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Research Article

Antibody Responses to BHV-1 Vaccination in Neonatal Calves and in Prime-Boost Programs

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Abstract

Four hundred newborn Angus base calves were randomly assigned to eight different treatment groups. Groups 1 - 4 received an intranasal (IN) vaccine containing modified live, temperature sensitive (MLV-TS) bovine herpesvirus -1 (BHV-1), bovine respiratory syncytial virus (BRSV) and parainfluenza -3 virus (PI3) on the day of birth. Groups 5 - 8 received no treatments at birth. At approximately 60, 210, 240 and 280 days of age, serum and nasal swabs were collected from all calves for BHV-1 antibody analysis (IgG and IgA). Beginning on day 60 and at each sampling day (except 280), calves in groups 1-7 were vaccinated with either the same IN vaccine administered to groups 1-4 at birth and a bovine viral diarrhea vaccine (BVDV) type 1 and 2 modified live viral vaccine in combination with a *Mannheimia haemolytica* (Mh) inactivated leukotoxoid vaccine or a MLV combination vaccine containing BVDV types 1 and 2, BHV-1, BRSV, PI3 virus and the same Mh vaccine (FivewayMH). Group 8 served as a control group throughout the study. The cow and calf pairs were in four independent pasture sites to avoid inadvertent viral transmission of the intranasal vaccine and pairs were reassigned to the different pastures depending on the vaccine the calves received. No evidence of wild BHV-1 exposure was seen in the control group throughout the study. Significantly higher systemic BHV-1 IgG titers were seen at day 60, 240 and 280 if the calves were administered the IN vaccine on the day of birth ($P < .05$). At days 240 and 280, the highest IgG responses were seen in calves receiving the IN as the first dose (either at birth and again at Day 60 or as the first dose at Day 60) followed by the systemically administered vaccine. Similar responses were not seen if the vaccines were alternated. Prime boost did not occur if the systemic vaccine was administered first, and a third dose of the IN in this short timeframe did not appear to continue to stimulate antibody production. Similar responses were not seen in nasal IgA, possibly due to the shorter duration of those antibodies.

Keywords: *Intranasal Vaccination; IgG; IgA; Beef Calves; Vaccination Programs*

Abbreviations

IN: Intranasal; MLV-TS: Modified Live, Temperature Sensitive; BHV-1: Bovine Herpesvirus-1; BRSV: Bovine Respiratory Syncytial Virus; PI3: Parainfluenza-3 Virus; BVDV: Bovine Viral Diarrhea Vaccine; Mh: *Mannheimia haemolytica*; FivewayMH: MLV Combination Vaccine Containing BVDV Types 1 and 2, BHV-1, BRSV, PI3 Virus and Mh Vaccine

Introduction

While a "prime-boost" vaccination program is required for many vaccines used today in animals, these programs entail the use of the same (homologous) vaccine for the booster. Heterologous prime-boost has been defined as: 1. same antigens being administered via different delivery methods [1-10] and 2. antigens from the same bacteria or virus being presented differently [1-10]. Most of the original studies in humans and animals utilized the former method of different vaccine delivery methods while using the same antigen, in a "heterologous" prime-boost format [1-3,7,9]. Many of the studies today combine two different antigen presentations and delivery systems/routes of administration to determine maximum immune responses and protection to various pathogens [1-10]. In veterinary medicine studies have utilized both definitions when describing heterologous prime-boost studies [11-23]. The majority of these studies have shown that the use of heterologous boosting can not only improve the immune response but increase the protection (degree and/or duration of protection) provided over traditional homologous boosting [1,2,5,6,12,14,15,19,21,23]. Very few of these studies determined when "priming" was done and the switch to the heterologous boost should occur i.e. does a three dose series with two priming doses stimulate even greater immunity/protection. This study investigated heterologous prime-boost (as defined by different delivery routes) antibody response to modified live BHV-1 vaccines administered IN and systemically in several vaccination program combinations.

