PEER REVIEWED

Immunologic, health, and growth responses of beef calves administered pentavalent modified-live virus respiratory vaccine in the presence of maternal antibody versus a traditional vaccination regimen

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Abstract

Crossbred beef calves (n = 253) from three breeding herds were used to determine the effects on health, performance, and immune response of different pentavalent (bovine herpesvirus-1, bovine viral diarrhea virus (BVDV) types 1 and 2, parainfluenza-3 virus, and bovine respiratory syncytial virus) modified-live virus (MLV) vaccine regimens administered initially at either 62 or 188 days of age. Calves were stratified by date of birth, gender, body weight and dam parity, then assigned randomly to one of two vaccine regimens. The early vaccination (EV) treatment group was administered a pentavalent MLV respiratory vaccine containing *Mannheimia haemolytica* leukotoxoid on day 0 (average calf age = 62 ± 17 days). The traditional vaccination treatment (TV) group received the same respiratory vaccine on day 126 (average calf age = 188 ± 17 days). Both treatment groups were revaccinated with the MLV respiratory vaccine on day 147 (weaning). A clostridial bacterin-toxoid was administered to all calves on days 0, 126, and 147. A subset of mixed-gender calves (n = 52/treatment) and steers (n = 10/treatment) were randomly selected to provide blood samples for measurement of the humoral and cell-mediated immune assays, respectively. To determine humoral immune response, blood was collected on days 0, 21, 126, 147 (weaning), 175, and 231, and serum was harvested for later determination of BVDV type 1a (Singer strain) serum neutralization antibody titers. Peripheral blood mononuclear cells were harvested from anticoagulated blood collected on days 0, 7, 21, 42, 126, and 189 to conduct cell population analysis indicative of CD25 expression index using a multi-parameter flow cytometry assay. No calves required treatment for bovine respiratory disease during the study, which ended after an 84-day post-weaning period. Interim and overall gain performance were similar ($P \geq 0.84$) between vaccine treatments throughout the study. On day 0, serum BVDV type 1a antibody titers were present, presumably from passive BVDV antibody transfer via colostrum, but did not differ ($P=0.50$) between vaccine treatments. However, BVDV antibody titers were greater for EV calves compared to TV calves on days 21, 126, and 147. There was a treatment × day interaction ($P=0.05$) for the CD25 expression index of CD8+ cells; EV calves had a greater expression index than TV calves on day 126. Differences in BVDV type 1a titer concentrations and CD25 expression indices observed in this study suggest that calves develop both humoral and cell-mediated immunity when vaccinated at 62 days of age. Furthermore, growth performance or health was not affected by vaccine regimen, which supports early vaccination as a cost-effective alternative to traditional calfhood vaccine regimens.

Key words: beef calves, bovine viral diarrhea virus, maternal antibody interference, modified-live virus vaccine

Résumé

Des veaux croisés de bovins de boucherie (n = 253) provenant de trois troupeaux reproducteurs ont servi à déterminer l'effet sur la santé, la performance et la réponse immunitaire de deux régimes de vaccination à vaccin vivant modifié pentavalent (herpèsvirus-1 bovin, types 1 et 2 du virus de la diarrhée virale bovine [VDVB], virus de la parainfluenza-3 et virus respiratoire syncytial bovin), l'un administré à l'âge de 62 jours et l'autre à l'âge de 188 jours. Les veaux ont été stratifiés selon la date de naissance, le sexe, le poids corporel et la parité

de leur mère, puis soumis de façon aléatoire à l'un de ces deux régimes. Le virus vivant modifié, contenant un leucotoxoïde de *M. haemolytica*, fut administré au jour 0 (à un âge moyen de veaux de 62 ± 17 jours) au groupe de vaccination hâtive et au jour 126 jours (à un âge moyen des veaux de 188 ± 17 jours), au groupe de vaccination traditionnelle. Les deux groupes ont été revaccinés avec ce même vaccin vivant atténué au jour 147 (sevrage). On administra à tous les veaux un vaccin bactérien à base de toxoïde clostridien aux jours 0, 126 et 147. On préleva le sang d'un sous-groupe de veaux des deux sexes (n = 52/traitement) et d'un autre de bouvillons (n = 10/traitement), sélectionnés au hasard, pour mesurer la réaction immunitaire des portions humorale et cellulaire, respectivement. Pour déterminer la réponse immunitaire humorale, du sang fut prélevé aux jours 0, 21, 126, 147 (sevrage), 175 et 231, et du sérum fut prélevé pour la détection ultérieure d'anticorps neutralisants contre le virus de la DVB de type 1a (souche de Singer). Des cellules mononucléées de sang périphérique ont été isolées du sang avec anticoagulant prélevé aux jours 0, 7, 21, 42, 126 et 189 pour en déterminer l'indice d'expression du récepteur CD25 dans la population cellulaire au moyen de la cytométrie de flux multiparamétrée.

