PEER REVIEWED

Serological effect of two concurrent IBRV, BVDV, BRSV, PI3V, and *Mannheimia haemolytica* vaccination protocols and time interval between the first and second dose on the subsequent serological response to the BRSV and *M. haemolytica* fractions in suckling beef calves

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Abstract

This study was conducted to compare the serologic response of suckling beef calves to the bovine respiratory syncytial virus (BRSV) and *Mannheimia haemolytica* fractions of 2 bovine respiratory disease (BRD) vaccination protocols when the second dose was given 153 days after the first dose. Calves in 1 group were vaccinated with intranasal (IN) modified-live virus (MLV) 3-way BRD vaccine plus separate subcutaneous (SC) injections of MLV bovine viral diarrhea virus (BVDV) type 1 and 2 vaccine and *M. haemolytica* bacterin-leukotoxoid. Calves in the other group were vaccinated with SC MLV 5-way BRD vaccine combined with *M. haemolytica* leukotoxoid. On day 153, all calves were revaccinated with SC MLV 5-way vaccine combined with *M. haemolytica* leukotoxoid. Both calf vaccination protocols stimulated a humoral immune response to the BRSV and *M. haemolytica* vaccine fractions. Vaccination with IN MLV 3-way BRD vaccine resulted in a significantly (P = 0.003) higher initial antibody response to BRSV and a significantly (P = 0.006) higher anamnestic response. No differences were seen between groups in initial *M. haemolytica* leukotoxoid antibody levels. Vaccination with SC MLV 5-way BRD vaccine combined with *M. haemolytica* leukotoxoid resulted in a significantly (P = 0.02) higher anamnestic response to *M. haemolytica* leukotoxoid.

Key words: beef calves, virus vaccine, intranasal vaccine, bovine respiratory syncytial virus, *Mannheimia haemolytica,* immune response, prime-boost strategy

Résumé

Cette étude a été menée afin de comparer la réponse sérologique de veaux de boucherie allaitants au virus respiratoire syncytial bovin et à *Mannheimia haemolytica* dans deux protocoles de vaccination pour le complexe respiratoire bovin (CRB) lorsque la seconde dose est administrée 153 jours après la première dose. Dans un groupe, les veaux ont été vaccinés par voie intranasale avec un vaccin trivalent à virus vivants modifiés pour le CRB et ont reçu en plus des injections sous-cutanées comportant des virus vivants modifiés du virus de la diarrhée virale bovine du type 1 et 2 et des bactérines et toxoïdes de *M. haemolytica*. Les veaux de l'autre groupe ont été vaccinés par voie sous-cutanée avec un vaccin pentavalent à virus vivants modifiés pour le complexe respiratoire bovin combinée avec des toxoïdes de *M. haemolytica*. Au jour 153, tous les veaux ont été vaccinés à nouveau par injection sous-cutanée du vaccin pentavalent à virus vivants modifiés pour le complexe respiratoire bovin combiné avec des toxoïdes de *M. haemolytica*. Les deux protocoles de vaccination ont stimulé une réponse immunitaire humorale au virus respiratoire syncytial bovin et à *Mannheimia haemolytica*. La vaccination par voie intranasale avec un vaccin trivalent à virus vivants modifiés pour le complexe respiratoire bovin a entrainé une production initiale d'anticorps significativement plus élevée au virus syncytial respiratoire bovin (P = 0.003) et une réponse anamnestique significativement plus élevée (P = 0.006). Il n'y a pas eu de différence entre les deux groupes dans la production initiale d'anticorps aux toxoïdes de *M. haemolytica*. La vaccination par voie sous-cutanée avec un

vaccin pentavalent à virus vivants modifiés pour le complexe respiratoire bovin combinée avec des toxoïdes de *M. haemolytica* a causé une réponse anamnestique significativement plus élevée ($P = 0.02$) aux toxoïdes de *M. haemolytica*.

