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Efficacy of a Non-adjuvanted, Modified-live Virus Vaccine in Calves with Maternal Antibodies against a Virulent Bovine Viral Diarrhea Virus Type 2a Challenge Seven Months following Vaccination

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Abstract

In the presence of maternal antibodies, 26 calves three days of age were either vaccinated (n=15) with a single dose of a non-adjuvanted, pentavalent, modified-live vaccine containing bovine herpesvirus-1, bovine respiratory syncytial virus, bovine viral diarrhea virus 1a (BVDV1a), bovine viral diarrhea virus 2a (BVDV2a), and bovine parainfluenza-3, or sham vaccinated with sterile saline (n=11). At approximately seven months post-vaccination, when vaccinates and non-vaccinates were seronegative to BVDV1a and BVDV2a antibodies, all calves were intranasally challenged with a virulent BVDV2a (strain 1373). Clinical signs of BVDV infection were monitored beginning three days prior to challenge and concluded on day 21 after challenge. Calves vaccinated in the face of maternal antibodies (IFOMA) were less impacted by the challenge. Based on clinical parameters, vaccinates had reduced clinical scores, rectal temperature, weight loss, and mortality compared to sham-vaccinated animals. Vaccinated animals had higher levels of circulating white blood cells and fewer animals were viremic than sham-vaccinated animals. This study demonstrated that a calf as young as three days of age when vaccinated IFOMA can be protected against a virulent BVDV2a challenge with a non-adjuvanted, pentavalent vaccine.

Keywords: bovine, bovine viral diarrhea virus, vaccine, vaccination, maternal interference

Résumé

Un total de 26 veaux âgés de trois jours ont été vaccinés en présence d'anticorps maternels soit avec une simple dose d'un vaccin pentavalent à virus vivants modifiés sans adjuvant (n = 15) contenant l'herpèsvirus bovin de type 1, le virus respiratoire syncytial bovin, le virus de la diarrhée virale bovine de type 1a (BVDV1a), le virus de la diarrhée virale bovine de type 2a (BVDV2a) et le virus parainfluenza bovin de type 3, ou soit avec une solution de saline stérile (n = 11). Sept mois approximativement suivant la vaccination, lorsque les individus vaccinés et non-vaccinés ne présentaient plus d'anticorps détectables contre le BVDV1a et le BVDV2a, tous les veaux ont été infectés expérimentalement par voie intranasale avec une souche virulente de BVDV2a (souche 1373). Les signes cliniques de l'infection au BVDV ont été surveillés sur une période s'étalant de trois jours avant l'infection expérimentale jusqu'à 21 jours plus tard. Les veaux vaccinés en présence d'anticorps maternels ont été moins influencés par l'infection expérimentale. Les scores cliniques, la température rectale, la perte de poids et la mortalité étaient moins élevés chez les individus vaccinés que chez les individus du groupe témoin. Les animaux vaccinés avaient une concentration plus élevée de leucocytes circulants et moins de virémie que les animaux du groupe témoin. Cette étude démontrait que des veaux d'à peine 3 jours peuvent être protégés contre une souche virulente de BVDV2a avec un vaccin pentavalent sans adjuvant en présence d'anticorps maternels.

Introduction

Bovine viral diarrhea virus (BVDV) infections result in severe losses to the cattle industry.[8] Vaccination can be an effective tool for control of BVDV.[10] For parenteral vaccine administration, vaccination in the presence of maternal antibodies has been reported to

reduce efficacy of the vaccine.[6] The ideal timing for vaccination of a calf can be difficult to determine due to a number of variables, such as age of the calf at the time of colostral feeding, total immunoglobulin ingested, method of intake (suckling vs esophageal feeder), breed, environmental temperature, calf vigor, and the cow's mothering ability.[9] Furthermore, timing of vaccination can be a double-edged sword; some calves will have become seronegative prior to vaccination, and thus are at risk for disease, and some calves will have lingering maternal antibodies, which may mitigate the response to vaccination, and thus limit vaccine efficacy.[3]

The objective of this study was to determine if a three-day old calf vaccinated in the face of maternal antibodies (IFOMA) with a single dose of a non-adjuvanted, pentavalent, modified-live virus vaccine containing bovine herpesvirus-1 (BHV-1), BVDV1a, BVDV2a, bovine parainfluenza-3 (PI-3), and bovine respiratory syncytial virus (BRSV), would be protected against an intranasally administered challenge with a virulent BVDV2a (strain 1373) after becoming seronegative to BVDV1a and BVDV2a antibodies at seven months of age.

