PEER REVIEWED

Evaluation of the Onset of Protective Immunity from Administration of a Modified-live, Non-adjuvanted Vaccine prior to Intranasal Challenge with Bovine Herpesvirus-1

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Abstract

Study objectives were to determine if subcutaneous administration of a modified-live, non-adjuvanted vaccine containing bovine herpesvirus-1 (BHV-1) at five, three, or two days pre-challenge, would reduce clinical signs, rectal temperatures, and viral shedding, and enhance serological response to BHV-1. Colostrum-deprived, neonatal calves (n = 48) were randomly assigned to six treatment groups, each containing eight calves. Treatment groups were based on administration of vaccine (VAC) or saline controls (CON) and day of administration (day -5, -3 or -2) relative to intranasal BHV-1 challenge (day 0). Following challenge, calves were monitored for clinical signs, rectal temperature, seroconversion, and quantity of BHV-1 recovered by virus isolation from nasal swabs. Data for the evaluation period (days 4-14) were analyzed using multivariable statistics. Day -5 and -3 VAC groups had fewer ($P < 0.05$) days of clinical illness compared to CON. Rectal temperatures were lower ($P < 0.05$) during days 4-8 for each of the VAC groups as compared to combined CON groups. CON calves shed BHV-1 for more days than calves vaccinated on day -5 ($P < 0.01$), day -3 ($P = 0.06$), or day -2 ($P = 0.06$). Mean concentrations of nasal BHV-1 also differed ($P < 0.05$) between combined CON groups and each of the VAC groups during at least one study day. Calves in the VAC groups (median = 10 days) seroconverted to BHV-1 ($P < 0.01$) sooner than CON calves (median = 14 days). This study demonstrated that the use of a non-adjuvanted MLV vaccine in neonatal calves can reduce the effects of BHV-1 challenge soon after vaccination.

Keywords: BHV-1, IBR, vaccine, immunity challenge studies

Résumé

Les objectifs de l'étude étaient de déterminer si un vaccin à virus vivants modifiés sans adjuvant, contenant l'herpèsvirus bovin 1 (BHV-1), administré cinq, trois ou deux jours avant l'infection expérimentale allait réduire les signes cliniques, la température rectale et l'excrétion virale tout en rehaussant la réponse sérologique au BHV-1. Des veaux nouveau-nés, qui n'avaient pas reçu de colostrum, ont été alloués au hasard dans l'un des six groupes de traitement chacun contenant huit veaux. Les groupes de traitement étaient basés sur la combinaison du traitement, soit le vaccin ou soit la saline, avec le jour d'administration (jours -5, -3 et -2) relatif à l'infection expérimentale par voie intranasale avec le BHV-1 au jour 0. Suite à l'infection expérimentale, les veaux ont été suivis pour déterminer les signes cliniques, la température rectale, la séroconversion et la quantité de BHV-1 recouverte par isolation virale dans des écouvillons nasaux. Les données pendant la période d'évaluation (jours 4 à 14) ont été analysées avec des statistiques multivariées. Les individus des groupes vaccinés au jour -5 et au jour -3 avaient moins ($P < 0.05$) de maladies cliniques par rapport aux veaux témoins. La température rectale était moins élevée ($P < 0.05$) chez

les individus de tous les groupes vaccinés que chez les veaux témoins durant les jours 4 à 8. Les veaux témoins excrétèrent BHV-1 sur une plus longue période que les veaux vaccinés au jour -5 ($P < 0.01$), au jour -3 ($P = 0.06$) ou au jour -2 ($P = 0.06$). La concentration moyenne de BHV-1 dans les écouvillons nasaux différait aussi ($P < 0.05$) entre les groupes témoins combinés et chacun des groupes vaccinés au moins pendant une journée de l'étude. La séroconversion pour le BHV-1 a pris place plus tôt ($P < 0.01$) chez les individus des groupes vaccinés (médiane = 10 jours) que chez les veaux témoins (médiane = 14 jours). L'étude démontrait que l'utilisation d'un vaccin à virus vivants modifiés sans adjuvant chez les veaux nouveau-nés peut réduire les effets d'une infection expérimentale avec BHV-1 peu après la vaccination.