Materials and Methods

Animals

The study consisted of 400 newborn calves that were part of the resident Angus base herd. The dams of these calves ranged in age from 3 to 12 years. Every other calf born was randomly assigned to treatment group 1 or 2 (Pasture A), treatment group 3 or 4 (Pasture B), treatment group 5 or 6 (Pasture C) or treatment group 7 or 8 (Pasture D). Table 1 shows assignment and outcomes of the calves during the study period. Ear tags and Day 0 treatments were applied at birth. Ear tags were applied as follows: Tags 1-50 to treatment group 1; Tags 51-100 to treatment group 2; Tags 101-150 to treatment group 3; Tags 151-200 to treatment group 4; Tags 201-250 to treatment group 5; Tags 251-300 to treatment group 6; Tags 301-350 to treatment group 7 and Tags 351-400 to treatment group 8. Four independent, non-adjoining, native range pastures (two treatment groups per pasture) were initially used at this location. To ensure that calves vaccinated intra-nasally were not comingled with non-intranasal vaccinated calves, at day 0 vaccination, calves were assigned to pastures as follows: treatment groups 1 and 2 were in pasture A; treatment groups 3 and 4 were in pasture B; treatment groups 5 and 6 were in pasture C and treatment groups 7 and 8 were in pasture D. At 60 (range 43 - 64 days) days of age, after vaccination, the cow calf pairs were moved into the appropriate pastures based on vaccines administered to their calf to avoid contact between the IN and non-IN vaccinated calves. Cows in Pastures A, B and D had not received a vaccination for IBRV, PI3 virus, BVDV or BRSV during 2015 or prior to calving in 2016. Cows in Pasture C had received a vaccination for IBRV, PI3 virus and BVDV (not BRSV) during the fall of 2015. They had not received any vaccination prior to calving in 2016. At 210 (range 197 - 218 days) days of age, all calves were weaned into pens consistent with the vaccines they received on days 210 and again on day 240 (range 232 - 253 days) on the home farm and were maintained in said pens until termination of the study on day 280 (Graph 1). A pen was left empty between each group to stop any direct contact between groups. All vaccine labels were followed and BQA injection techniques were followed. All systemic vaccinations were administered subcutaneously. Calves had final samples taken on day 280 (range 273 - 294 days) and the study was terminated.

Vaccination

Table 2 shows the assignment of the calves and vaccination, sampling and other management at each time point in the study. On the day of birth all calves in groups 1 - 4 received 2 ml of an intranasal vaccine containing modified live, temperature sensitive BHV-1 and PI3 virus and a modified live BRSV[1] (IN). Groups 5-8 did not receive any vaccination on the day of birth. On approximately day 60 (range 53

[1]Inforce™ 3 Zoetis, Parsippany, NJ 07054, USA.

Treatment Group	Day 0 Birth	~Day 60	~Day 210	~Day 240	~Day 280
T01 (Tags 1-50)	50 calves	50 calves	50 calves	50 calves	50 calves
T02 (Tags 51-100)	50 calves	50 calves	50 calves	50 calves	50 calves
T03 (Tags 101-150)	50 calves	49 calves* #116	49 calves	49 calves	49 calves
T04 (Tags 151-200)	50 calves	50 calves	49 calves** #176	49 calves	48 calves# #188
T05$ (Tags 201-250)	50 calves	49 calves*** #227	47 calves**** #234 and 240	47 calves	47 calves
T06$ (Tags 251-300)	50 calves	49 calves***** #253	49 calves	49 calves	49 calves
T07 (Tags 301-350)	50 calves	50 calves	49 calves! #344	49 calves	49 calves
T08 (Tags 351-400)	50 calves	50 calves	50 calves	49 calves+ #388	48 calves!! #389

Table 1: *Number of animals by treatment group present at each treatment event and reason for missing animal(s).*

**Calf 116 orphan, **Calf 176 orphan, #Calf 188 died of cellulitis/septicemia, ***Calf 227 died of H. somnii infection, ****Calves 234 and 240 found autolyzed in pasture, *****Calf 253 died of enterotoxaemia, !Calf 344 found autolyzed in pasture, +Calf 388 died of pneumonia, !!389 died of urolithiasis.*

$Twenty doses of FivewayMH had inadvertently been used on some of these calves instead of BVDMh, only 13 calves from Group 5 and 17 calves from Group 6 were included in the analysis to ensure no double vaccinated calves were included in the analysis.