Materials and Methods

Animals – Twenty-six newborn, non-suckled Holstein bull calves were acquired for the study and randomly assigned to one of two groups (15 vaccinates, 11 controls) 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). On arrival to the rearing facility, each calf was weighed, had an ear notch biopsy taken to test for persistent infection (PI) with BVDV using immunohistochemistry[m] (IHC), had serum protein measured with a refractometer, and was fed 2.3 L of colostrum containing a known quantity of BVDV antibody (110g of total immunoglobulin G, BVDV1a titer of 1:3200 and a BVDV2a titer of 1:1600). Calves were housed individually within calf hutches until weaning at approximately nine weeks of age. At the time of commingling, all calves were weighed, dehorned, and placed in a single lot with access to shelter in a barn. Calves were fed an age-appropriate grain and hay ration ad libitum throughout the study period. All protocols were reviewed and approved by the Rural Technologies, Inc. (RTI) Institutional Animal Care and Use Committee (IACUC).

Vaccination – Calves in all treatment groups were vaccinated at three days of age according to randomization. Calves in Treatment Group 1 were vaccinated subcutaneously according to the manufacturer's recommendation with a single 2-mL dose of a commercially available, pentavalent, modified-live virus (MLV) vaccine[a] containing BHV-1, BVDV1a, BVDV2a, PI-3, and BRSV. Calves in Treatment Group 2 served as controls and were sham vaccinated subcutaneously with a single 2-mL dose of sterile saline.[b] Animals were checked once daily for post-vaccination adverse events for seven days.

Pre-vaccination serology assays – Blood was collected via jugular venipuncture from calves at birth and on day 3 of age just prior to vaccination. Serum samples were tested for BVDV1a (Singer[c]) and BVDV2a (A125[c]) serum neutralizing (SN) antibody titers by use of the constant virus-decreasing serum assay.[1] Twofold 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[d] in microtiter tissue culture plates.[e] 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 BVDV1a and BVDV2a. 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.

Serologic assays after vaccination – Blood was collected from all calves for BVDV1a and BVDV2a SN testing at 14, 28, 42, and 56 days post-vaccination, and at approximately 70, 91, 120, 163, and 192 days of age until all calves were serologically negative (<1:2) to BVDV1a and BVDV2a.

Challenge – Twenty-six seronegative calves (15 vaccinates, 11 controls) were challenged intranasally with BVDV2a (strain 1373)[2] using an atomizer[f] approximately 210 days after vaccination (age range 203 – 216 days). The challenge inoculum contained 2.3 X 10^5 virus/mL, and 2 mL were atomized into each naris (total volume, 4 mL/calf).

Post-challenge observations – Daily clinical observations were performed by the same person each day beginning three days prior to challenge and continuing through day 21 after challenge. The person performing the clinical observations was blinded to treatment group assignment. Each calf was visually examined and scored in the pen prior to handling for signs of abnormal respiration, nasal and ocular discharge, diarrhea, and depression using a scale of 0 to 5, with the absence of a clinical sign scored as 0 and the most severe clinical sign 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; 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[g] and examined for oral cavity ulcers. Calves that died or were euthanatized during the observation period were given an additional score of 4. On the day of death, 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 on day -1 (prior to challenge), day 14, and day 21 following challenge using a portable livestock scale[h] 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 at one day prior to challenge (day -1) and on days 2, 4, 6 – 10, 12, and 14 after challenge for virus isolation. White blood cells were isolated according to a previously described technique.[4] The isolated WBCs were resuspended in 2 mL of media[i] supplemented with equine serum[j] and tested for BVDV using a modification of an isolation assay previously described.[13] 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 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 an immunoperoxidase assay for the detection of BVDV.[13] 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.[k] White blood cell and platelet counts were determined for each animal. Additional blood samples for serologic tests were collected on the day prior to challenge (-1) and 7, 14, and 21 days after challenge. Serum neutralizing antibody titers against BVDV1a (Singer) and BVDV2a (A125) were determined.