Introduction

Bovine respiratory disease (BRD) is a common syndrome that affects cattle of all ages. It is the major cause of mortality in weaned dairy heifers[15] and an extremely important disease complex in beef calves as well.[2,10] Viral agents commonly involved with BRD include bovine herpesvirus-1 (BHV-1, causative agent of infectious bovine rhinotracheitis), bovine viral diarrhea virus type 1 (BVDV1), bovine viral diarrhea virus type 2 (BVDV2), bovine respiratory syncytial virus (BRSV), and parainfluenza-3 virus (PI-3). Immunizing calves early in life against the major viruses involved with the BRD complex is one management strategy that veterinarians and producers have chosen to help prevent BRD in young calves, and to prime calf immunity for repeat inoculations with viral vaccines.

Despite studies that examined the effects of modified-live virus (MLV) vaccines on neonatal calves,[13,17,18] there is limited information regarding how early in life, and how much in advance of field viral challenge, calves need to be vaccinated in order to respond adequately and thus be protected at least partially from viruses causing BRD. Bovine fetuses demonstrated antibody and cell mediated (basic lymphocyte proliferation) responses to tetanus toxoid comparable to adult cattle when those fetuses were stimulated with tetanus toxoid in the third trimester of gestation.[12] As evidenced by a positive precolostral serum neutralization (SN) antibody titer, a bovine fetus in the third trimester responded serologically to BHV-1 virus.[8]

Early development of immune responses after vaccination is considered important in advance of, and during, stressful situations including inclement weather, crowded calving or commingling areas, weaning, and transport. Providing early protection against common BRD viruses, such as BHV-1, may allow calves to respond more satisfactorily to challenges accompanying non-viral stressors. Numerous studies have verified that protection can be elicited soon post-vaccination, as early as 24 hours.[4,5,14] In one study, calves seronegative to BRSV that had positive BRSV-specific T-cell memory responded more rapidly (virus-specific IgG, SN antibodies, and T-cell recall) to MLV BRSV vaccination than did calves that were both seronegative and T-cell memory-negative.[9] This demonstrated that T-cell responses can occur in the absence of an antibody response.[9] Previous studies have also provided evidence that calves can develop protective immune responses following vaccination, whether or not maternal antibodies are present.[7,9,18]

Our study objectives were to determine if subcutaneous administration of a modified-live, non-adjuvanted vaccine containing BHV-1 at five, three, or two days pre-challenge, would reduce clinical signs, rectal temperature, and viral shedding, and enhance serological response to BHV-1 in colostrum-deprived calves that were 30 days of age or younger.

Materials and Methods

Animals

Animal handling within this study followed the Guide for the Care and Use of Agricultural Animals in Agricultural Research and Teaching. Forty-eight colostrum-deprived dairy calves were acquired within a day of their birth from a commercial dairy farm located approximately 75 miles (120 km) from the study site. On the day of birth, ear tags were applied to calves for identification purposes. In addition, ear notches (skin biopsies) were tested by immunohistochemistry (IHC) for BVDV persistent infection status at a regional diagnostic laboratory.[a] Cooperators at the dairy farm of origin used an esophagogastric tube to feed a commercial milk replacer[b] to calves shortly after their birth. Calves were then bucket-fed two quarts (~two liters) of milk replacer twice daily at the cooperating dairy, where they remained for no longer than ~24 hours following birth, and also at the study site. Upon arrival at the study site, calves were housed in individual huts where they remained through the duration of the study. Calves were fed an age-appropriate commercial starter ration[c] beginning at 10 days of age, and hay was fed ad libitum starting at one month of age. Calves were processed and managed according to study site standard operating procedures. Calves, eight to 30 days old (mean = 20 days (SD = 5.19)), were randomly assigned to one of six treatment groups; randomization was completed within blocks defined by birth order, using commercial software.[d]