Introduction

Spring-born beef calves are routinely vaccinated at a young age to aid in prevention of specific infectious diseases. Vaccination protocols often include 7-way clostridial bacterin-toxoid and other viral and bacterial vaccines to help reduce the risk of clostridial and respiratory disease. Although suckling beef calves have a functioning immune system, a number of variables, including age of the calf at the time of colostrum intake, total immunoglobulin ingested, breed, ambient temperature, calf vigor, and the cow's mothering ability, can compromise development of a strong immune response.[1,3,7–9,12,13] Some calves become seronegative prior to vaccination and are susceptible to infection, whereas others with lingering antibodies from the dam may not be able to develop a humoral response to vaccination.[4] The presence of high levels of maternal antibodies can inhibit the humoral response of suckling calves to vaccination; however, the lack of seroconversion after vaccination of calves with maternal antibodies has previously been shown to be an unreliable indicator that vaccination failed to protect.[4,6,8,12,14,16,19,23] In these studies, although colostral antibodies inhibited some of the humoral response to vaccination, it was demonstrated that memory immune cells were recruited and replicated, and were capable of inducing protective immunity.[4,6,8,12,14,16,19,23] Investigators also have shown that vaccination of calves with maternal antibodies can induce an anamnestic response when the calves are revaccinated later in life,[1,3,18] that vaccination can prolong the persistence of antibodies,[11] and that vaccination can prime for T cell responses even when calves do not seroconvert.[6,8]

Although 2 doses of BRD vaccine are often required for successful vaccination in young calves with colostral antibodies, these vaccines are not typically evaluated to establish ideal dose intervals. In practice, the second, or booster, dose is rarely given according to label directions. Unless compelling evidence exists for administering a second dose within the label-specified time frame, the booster dose typically is not administered for at least 90 to 150 days following the first dose to coincide with conventional processing events of beef herds, such as branding and weaning.

The purpose of the current field study was to provide additional information on the serologic response to the bovine respiratory syncytial virus (BRSV) and *Mannheimia haemolytica* components of 2 BRD vaccination protocols when the first dose is given to suckling calves at approximately 2 months of age, and the second dose in each protocol is given to weaned calves 153 days following the first dose.

Materials and Methods

Study Facility

This study was conducted at the Central Grasslands Research Extension Center, Streeter, North Dakota, a university extension research livestock operation in south-central North Dakota. Native grass pastures were utilized and water was provided by way of wells and sloughs. The predominantly Angus-based dams of enrolled calves were individually identified and had received a multivalent MLV vaccine containing infectious bovine rhinotracheitis virus (IBRV), bovine viral diarrhea virus (BVDV) types 1 and 2, parainfluenza type-3 virus (PI3V) antigens that also included *Campylobacter fetus* and 5 serovars of *Leptospira*[a] prior to the previous breeding season (spring 2012).

Animals

During the spring of 2013, 190 spring-born calves native to the ranch were screened for IBRV, BRSV, and *Mannheimia haemolytica* leukotoxoid colostral antibody titers on day -30. Only calves determined to have low IBRV titers, defined as IBRV serum neutralization (SN) antibody titer of ≤ 1:4, were included in the study. A total of 150 calves ranging in age from 55 to 99 days, with mean age of 74 days on day 0, qualified for the study. The calves were blocked by age and sex and randomly assigned to 1 of 2 treatment groups (Table 1). On day 0, each calf in both treatment groups was individually

Table 1. Vaccine treatments (Yes = vaccine administered; No = vaccine not administered).

Treatment group	Vaccine treatment description	Day 0	Day 153
T1	MLV Intranasal 3-way* plus MLV BVDV† + *M. haemolytica* leukotoxoid‡ (separate injections)	Yes	No
	MLV 5-way + *M. haemolytica* (combination)§	No	Yes
T2	MLV 5-way + *M. haemolytica* (combination)§	Yes	Yes

*INFORCE 3®, Zoetis, Florham Park, NJ
†Bovi-Shield GOLD® BVD, Zoetis, Florham Park, NJ
‡One Shot®, Zoetis, Florham Park, NJ
§Bovi-Shield GOLD® One Shot®, Zoetis, Florham Park, NJ

identified and treated following the respective vaccination protocol, and a serum sample was collected. On days 14 and 27, calves were again processed, individually identified, and serum was collected. Following each processing event, calves in both treatment groups were reunited with their dams and all animals were commingled on the same pasture until the next processing event. On day 112, calves were weaned, administered tulathromycin[b] and placed in a drylot at the research facility. On day 153, calves were identified, serum was collected, and were treated following the described vaccination protocols. On day 174, calves were again identified and serum was collected.