Aucun veau n'a dû recevoir de traitement contre la maladie respiratoire bovine au cours de l'étude, qui s'est terminée 84 jours après le sevrage. Le gain de poids graduel et total s'est avéré similaire ($P \geq 0.84$) pour les deux dates de vaccin. Au jour 0, nous avons détecté des anticorps contre la souche 1a du VDVB, vraisemblablement par suite du transfert d'anticorps contre le VDVB via le colostrum, mais en titres similaires ($P = 0{,}50$) entre les deux traitements de vaccins. Toutefois, aux jours 21, 126 et 147, le titre d'anticorps contre le VDVB s'est révélé plus élevé chez le groupe de vaccination hâtive que chez le groupe de vaccination traditionnelle. Nous avons noté une interaction « jour du traitement x jour d'analyse » ($P = 0{,}05$) en ce qui concerne l'indice d'expression du récepteur CD25 des cellules cytotoxiques CD8+ : les veaux vaccinés tôt avaient un indice d'expression plus élevé que les veaux vaccinés à la période traditionnelle (jour 126). Ces différences en ce qui a trait à la concentration d'anticorps contre le VDVB de type 1a et à l'indice d'expression CD25 suggèrent que les veaux vaccinés à l'âge de 62 jours développent une immunité à la fois humorale et cellulaire. De surcroît, la croissance et la santé des veaux n'ont pas été affectées par l'un ou l'autre régime de vaccination, ce qui milite en faveur de la vaccination hâtive comme alternative rentable à la vaccination traditionnelle des veaux.

Introduction

With an estimated cost of more than $1 billion annually,[1] bovine respiratory disease (BRD) is the most expensive disease associated with cattle production in the US. Economic losses are a result of not only increased antibiotic treatment and death loss, but also reduced animal performance and carcass value.[8] Preconditioning has been shown to reduce BRD morbidity because this comprehensive management practice is designed to mitigate predisposing disease factors by reducing physiological stress, and improve immunity through appropriate timing of vaccination before marketing and disease challenges occur.[7,9,11]

Typically, preconditioning guidelines recommend calves be vaccinated against respiratory and other pathogens at or near weaning (calf age approximately 180 to 210 days) in consideration of the historical belief that maternal antibodies present from colostrum transfer interfere with the immune response to vaccination. However, although maternal antibodies received passively in colostrum may interfere with humoral (antibody) response to vaccination, cell-mediated (T-cell) immunity during this time is clearly activated.[6] Furthermore, recent experimental challenge studies investigating vaccination during the presence of maternal antibodies suggest that neonatal calves vaccinated with a modified-live virus (MLV) vaccine are protected from subsequent bovine viral diarrhea virus (BVDV) challenge,[10,12] and immature calves vaccinated at 67 days of age develop both a primary and anamnestic antibody response.[2] If field efficacy of MLV vaccination in immature beef calves during the presence of maternal antibody is established, it affords new opportunity to administer an initial MLV vaccine during the management event known as "branding" (calf age approximately 60 days) and modify existing vaccination guidelines within preconditioning programs.

Our objective was to determine the effects of administering initial pentavalent MLV respiratory vaccine, *Mannheimia haemolytica* leukotoxoid, and clostridial bacterin-toxoid to calves at approximately 60 days of age versus a traditional vaccination regimen administered near weaning age on health, growth performance, BVDV type 1a antibody titers (humoral immunity), and *in vitro* IFN-γ expression and BVDV-specific T cell activation (cell-mediated immunity).

Materials and Methods

Animals and treatment assignment

This study was conducted from May 2010 until July 2011 at the University of Arkansas Division of Agriculture Experiment Station located in Savoy, and the University of Arkansas Division of Agriculture Livestock and Forestry Research and Extension Station located near Batesville. A total of 253 Angus-cross beef calves from three herds (58 in a spring-calving herd and 102 in a fall-calving herd at the Savoy unit, and 93 in a spring-calving herd at the Batesville unit) were stratified by

birth date, gender (heifers or steers castrated at birth), calf body weight and cow body weight, body condition score and parity, then assigned randomly to one of two treatment groups using a computer generated randomization chart. Calves assigned to the early vaccination treatment (EV) were administered a pentavalent (bovine herpesvirus-1 (BHV-1), BVDV type1a and type 2a, parainfluenza-3 virus (PI_3), and bovine respiratory syncytial virus (BRSV)) MLV respiratory vaccine containing *Mannheimia haemolytica* leukotoxoid[a] and a multivalent clostridial bacterin-toxoid[b] on day 0 of the study when the mean age of calves was 62 ± 17 days. Calves assigned to the traditional vaccination treatment (TV) were administered a pentavalent MLV respiratory vaccine containing *M. haemolytica* leukotoxoid[a] and a multivalent clostridial bacterin-toxoid[c] on day 126 of the study (21 days prior to weaning; mean age = 188 ± 17 days). Both vaccine treatment groups were revaccinated with a pentavalent MLV respiratory vaccine containing *M. haemolytica* leukotoxoid[a] and received a multivalent clostridial bacterin-toxoid[c] booster on day 147 (day of weaning; mean age = 209 ± 17 days).