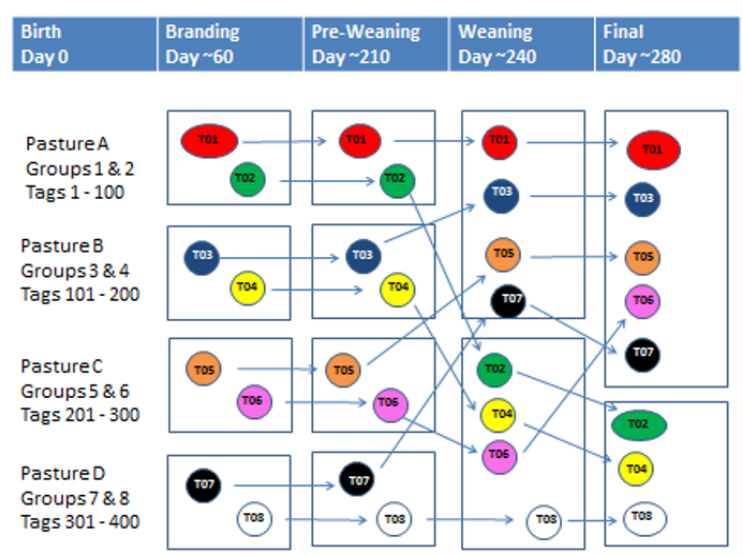
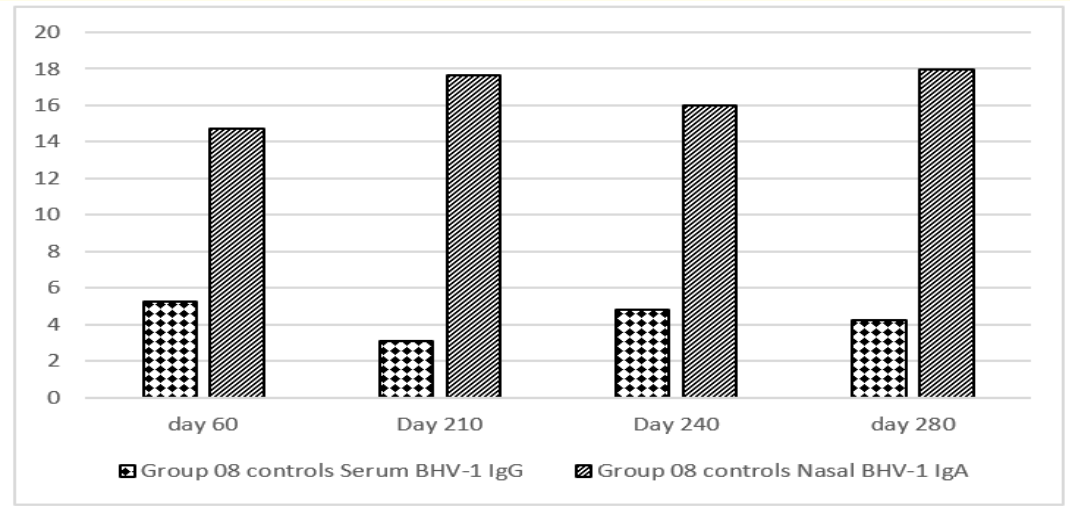


Figure 1: *Group movement based on vaccination administered to calf (pasture or pen)*.*