Serology

Blood for BHV-1, BVDV1, BVDV2, and BRSV serology was collected from all calves immediately prior to administration of vaccine or saline and was submitted to

the Iowa State University Veterinary Diagnostic Laboratory, where personnel masked to treatments evaluated the samples per their standard methods. Serum neutralizing antibody titers were determined for BHV-1 (Colorado strain), BVDV1 (Singer strain), BVDV2 (125A strain), and BRSV (A51908 (ATCC VR-794)). Constant virus was incubated with two-fold serial dilutions (1:2-1:256 for BHV-1, 1:2-1:4096 for BVDV1 and BVDV2, and 1:2-1:512 for BRSV) of sera before inoculation of Madin-Darbin bovine kidney cells (for BHV-1) or bovine turbinate cells (for BVDV1, BVDV2, and BRSV) in microtiter tissue culture plates. Plates were incubated at 98.6°F (37°C) with 5% carbon dioxide for three days (BHV-1), five days (BVDV1 and BVDV2), or seven days (BRSV) before visual assessment of virus-induced cytopathic effect. Blood for BHV-1 serology was also collected from all calves on day 0 (day of challenge), day 5 or 7, and days 10 and 14 post-challenge. For all samples, blood was allowed to clot for approximately two hours, and then serum was harvested by centrifuging for 15 minutes @ 1200 x g. Serum was split into two aliquots and stored in tubes labeled with animal ID and date of collection at -4°F (-20°C) until serological evaluation.

Vaccination

Calves received either a commercially available, non-adjuvanted modified-live combination vaccine[e] containing BHV-1, BVDV1, BVDV2, PI-3 virus, and BRSV subcutaneously (VAC), or saline intranasally (CON). Treatments were administered on day -5 (five days before BHV-1 challenge), day -3, and day -2. On each administration day, there were eight calves in VAC groups and eight calves in CON groups based on the previously described random assignment. All calves were observed daily after vaccination for vaccine-related adverse events.

Challenge

On day 0, all calves were challenged intranasally with BHV-1[f] using an atomizer.[g] The study was run in two study periods with equal numbers of calves, four in each treatment group for each study period, in order to accommodate the desired number of study animals in the research facilities at any one time. The challenge inoculum contained 3.2 x $10^{5.5}$ $TCID_{50}$/mL for the challenge in the first study period. In order to increase clinical expression of disease, the challenge inoculum for the second study period was increased to 3.2 x $10^{6.5}$ $TCID_{50}$/mL. On both challenge dates, 2 mL of challenge virus inoculum were atomized into each nares (total volume 4 mL/calf).

Post-challenge Observations

Clinical observations were performed daily from one day prior to challenge through 14 days after challenge. Individuals that performed clinical observations were masked (blinded) to treatment assignment. Each calf was visually examined and scored for attitude (scale of 0-2), appetite (scale of 0-2), cough (scale of 0-2), fecal consistency and/or containing blood (scale of 0-2), nasal discharge (scale of 0-4), nasal lesions (scale of 0-2), ocular discharge (scale of 0-4), ocular lesions (scale of 0-1), and respiratory character (scale of 0-2) (Table 1). The absence of a clinical sign was scored as a 0, and a severe clinical sign was scored as the highest number for that particular clinical sign.

Virus Isolation

Nasal swabs were collected from all calves on days 0, 2, 4, 6, 8, 10, 12, and 14. One polyester-tipped, individually packaged, dry, sterile swab, per calf, was used to sample both of the calf's nares and then placed in a cryovial tube (each containing 2 mL of sterile Dulbecco's Modified Eagle Medium) that was marked with the study number, calf identification number, date of collection, and day of study. These samples were held at -112°F (-80°C) until shipped on dry ice to Intervet/Schering-Plough Animal Health's (ISPAH) De Soto, KS facility to be assessed for virus quantity by the following described methodology.[16] All personnel at the ISPAH facility were masked to treatment. Samples from the first study period were evaluated over an 11 day period following that study period, and samples from the second study period were evaluated over an 11 day period following that study period. All samples were diluted 10-fold, and each was added to bovine turbinate cell monolayers in microtiter tissue culture plates. Plates were then incubated at 98.6°F for five days with 5% carbon dioxide before being evaluated for cytopathic effect. Plates not showing cytopathic effect were fixed with 80% acetone and processed for immunofluorescent assay. Murine anti-BHV-1 monoclonal antibody (R54) was added to the fixed plates (50 microliters per well) and then incubated for one hour. After three washes with phosphate buffered saline (PBS), fluorine-labeled goat anti-mouse IgG was added to the plates which were then incubated for another hour. The plates were then washed three more times with PBS and examined under fluorescent microscopy. Samples were considered BHV-1 negative if no cytopathic effect or virus-specific fluorescence was observed after one blind passage.