Throughout the study, all animals were housed and cared for in compliance with procedures approved by the University of Arkansas Animal Care and Use Committee. Careful consideration of Beef Quality Assurance guidelines and manufacturer recommendations were followed with regard to vaccine handling and administration. All cows were vaccinated with a multivalent MLV vaccine containing BHV-1, BVDV type1a and type 2a, PI-3V, BRSV and five serovars of leptospira[d] and administered a pour-on anthelmintic[e] on day 0 (branding). Cows and calves were separated into pasture groups based on calf treatment and grazed on replicated pastures until weaning (two to four pasture replications/treatment within each herd). This reduced the potential for shedding of virus from the MLV respiratory vaccine, although the risk is minimal.[3]

All cows and calves were weighed seven days prior to the initiation of the experiment to determine appropriate allocation to treatments. At this time, all calves assigned to the study were ear notched and tested for BVDV-persistent infection using the antigen-capture ELISA procedure at a commercial laboratory.[f] Calves were weighed individually at the initiation of the experiment (day 0; branding), day 21, day 126 (21 days prior to weaning), day 147 (day of weaning), and every 28 days throughout the backgrounding phase until the study ended on day 231. In addition to the appropriate vaccinations at weaning, a pour-on anthelmintic[e] was administered to calves at this time.

Backgrounding phase

Calves were weaned according to standard protocol at the respective study locations. During weaning at the Savoy location, pairs were split and calves were transported to the Savoy Stocker Cattle Unit and penned according to treatment, sex, and body weight, resulting in four pens/treatment. The feed management approach was designed to provide cattle with ad libitum hay and supplemental ration intended to achieve approximately 1.5 lb (0.68 kg) of gain per day over the 28-day weaning period. After this 28-day period, treatments were commingled and calves were moved to mixed-grass pastures to be maintained for the duration of the backgrounding phase of the study. During weaning at Batesville, calves were weaned as groups maintained by treatment and offered Bermudagrass hay for three days in large holding pens. They were then moved to non-toxic novel endophyte-infected tall fescue pastures for the remainder of the backgrounding period. Equal representation of vaccination treatments was maintained within each pasture. Grain supplementation (0.5% of average body weight) was used to maintain calf growth as forage quality declined. Health was monitored daily for all calves throughout the cow-calf and backgrounding phases of the study.

Sample Collection and Immune Analyses

Blood samples, used to determine serum antibody concentrations for BVDV, were collected into evacuated tubes[g] via jugular venipuncture from 16 randomly selected calves (eight steers and eight heifers) in each treatment group within each of the three herds. An additional four steers/treatment from the fall-calving herd at Savoy were bled so that titers would be known for all calves that were also sampled for the cell-mediated immune response assay, which resulted in BVDV antibody concentrations being analyzed for a total of 104 calves. On the day of blood collection, blood was stored in an insulated container and transported to a laboratory, centrifuged at 2,100 × g for 20 minutes at 68°F (20°C), and serum was decanted and stored frozen at -4°F (-20°C). Subsequently, serum samples from days 0, 21, 126, 147, 175, and 231 were shipped on ice via overnight parcel service to the Iowa State University Veterinary Diagnostic Laboratory for determination of serum neutralizing antibody titer concentration against the homologous BVDV type 1a strain contained in the pentavalent MLV respiratory vaccine (Singer strain).

Blood samples from 33 steer calves in the fall-calving herd at Savoy were obtained between 24 and 48 hours of birth to verify that steers being used for the cell-mediated immune assay had received maternal antibodies from colostrum. The 20 steers selected to be sampled had BVDV antibody titers ≥ 1024, indicating sufficient colostrum transfer occurred. To measure cell-mediated immune responses, jugular blood was collected on days 0 (branding), 7, 21, 42, 126, 147 (weaning), and 189 from a

subset of 10 steers from each vaccination timing regimen in the fall-calving herd at Savoy. Blood samples were drawn into a sterile disposable syringe, the needles were removed from the hubs, and the contents were carefully transferred into 15-mL conical tubes containing 2 mL of 2X acid citrate dextrose to be utilized for flow cytometry analysis of T-cell populations, and in vitro IFN-γ expression analyses according to procedures described previously.[5] Briefly, after centrifugation (1,000 × g for 20 min at 77°F (25°C)) buffy coats were harvested and red blood cells were lysed. After appropriate wash steps with phosphate buffered saline, the peripheral blood mononuclear cell (PBMC) pellets were re-suspended in 3 mL of RPMI solution. The PBMC pellets harvested on day 147 had significant red blood cell contamination and were excluded from the analysis. Cells were counted using an automated cytometer.[h] The lymphocyte and monocyte populations were summed and used to re-suspend to a concentration of 5×10^6 mononuclear cells/mL in RPMI. Cells (10^6/well) were incubated in a 96-well flat-bottomed microtiter plate for four days with either an undiluted supernatant of live BVDV type 1b (TGAN strain; provided by Dr. Julia Ridpath, USDA, National Animal Disease Center, Ames, IA) propagated in Madin-Darby bovine kidney (MDBK) cells or a mock antigen consisting of uninfected MDBK cell culture supernatant (also provided by Dr. Ridpath).