Treatment Groups

Seventy-five calves were assigned to treatment group T1 (intranasal (IN) MLV IBRV, PI3V, and BRSV vaccine[c] plus a separate subcutaneous (SC) injection of MLV BVDV (type 1 and 2) vaccine[d] and *M. haemolytica* bacterin-leukotoxoid[e]), and 75 calves were assigned to treatment group T2 (SC MLV IBRV, BVDV (type 1 and 2), BRSV, PI3V respiratory vaccine combined with *M. haemolytica* leukotoxoid[f]). On day 0, at a mean age of 74 days, calves were administered the vaccine or vaccines specified in their respective protocol and blood samples were collected. Calves received no additional vaccines, bacterins, or toxoids prior to or during the study other than a SC dose of 7-way clostridial bacterin-toxoid[g] on day 0.

On day 153, all calves in each treatment group were given a SC dose of the same MLV 5-way respiratory vaccine combined with *M. haemolytica* leukotoxoid.[f]

Sample Collection and Analysis

Following the collection of blood from all calves in the study on days 0, 14, and 27, the blood was processed, and the serum was held frozen at the North Dakota State University Veterinary Diagnostic Laboratory. Following day 27, the serum samples from days 0, 14, and 27 were submitted to laboratories at Oklahoma State University for evaluation of IBRV and BRSV antibody titers and *M. haemolytica* leukotoxoid levels. Blood samples collected from calves on days 153 and 174 were processed and held frozen in the same manner at the North Dakota State University Veterinary Diagnostic Laboratory, and following day 174 were submitted to the same Oklahoma State University laboratories for evaluation of IBRV and BRSV antibody titers and *M. haemolytica* leukotoxoid levels.

Statistical Methods

All data were recorded using specifically designed forms supplied by Zoetis. Data and laboratory results were analyzed by the sponsors of the study.[h]

IBRV and BRSV serum antibody titers were transformed to the log scale and analyzed with a linear mixed model that included the fixed effects of treatment, day of study, and the interaction, along with the random effects of block and block by treatment. Day -30 antibody titers were included as a covariate ($P \leq 0.05$) in the analysis of all antibody titer results. Resulting least squares means (LSM) were back-transformed to geometric means for presentation.

Leukotoxoid data were transformed to log + 1 scale and analyzed with a linear mixed model that included the fixed effects of treatment, day of study, and the interaction, along with the random effects of block and block by treatment. Day -30 antibody titers were tested for inclusion in the model as a covariate ($P \leq 0.05$) in the analysis of all antibody titer results. Resulting LSM were back-transformed for presentation.

A significant ($P \leq 0.05$) treatment or treatment by day of study interaction was required before conducting pairwise tests of treatment differences.

The study was conducted in accordance with the guidelines of the North Dakota State University Animal Care and Use Committee.

Results

One calf from the T1 group and 1 calf from the T2 group were not present on day 153 and were removed from the study. All other calves completed the study.

IBRV Antibody Titers

The LSM antibody titers for IBRV of calves in the T1 group were 3.0 on day 0, 2.8 on day 27, and 2.1 on day 153, whereas calves in the T2 group had titers of 3.7, 3.4, and 2.1, respectively, on days 0, 27, and 153. The IBRV titers were significantly higher for calves in the T2 group compared with calves in T1 group on day 0 ($P = 0.049$) and day 27 ($P = 0.038$), but for the duration of study the IBRV titers remained below the enrollment threshold of < 1:4.

