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Research Article

Impact of Maternal Antibody and Concurrent Vaccination on Serologic Responses to Clostridial Vaccination in Calves

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Abstract

At birth, one hundred and fifty beef calves were randomly assigned to one of three groups. Dams of the calves had received no vaccines for at least two years. At day sixty of age, serum was drawn for antibodies against exotoxins from *Clostridium perfringens* (Clp) type C and D, *Cl. novyi*(Cln) and *Cl. sordellii* (Cls). Calves in group one were administered 2ml of an intranasal vaccine containing modified live BHV-1, BRSV and PI3 virus[a] (IN), a modified live viral vaccine containing bovine viral diarrhea virus (BVDV) types 1 and 2 in combination with a *Mannheimia hemolytica* (BVDMh) inactivated leukotoxoid[c] and a Clostridium Chauvoei-Septicum-Novyi-Sordellii-Perfringens Types C and D Bacterin-Toxoid vaccine (7way).[b] Calves in group two received a systemic MLV combination vaccine containing BVDV types 1 and 2, BHV-1, BRSV, PI3 virus and the same Mh vaccine[d] (FivewayMH). Group three were vaccinated with only the 7 way Clostridium vaccine. At 210 days of age serum was drawn and calves in group 1 received the FivewayMH and 7way vaccines. Calves in group 2 were administered the IN, BVDMH and 7way vaccines and group 3 calves were vaccinated with only the 7way. At 240 days of age sera was drawn for final SN titers. At 60 days of age high levels of maternal antibody against all the exotoxins tested were detected. Serologic responses in all groups indicated maternal antibody interference after 7way vaccination to *Cl. Perfringens* C and D and in

group 3 to *Cl. novyi and sordellii*. Interference of the immune response to *Cl. novyi* by systemic MLV BHV-1 viral vaccination was seen on day 60 and 210 to *Cl. sordellii*. This study indicates that both maternal antibody and concurrent systemic BHV-1 vaccination may interfere with serologic responses to Clostridial exotoxin vaccination. The impact of the decreased antibody levels on protection could not be determined in this study.

Keywords: Interference; Clostridium vaccination; Calf vaccination; Maternal antibodies

Abbreviations: Clostridium perfringens (Clp) type C and D, Cl. novyi(Cln) and Cl. sordellii (Cls)

Introduction

Clostridia bacteria are spore forming gram positive rods. They are anaerobic and over 200 species are found in nature [1,2]. The bacteria are usually found in dead or dying plant and animal material where their potent enzymes digest the material, breaking it down and using it to as a growth media [2,3]. These potent enzymes, synthesized and released by the bacteria, are called exotoxins. These exotoxins are some of the most potent toxins known to man [3].

Clostridia also have the ability to form highly durable spores when the conditions are not favorable for bacterial growth [3,4]. Clostridial spores can remain viable in the soil for 25 years and are resistant to heat, cold and sunshine [1-4]. These spores can be picked up in the course of normal eating, circulate through the animal and be re-excreted [5]. If there is disruption of blood flow to an area in the body these spores can lodge, and in the anaerobic environment begins to replicate and release toxins causing sudden death [2,6,7]. Even though outbreaks due to Clostridia are rare, after an infection mortality rates are high. Furthermore, the spores are readily found in the soil [1-6]. For these reasons, Clostridial vaccines are among some of the oldest and most widely used bacterins in cattle, sheep and horses [1-4]. These vaccines have been used for well over 60 years in cattle [4,8]. The availability and use of efficacious bacterins, toxoids, and bacterin-toxoids has not eliminated clostridial infections.

However, little research has been done evaluating immune responses with these vaccines in recent years. This study was done to assess the effect of maternal antibody and concurrent vaccination with viral vaccines on serologic responses response of calves to a multivalent Clostridial vaccination program.