**Cow calf pairs in a pasture or calves in a pen where the calf receives an IN vaccination on a given treatment day, the pair or calf is immediately moved to the next pasture-pen with like-vaccinated calves until the next treatment event.*

- 64 days), two serum tubes and two nasal swabs were collected from each calf and all calves received a 5 ml Clostridium Chauvoei-Septicum-Novyi-Sordellii-Perfringens Types C and D Bacterin-Toxoid vaccination[2] administered subcutaneously. Calves in groups 1, 2, 5 and 6 received the IN vaccination and a modified live viral vaccine containing bovine viral diarrhea virus (BVDV) types 1 and 2 in combination with a *Mannheimia haemolytica* (Mh) inactivated leukotoxoid[3] (BVDMh, 2 ml subcutaneously). Groups 3, 4 and 7 were administered an MLV combination vaccine containing BVDV types 1 and 2, BHV-1, BRSV, PI3 virus and the same Mh vaccine[4] (FivewayMH, 2 ml subcutaneously). Calves in group 8 received only the clostridial vaccination and served as viral non-vaccinated controls throughout the study. After vaccinating 70 calves from Groups 5 and 6 at Day 60, it was determined that 20 doses of FivewayMH had inadvertently been included in the vaccine inventory to be used that day and some of these calves had received this product instead of BVDMh. In that FivewayMH was the current product in the syringe and approximately 5 doses remained, it was determined that approximately 15 calves +/- one head had been vaccinated with the incorrect product. Thus, to ensure that no calves that had been incorrectly vaccinated were included in the analysis the 70 calves were removed from the study. Starting with the 71st calf, the remaining 30 calves were correctly vaccinated, and their identification noted. Therefore, only 13 calves from Group 5 and 17 calves from Group 6 were included in the analysis even though the other 70 calves remained with their respective groups throughout the remainder of the study and were processed according to the original protocol. On day 210 all calves were weaned into pens. Calves in groups 1, 3, 5 and 7 received the IN and BVD/Mh vaccination and calves in groups 2, 4 and 6 were administered the FivewayMH vaccine. All calves including group 8 were vaccinated with the seven-way clostridial vaccine. On day 240 calves in groups 1, 3, 5, 6 and 7 received the IN and BVD/Mh vaccination and calves in groups 2 and 4 were administered the FivewayMH vaccine. Throughout the study groups were moved into various pastures and pens to ensure that all IN vaccinated calves were housed together to minimize the risk of shedding post vaccination to calves not receiving the IN vaccination (Graph 1).



Graph 1: *Control group (T08) IgG and IgA LSM ELISA titers (optical density units) throughout the study period.*

[2]Ultrabac® 7, Zoetis, Parsippany, NJ 07054, USA.

[3]OneShot® BVD, Zoetis, Parsippany, NJ 07054, USA.

[4]Bovi-Shield Gold OneShot®, Zoetis, Parsippany, NJ 07054, USA.