Statistical Analysis

Data were analyzed with JMP[h] and SAS[i] software. Individual calf was the unit of analysis for all comparisons. Overall, the six treatment groups were based on VAC or CON and day of administration (day -5, -3, or -2). Calves were challenged during two periods; therefore, study period was evaluated as a potential factor (covariate) in statistical analyses. Prior to statistical

Table 1. Definition of scoring system used for clinical observations of calves challenged with BHV-1.

Clinical observation	Scores and descriptions				
Attitude	0 = normal, bright, vigorous and aware of surroundings	1 = mild/moderate depression, slow movements and responses, reluctant to move	2 = severe depression, very weak and unable to stand		
Appetite	0 = normal, calf anxious to eat	1 = reduction in feed consumption	2 = no feed intake		
Nasal discharge	0 = none	1 = mild/ moderate serous	2 = severe serous	3 = mild/ moderate purulent	4 = severe purulent
Nasal lesions	0 = normal, no lesions present	1 = hyperemia of nares/muzzle	2 = nasal mucosal plaques		
Ocular discharge	0 = none	1 = mild/ moderate serous	2 = severe serous	3 = mild/ moderate purulent	4 = severe purulent
Ocular lesions	0 = none	1 = plaques/lesions present			
Respiratory character	0 = normal	1 = rapid breathing	2 = dyspnea		
Cough	0 = none	1 = soft, mild, intermittent	2 = harsh, repetitive cough		
Fecal score	0 = normal formed stool	1 = watery feces	2 = watery or formed with blood		

analysis, investigators determined to only analyze data collected during a predefined evaluation period (days 4 to 14 post-challenge). Since most outcomes were recorded multiple times on the same calf, repeated measures analyses were performed for such comparisons. Besides exceptions described below, data were analyzed using general and generalized linear mixed models. In these models, study period was considered as a random effect, and a first-order autoregressive correlation structure was defined to account for repeated measurements on calves over time. Treatment groups were independent variables in all analyses. Contrast statements were used for individual pair-wise comparisons when overall treatment effects were observed. A significance level of $P < 0.10$ was used for all comparisons. A less conservative significance level was used, because sample sizes were limited for the specific individual comparisons (e.g., by day of vaccine administration) of primary interest. Serology data were normalized prior to analysis. Data on clinical signs could not be appropriately evaluated with mixed models; thus, nonparametric permutation tests were used to compare means among treatment groups. The number of days until an animal stopped shedding BHV-1 and the number of days until seroconversion occurred were analyzed using nonparametric survival analyses and Wilcoxin tests to compare outcomes among treatment groups.

Results

Clinical Signs

The 48 calves were observed for 11 days during the evaluation period (528 calf-days of clinical observations). Overall, there were 198 calf-days in which at least one clinical sign was observed; most common were nasal discharge (n = 176) and ocular discharge (n = 53). Clinical signs related to attitude (n =17), appetite (n = 3), nasal lesions (n = 0), ocular lesions (n = 0), respiratory character (n = 7), cough (n = 4), and fecal score (n = 0) were rarely observed. A daily measure of the presence or absence of ≥1 clinical sign and a cumulative sum (for each calf) of the number of days with ≥1 clinical sign were further evaluated. Comparisons among CON calves indicated that day of administration (day -5, -3, or -2) was not associated with the daily clinical illness measure (≥1 clinical sign) or the sum of days with ≥1 clinical sign (all

$P > 0.35$). There were no significant differences in the daily number of clinically ill calves between VAC groups (day -5, -3, or -2) and CON (all $P > 0.50$). However, the sum of days in which calves were clinically ill differed among groups; the results demonstrating differences in the sum of clinically ill days are shown in Table 2.

Differences in the number of clinically ill days demonstrated in Table 2 appeared to be largely driven by differences in the numbers of days when calves had nasal discharge. CON calves had more days with nasal discharge (mean = 5.33 (SD = 3.82)) than calves vaccinated at day -5 (mean = 1.5 (SD = 1.2)) and day -3 (mean = 2.0 (SD = 2.27)), P-values were 0.03 and 0.07 respectively, but not more ($P \sim 1$) than day -2 VAC calves (mean = 5.25 (SD = 4.68)). No significant differences were observed for comparisons among any other clinical observation data, including data on observed ocular discharge (all $P > 0.50$).