Materials and Methods

Animals

The study consisted of 150 newborn calves that were part of the resident Angus base herd. The dams of these calves ranged in age from 3 to 12 years. At birth, calves were individually identified with an ear tag and randomly assigned to treatment group 1, 2 or 3. To ensure that calves vaccinated intra-nasally were not comingled with non-intranasal vaccinated calves, treatment groups were maintained in separate pastures for thirty days after vaccination to minimize the risk of shedding post vaccination to calves not receiving the IN vaccination.

Vaccination

Table 1 shows the assignment of the calves and vaccination, sampling and other management at each time point in the study. On approximately day 60 (range 53-64 days), two serum tubes were collected from each

calf and all calves received a *Clostridium Chauvoei-Septicum-Novyi-Sordellii-Perfringens* Types C and D Bacterin-Toxoid vaccination[a].

Group	60 Days of Age	210 Days of Age	240 Days of Age
Group 1	Serum drawn, nasal swab, IN, BVDMh, 7way	Serum drawn, nasal swab, FivewayMH. 7 way	Serum drawn, nasal swab,
Group 2	Serum drawn, nasal swab, FivewayMH, 7 way	Serum drawn, nasal swab, IN, BVDMh, 7way	Serum drawn, nasal swab,
Group 3	Serum drawn, nasal swab, 7 way	Serum drawn, nasal swab, 7 way	Serum drawn, nasal swab,

Table 1: Schedule of events and products administered

Calves in groups 1, received 2ml of an intranasal vaccine containing modified live BHV-1, BRSV and PI3 virus[b] (IN) vaccination and a modified live viral vaccine containing bovine viral diarrhea virus (BVDV) types 1 and 2 in combination with a *Mannheimia hemolytica* (Mh) inactivated leukotoxoid[c]. Group 2 was administered a MLV bovine viral diarrhea virus (BVDV) types 1 and 2, BHV-1, BRSV, PI3 virus and the same Mh vaccine[d] (FivewayMH)[d]. On day 210, All calves were vaccinated with the seven way clostridial vaccine and serum was drawn. Calves in group 1 were administered the FivewayMH vaccine and calves in group 2 received the IN and BVD/Mh vaccination. On day 240 serum was collected from all the calves. BHV-1 serology was monitored during the study to for the possibility of viral transmission from the intranasal vaccinates.

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 ^{o}C until the completion of the study and shipped to the diagnostic laboratory for laboratory analysis. Antibody to *Clostridium perfringens* beta and epsilon exotoxins (*Cl. perfringens* C and D), *Cl. novyi* and *sordellii* toxin were determined using an ELISA with the following format: beta or epsilon toxin was coated to 96-well plates. The serum samples were tested using a series of two-fold dilutions to determine the endpoint titer. Bound calf antibody was detected using a secondary

antibody conjugate. Each serum sample was tested twice to determine titers. One calf's sample was retested as the initial result was an outlier for beta and epsilon toxin levels.[e]

a. Ultrarace 7, Zoetis, Parsippany, NJ
b. Inforce 3, Zoetis, Parsippany, NJ
c. OneShot BVD Zoetis, Parsippany, NJ
d. BoviShield Gold 5/OneShot, Zoetis, Parsippany, NJ
e. Benchmark Biolabs, f. SAS v9.4, Cary, NC

Statistical Analysis

Since the study involved repeated observations of titers on the same animal, the basic analysis was done using a repeated measures model. Due to the nested structure of treatment groups within pasture, the basic model included fixed effect of treatment group within pasture, day of serum collection and the interaction of treatment group by time within pasture. The calf was considered a random effect. All analyses for the current study were conducted using a commercial statistical program.[f]

Results

Animals

Four calves died during the study period, three were discovered autolyzed in the pasture (one from each group) and the cause of death was not determined and one calf died from pneumonia (group 3). Control calves had BHV-1 serum IgG levels of less than 1:8 during the study period indicating no BHV-1 exposure from vaccinates or wild virus occurred (Figure 1).