Treatment Group	Birth (Day 0)	~Day 60*	~Day 210*	~Day 240	~Day 280
T01	Intranasal (IN) vaccination, tagged	IN and BVDMh vaccination Serum and nasal swabs obtained	Wean, IN and BVDMh vaccination Serum and nasal swabs obtained	IN and BVDMh vaccination Serum and nasal swabs obtained	Serum and nasal swabs obtained
T02	Intranasal (IN) vaccination, tagged	IN and BVDMh vaccination Serum and nasal swabs obtained	Wean, FivewayMh vaccination Serum and nasal swabs obtained	FivewayMh vaccination Serum and nasal swabs obtained	Serum and nasal swabs obtained
T03	Intranasal (IN) vaccination, tagged	FivewayMh vaccination Serum and nasal swabs obtained	Wean, IN and BVDMh vaccination Serum and nasal swabs obtained	IN and BVDMh vaccination Serum and nasal swabs obtained	Serum and nasal swabs obtained
T04	Intranasal vaccination, tagged	FivewayMh vaccination Serum and nasal swabs obtained	Wean, FivewayMh vaccination Serum and nasal swabs obtained	FivewayMh vaccination Serum and nasal swabs obtained	Serum and nasal swabs obtained
T05[$]	Tagged	IN and BVDMh vaccination Serum and nasal swabs obtained	Wean, IN and BVDMh vaccination Serum and nasal swabs obtained	IN and BVDMh vaccination Serum and nasal swabs obtained	Serum and nasal swabs obtained
T06[$]	Tagged	IN and BVDMh vaccination Serum and nasal swabs obtained	Wean, FivewayMh vaccination Serum and nasal swabs obtained	IN and BVDMh vaccination Serum and nasal swabs obtained	Serum and nasal swabs obtained
T07	Tagged	FivewayMh vaccination Serum and nasal swabs obtained	Wean, IN and BVDMh vaccination Serum and nasal swabs obtained	IN and BVDMh vaccination Serum and nasal swabs obtained	Serum and nasal swabs obtained
T08	Tagged	Serum and nasal swabs obtained	Wean, Serum and nasal swabs obtained	Serum and nasal swabs obtained	Serum and nasal swabs obtained

Table 2: *Schedule of events.*

**All calves included in the study received a dose of 7-way clostridial bacterin-toxoid at Day 60 and 210.*

[$]Twenty doses of FivewayMH had inadvertently been used on some of these calves instead of BVDMh, only 13 calves from Group 5 and 17 calves from Group 6 were included in the analysis to ensure no double vaccinated calves were included in the analysis.

Sample collection and processing

All samples were transported on ice packs to the point of serum separation and sample freezing. Serum was separated, and all samples were stored frozen at -20°C until the completion of the study and shipped to the diagnostic laboratory for BHV-1 serum IgG and nasal IgA ELISA antibody evaluation as previously described [24-26].

Statistical analysis

Since the study involved repeated observations of titers on the same animal, the basic analysis was done using a repeated measures model for continuous response variables using PROC MIXED (SAS, v9.4, Cary, NC). Dependent variables analyzed included serum (IgG) and nasal (IgA) antibody titers for BHV-1. Pasture contemporary groups were defined at the beginning of the study and maintained such that IN vaccinated and non-vaccinated calves were not in the same pasture at the same time to prevent vaccine shedding. Due to this nested structure of treatment groups within pasture, the basic model for all dependent variables included fixed effect of treatment group within pasture, day of swab or serum collection (60, 210, 240 or 280) and the interaction of treatment group by day within pasture. Calf was considered as a random effect and used as subject in the repeated analysis across days (the variance-covariance structure was the default which is based on estimates of the corresponding variance components). Significance of treatment effects was considered at $P < .05$ and when observed, pairwise comparisons were examined to determine specific treatment differences. In addition, linear contrasts of some treatment group means and combinations of treatment group means were examined using an estimate statement in PROC MIXED.

Results

Animals

A total of ten calves were removed from the study (Table 1). One calf (#116) from group 3 was orphaned early, removed from the study and hand raised. One calf (#176) was orphaned later in the study (prior to weaning), was kept with its group, but the data was not included in the analysis. Eight calves died during the study. One calf (253) from group 6 died of enterotoxaemia prior to the day 60 processing, two calves (234 and 240) from group 5, as well as one calf (344) from group 7 were discovered autolyzed in the pasture and the cause of death was not determined. One calf (388) died from pneumonia and one calf (389) died from urolithiasis, both calves were from group 8. One calf (188) from group 4 died of lower abdominal abscess and septicemia and one calf (227) from group 5 died of thromboembolic encephalitis, both following weaning. Only one calf that died was from the groups vaccinated at birth and that calf (188) died of a non-respiratory related cause between day 240-280. Any samples collected before death were included in the analysis and the numbers were adjusted for analysis for samples collected after an animal died.