After four days of incubation in a 5% CO_2 humidified incubator at 98.6° F (37° C), in vitro responses of PBMC were evaluated by comparing CD25 up-regulation on lymphocytes by flow cytometry as described previously.[4] Briefly, primary antibodies used were a mouse anti-bovine CD8 (isotype IgM[i]), a mouse anti-bovine γδ TCR (isotype IgG2b[i]), and a mouse anti-bovine CD25 (isotype IgG3[i]). Secondary antibodies used were a FITC-conjugated goat anti-mouse IgM (μ chain specific[j]), an Alexa-Fluor 647 conjugated goat anti-mouse IgG2b,[k] and a R-PE conjugated goat anti-mouse IgG3.[j] Unlabeled isotype control mouse monoclonal antibodies[l] with unrelated binding specificity were included to determine nonspecific binding of primary and secondary antibodies. Unlabeled cells were used as a negative control for innate fluorescence detectable in cell suspensions, and to conduct compensation, single-color-labeled cell suspensions were used. The percentage of various cell populations was determined using a flow cytometer equipped with analysis software[m]. The CD25 expression index was calculated by dividing the product (percentage CD25+ × mean fluorescent intensity) from BVDV-stimulated cells by the product from mock antigen-stimulated cells of the same cell subset from the same animal.[4]

Supernatant from BVDV-cultured PBMC after the four-day incubation period were transferred into sealed 96-well storage plates and stored frozen at -4°F (-20°C) until subsequent analysis using a custom bovine-specific IFN-γ ELISA kit[n] according to procedures provided by the manufacturer. Optical density (450 nm wavelength) of known standards and unknown samples were acquired using a microtiter plate reader.[o] A standard curve using the known IFN-γ concentrations provided in the ELISA kit and concentrations (ng/mL) of unknown samples for each microtiter plate was developed using a computer software program.[o] The mean concentration of IFN-γ in supernatant from each vaccination timing regimen was compared on days 0, 7, 21, 42, 126, 147 (weaning), and 189.

Statistical analyses

Results were analyzed using the PROC MIXED procedure of SAS[p]. Statistical significance was considered for a *P*-value of less than or equal to 0.05. The statistical model for body weight and ADG included treatment, gender, and the treatment × gender interaction as fixed effects, and herd as a random effect. The Kenward Rogers test was used as the degrees of freedom selection method. Antibody titer data were $\log_2$-transformed and analyzed as repeated measures with calf as the subject. The model included treatment, day, and the treatment × day interaction as fixed effects and herd as a random effect. The covariance model structure used was SP(POW). Flow cytometry and IFNγ data were also analyzed as repeated measures. The model included treatment, day, and the treatment × day interaction; the Kenward Rogers test was used as the degrees of freedom selection method. The covariance model structure specified was SP(POW) and the subject of the repeated statement was calf.

Results and Discussion

Health and growth performance

No calves were found to be persistently infected with BVDV, nor were any calves treated for BRD at either location pre- or post-weaning. At no time during the experiment did body weight (Table 1) differ ($P \geq 0.51$) between vaccination timing treatments. Similarly, neither pre-weaning nor post-weaning average daily gain, nor the combined average daily gain for the entire 231 days of the study were impacted ($P \geq 0.84$) by the timing of vaccination; power analysis for growth data indicated that this project could detect a difference of approximately 4% between treatments. Therefore, growth performance of calves was not negatively impacted by MLV vaccination at 62 days of age.

Serology

Bovine viral diarrhea virus type 1a antibody concentrations (Figure 1) were affected by the timing of vaccination (treatment × day interaction; $P<0.001$). Calves on both treatments (EV and TV) had equivalent titers

Table 1. Effect of vaccination timing regimen on growth performance of beef calves (least squares means ± SE; n = 253).