Statistical analysis – The experimental unit was the individual animal. Statistical significance was established at or equal to the 5% level ($P \leq 0.05$). Discrete or ordinal repeated measures data were modeled initially with Genmod procedures in SAS[l] (Version 9.2) to assess treatment x time interactions using animal as the repeated subject. A multinomial distribution was used for clinical scores (scores 0 to 9), whereas a binomial distribution was used for virus isolation score (score 0 or 1). If the Genmod model was not solved, the sum score across time was calculated, ranked using the Rank procedure, and ranked data were subjected to the Kruskal-Wallis test in the Npar1way procedure. These data were also summarized graphically by day for illustrative purposes. Mortality data were analyzed using a binomial distribution in Genmod.

Remaining continuous data (rectal temperature, platelet counts, white blood cell counts, body weight, and average daily gain (ADG), $\log_2$-transformed serum neutralization titers) were analyzed using Mixed procedures. For repeated measures, the model included the fixed effects of treatment and day and the random effect of animal. Measures taken before treatments were applied were utilized as covariates and remained in the model when significant ($P < 0.10$). If continuous data did not meet the requirements for analysis of variance, data were ranked and analyzed with a Kruskal-Wallis test as described.

Results

Clinical Scores, Mortality, and Body Weight

No adverse vaccine reactions were noted in any of the study calves. Additionally, no study calves were PI with BVDV as assessed by IHC. Vaccinates had statistically lower ($P < 0.05$) mean rectal temperatures than controls on 7, 10 – 12, and 14 – 17 days after challenge (Figure 1). The composite clinical scores were measured in all calves from three days prior to challenge through 21 days after challenge (Figure 2). The ranks of subjective clinical scores were statistically lower ($P = 0.01$) in vaccinated animals than for non-vaccinated animals (Table 1). The onset of clinical signs after challenge was similar between both groups (day 7 after challenge); however, the impact of the challenge on clinical scores was different between the two groups after the onset of clinical disease. Specifically, the mean clinical scores for vaccinated animals peaked on day 11 after challenge (mean clinical score on day 11 = 3.3, range 1- 6). In contrast to the vaccinated animals, the mean clinical scores for the non-vaccinated animals continued to increase after day 11, and peaked on day 13 after challenge (mean clinical score on day 13 = 5.8, range 4 – 9; Figure 2). For vaccinated animals, the mean peak clinical score was followed by a steady decline in mean clinical scores, which ultimately culminated in the absence of clinical signs at 21 days after challenge (Figure 2). After reaching mean peak clinical score, non-vaccinated animals

maintained elevated mean clinical scores until 18 days after challenge (Figure 2).

Total mortality was significantly lower for vaccinated animals ($P = 0.02$; Table 1). Three of 15 vaccinated animals died or were euthanized after challenge, which represented a total mortality rate of 20% (3/15). Seven of the 11 non-vaccinated animals died or were euthanized, which represented a total mortality rate of 63.6 % (7/11). All calves that died were necropsied at a veterinary diagnostic laboratory[n] by a trained pathologist. Based on histopathology, gross pathology, virus isolation, and clinical pathology (complete blood count), all mortality was consistent with BVDV as the primary pathogen, regardless of euthanasia or pen death.