Serologic Responses to MLV BRSV Vaccination

BRSV antibodies detected on day 0 in both groups of calves were assumed to be of maternal origin because the herd's 2012 pre-breeding vaccination program did not include a BRSV antigen,[a] consequently, stimulation of the dams' immune systems and subsequent transfer of BRSV antibodies to the colostrum likely resulted from BRSV infection of the dams. No significant differences in day 0 BRSV SN titers were found between calf groups (Table 2). Following the day 0 sampling and IN or SC administration of an MLV BRSV vaccine fraction to calves with BRSV colostral antibody present, BRSV antibody titers continued to decline until day 27, when both groups showed an increase in titers. A significantly ($P = 0.003$) greater increase was observed on day 27 in the T1 group calves administered IN MLV BRSV vaccine fraction on day 0, as compared with calves in the T2 group that had received the SC MLV BRSV vaccine fraction. A significantly ($P = 0.001$) higher immune response to the BRSV fraction was also observed on day 153, as was a significantly ($P = 0.006$) higher anamnestic response on day 174 following

revaccination with the SC MLV 5-way respiratory vaccine combined with *M. haemolytica* leukotoxoid. The percent of calves that responded serologically at multiples of their individual baseline BRSV antibody titers was similar within each treatment group (Table 3).

Serologic Responses to M. haemolytica Leukotoxoid

Compared with calves administered the T1 protocol on day 0, calves administered the T2 vaccination protocol had a numerically but not statistically higher serological *M. haemolytica* leukotoxoid response on days 14, 27, and 153 (Table 4). Administration of a second dose of the SC MLV 5-way plus *M. haemolytica* leukotoxoid resulted in a significantly ($P = 0.02$) higher anamnestic response on day 174 in calves in the T2 group compared with calves in the T1 group. The percent of calves that responded serologically at multiples of their individual baseline *M. haemolytica* leukotoxoid levels was similar within each treatment group (Table 5).

Discussion

The BRSV antibody response in calves vaccinated following the T1 protocol wherein suckling calves with colostral antibodies to BRSV were initially vaccinated with an IN MLV BRSV vaccine fraction and then revaccinated 153 days later with a SC MLV BRSV vaccine fraction is of particular interest. As other investigators have previously demonstrated, parenteral MLV BRSV vaccination stimulates production of little or no systemic antibody when maternal antibodies to BRSV are present;[1,9] however, priming of the immune system

Table 2. Least squares means of BRSV neutralizing antibody at days 0, 14, 27, 153, and 174.

Treatment group	Vaccine	Day 0	Day 14	Day 27	Day 153	Day 174
T1	Intranasal MLV 3-way + subcutaneous BVDV + *M. haemolytica*	14.1	8.7	24.1	2.8	15.3
T2	Subcutaneous MLV 5-way + *M. haemolytica* (combination)	12.9	7.4	14.7	2.1	7.2
T1 vs T2		$P = 0.64$	$P = 0.33$	$P = 0.003$	$P = 0.001$	$P = 0.006$

T1 = INFORCE 3®; Bovi-Shield GOLD® BVD; One Shot®, Zoetis, Florham Park, NJ

T2 = Bovi-Shield GOLD® 5, Zoetis, Florham Park, NJ

Table 3. Percent of animals within each treatment group that responded serologically at multiples of their individual baseline BRSV antibody titers.

Treatment group	Day 27 vs Day 0					
	< 2X	2X	3X	4X	5–6X	7–8X
T1	76	16	5.3	0	1.3	1.3
T2	80	10.7	6.7	1.3	1.3	0
	Day 153 vs Day 0					
T1	97.2	1.4	0	1.4	0	0
T2	100	0	0	0	0	0
	Day 174 vs Day 153					
T1	31.9	23.6	13.9	8.3	15.3	6.9
T2	45.9	6.8	18.9	10.8	14.9	2.7

T1 = INFORCE 3®; Bovi-Shield GOLD® BVD; One Shot®, Zoetis, Florham Park, NJ

T2 = Bovi-Shield GOLD® 5, Zoetis, Florham Park, NJ

Table 4. Least squares means serological levels (µg/mL) of *Mannheimia haemolytica* leukotoxoid at days 0, 14, 27, 153, and 174.

Treatment group	Vaccine	Day 0	Day 14	Day 27	Day 153	Day 174
T1	Intranasal MLV 3-way + subcutaneous BVDV + *M. haemolytica*	0.25	0.40	0.38	0.62	1.19
T2	Subcutaneous MLV 5-way + *M. haemolytica* (combination)	0.26	0.47	0.43	0.69	1.43
T1 vs T2		$P = 0.51$	$P = 0.10$	$P = 0.15$	$P = 0.24$	$P = 0.02$

T1 = INFORCE 3®; Bovi-Shield GOLD® BVD; One Shot®, Zoetis, Florham Park, NJ

T2 = Bovi-Shield GOLD® 5, Zoetis, Florham Park, NJ

Table 5. Percent of animals within each treatment group that responded serologically at multiples of their individual baseline *M. haemolytica* leukotoxoid antibody titers.