Item	Treatment		*P*-value
	EV[a]	TV[b]	
Body weight, lb			
Day 0	191 (± 4.3)	187 (± 4.3)	0.51
Day 21	222 (± 4.8)	224 (± 4.8)	0.68
Day 126	391 (± 7.5)	387 (± 7.6)	0.69
Day 147 (weaning)	424 (± 7.2)	422 (± 7.3)	0.80
Day 175	469 (± 7.3)	468 (± 7.4)	0.95
Day 203	481 (± 7.4)	477 (± 7.5)	0.72
Day 231	524 (± 7.6)	519 (± 7.7)	0.64
Average daily gain, lb			
Day 0 to 147	1.59 (± 0.033)	1.60 (± 0.033)	0.84
Day 147 to 231	1.18 (± 0.038)	1.17 (± 0.039)	0.88
Day 0 to 231	1.44 (± 0.025)	1.44 (± 0.025)	0.97

[a]Early vaccination calves received a pentavalent modified-live virus respiratory vaccine containing *M. haemolytica* leukotoxoid (Pyramid® 5 + Presponse® SQ) and a multivalent clostridial bacterin-toxoid (Alpha 7®) on day 0 (62 days of age). Calves were revaccinated on day 147 (weaning) with a pentavalent modified-live virus respiratory vaccine containing *M. haemolytica* leukotoxoid (Pyramid® 5 + Presponse® SQ) and received a multivalent clostridial (Caliber® 7) booster.
[b]Traditional vaccination calves received a pentavalent modified-live virus respiratory vaccine containing *M. haemolytica* leukotoxoid (Pyramid® 5 + Presponse® SQ) and a multivalent clostridial bacterin-toxoid (Caliber® 7) on day 126 (188 days of age). Both vaccine treatment groups were revaccinated on day 147 (weaning) with a pentavalent modified-live virus respiratory vaccine containing *M. haemolytica* leukotoxoid (Pyramid® 5 + Presponse® SQ) and received a multivalent clostridial (Caliber® 7) booster.

on day 0 (pre-vaccination), which can be attributed to maternal antibody transfer in the calves. However, the EV calves exhibited greater BVDV antibody concentrations three weeks after their initial vaccination (day 21; calves averaged 83 days of age). Although BVDV titers increased for EV from days 0 to 21, they did not for calves that had not been vaccinated (TV). Furthermore, EV calves continued to show increased BVDV titers on day 126, whereas the titers declined for TV calves. The lowest serum anti-BVDV antibody concentrations for either vaccination treatment during the entire study were observed for the TV calves on day 126. Calves on the TV treatment were initially vaccinated on day 126 and BVDV antibody concentrations increased on day 147; however, at weaning (day 147) EV calves still exhibited greater ($P \leq 0.01$) BVDV titer concentrations, even though TV had been vaccinated more recently (21 vs 147 days since initial vaccination for TV and EV, respectively). The differences in serum BVDV antibody titers were likely due to differences in vaccine regimen, because the closed herds used in this study were PI-BVDV negative, which limited the chance for confounding effects of wild-type BVDV transmission. During the 84-day backgrounding period, all calves exhibited high serum BVDV antibody titers and vaccination treatment differences were not evident ($P \geq 0.07$). In a similar study,[2] BVDV type 1 antibody titers measured three weeks prior to weaning were greatest for calves receiving an initial MLV vaccine at 67 days of age, intermediate for calves initially vaccinated three weeks prior to weaning, and least for unvaccinated control. Additionally, the authors reported that BVDV type 1 titers on the day of weaning and 42 days after weaning when calves were shipped to a feedlot, were least for unvaccinated control calves, but similar for either vaccination timing regimen.

Cell-mediated immunity

Vaccination regimen had no effects on the net increase of percentage of isolated lymphocytes expressing CD25, a molecule up-regulated in activated T- and B-lymphocytes, after four days of incubation with BVDV compared to mock antigen (Table 2). There was a tendency for a treatment × day interaction ($P=0.10$), the EV calves had an increase ($P=0.04$) in the percentage of CD25+ cells compared to the TV group on day 7, but treatments did not differ on other sampling days. Likewise, the percentage of γδ-TCR+ cells that expressed CD25 was also not affected by treatment ($P=0.72$). However, the percentage of CD8+ cells that expressed CD25 was greater ($P<0.001$) for EV calves compared to TV

