lb (250 kg) for Group 3. Calves in Group 2 were the only calves that gained weight (gain of 18.9 lb; 8.6 kg) during the challenge phase of the study. Calves in Group 1 lost 5.1% of their body weight (-27.3 lb; -12.4 kg), and calves in Group 3 lost 10% of their body weight (-54.8 lb; -24.9 kg) during the challenge phase of the study. Calves in Groups 1 and 3 did not regain enough weight during the 21-day study period to meet or exceed the initial body weights taken on the day prior to challenge (day -1). On day 14 following challenge, calves in Group 1 had lost more (P=0.02) body weight (loss of 62.5 lb; 28.4 kg) when compared to calves in Group 2 (gain of 3.5 lb; 1.6 kg), but had similar (P=0.12) body weights to calves in Group 3 (loss of 55.2 lb; 25.1 kg). There was a trend toward a significant difference (P=0.07) between Groups 2 and 3 at the same time point. Between days 14 and 21 following challenge, calf weight gain between all groups was not significantly different. All six calves that died or were euthanized following BVDV challenge lost weight ranging from 33 to 115.7 lb (15 to 52.6 kg).

Virus Isolation

Virus isolation was performed on buffy coat cells from all calves from the day before challenge through 21 days after challenge. Significantly fewer calves (38%; three of eight) in Group 2 were viremic on day 4 ($P \leq 0.05$) than calves is Group 1 (83%) and Group 3 (75%). On days 7 to 10 after challenge, significantly fewer ($P \leq 0.05$) calves in Group 2 were viremic compared to those in Groups 1 and 3 (day 7: Group 2: 25% vs 83% and 100% in Groups 1 and 3, respectively; day 8: 0% vs 83% and 75%; day 9: 38% vs 67% and 100%; day 10: 0% vs 17% and 75%). Calves in Group 1 had detectable viremia beginning on day 3 and continuing through day 12 following challenge. Most calves in this group were viremic on days 4, 6, 7, and 8, when five of the six calves were positive, and on days 5 and 9 when four of the six calves were positive. One calf in Group 1 was viremic for nine consecutive days; however, the group average was five consecutive days. Two of the eight calves in Group 2 were viremic on day 3; however, the average length of viremia was only two consecutive days. A majority of calves in Group 2 were viremic on day 6, when six of the eight calves were positive. Calves in Group 3 were viremic beginning on day 3, when three of the eight calves were positive. These calves were viremic from day 4 through day 10 (six out of eight calves were viremic on days 4, 5, 6, and 8; eight out of eight calves were viremic on days 7 and 9). Most calves in Group 3 were no longer viremic by day 12 following challenge; however, one calf was consistently viremic from day 3 through day 21 following challenge.

Discussion

Vaccination continues to be an effective way to control the spread of BVDV in cattle herds. Vaccinating calves in the face of maternal antibody is one way to close the window of susceptibility that occurs when calves are vaccinated after maternal antibodies have declined. In this study, one dose of an adjuvanted MLV BVDV vaccine administered in the presence of BVDV neutralizing maternal antibodies was able to significantly reduce clinical signs of disease seven months following vaccination when maternal antibodies had decreased to $\leq 2_{log2}$ (≤ 4).

Additional research has shown that vaccines can be effective at stimulating an immune response while maternal antibodies are still present, potentially protecting calves from disease following challenge. Many of these studies have shown that calves can mount an immune response following vaccination, but did not include challenge with the disease after vaccination to prove efficacy. Endsley[8] vaccinated calves with either an inactivated or MLV vaccine while they had high levels of circulating maternal antibodies, and concluded a memory response was stimulated. Antibody titers did not increase after the initial vaccination; however, following the booster 14 weeks later, calves demonstrated a memory response to BVDV. This suggests that even though there was no neutralizing antibody response after the first vaccination, calves developed a T cell-mediated immune