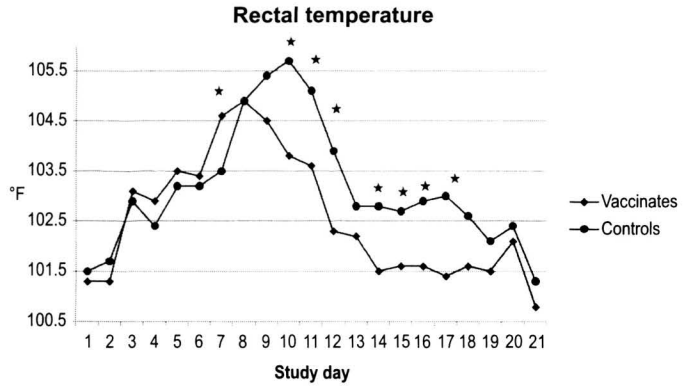

Figure 1. Mean rectal temperature observations in two groups of calves after challenge with virulent BVDV2a. Controls (closed circles; n=11) consisted of calves that were seropositive for anti-BVDV antibodies and that were sham vaccinated. Vaccinates (closed diamonds; n=15) consisted of calves that were seropositive for anti-BVDV antibodies and were vaccinated with vaccine. The asterisks indicate significant differences. Vaccinates had statistically lower ($P < 0.05$) mean rectal temperatures than controls on 7, 10 – 12, and 14 – 17 days after challenge.

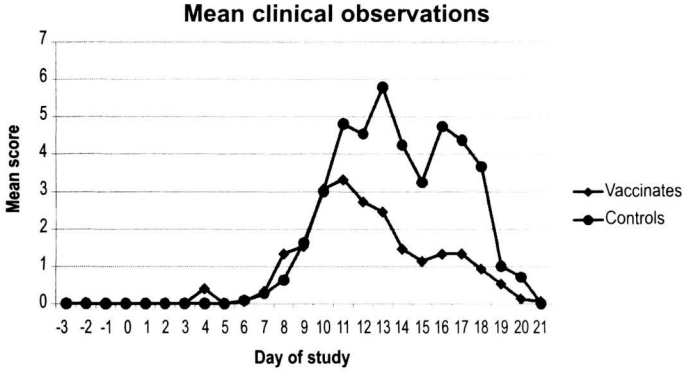

Figure 2. Mean clinical observations in two groups of calves after challenge with virulent BVDV2a. Controls (closed circles; n=11) consisted of calves that were seropositive for anti-BVDV antibodies and that were sham vaccinated. Vaccinates (closed diamonds; n=15) consisted of calves that were seropositive for anti-BVDV antibodies and were vaccinated with vaccine. The composite clinical scores were measured in all calves from three days prior to challenge through 21 days. The ranks of subjective clinical scores were statistically lower ($P = 0.01$) in vaccinates than for controls.

Vaccinated animals had a significantly higher final body weight than non-vaccinated animals ($P = 0.05$; Table 1). The average body weight of vaccinated animals at the time of challenge was 510 lb (231 kg), while non-vaccinated animals weighed 493 lb (223 kg; $P = 0.41$). ADG was significantly higher for vaccinated animals compared to non-vaccinated animals ($P = 0.004$). Vaccinated animals gained 1 lb (0.5 kg (ADG = 0.07 lb or 0.032 kg/day)) over the challenge period; whereas, non-vaccinated animals lost 28 lb (12.7 kg (ADG = -1.34 lb or -0.608 kg/day)) over the same period (Table 1).

Hematology

White blood cell and platelet counts were obtained daily, beginning at three days prior to challenge and concluding on day 21 after challenge (Figure 3). Overall, vaccinated animals had a higher white blood cell ($P = 0.02$) count than non-vaccinated animals during the challenge period. In addition to an overall treatment effect, significant differences were found between treatment groups within a day throughout the study period. Specifically, compared to non-vaccinated animals, vaccinated animals had significantly higher ($P < 0.05$) white blood cell counts on days 8 – 18 after challenge (Figure 3).

Although non-vaccinated animals had numerically higher platelet counts than vaccinated animals, they were not significantly different ($P = 0.58$; Table 1). Platelet counts for vaccinated animals remained stable during the challenge period; however, non-vaccinated animals tended to have a total numerical decrease in platelets on days 9 – 13 after challenge. Regardless of treatment group, all calves had increased platelet production beginning on day 14 after challenge.