Rectal Temperature

Rectal temperatures for all VAC and CON calves during the evaluation period ranged between 99.3°F (37.4°C) and 105.4°F (40.8°C), with a mean and median of 101.5°F (38.6°C) and 101.1°F (38.4°C), respectively. Rectal temperatures were significantly associated ($P < 0.05$) with treatment groups (vaccine and day of administration), day of temperature evaluation, and their interactive effects. Mean rectal temperatures were lower ($P < 0.01$) for all VAC combined as compared to CON, but effects varied by administration day (day -5, -3, -2) and by day of evaluation (day 4 – 14) (Figure 1). For CON calves, effects varied significantly by evaluation day ($P < 0.01$), but not administration day.

BHV-1 Shedding

All animals were shedding virus on day 4 following challenge (mean = 6.29 (SD =1.46), median = 6.5; all as $TCID_{50}$/mL). One calf (CON) was still shedding virus on the final sampling day (day 14 post-challenge), whereas all other calves had ceased shedding virus by day 14. Overall, CON calves shed virus (median = 12 days) significantly ($P < 0.01$) longer than VAC calves (median = 10 days). For CON calves, the duration of viral shedding was not significantly affected by administration day ($P < 0.35$), but administration day affected shedding for VAC calves ($P < 0.08$). Calves vaccinated on day -5 ($P < 0.01$), day -3 ($P = 0.06$), or day -2 ($P = 0.06$) shed virus for a shorter duration than CON calves. The percent of calves in the three VAC groups (eight calves/group) and three CON groups combined (24 total calves) shedding BHV-1 at each of the six sampling days is depicted in Figure 2.

There were significant ($P < 0.05$) interactions among sampling day and treatment groups for viral shedding quantities ($TCID_{50}$/mL). All VAC calves combined shed significantly less virus ($P < 0.01$) than CON cattle; however, the effects of vaccination varied by day of administration and day of nasal swab sampling ($P < 0.01$ for interactive effects) (Figure 3). There were no significant differences between different administration times for CON calves.

Serology

Prior to study initiation, none of the study calves had detectable antibodies to BRSV, BVDV1, BVDV2, and BHV-1, and all were negative for BVDV PI (based on pre-study IHC evaluation). None of the calves had detectable antibodies to BHV-1 at day of challenge (day 0) or by the mid-study monitoring period (days 5-7), but there was evidence of seroconversion by day 10. All VAC and all but three CON calves had seroconverted by the end of the evaluation period (day 14). Calves in the VAC groups had higher ($P = 0.04$) titers than CON cattle overall (across all sampling days). However, there tended to be a significant interaction ($P = 0.07$) among sampling day and treatment groups; thus, mean titer values for each sampling day and treatment group are displayed in Figure 4. No significant differences occurred among administration times (days -5, -3, and -2) for CON calves (P-value = 0.90); i.e., the

Table 2. Comparison of the cumulative number of days when calves were clinically ill (≥1 clinical signs) among treatment groups for the evaluation period (days 4 – 14) following challenge with BHV-1 virus. Data for Control groups were pooled as there were no statistical differences among them.

Treatment groups	N	Median	Mode	Mean (standard deviation)	P-value[a]
Controls	24	5	4	5.17 (2.70)	
Vaccinates day-5	8	2	3	1.875 (1.13)	< 0.01
Vaccinates day-3	8	1.5	1	2.25 (2.38)	0.02
Vaccinates day-2	8	5	6	5.13 (2.64)	0.99