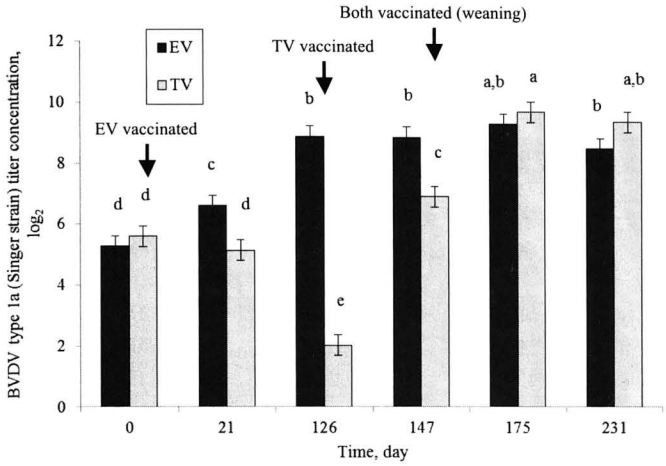

Figure 1. Effect of vaccination timing regimen on serum bovine viral diarrhea virus type 1a antibody titers of beef calves (n = 52/treatment). Effect of treatment, $P<0.001$; day, $P<0.001$; treatment × day interaction, $P<0.001$. EV = Early vaccination; calves received a pentavalent modified-live virus respiratory vaccine containing *M. haemolytica* leukotoxoid (Pyramid® 5 + Presponse® SQ) and a multivalent clostridial bacterin-toxoid (Alpha 7™) on day 0 (62 days of age). TV = Traditional vaccination; calves received a pentavalent modified-live virus respiratory vaccine containing *M. haemolytica* leukotoxoid (Pyramid® 5 + Presponse® SQ) and a multivalent clostridial bacterin-toxoid (Caliber® 7) on day 126 (188 days of age). Both vaccine treatment groups were revaccinated with a pentavalent modified-live virus respiratory vaccine containing *M. haemolytica* leukotoxoid (Pyramid® 5 + Presponse® SQ) and received a multivalent clostridial (Caliber® 7) booster at weaning (day 147).
[a,b,c,d,e]Least square means without a common superscript differ ($P<0.05$).

calves, but again there was not a significant treatment × day interaction ($P=0.37$).

There tended to be a treatment × day interaction ($P=0.08$) for the CD25+ expression index (EI). Isolated PBMC from EV calves had a greater CD25 EI than isolated PBMC from TV calves on day 126 (data not shown); this was 126 days from the initial vaccination of the EV calves and immediately prior to initial MLV vaccination for the TV calves. There was a treatment × day interaction ($P=0.05$) for the CD25 EI for CD8+ cells (Figure 2). Again, isolated PBMC from EV calves had a greater CD25 EI than isolated PBMC from TV calves on day 126. The increase in various CD25 EI parameters on day 126 is suggestive of stimulation of the cell-mediated immune response for EV. Platt and coworkers[6] observed an increase in CD25 EI for vaccinates, which corresponded with protection from subsequent challenge with BVDV. There was no effect of vaccination regimen ($P=0.66$) or a treatment × day interaction ($P=0.22$) on the CD25 EI for γδ-TCR+ cells (Table 2) in the current study. Other researchers reported more consistent increases in CD25 EI for each PBMC population (CD4+, CD8+, and γδ-TCR+ cells) from calves that had been vaccinated at 110[4] or 52[6] days of age versus unvaccinated controls. Our study is among the first to quantify the cell-mediated immune response from calves vaccinated and housed under field conditions. Furthermore, there was not a BVDV challenge component in the current study. Previous efforts sampled calves more frequently after vaccination and subsequent BVDV challenge, and calves were housed in a more controlled environment, possibly reducing variability in CD25 EI responses observed. Although numerical increases were observed for EV, there were no statistical differences (treatment, $P=0.23$; treatment × day, $P=0.31$) in IFN-γ concentrations of supernatant after in vitro culture of PBMC for four days with BVDV (Figure 3). Nevertheless, increases observed for some cell-mediated immune parameters in EV calves would suggest that MLV respiratory vaccine administered during the presence of maternal antibody stimulated the cell-mediated immune response.

Study implications

The biological responses observed in the current study suggest onset of immunity to BVDV is earlier when vaccine is administered at branding, even in the presence of maternal antibodies. These data support initial vaccination at branding as an efficacious alternative to initial vaccination at, or immediately prior to, weaning. However, serologic and cell-mediated data from the current study only included responses to BVDV, and these results may or may not apply to other respiratory viral antigens, such as BHV-1, PI3V, or BRSV.

A potential confounding factor in the model for the current study was that two different clostridial bacterins were used during initial vaccination of calves. The possibility exists that one clostridial bacterin may have had a greater immunostimulatory effect than the other, thereby indirectly influencing the humoral or CMI response to the BVDV component contained in the respiratory vaccine that was administered concurrently. However, the authors are not aware of data to support or contradict this potential effect. Furthermore, we would note that both the EV and TV calves exhibited a similar magnitude of humoral BVDV response 21 days following their initial vaccination with the respiratory vaccine, regardless of which clostridial bacterin was administered. In retrospect, the same clostridial bacterin should have been selected for initial vaccination.

Table 2. Effect of vaccination timing regimen on net increase of %CD25+ peripheral blood mononuclear cell populations of beef calves after being cultured in vitro with bovine viral diarrhea virus or mock antigen for four days (least squares means ± SE; n = 10/treatment on each of six sampling days).