Virus Isolation

The ranks of virus isolation were statistically lower ($P = 0.01$) in vaccinated animals than in non-vaccinated animals (Table 1). The first positive virus isolation for both treatment groups occurred on day 4 after challenge. The percentage of viremic vaccinated animals (80%) peaked on day 6, and the subsequent viremia began to steadily decline on day 7 after challenge (Table 2). Three of 15 (20%) vaccinated animals were negative for virus isolation at all sampling points after challenge (i.e. they were not positive at any point during the sam-

Table 1. Effects of vaccination before a viral challenge on body weight, health, and mortality.

Item	Vaccinated	Non-Vaccinated	SE	*P*-value
Animals enrolled	15	11	-	-
Initial wt, lb	510	493	15	0.41
Final wt, lb[a]	511	465	17	0.05
Average daily gain, lb[a,b]	0.07	-1.34	0.33	0.004
Clinical score rank[c]	10	18	-	0.01
Virus isolation score rank[c]	10	18	-	0.01
Platelet count[d]	363	385	24	0.58
Time of vaccination titers				
BVDV1, $\log_2$	8.5	8.8	0.5	0.14
BVDV2, $\log_2$	8.2	8.0	0.2	0.34
Day 7 to 21 titers				
BVDV1, $\log_2$[e]	1.3	1.2	0.2	0.66
BVDV2, $\log_2$[e]	4.9	5.2	0.2	0.16
Mortality, %	20.0	63.6	-	0.02

[a]Dead weight was not determined, so weight recorded closest to the date of death was used as final weight for dead cattle.
[b]Data did not fit a normal distribution by the Shapiro-Wilk test and/or displayed unequal variances by Levene's and Bartlett's test, so data were ranked and subjected to a Kruskal-Wallis test.
[c]The full model did not converge, so the sums scores across time were ranked and subjected to a Kruskal-Wallis test.
[d]The day 0 measurement remained in the model as a covariate ($P < 0.0001$), and covariate-adjusted means are presented.
[e]Treatment x day ($P \geq 0.15$).

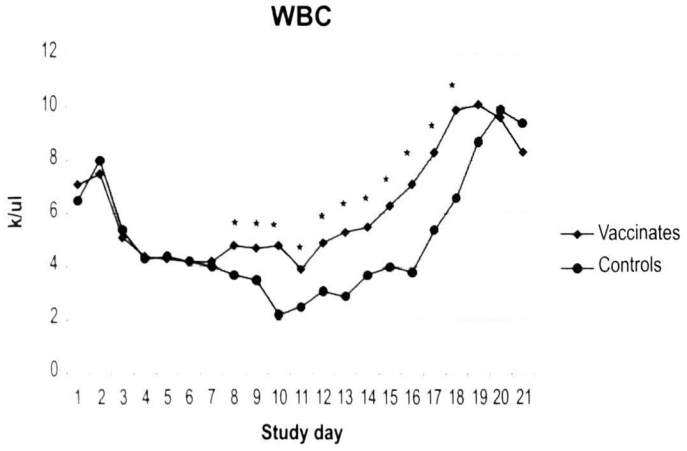

Figure 3. Mean white blood cell (WBC) counts in two groups of calves after challenge (day 0) with virulent BVDV2a. Controls (closed circles; n=11) consisted of calves that were seropositive for anti-BVDV antibodies and that were sham vaccinated. Vaccinates (closed diamonds; n=15) consisted of calves that were seropositive for anti-BVDV antibodies and were vaccinated with vaccine. The asterisks indicate significant differences. There was a significant ($P < 0.05$) decrease in leukocyte numbers in controls as compared to vaccinates from day 8-18.

pling period). The percentage of viremic non-vaccinated animals (100%) peaked on day 7, and continued at that level for the next two sampling days (days 8 and 9 after challenge). Viremia in non-vaccinated animals began to steadily decline on day 10 after challenge.