Treatment group	< 2X	2X	3X	4X	5–6X	7–8X	≥ 9X
				Day 14 vs Day 0			
T1	65.3	22.7	9.3	2.7	0	0	0
T2	61.3	18.7	10.7	4.0	5.3	0	0
				Day 27 vs Day 0			
T1	66.7	24.0	8.0	1.3	0	0	0
T2	65.3	18.7	8.0	6.7	1.3	0	0
				Day 153 vs Day 0			
T1	37.5	20.8	20.8	6.9	6.9	5.6	1.4
T2	31.1	25.7	17.6	9.5	9.5	6.8	0
				Day 174 vs Day 153			
T1	43.7	25.4	15.5	11.3	2.8	0	1.4
T2	39.2	33.8	13.5	6.8	2.7	0	4.1

T1 = INFORCE 3®; Bovi-Shield GOLD® BVD; One Shot®, Zoetis, Florham Park, NJ

T2 = Bovi-Shield GOLD® 5, Zoetis, Florham Park, NJ

for subsequent parenteral vaccination was established.[1] The IN BRSV vaccine used in the previous study demonstrated a lack of maternal interference, which was also demonstrated in the current study.[8] Not all IN BRSV vaccines have this ability in the face of BRSV maternal antibody.[7] In the current study, not only did the IN BRSV vaccination appear to be superior to parenteral vaccination in calves with high concentrations of circulating BRSV antibodies, it also appeared to provide superior priming of the immune system to the target BRSV antigen when administered the second dose of antigen parenterally. This sequential administration of vaccines using different antigen delivery systems (or heterologous boosting) is referred to as a "prime-boosting" strategy.[16–18] Key advantage of the strategy in certain circumstances is that greater levels of immunity can be established by heterologous prime-boosting than can be established by a single vaccine administration or a homologous boosting strategy.[15,24,27] The synergistic enhancement of immunity to a target antigen was initially thought to result from an increased number of antigen-specific T cells, selective enrichment of the efficacy of T cells, and increased efficacy against pathogen challenge.[10,17] More recent studies have established that both $CD4^+$ and $CD8^+$ T cells can be induced using appropriate prime-boost strategies.[27] In 1 study reported in 2015, a new respiratory syncytial virus (RSV) antigen delivered by genetic vaccine vectors using a combination of routes and a heterologous prime-boost regimen induced a full array of immune responses that potentially could address the different attributes required to protect human infants and adults against RSV infection. In that study, the experimental genetic vaccine was based on chimpanzee adenovirus (PanAd3-RSV) and modified vaccinia Ankara RSV (MVA-RSV) encoding the F, N, and M2-1 proteins for induction of neutralizing antibodies, and broad-based cellular immunity was evaluated in rodents and nonhuman primates. Whereas single IN or intramuscular vaccination completely protected mice and cotton rats against RSV replication in the lungs, only IN administration prevented infection in the upper respiratory tract. Intramuscular vaccination with MVA-RSV also protected cotton rats against lower respiratory tract infection in the absence of detectable neutralizing antibodies. Either IN or IM priming with the PanAd3-RSV and IM boosting with MVA-RSV induced high levels of neutralizing antibodies as well as potent and broad RSV-specific T cell responses in nonhuman primates. Interestingly, only animals primed in the nose developed mucosal IgA against the F protein, suggesting that IN delivery of RSV antigen was responsible for eliciting mucosal immunity.[22] As this study illustrates, the prime-boosting strategy is emerging as a powerful approach to establishing immunity. Further development of the strategy for use in human and animal vaccination programs likely will depend upon advances in basic understanding of the mechanisms of how systemic and mucosal T-cell memory is established, maintained at different body sites, and recalled in the face of a subsequent infection.[27]