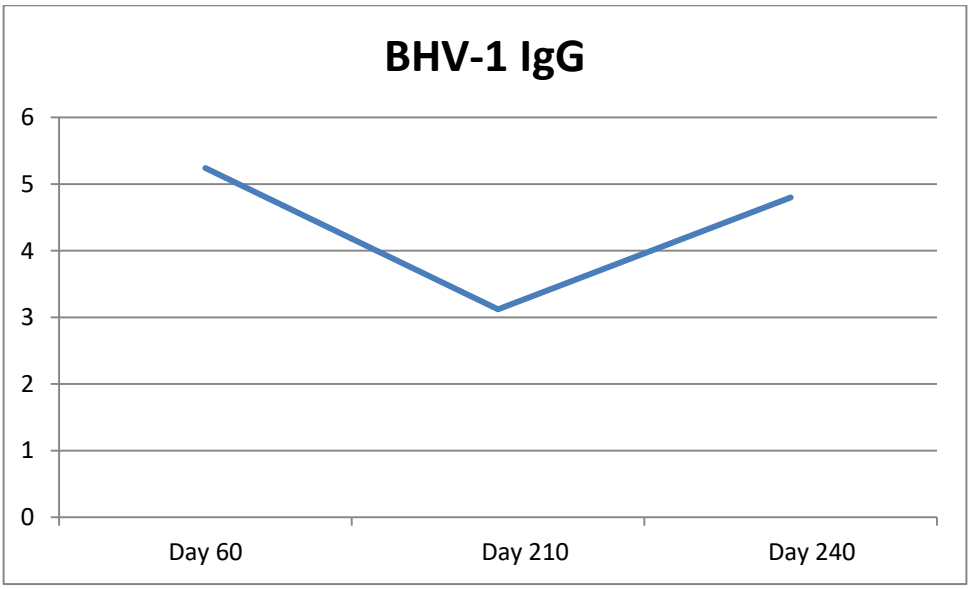

Figure 1: Group 3 IgG geometric mean BHV-1 ELISA titers throughout the study period

Clostridium perfringens C and D

Analysis was performed on actual and log transformed antibody levels. Similar statistical results were seen on both analysis (Tables 2, 3 Figures 2, 3). High levels of *Cl. perfringens* titers were seen across all three groups prior to vaccination on day 60. Each group had significant decreases from the day 60 antibody levels and significant increases following their second vaccination (on day 210) by day 240 to both *Clostridium perfringens C and D*.

Group 1	60	3029.33	425.13	<.0001
Group 1	210	1034.67	425.13	0.0155
Group 1	240	3664.42	599.87	<.0001
Group 2	60	2713.6	329.3	<.0001
Group 2	210	1837.36	332.64	<.0001
Group 2	240	3645.23	599.34	<.0001
Group 3	60	3302.4	329.3	<.0001
Group 3	210	1472	329.3	<.0001
Group 3	240	3025.06	599.33	<.0001