	Treatment		*P*-value		
Item	**EV[a]**	**TV[b]**	**Treatment**	**Day**	**Treatment × Day**
CD25+, %[c]	-3 (± 2.1)	-4 (± 2.1)	0.78	0.18	0.10
γδ-TCR+CD25+, %[c]	-4 (± 2.4)	-6 (± 2.5)	0.72	0.16	0.34
CD8+CD25+, %[c]	-7.6	-8.4	<0.001	0.08	0.37
CD25, EI[d]	2.4 (± 0.66)	1.8 (± 0.068)	0.56	0.01	0.08
γδ-TCRCD25, EI	3.1 (±1.4)	2.2 (± 1.4)	0.66	0.01	0.22
CD8+CD25, EI	1.5 (± 0.37)	1.1 (± 0.38)	0.47	0.24	0.05

[a]Early vaccination calves received a pentavalent modified-live virus respiratory vaccine containing *M. haemolytica* leukotoxoid (Pyramid® 5 + Presponse® SQ) and a multivalent clostridial bacterin-toxoid (Alpha 7®) on day 0 (62 days of age). Calves were revaccinated on day 147 (weaning) with a pentavalent modified-live virus respiratory vaccine containing *M. haemolytica* leukotoxoid (Pyramid® 5 + Presponse® SQ) and received a multivalent clostridial (Caliber® 7) booster.

[b]Traditional vaccination calves received a pentavalent modified-live virus respiratory vaccine containing *M. haemolytica* leukotoxoid (Pyramid® 5 + Presponse® SQ) and a multivalent clostridial bacterin-toxoid (Caliber® 7) on day 126 (188 days of age). Both vaccine treatment groups were revaccinated on day 147 (weaning) with a pentavalent modified-live virus respiratory vaccine containing *M. haemolytica* leukotoxoid (Pyramid® 5 + Presponse® SQ) and received a multivalent clostridial (Caliber® 7) booster.

[c]Difference between percentage for BVDV-stimulated cells minus percentage for mock antigen-stimulated cells of the same cell subset from the same animal.

[d]Expression index calculated by dividing the product (percentage CD25+ × mean fluorescent intensity) from BVDV-stimulated cells by the product from mock antigen-stimulated cells of the same cell subset from the same animal.

Clinical Relevance

Clinically relevant outcomes that can be concluded from this study are that no differences existed between EV and TV groups in regards to gain performance, morbidity rates, and mortality rates, yet the onset of BVDV-specific immunity was earlier for EV. However, it is important to note that there was not a significant natural disease challenge in this study based on the absence of clinical observations; this is likely a result of exceptional herd health management factors. These health management factors included the herds being closed to animal introduction or contact and other biosecurity measures, as well as castration being performed at birth to eliminate this potential stressor at branding or weaning. Our implications may not apply to every scenario a practitioner might encounter, but for herds managed similarly to those in the current study, vaccination at branding may afford earlier protection because BVDV-specific immune responses were earlier for EV. Bovine practitioners could recommend either vaccine timing regimen to cow-calf clients that manage a closed, biosecure operation and expect few health or performance consequences through weaning and a ranch-origin stocker period. Clinically relevant outcomes after the calves were sold are unknown and warrant further investigation. Furthermore, the data may suggest that respiratory vaccines administered to cows on similarly managed operations also have significance, because both vaccine treatments exhibited abundant BVDV-specific maternal antibody transfer, and clinical respiratory disease was not observed prior to weaning.

Conclusions

Growth performance and health did not differ due to vaccination timing regimen for calves that remained on their origin ranch through an 84-day backgrounding period after weaning. Differences in serum BVDV type 1a antibody concentrations indicate that calves vaccinated at branding developed a significant humoral antibody response despite maternal antibodies being present. Calves vaccinated early in life had greater BVDV type 1a titers during the pre-weaning phase, and, during the backgrounding phase, were equivalent to calves vaccinated in a traditional preconditioning regimen. Furthermore, vaccination with MLV respiratory vaccine during the presence of maternal antibodies stimulated the cell-mediated immune response.

Endnotes

[a]Pyramid® 5 + Presponse® SQ, Boehringer Ingelheim Vetmedica, Inc., St. Joseph, MO