Also of interest in the current study is the observed increase in *M. haemolytica* leukotoxoid antibodies over day 0 baseline levels when measured at days 14, 27, and 153, and the greater increase in leukotoxoid antibodies following revaccination of calves administered the T2 protocol. Previously, multiple field-based[5,26] and experimental studies[2,20,21] demonstrated that *M. haemolytica* antibody levels decreased when SC MLV IBRV vaccine was administered concurrently with *M. haemolytica* bacterin-leukotoxoid. A decrease in *M. haemolytica* antibody levels was also demonstrated when a temperature-sensitive IBRV vaccine was administered parenterally at the same time; however, the decrease in response was less than that observed when other MLV IBRV vaccines were given.[5] In the current study, *M. haemolytica* antibodies not only increased in T2 group calves administered a reformulated *M. haemolytica* fraction containing additional

antigen, but also in T1 group calves administered a TS IBRV fraction and an *M. haemolytica* fraction containing comparatively less antigen.

Regardless of the vaccination protocol followed in the current study, the antibody response seen in both treatment groups at day 174 following the second dose of *M. haemolytica* leukotoxoid appeared to be an anamnestic response to the dose administered at day 0. Results obtained with the T1 protocol are similar to responses observed in a previous study[25] when beef calves approximately 11 weeks of age were vaccinated at day 0 with IN MLV IBRV, PI3V, and BRSV vaccine[c] and *M. haemolytica* bacterin-leukotoxoid,[e] and were revaccinated parenterally at day 91 with a MLV 5-way BRD vaccine[i] and a separate *M. haemolytica* bacterin-leukotoxoid.[e] Results obtained with the T2 protocol suggest that vaccination with a reformulated *M. haemolytica* fraction containing additional antigen also is capable of overcoming antigen interference associated with concurrent administration of MLV IBRV antigen. Because it is possible that the greater antibody response to the *M. haemolytica* fraction of the vaccination protocols resulted from a more mature immune system in the calves at revaccination, challenge studies likely are needed to further define the effect of antigen interference on disease prevention.

Conclusions

Results of the laboratory evaluations suggest that both the T1 and T2 vaccination protocols administered to suckling beef calves at approximately 2 months of age successfully stimulated a humoral response to both the BRSV and *M. haemolytica* vaccine fractions. Vaccination of calves with the IN MLV IBRV, PI3V, BRSV component of the T1 protocol resulted in a significantly higher BRSV antibody response initially, 153 days later, and again following revaccination with a SC MLV 5-way viral BRD vaccine combined with a *M. haemolytica* leukotoxoid. In comparison, vaccination of calves with the SC MLV 5-way viral BRD vaccine combined with a *M. haemolytica* leukotoxoid resulted in the highest initial leukotoxoid response at 14, 27, and 153 days after vaccination, and a significantly higher anamnestic response on day 174 following administration of the second SC dose of the MLV 5-way vaccine plus *M. haemolytica* leukotoxoid on day 153.

For spring-born calves, the time interval from turnout and initial clostridial and BRD vaccinations to preweaning or weaning vaccinations can range from 120 to 180 days. The serological results reported in this study provide beef producers and veterinarians with evidence that vaccination of suckling calves likely helps prime the calves' immune systems so that subsequent vaccination can elicit an immune memory response at 153 days following administration of the initial dose. This information can help further define how beef calf vaccination protocols might be designed to maximize immune responses to both viral and bacterial antigens.

Endnotes

[a]PregGuard® GOLD FP®, Zoetis, Florham Park, NJ
[b]Draxxin®, Zoetis, Florham Park, NJ
[c]INFORCE 3®, Zoetis, Florham Park, NJ
[d]Bovi-Shield GOLD® BVD, Zoetis, Florham Park, NJ
[e]One Shot®, Zoetis, Florham Park, NJ
[f]Bovi-Shield GOLD® One Shot®, Zoetis, Florham Park, NJ
[g]Ultrabac® 7, Zoetis, Florham Park, NJ
[h]Dr. Daniel Weigel of Outcomes Research Group, Zoetis, Kalamazoo, MI
[i]Bovi-Shield GOLD® 5, Zoetis, Florham Park, NJ

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