Table 2: LSMean Cl. Perfringens type D (beta) exotoxin neutralizing antibodies

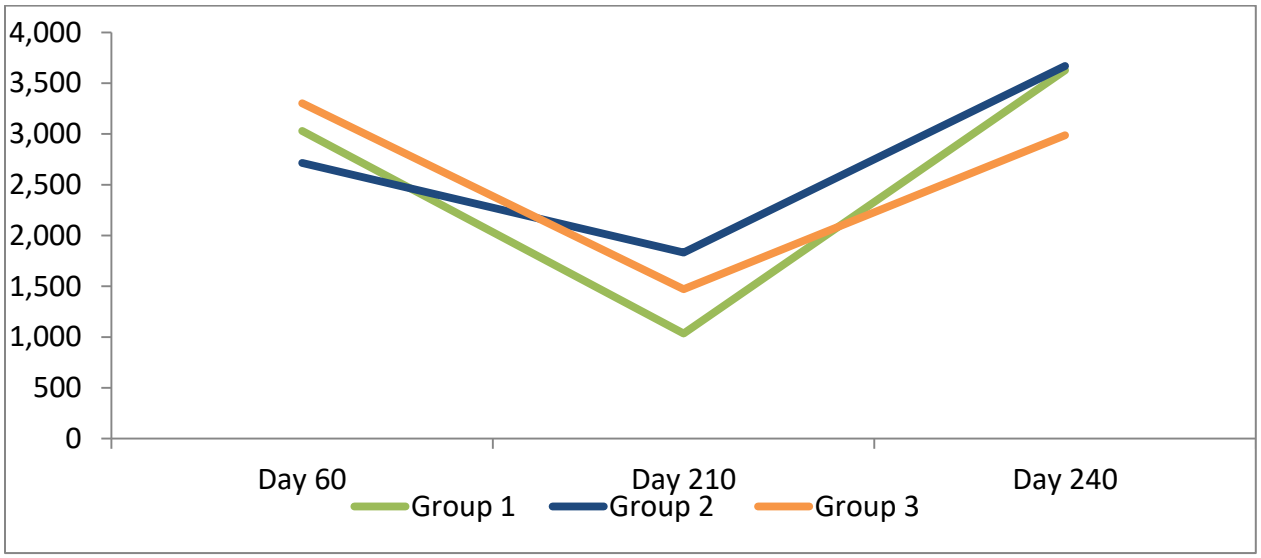

Figure 2: Back transformed geometric mean Cl. perfringens type D (beta) exotoxin neutralizing antibodies

Group	Day	Estimate	Std Err	Pr>t
Group 1	60	901.33	156.43	<.0001
Group 1	210	493.33	156.43	0.0018
Group 1	240	1367.95	221.79	<.0001
Group 2	60	756.8	121.17	<.0001
Group 2	210	486.15	122.41	<.0001
Group 2	240	1098.89	222.02	<.0001
Group 3	60	1440	121.17	<.0001
Group 3	210	366.4	121.17	0.0027
Group 3	240	801.4	222.02	0.0004

Table 3: Mean Cl. Perfringens type D (epsilon) exotoxin neutralizing antibodies

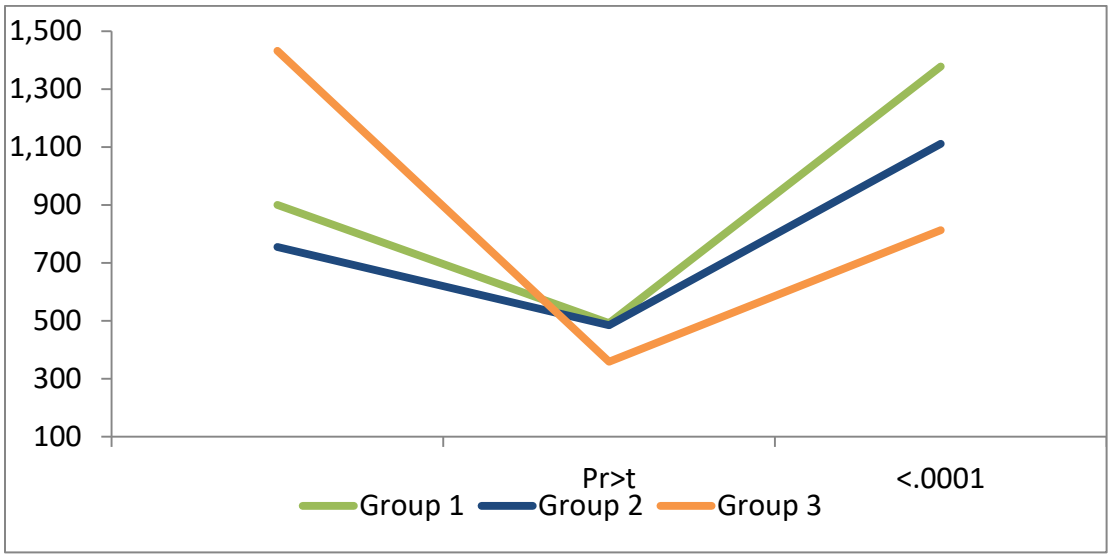

Figure 3: Back transformed geometric mean Cl. perfringens type D (epsilon) exotoxin neutralizing antibodies