[a]As compared to Controls; from a nonparametric (permutation) test of means

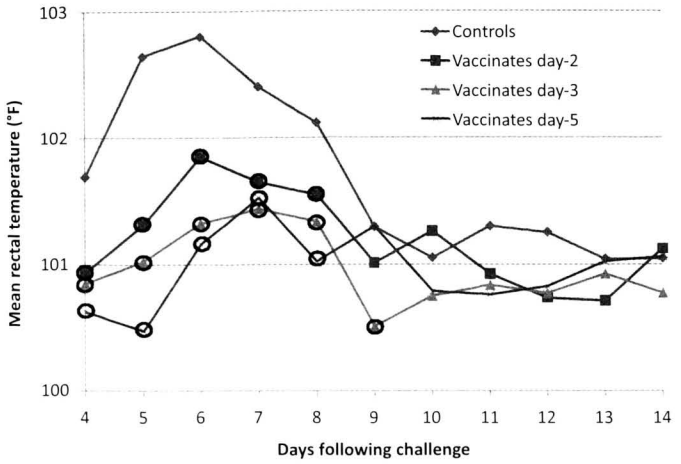

Figure 1. Model-adjusted[a] mean rectal temperatures for Controls and Vaccinates that were administered vaccine containing BHV-1 on day -5, -3, or -2 (prior to BHV-1 challenge). Means that differed statistically ($P < 0.05$) from Control means within day are designated by O.

[a]From a linear mixed model with a random effect for study period and a first order autocorrelation structure to adjust for repeated observations on calves within groups.

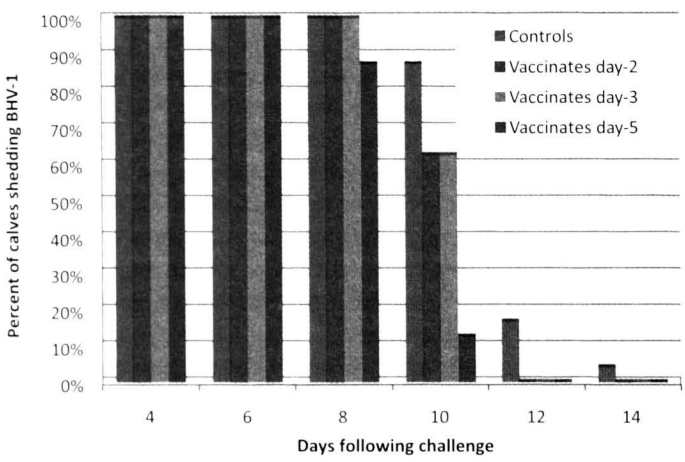

Figure 2. Percent of calves in each Vaccinate group and the three combined Control groups shedding BHV-1 virus on each sampling day following challenge. Percent of calves is based on eight calves per group in each vaccinate group (one calf = 12.5%), and 24 calves in the combined control groups (one calf = 4.2%). The median number of days until BHV-1 shedding ceased tended to be greater for calves in the combined Control groups than for Vaccinates that were administered vaccine on day -5 ($P < 0.01$), day -3 ($P = 0.06$), and day -2 ($P = 0.06$).

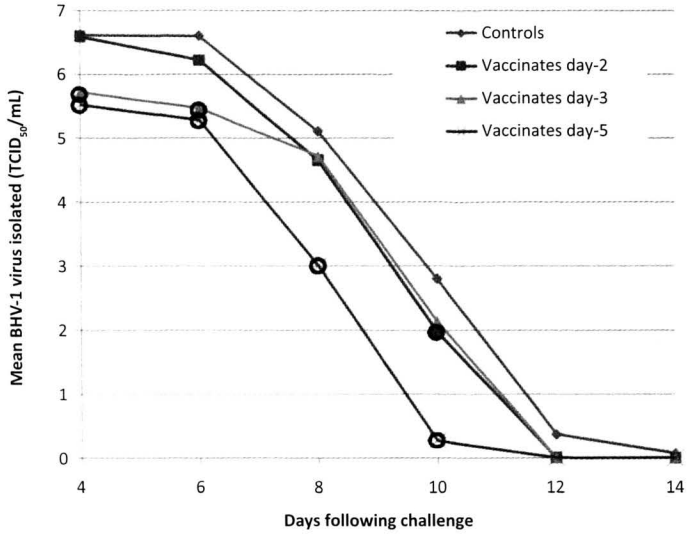

Figure 3. Model-adjusted[a] mean concentration ($TCID_{50}$/mL) of BHV-1 isolated from nasal swabs collected from Control calves and Vaccinates that were administered vaccine on day -5, -3, or -2 prior to BHV-1 challenge. Means for Vaccinates that differed statistically ($P < 0.05$) from means for Controls within the same sampling day are designated by O.