[b]Alpha 7®, Boehringer Ingelheim Vetmedica, Inc., St. Joseph, MO

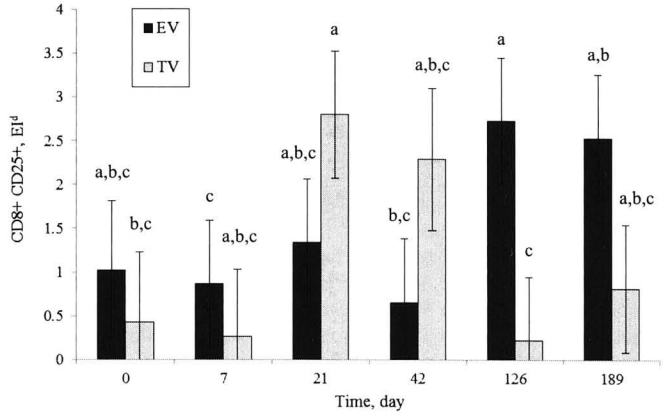

Figure 2. Effect of vaccination timing regimen on CD8+ CD25 expression index (EI) of isolated peripheral blood mononuclear cells after four days of incubation with either bovine viral diarrhea virus or a mock antigen (n = 10/treatment). Treatment, P=0.47; Day, P=0.24; Treatment × day interaction, P=0.05. EV = Early vaccination; calves received a pentavalent modified-live virus respiratory vaccine containing *M. haemolytica* leukotoxoid (Pyramid® 5 + Presponse® SQ) and a multivalent clostridial bacterin-toxoid (Alpha 7™) on day 0 (62 days of age). TV = Traditional vaccination; calves received a pentavalent modified-live virus respiratory vaccine containing *M. haemolytica* leukotoxoid (Pyramid® 5 + Presponse® SQ) and a multivalent clostridial bacterin-toxoid (Caliber® 7) on day 126 (188 days of age). Both vaccine treatment groups were revaccinated with a pentavalent modified-live virus respiratory vaccine containing *M. haemolytica* leukotoxoid (Pyramid® 5 + Presponse® SQ) and received a multivalent clostridial (Caliber® 7) booster at weaning (day 147).

[a,b,c]Least square means without a common superscript differ (P<0.05).

[d]Expression index(EI) calculated by dividing the product (percentage CD25+ × mean fluorescent intensity of CD8+CD25+ cells) from BVDV-stimulated cells by the product from mock antigen-stimulated cells of the same cell subset from the same animal.

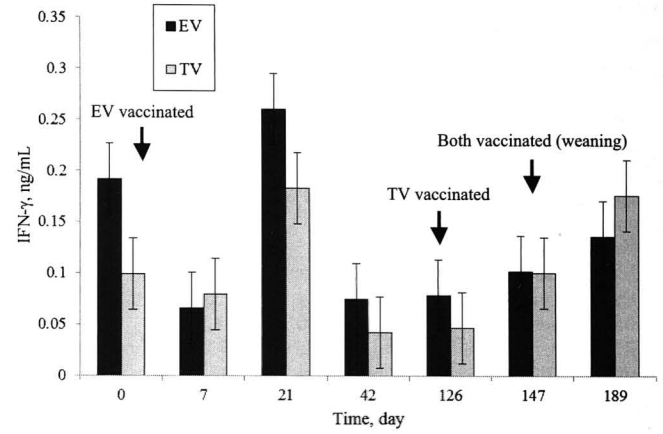

Figure 3. Effect of vaccination timing regimen on interferon-gamma concentrations of supernatant of peripheral blood mononuclear cells cultured *in vitro* with bovine viral diarrhea virus for four days (n = 10/treatment). Treatment, P=0.23; Day, P<0.001; Treatment × day interaction, P=0.31. EV = Early vaccination; calves received a pentavalent modified-live virus respiratory vaccine containing *M. haemolytica* leukotoxoid (Pyramid® 5 + Presponse® SQ) and a multivalent clostridial bacterin-toxoid (Alpha 7™) on day 0 (62 days of age). TV = Traditional vaccination; calves received a pentavalent modified-live virus respiratory vaccine containing *M. haemolytica* leukotoxoid (Pyramid® 5 + Presponse® SQ) and a multivalent clostridial bacterin-toxoid (Caliber® 7) on day 126 (188 days of age). Both vaccine treatment groups were revaccinated with a pentavalent modified-live virus respiratory vaccine containing *M. haemolytica* leukotoxoid (Pyramid® 5 + Presponse® SQ) and received a multivalent clostridial (Caliber® 7) booster at weaning (day 147).

[c]Caliber® 7, Boehringer Ingelheim Vetmedica, Inc., St. Joseph, MO

[d]Pyramid® 10, Boehringer Ingelheim Vetmedica, Inc., St. Joseph, MO

[e]Cydectin® Pour-on, Boehringer Ingelheim Vetmedica, Inc., St. Joseph, MO

[f]Cattle Stats, LLC, Oklahoma City, OK

[g]Vacutainer® SST Ref# 367985; Becton-Dickinson, Inc., Franklin Lakes, NJ

[h]Hemavet® 950FS, Drew Scientific Inc., Oxford, CT

[i]Veterinary Medical Research and Development, Inc., Pullman, WA

[j]Southern Biotechnology Associates, Inc., Birmingham, AL

[k]Invitrogen™ A21242, Life Technologies Corp., Grand Island, NY

[l]Mouse IgM and Mouse IgG2b, Sigma-Aldrich Corp., St. Louis, MO

[m]FACSort/FACSCalibur with CellQuest™ software, Becton-Dickinson Immunocytometry Systems, San Jose, CA

[n]Bethyl Laboratories, Inc., Montgomery, TX

[o]Spectramax® 250 with Soft Max Pro software, Molecular Devices, LLC, Sunnyvale, CA

[p]SAS Institute Inc., Cary, NC, Software Version 9

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