Clostridium novyii

For *Cl. novyi* antibodies levels, Treatment Group 3 had significantly higher day 60 maternal antibody levels than the other two groups. While not statistically different, there was a trend (P=.11) for Treatment Group 1 to have higher exotoxin antibody levels than Treatment Groups 2 and 3 on day 210, and group 2 to have higher titers on day 240(p=.17) (Table 4, figure 4). All groups had significantly higher titers at day 240 than day 210.

Clostridium novyii	Day 60	Day 210	Day 240
Group 1	400[a]	512	1109
Group 2	395[a]	402	1280
Group 3	858[b]	429	747
	P<.0001	P<.11	P<.17

Table 4: Back transformed lsmean Cl. novyii exotoxin neutralizing antibodies

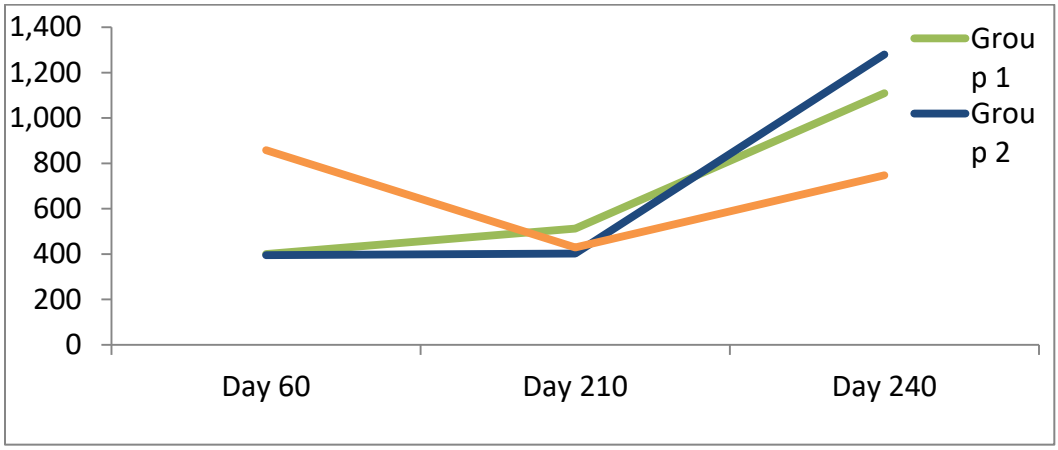

Figure 4: Back transformed LSmean Cl. novyi exotoxin neutralizing antibodies

Clostridium sordellii

Although maternal antibody was higher in the Group 3 calves for *Cl. sordellii*, it was not significant. Both the Group 1 and Group 2 calves had significantly higher day 210 and day 240 antibody titers than Group 3. At day 210, group one had numerically (but not significant) higher antibody levels than group 2. At 240 days, Group 2 had significantly higher antibody levels than Group 1(Table 5, figure 5). All groups had significantly higher titers at day 240 than day 210.

Clostridium sordelli	Day 60	Day 210	Day 240
Group 1	704	533[a]	1835[a]
Group 2	747	470[a]	2219[ab]
Group 3	883	342[b]	1109[ac]
	P<.26	P<.004	P<.05

Table 5: Back transformed lsmean *Cl. sordellii* exotoxin neutralizing antibodies