Serology

At the time of vaccination (three days of age), vaccinated animals had a mean antibody titer against BVDV1a of $8.5_{\log 2}$ (range: $8 - 9_{\log 2}$) and a BVDV2a antibody titer of $8.2_{\log 2}$ (range: $8 - 9_{\log 2}$; Tables 1 and 3). When sham vaccinated with sterile saline, non-vaccinated animals had a mean antibody titer against BVDV1a of $8.8_{\log 2}$ (range: $8 - 9_{\log 2}$) and a BVDV2a antibody titer of $8.0_{\log 2}$ (range: $7 - 9_{\log 2}$; Tables 1 and 3). At the time of challenge, all calves were seronegative (<1:2) to both BVDV1a and BVDV2a antibodies. The BVDV2a titers increased the most following a BVDV2a challenge, with both groups having similar titers beginning 14 days after challenge. The BVDV1a titers were lower than the BVDV2a titers, and there was no difference in the BVDV1a titers between the groups. Following challenge, the kinetics and magnitude of the humoral responses were similar between vaccinates and non-vaccinates for the mean antibody titers against BVDV1a ($P = 0.66$) and BVDV2a ($P = 0.16$; Tables 1 and 3).

Table 2. The raw data and percentages of animals positive for BVDV virus following BVDV challenge. Controls (n=11) consisted of calves that were seropositive for anti-BVDV antibodies and that were sham vaccinated. Vaccinates (n=15) consisted of calves that were seropositive for anti-BVDV antibodies and were vaccinated with vaccine. The ranks of virus isolation were statistically lower ($P = 0.01$) in vaccinated calves than the control calves.

Group		Day of study											
		-1	2	4	6	7	8	9	10	12	14	19	21
Vaccinates	Raw data	0/15	0/15	5/15	12/15	10/15	7/15	6/15	5/15	2/15	1/14	0/12	0/12
	%	0	0	33	80	67	47	40	33	13	7	0	0
Non-vaccinates	Raw data	0/11	0/11	8/11	10/11	11/11	11/11	11/11	10/11	5/11	2/8	0/4	0/4
	%	0	0	73	91	100	100	100	91	45	25	0	0

Table 3. The range and mean of serum neutralizing BVDV antibody titers (log_2) following BVDV challenge. Controls (n=11) consisted of calves that were seropositive for anti-BVDV antibodies and that were sham vaccinated. Vaccinates (n=15) consisted of calves that were seropositive for anti-BVDV antibodies and were vaccinated with vaccine. Following challenge, the kinetics and magnitude of the humoral responses were similar between vaccinates and controls for the mean antibody titers against BVDV1a ($P = 0.66$) and BVDV2a ($P = 0.16$).

Day of Study				At vaccination	-1	7	14	21
Group	Vaccinates	BVDV1a	Average	8.5	0	0	1.4	2.4
			Range	8 - 9	0	0	0 - 4	1 - 4
		BVDV2a	Average	8.2	0	0	5.9	8.8
			Range	8 - 9	0	0	3 - 7	8 - 9
	Non-vaccinates	BVDV1a	Average	8.8	0	0	0.8	2.8
			Range	8 - 9	0	0	0 - 3	2 - 3
		BVDV2a	Average	8	0	0	5.8	10
			Range	7 - 9	0	0	4 - 8	9 - 11

Discussion

In the current study, we observed a significant decrease in clinical scores and mortality rates in animals vaccinated parenterally at three days of age IFOMA with a single dose of a non-adjuvanted, pentavalent, modified-live virus vaccine containing BVDV1a and BVDV2a, when challenged at seven months of age. Challenged animals that were vaccinated had lower clinical scores, decreased viremia, elevated white blood cell counts, no loss of weight, better ADG, and a lower mortality rate than their age-matched, sham-vaccinated animals.

Clinically relevant studies have been completed to determine whether BVDV vaccination IFOMA can protect calves from disease.[2,5,11,12,15,16] In the four more recent and contemporary studies,[5,12,15,16] all calves were vaccinated with a single dose of an adjuvanted[12,15,16] or non-adjuvanted[5] BVDV vaccine IFOMA and subsequently challenged with a virulent BVDV2a (strain 24515[5] or 1373[12,15,16]). However, there were several important design differences between the current study and previous studies. In the current study: 1) at the time of vaccination, all calves were three days of age, which is younger than previously reported in the literature; 2) all calves were seronegative at the time of challenge to BVDV1a and BVDV2a (i.e., < 1:2); and 3) all calves were challenged at approximately 210 days post-vaccination. One major difference in the outcome of the current study was the 20% mortality rate, which was higher than in three of the previous studies (mortality rate was 0%[12,15,16]), but was lower than the 67% mortality observed in the fourth study.[5]