[a]From linear mixed model with a first order autocorrelation structure to adjust for repeated observations on calves within groups.

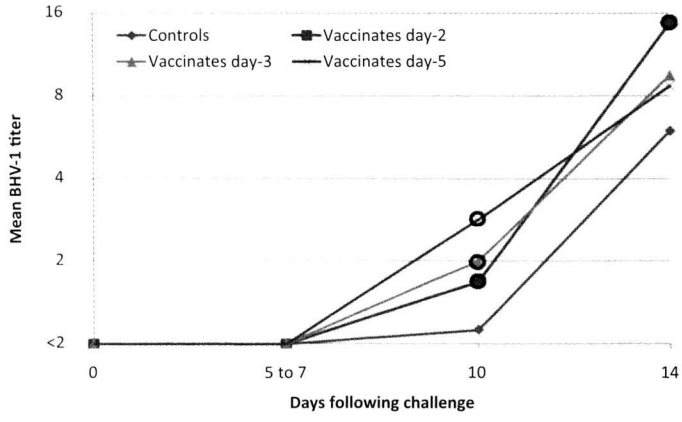

Figure 4. Mean[a] antibody titer values for BHV-1 over the period of evaluation for Control calves and Vaccinates that were administered vaccine on day -5, -3, or -2 prior to BHV-1 challenge. Means for Vaccinates that differed statistically ($P < 0.05$) from means for Control calves within the same sampling day are designated by O.

[a]From a linear mixed model with an autocorrelation structure to adjust for repeated observations on calves within groups. Data for titer values were normalized for analysis.

timing of saline administration had no effect on BHV-1 antibody titer values.

The probability of seroconversion by day 14 did not differ statistically among treatment groups or study periods (P-values > 0.90). However, treatment group (VAC or CON) was significantly associated ($P < 0.01$) with the time period (day) at which seroconversion occurred. The day of product administration (-5, -3, or -2) did not significantly affect time of seroconversion when comparing among VAC ($P = 0.22$) or among CON calves ($P = 0.13$). However, all VAC calves combined (median = 10 days) seroconverted ($P < 0.01$) sooner than CON calves (median = 14 days).

Discussion

Vaccination of neonatal calves is widely practiced in the US dairy and beef industries, and many early vaccinations are intended to help prevent and control BRD. Despite the fact that appropriate vaccinations administered well in advance of pathogen insult is championed as a means of reducing BRD, beef and dairy producers may not provide calves sufficient time to respond to vaccines prior to pathogen challenge. Dairy calves may be relocated to another farm and commingled with other calves on the day of birth, without the advantages of adequate colostral intake and preventive vaccinations. Beef calves are often abruptly weaned, transported to auction markets where they are commingled with other calves, and transported to central gathering points. Further commingling is common, and calves may or may not be vaccinated prior to transport to a stocker operation or feedlot. In this situation, it is important that vaccines intended to prevent BRD provide a rapid protective immune response.

There is limited information on the response of neonatal calves to virus vaccines, and the length of time it takes neonatal calves to develop immunity following vaccination. Calves in the present study were less than 30 days of age when vaccinated with a non-adjuvanted, multivalent virus vaccine containing BHV-1. When challenged with BHV-1 two, three, or five days following vaccination, clinical signs of BHV-1 infection were lessened in calves vaccinated five or three days prior to the challenge as compared to controls. Calves in all VAC groups had lower rectal temperatures, less shedding of BHV-1, and higher SN titers to BHV-1 than calves in the CON groups. BHV-1 experimental challenges typically cause relatively subtle signs of disease. It was deemed advantageous to increase the challenge level of BHV-1 virus for the second period of the study in order to further demonstrate differences in clinical signs and incidence of BHV-1 virus isolation between treatments.

These calves were colostrum deprived, not unlike a significant proportion of dairy calves in the US.[15] In an earlier study, the MLV vaccine used in this study elicited cell-mediated immune (CMI) responses in three- to four-month old calves,[6] but the CMI response to this product has not been studied in calves less than 30 days of age. Further evidence of the ability of CMI to protect young calves was evident in another study after maternal antibodies had been depleted prior to a BVDV type 2 challenge.[3] Although the CMI response was not measured in the present study, it is possible that CMI contributed to the protection afforded the vaccinated calves.