response to BVDV. Kaeberle[9] reported that one of the three inactivated virus vaccines tested stimulated a high antibody titer following vaccination of calves with maternal antibody. The authors concluded that this response may be due to the vaccine formulation or the type of adjuvant used in that specific vaccine, since the other two vaccines were not able to overcome maternal interference, based on serum neutralizing antibody titers. Two additional studies[1,11] involved vaccination with MLV infectious bovine rhinotracheitis vaccine while calves had high levels of circulating maternal antibodies, followed by a second dose six to eight months later. Neither study showed an increase in antibody titers after the first vaccination; however, in both studies calves developed an anamnestic response following the second vaccination while control calves did not. The studies cited above suggest some vaccines are capable of overcoming maternal interference and stimulating an immune response, even when there is no increase in antibody titers following the initial vaccination.[1,8,11] Additionally, these studies[1,8,11] showed that calves did develop an anamnestic response following booster vaccinations, further confirming that calves can develop a T cell-mediated immune response when vaccinated in the face of maternal antibodies. These results are comparable to the current study, in that calves did not produce antibody titers following vaccination (based on estimated end-point titers); however, the vaccine was able to overcome maternal interference and stimulate a memory response based on protection from disease following challenge.

A weakness of our study was that end point titers to BVDV1 and BVDV2 were not determined on day of vaccination to demonstrate the precise level of maternal antibody present when the calves were vaccinated; these serum samples were discarded following completion of the study. However, estimated end-point titers were determined by back-calculating titers from either day 116 or day 154 of age using a 21-day half-life for BVDV2.[14] At time of vaccination (day 28 of age), the calves would have estimated titers of 10_{log2} to 13_{log2} (1024-8192). The estimated mean of treatment Group 2 and treatment Group 3 on the day of vaccination is 12_{log2} (4096).

Stimulation of an immune response is important as protection from disease is the ultimate goal of any vaccination program. A BVDV study[6] showed that calves with maternal antibodies vaccinated at 10-14 days of age and challenged at four months of age developed severe disease, whereas calves without maternal antibodies vaccinated at 10-14 days of age and challenged at four months of age only developed mild clinical signs of disease. This study suggests that maternal interference could potentially affect vaccine efficacy when administered at less than two weeks of age. Two studies[7,16] exposed calves to BVDV virus at two to five weeks of age and challenged the calves seven to nine months later. Both studies showed that exposed calves were protected from disease, and one of the studies[16] showed that the calves were protected from viral shedding following challenge. These studies are similar to the current study in that calves had similar antibody decay, the same challenge model was utilized, and the time frames for vaccination/exposure and challenge were the same; however, these studies were looking only at a memory response and not evaluating resistance to disease following challenge. The only vaccine study[19] that demonstrated complete protection from viral shedding after challenge used an adjuvanted MLV BVDV vaccine administered at five weeks of age to calves with or without BVDV maternal antibodies. Calves were challenged three months following vaccination; vaccinates showed significantly fewer clinical signs and did not shed virus following challenge. This study was similar to the current study as the same vaccine, timing of vaccination, and challenge model were used; however, the current study did not challenge calves until they were seronegative seven months following vaccination instead of three months.

Conclusion

This study showed significant reduction in clinical disease in challenged calves after only one vaccination with BVD vaccine at 4.5 weeks of age. Calves vaccinated against BVD were protected from severe disease when challenged, did not develop leukopenia, and had reduced viral shedding. This indicates that the adjuvanted MLV vaccine used in this study was able to stimulate an immune response and provide good protection from disease. Further studies using a booster at six months of age are warranted, and would most likely enhance the protection afforded by this vaccine.

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Endnotes

[a]Sterling Technology, Brookings, SD
[b]Land O'Lakes Animal Milk Products Co, Shoreview, MN
[c]Midland Bioproduct Corp, Boone, IA
[d]Triangle® 9 + Type II BVD, Fort Dodge Animal Health, Fort Dodge, IA
[e]National Veterinary Services Laboratory, Ames, IA
[f]American Type Culture Collection, Manasses, VA

[g]Greiner Bio-ONE, Frickenhausen, Germany
[h]Pyramid® 5, Fort Dodge Animal Health, Fort Dodge, IA
[i]Vedco Inc., St. Joseph, MO
[j]Gelman Sciences, Ann Arbor, MI
[k]GLA Agriculture Electronics, San Luis Obispo, CA
[l]Tru-Test Inc., Mineral Wells, TX
[m]Cellgro, Mediatech Inc., Herndon, VA
[n]Atlanta Biologicals, Norcross, GA
[o]Sanford Health, Sioux Falls, SD
[p]SAS Institute Inc., Cary, NC

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