Although vaccinated animals in the present study had lower mortality rates and less clinical disease than the non-vaccinated animals, an amnestic humoral response following challenge was not detected (Table 3). The magnitude and kinetics of seroconversion to BVDV2a were nearly identical between vaccinated and

non-vaccinated animals. In a study by Platt *et al*,[12] researchers administered a single dose of an adjuvanted commercial vaccine to three distinct age groups of cattle: 1) one-to-two weeks of age; 2) four-to-five weeks of age; and 3) seven-to-eight weeks of age. Vaccinates and age-matched controls were challenged at 84 days post-vaccination. Interestingly, there was no humoral memory response in calves vaccinated at one-to-two weeks of age. Although the calves in the present study were younger than those in the Platt study at the time of vaccination (three days of age vs one-to-two weeks of age), calves used in the study by Platt and co-workers had a similar mean $\log_2$ SN titer to BVDV (BVDV1a = 7.6, BVDV2a = 7.4) as the calves in this study (BVDV1a = 8.8 and BVDV2a = 8.0) at the time of vaccination. Given that maternal SN titers at vaccination were similar between the two studies, there are at least two possible reasons for the inability to detect a humoral memory response, compared to the Platt study calves that were vaccinated at four-to-five weeks or seven-to-eight weeks of age: 1) interference of maternal antibody with the development of B-cell memory; and/or 2) the immaturity of the immune system in very young neonatal calves.[4]

In the current study, 80% of vaccinated animals were viremic four days after challenge. In another study IFOMA, Zimmerman and co-workers administered a single dose of a pentavalent MLV vaccine containing BVDV1a (Singer) and BVDV2 (5912) to approximately five-week-old calves and challenged the calves at 3.5 months (approx. 105 days) of age.[16] Results of the study showed that the vaccine induced a strong immune response IFOMA that was capable of providing protection. As in the study by Platt *et al*, calves in the study were not seronegative at the time of challenge. An important finding by Zimmerman *et al* was that vaccinated calves were completely protected against viral shedding after challenge. In a follow-up study using the same vaccine, Zimmerman *et al* changed their study design by delaying the challenge phase (215 days post-vaccination) until the calves were seronegative to BVDV1a and BVDV2a.[15] In that study, calves were protected from a virulent BVDV2a challenge; however, unlike the previous study, the vaccinated calves were viremic during the challenge phase in the follow-up study. The finding of viremia in the present study is consistent with the results from the second study by Zimmerman and co-workers.[15] The viremia in the study calves is likely attributable to the duration of time between vaccination and challenge.

Given the absence of B-cell memory, the non-adjuvanted vaccine used in this study most likely primed antigen-specific T-cells, which provided an appropriate response following challenge. However, the exact mechanism of response in the presence of maternal antibody is not known. At least two factors may contribute to the protective response measured in calves in the present study. The first is the age of the animal at the time of vaccination. Neonatal calves have all the components necessary to develop an adaptive immune response and B and T-cell memory. However, the B and T-cell responses will not be completely functional until calves are at least two-to-four weeks of age, and the responses will continue to mature until they reach puberty. This decreased response is supported by the lower level of protection in this study compared to previous studies when calves where vaccinated at several weeks of age,[12,15,16] which is particularly well documented in the study by Platt *et al* where calves vaccinated at one-to-two weeks of age had more severe leukopenia and higher clinical scores than calves vaccinated at four-to-five weeks or seven-to-eight weeks of age.[12]