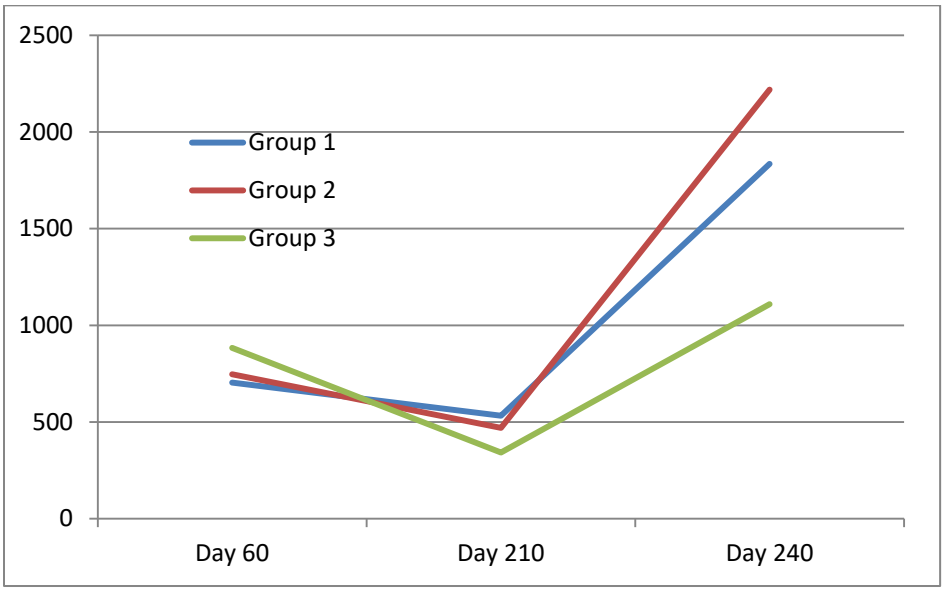

Figure 5: Back transformed lsmean *Cl. sordellii* exotoxin neutralizing antibodies

Discussion

The herd was chosen for this study, in part, because the cows in this herd had received no vaccine for several years. However, the high titers against *Clostridium perfringens* exotoxins, seen in the sixty day old calves' pre-vaccination, were most likely due to maternal transmission. While it cannot definitively be attributed to colostral transfer and not exposure, the fact the antibody levels declined until a second dose of vaccination was given on day 210, when the antibody levels had dropped, would indicate continual maternal decay not active immunity in the calves. While the maternal antibody transmission was not as high for *Cl. novyi* and *sordellii*, it was still high enough to block serologic responses in some of the calves. Whether the high level of maternal antibody is an indication of the ongoing exposure in the cows or persistence of previous vaccine responses could not be determined in this study but has been reported previously [9,10].

The lack of response to the initial doses is most likely due to maternal blockage of vaccination. It is important to note that the two groups with lower 60 day *Cl. novyii* and *sordellii* (group 1 and 2) did not show the decline of antibody following the sixty day vaccination and over the long time frame between vaccinations actually had some increase in antibody responses. It can be assumed that higher levels of maternal antibody can block clostridial vaccines responses, whereas moderate to low maternal antibody levels do not. The level of antibody in which blockage of vaccination occurs may vary by the vaccine antigen and/or formulation with different adjuvants [11]. For protection against early clostridial infections it may be more efficient to vaccinate the cows to increase colostral transmission of the colostridial antibodies and wait to vaccinate calves until they are older [10].

While only significant on the day 240 *Cl. sordellii* antibody levels, it is important to note the responses and the correlation to which vaccines were administered at each timeframe. For both *Cl. novyii* and *Cl. sordellii*, the higher immune response was seen at both time-points following vaccination if it was co-administered

with the intranasal MLV vaccine. Normally the higher booster response to the same vaccine would be anticipated with the calves that had the higher initial response to the vaccine, but the groups flipped as to the higher response and the calves with the numerically lower initial response to vaccination had the higher booster response. Interference of the serologic response to bacterins co-administered with systemic BHV-1 vaccination has been previously published as has the lack of interference when the co-administration of an intranasal of the BHV-1 response. This is another example of the ability of systemic BHV-1 vaccines to decrease the immune response to co-administered bacterins. If a maximum response to clostridial vaccines is desired, then co-administration with an intranasal BHV-1 vaccine should be considered.

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