Serum BHV-1 IgG results

Table 3 shows BHV-1 serum levels (IgG) by treatment group and day of sampling. Control calves (vaccination group 8) maintained serum BHV-1 IgG levels below 1:6 throughout the study period indicating no BHV-1 exposure from vaccinates or wild virus (Graph 1). Across sampling days (i.e. the main effect of experimental treatment), there was a significant effect of treatment group on BHV-1 IgG serum levels ($p < 0.0001$) and many differences were detected among treatment groups. Also, there was a significant effect of day of sampling ($p < 0.0001$) and the interaction between treatment group and day of sampling ($p < 0.0001$). To further examine these multiple treatment differences, alternative combinations of treatment groups were examined to determine the effect on BHV-1 titers. One such comparison included treatment groups where calves were or were not vaccinated with IN at birth. Calves vaccinated IN at birth had significantly lower serum IgG at day 60 ($p < 0.04$), were not different at day 210 ($p < 0.09$) but then had higher levels of antibodies throughout the remainder of the study with significant increases seen after revaccination at days 240 and 280 ($p < 0.0001$, Table 4) when compared to groups of calves not vaccinated at birth. While group 5 is different in responses seen, they are an outlier group when included in the analysis. The highest serum BHV-1 IgG levels were seen in groups 2 and 4 (one or two initial IN doses) when the modified live systemic vaccination followed initial IN vaccination (Graph 2). The negative control group differed from all treatment groups receiving IN at birth except for treatment group 1 ($p < 0.05$) and did not differ from other treatment groups not receiving IN at birth ($p > 0.16$).

Nasal BHV-1 IgA results

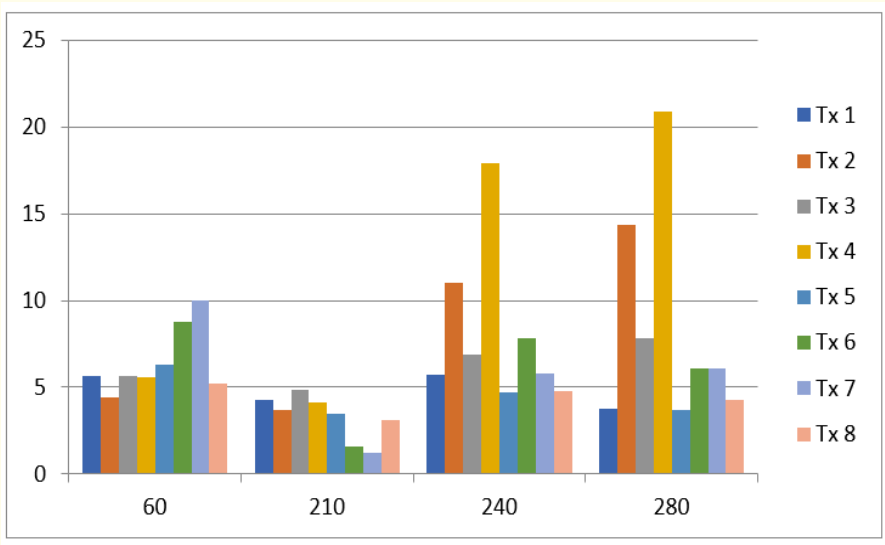
Titers in the negative control group had a slight increase in nasal BHV-1 IgA titers between 60 and 210 days that remained below 1:18 throughout the study. The minimal response of nasal IgA in the control calves to BHV-1 confirmed the serum BHV-1 IgG results that