Originally, 72 calves were included in the study. However, 24 calves administered an intranasal vaccine[j] were not included in the analysis because the vaccine is no longer marketed. Intranasal saline was selected as the control for both the intranasal[j] and subcutaneous vaccinate[e] groups (data on the former not shown). In retrospect, the decision to administer saline by the intranasal route to the control calves rather than subcutaneously (same route as vaccinated group) was less than optimal, but that decision was made when calves vaccinated with the intranasal vaccine were to be included in the analysis. However, administration of saline, regardless of route, provides for a suitable control as it is physiologically neutral, and has been used in other vaccine studies.[11,17,18]

An option for future studies is to delete the BHV-1 antigen from the multivalent test vaccine for use as the control product. By doing so, the control would differ from the test vaccine by only a single parameter, thus minimizing potential clinical or immunological differences caused by other antigens or extraneous material in the vaccine.

Space constraints at the study site dictated that the study be conducted in two periods and to limit the total number of animals during each of the two study periods to maintain adequate biosecurity and separation of individual calves, as well as separation of VAC calves from CON. By utilizing two study periods, we were able to include more calves per treatment group, thereby increasing statistical power to demonstrate any differences in outcomes.

A four-fold increase in antibody titer is commonly accepted as an indicator that a calf has responded serologically to a virus.[1] The mean BHV-1 SN titers for VAC were not four-fold greater than CON during the 14 day post-challenge period; however, statistical analysis demonstrated that VAC calves developed higher mean titers than did CON. BHV-1 virus does not typically stimulate production of high antibody titers, and the relatively low titers at 10 and 14 days post-challenge are not surprising. The purpose of measuring the serological response was not necessarily to demonstrate a four-fold or greater seroconversion, but rather to evaluate differences between treatments. In spite of the short time from vaccination to BHV-1 challenge, results demon-

strated that calves in the VAC groups had been sensitized by vaccination, and were able to mount a higher mean antibody response post-challenge as compared to calves in the CON groups. It can be argued that two-fold titer changes, e.g. 1:2 to 1:4, are not biologically different due to inherent characteristics of SN testing, but means for the VAC treatment groups were statistically different than those of the CON (multiple titer evaluations), thus, the data are meaningful for comparative purposes.

Conclusions and Clinical Relevance

This study demonstrated that using one dose of a non-adjuvanted MLV vaccine in colostrum-deprived calves less than 30 days of age when vaccinated five, three, or two days in advance of a direct BHV-1 challenge, reduced viral shedding and rectal temperature as compared to non-vaccinated animals. Additionally, clinical signs of IBR were reduced in calves vaccinated five days or three days before challenge, and those same calves developed higher SN mean titers to BHV-1 by 10 days post-challenge than did non-vaccinates. The rapid protective immune response observed in calves averaging 20 days of age suggests that vaccinating with this non-adjuvanted MLV vaccine could be beneficial when a quick immune response is crucial.

Endnotes

[a]Procedure for BVDV DAB, Bovine Viral Diarrhea Virus IHC Stain, Veterinary Diagnostic Laboratory, Iowa State University, Ames, IA
[b]Amplifier Max, Land O' Lakes, Shoreview, MN
[c]Purina Startina, Ralston Purina, Inc., St. Louis, MO
[d]Microsoft Excel, Microsoft, Inc., Redmond, WA
[e]Vista 5, Intervet/Schering-Plough Animal Health, Inc., Summit, NJ
[f]Cooper strain, Center for Veterinary Biologics, USDA, Ames, IA
[g]Model 163, DeVilbiss HealthCare, Somerset, PA
[h]JMP version 8.0.2, SAS Institute Inc., Cary, NC
[i]SAS version 9.2, SAS Institute Inc., Cary, NC
[j]Onset 5 IN, Intervet/Schering-Plough Animal Health, Inc., Summit, NJ

Acknowledgements

The authors thank Iowa State University Veterinary Diagnostic Laboratory personnel for serology test procedures and Veterinary Resources, Inc. for animal-related procedures. This study was supported by Intervet/Schering-Plough Animal Health, Inc.

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