In addition to age of the calf at the time of vaccination, calves in the present study received colostrum within six hours of birth. In the absence of an adjuvant, it is plausible that several components of colostrum may have been beneficial for assisting the immune system to generate an appropriate antigen-specific T-cell response IFOMA: specifically, the pro-inflammatory cytokines interleukin-1β, tumor necrosis factor-α, and interleukin-6, which are important for the development of immune responses and memory. Additionally, γ-interferon is present in high levels in bovine colostrum,[14] and may be beneficial in the face of maternal antibody for the successful priming of T-cells. These cytokines are absorbed by the calf, and the blood levels of these cytokines peak at 24 hours and then decline to almost non-detectable levels by 14 days of age. Interestingly, the levels of the aforementioned cytokines are still at 30-70% of peak levels at three days of age, while peak levels of γ-interferon are at 76%. It has been demonstrated that administration of interleukin-1β orally increases T-cell activation in the newborn calf, and that calves fed colostrum have higher lymphocyte activity than calves not fed colostrum.[7] Although the other four cited studies[5,12,15,16] had groups of calves that received colostrum, the levels of these cytokines would have been lower at the time of vaccination, since the calves were vaccinated between seven days of age and eight weeks of age, depending on the study. This age at vaccination issue is particularly interesting. Ellis *et al* vaccinated calves at 10-14 days of age with a non-adjuvanted vaccine IFOMA.[5] These animals were highly susceptible to disease and mortality (67% mortality) when challenged 4.5 months later, whereas calves in the present study, vaccinated at three days of age with a non-adjuvanted vaccine and challenged at seven months, had a 20% mortality.

The second factor for consideration is the strain of virus in the vaccine. The vaccines used in three previous studies[12,15,16] and the current study contained the Singer strain of BVDV, which is a type 1a cytopathic strain. Although the BVDV1 strains were similar be-

tween vaccines, the BVDV2 strains were different. The vaccine used by Platt *et al* contained strain 296-CP, whereas vaccines used in the Zimmerman studies contained strain 5912, and the vaccine used in the current study contained strain A125. Like other type 1 vaccine strains,[5,10] the Singer strain of BVDV has been shown to provide cross protection against highly virulent type 2 strains.[6] However, it is interesting that only the vaccines that contained Singer provided protection IFOMA. Since the other BVDV strain, NADL, demonstrated cross protection against a virulent type 2 strain in animals that were seronegative, but not IFOMA,[5] the strain effect cannot be discounted. The argument can be made that three of the vaccine studies that used the Singer strain of BVDV[12,15,16] contained an adjuvant, which could be responsible for protection IFOMA. The outcome would have been clearer if these studies immunized animals with the same vaccine without adjuvant to clarify adjuvant effect. Although it is likely that adjuvant contributed to protection IFOMA, this question cannot be answered until a clinical challenge trial with adjuvanted vs non-adjuvanted Singer vaccine is completed. In addition to the removal of the adjuvant, it would be appropriate in future studies to sham vaccinate control animals with a multivalent vaccine which does not contain BVDV. This would limit the potential impact of other vaccine components on the outcome of the trial.

Conclusion

This study demonstrated that calves vaccinated at three days of age IFOMA can be protected against a virulent BVDV2a challenge with a non-adjuvanted, pentavalent vaccine. Calves vaccinated IFOMA were less impacted by intranasal challenge with BVDV than control calves. Based on clinical parameters, vaccinates had reduced clinical scores, rectal temperatures, weight loss, and mortality compared to sham-vaccinated animals. Further evidence that protection was conferred was based on hematological parameters as well. Specifically, vaccinated animals had higher levels of circulating white blood cells and fewer were viremic, compared with control animals.

Endnotes

[a]Vista® 5 SQ, Intervet/Schering-Plough Animal Health, De Soto, KS
[b]Vedco Inc., St. Joseph, MO
[c]National Veterinary Services Laboratory, Ames, IA
[d]American Type Culture Collection, Manasses, VA
[e]Greiner Bio-ONE North America, Inc, Monroe, NC
[f]Gelman Sciences, Ann Arbor, MI
[g]GLA Agricultural Electronics, San Luis Obispo, CA
[h]Tru-Test Inc., Mineral Wells, TX
[i]Cellgro, Mediatech Inc., Herndon, VA
[j]Atlanta Biologicals, Norcross, GA
[k]Sanford Health, Sioux Falls, SD
[l]SAS Institute Inc., Cary, NC
[m]University of Nebraska Veterinary Diagnostic Center, Lincoln, NE
[n]Animal Disease Research and Diagnostic Laboratory, South Dakota State University, Brookings, SD

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