Group	Day 60	Day 210	Day 240	Day 280	LS Means across time
TO1	5.66 ± 1.21	4.26 ± 1.21	5.72 ± 1.21	3.78 ± 1.21	4.9 ± 0.71
T02	4.44 ± 1.21	3.72 ± 1.21	11.04 ± 1.21	14.34 ± 1.21	8.4 ± 0.71
T03	5.63 ± 1.22	4.82 ± 1.22	6.90 ± 1.22	7.84 ± 1.22	6.3 ± 0.71
T04	5.59 ± 1.22	4.14 ± 1.22	17.88 ± 1.22	20.89 ± 1.24	12.1 ± 0.71
T05	6.31 ± 2.38	3.46 ± 2.38	4.69 ± 2.38	3.69 ± 2.38	4.5 ± 1.38
T06	8.76 ± 2.08	1.59 ± 2.08	7.82 ± 2.08	6.12 ± 2.08	6.1 ± 1.21
T07	10.02 ± 1.21	1.20 ± 1.22	5.79 ± 1.22	6.06 ± 1.22	5.8 ± 0.71
T08	5.24 ± 1.21	3.12 ± 1.22	4.80 ± 1.22	4.26 ± 1.24	4.4 ± 0.71

Table 3: *Serum IgG antibody ELISA levels (optical density units) for Bovine Herpes Virus-1 (LS Means ± standard errors). Overall effect of treatment, p < 0.0001; day of sampling, p < 0.0001 and treatment group by day of sampling, p < 0.0001.*

BHV-1 IgG	Day 60	Day 210	Day 240	Day 280
IN @ birth (T01-T04)	5.33 ± 0.91	4.24 ± 0.91	10.39 ± 0.91	11.68 ± 0.91
No IN @ birth (T05-T08)	7.67 ± 1.27	2.29 ± 1.27	5.88 ± 1.28	5.12 ± 1.28
	P < 0.04	P < 0.09	P < 0.0001	P < 0.0001

Table 4: *Serum IgG ELISA levels (optical density units) for Bovine Herpes Virus-1 (LS Means ± standard errors) for calves either receiving or not receiving IN at birth.*



Graph 2: *Serum IgG LSmean BHV-1 titers (optical density units) by vaccination group across the 4 sampling days.*

no natural exposure to BHV-1 occurred during the study. Control calves started with the second highest initial BHV-1 IgA levels and this higher level was seen at other timepoints when compared to some vaccine groups (Table 5). The nasal BHV-1 IgA levels were less consistent than the IgG levels, presumably due to the shorter half-life of IgA and the long intervals between sampling in this study. Nasal BHV-1 IgA levels were higher in the calves not vaccinated at birth and remained significantly higher until day 280 (Table 6).

Group	Day 60	Day 210	Day 240	Day 280	LS Mean across times
T01	5.96 ± 3.18	10.40 ± 3.18	15.46 ± 3.18	15.52 ± 3.18	11.84 ± 1.88
T02	8.48 ± 3.18	14.32 ± 3.18	8.32 ± 3.18	15.86 ± 3.21	11.75 ± 1.88
T03	10.56 ± 3.28	11.31 ± 3.28	19.34 ± 3.25	26.00 ± 3.22	16.80 ± 1.91
T04	7.47 ± 3.22	9.06 ± 3.22	20.27 ± 3.25	22.37 ± 3.22	14.79 ± 1.90
T05	41.08 ± 6.24	20.08 ± 6.24	39.38 ± 6.24	22.38 ± 6.24	30.73 ± 3.68
T06	8.71 ± 5.46	16.00 ± 5.46	17.82 ± 5.46	13.19 ± 5.62	13.93 ± 3.24
T07	13.16 ± 3.18	21.60 ± 3.18	16.36 ± 3.18	21.62 ± 3.22	18.18 ± 1.89
T08	14.72 ± 3.18	17.62 ± 3.18	15.97 ± 3.22	17.96 ± 3.25	16.57 ± 1.89

Table 5: *Nasal IgA antibody ELISA levels (optical density units) for Bovine Herpes Virus-1 (LS Means ± standard errors). Overall effect of treatment, $p < 0.0003$; day of sampling, $p < 0.004$; treatment group by day of sampling, $p < 0.0088$.*

BHV-1 IgA	Day 60	Day 210	Day 240	Day 280
IN @ birth	8.11 ± 2.28	11.27 ± 2.28	15.85 ± 2.29	19.95 ± 2.28
No IN @ birth	11.27 ± 3.20	18.63 ± 3.20	21.67 ± 3.21	18.51 ± 3.25
	$p < 0.0004$	$p < 0.0097$	$p < 0.04$	$p < 0.62$

Table 6: *Nasal IgA ELISA levels (optical density units) for Bovine Herpes Virus-1 (LS Means ± standard errors) for calves either receiving or not receiving IN at birth.*

Treatment groups 1 and 5, due to the sequence of products administered, did not have an opportunity to exhibit a prime-boost effect. In these groups a third IN dose after 60 days (within six months) did not stimulate further BHV-1 IgA. However, treatment groups 2, 3, 4, 6 and 7 did exhibit increased BHV-1 IgA responses (Table 5).

Discussion

As different vaccine technologies have become available, combining the various vaccines in a program has become the focus of heterologous prime boost research [8,27]. Initially, the research focused on utilizing the same antigens delivered in different ways and/or routes [20]. More recent research, has focused on antigens from the same pathogen being presented in different ways and also combining different antigen presentations with different routes of administration. Studies are utilizing different routes of administration to design programs for some antigens, particularly in veterinary medicine, since the use of different presentations is often against the label indications for approved vaccine products. Studies in Europe (BRSV and PI3) [20,22] and US (BRSV) [18,20] have shown increased serum IgG responses when IN and systemic MLV vaccination technologies were used in calves. This study utilized modified live BHV-1 vaccines being delivered via two different routes to investigate the potential for heterologous prime boost in calves.

In these and other prime boost studies that have demonstrated increased efficacy, the combination of technologies was better than the individual vaccines, however, few of the studies determined how many doses of the priming vaccine were needed before switching to the second vaccine to maximize the heterologous prime boost response [4,5].

It is important to note that this study did not include a challenge and only looked at antibody responses. Including a cell mediated response evaluation or challenge would provide further insight into optimum vaccination schedules, however the size of this study did not allow for those evaluations. Also, it should be noted that an experimental design using replicate pastures for each treatment could have been considered to mitigate the concern for pasture/environment effect was responsible for any differences in treatment group outcomes. However, since calves shared pastures, depending on their vaccination and the pastures were all in the same geographic area it was felt that there was no pasture/environment affect and the calf was the experimental unit.

The longer half-life of IgG provided the most consistent responses for evaluation. Based on IgG, a single or two IN doses, at birth and 60 days later or only at ~60 days of age, both provided sufficient priming for subsequent MLV systemic vaccination 150 days later. When evaluated as a group, while there was some individual group variation following the second BHV1 vaccination administered at 60 days of age, calves vaccinated at birth had significantly higher BHV-1 IgG levels throughout the remainder of the study. Incorporating an initial dose at birth may help to increase immunity rather than waiting to start vaccination at the common vaccination period close to 60 days of age. While the cause of this higher BHV-1 IgG levels though day 280 was not ascertained, it may be due to antigen presentation teaching of the maternal cells by this early vaccination [28]. However, a third IN dose administered after sixty days, within this timeframe did not stimulate further priming. The highest immune responses were seen when IN was followed by systemic MLV BHV-1. It is interesting to note that some studies have demonstrated the importance of the sequence of vaccines in the program [12] while others showed that which vaccine was administered first didn't change the immune response [29]. In this study, initial systemic vaccine followed by IN vaccine did not stimulate similar antibody responses to initial IN vaccination followed by systemic.

Conclusion

This study provides further insight into utilization of prime boost technologies in calves. Furthermore, it demonstrates that vaccination at a very young age, with the intranasal BHV-1 vaccine can significantly impact subsequent vaccination responses for the next eight months. Delaying the initial vaccination until 60 days of age, while able to prime the immune system) did not achieve the same BHV-1 IgG levels.

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