Effects of gastrointestinal parasites on parasite burden, rectal temperature, and antibody titer responses to vaccination and infectious bovine rhinotracheitis virus challenge^1

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**ABSTRACT:** Thirty-three colostrum-deprived Holstein bull calves (initial BW of 131 ± 4 kg) were used to determine the effect of timing of anthelmintic administration relative to vaccination on antibody titer response to vaccine component antigens. When calves were at least 3 mo of age, they were sorted randomly into individual pens and assigned to 1 of 3 treatment groups, treatments consisted of 1) dewormed 2 wk before vaccination (DPV), 2) dewormed at the time of vaccination (DV), or 3) control, vaccinated but not dewormed (CONT). All calves were inoculated with infective larvae of brown stomach worms (Ostertagia ostertagi) and intestinal worms (Cooperia spp.) on d 1, 7, 10, 14, and 18 for a total dose of 235,710 infective larvae per calf. Calves (DPV and DV) were dewormed on d 21 or 35 with a 10% fenbendazole suspension at 5 mg/kg of BW. On d 35, all calves were vaccinated with a modified-live virus respiratory vaccine containing IBRV (infectious bovine rhinotracheitis virus), BVDV-1, BVDV-2, and PI-3 and cytokine levels for IL-4, IL-6, TNF-α (tumor necrosis factor-α), and IFN-γ (interferon-gamma). There was a tendency (P < 0.09) for CONT calves to have greater IL-4 concentrations. By design, control calves had greater (P < 0.01) fecal egg counts during the experiment. All calves developed antibody titers to IBRV and blood samples were obtained on d 88, 89, 90, 93, 95, 98, 99, and 103. All calves had increased rectal temperatures during the final 7 d of the IBRV challenge. However, the CONT group had greater (P < 0.01) rectal temperatures on each sampling day except d 90 compared with the DPV and DV treatments. Therefore, deworming before or at vaccination reduced parasite burden and decreased rectal temperature increase after an IBRV challenge. Deworming strategy had no effect on antibody response to vaccination or IBRV challenge.

**Key words:** calf, gastrointestinal parasite, immunity, titer, vaccination

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**INTRODUCTION**

Gastrointestinal parasite burden is one of the largest health concerns for ruminants worldwide (Armour, 1980). Performance has been shown to decline in relationship to parasite burden (Lee, 1955; Reinhardt et al., 2006). Gastrointestinal parasitism results in a wide range of effects from subclinical disease to death depending on parasite load, animal age and breed,
plane of nutrition, and overall health status of the animal (Hawkins, 1993). Calves treated with anthelmintics resulted in an improvement of $0.08 to $0.14/kg of BW gain after 41 d posttreatment (Leland et al., 1980). Grimson et al. (1987) reported that average sale prices were greater for calves given antiparasitic treatments vs. untreated calves, which reflects the perception of buyers that treated calves will outperform untreated calves.

Vaccinations are arguably the most cost-effective means for preventing disease, especially in feedlot environments. Of the schedules most currently used, the most effective deworming and vaccination program to prepare calves for entry into the feedlot has not been determined. It is critical that good management practices are implemented in conjunction with vaccination programs to ensure vaccine efficacy is not compromised. Most calves are vaccinated for respiratory-type infections and dewormed at weaning just before entering an on-site background facility or being transported to a feedlot (Bagley, 2001). Cytokine release associated with parasitic infestation can interfere with the immune response to foreign antigens, potentially affecting the ability of an animal to immunologically respond to vaccination (Kullberg et al., 1992; Urban et al., 2007). The objective of the present study was to determine whether the timing of anthelmintic administration relative to vaccination influences antibody titer response to vaccine components, rectal temperature, and antibody titer response to an intranasal challenge with infectious bovine rhinotracheitis virus (IBRV).

MATERIALS AND METHODS

Before the initiation of this experiment, care, handling, and sampling of the animals defined herein were approved by the Colorado State University Institutional Animal Care and Use Committee.

To ensure that no passive immunoglobulin transfer occurred and that all calves had similar immune responses, 33 colostrum-deprived Holstein bull calves (BW 131 ± 4.0 kg) were used in this experiment. Calves were obtained during the winter from a single local dairy immediately after birth and transported to Colorado State University’s Agricultural Research Development and Education Center (ARDEC) located in Fort Collins. Upon arrival, all calves were weighed, given a unique numerical identification ear tag, given 1.0 mL of a vitamin A and D solution (AgriPharm, Memphis, TN), and rectal temperatures were obtained. Calves were then housed in individual calf huts (1.5 × 1.9 m).

Growing Phase

At 2 wk of age, a jugular blood sample (10 mL) was collected in a nonheparinized Vacutainer tube (Becton Dickinson Co., Franklin Lakes, NJ) for the determination of infectious bovine rhinotracheitis virus (IBRV), bovine viral diarrhea virus genotype (BVDV)-1, BVDV-2, and parainfluenza-3 (PI-3) antibody titers. Calves were bottle-fed whole milk twice daily for the first 2 wk of life. Calves were then gradually transitioned to a milk replacer diet (Maxi Care 22-20 NT Medicated dairy herd and beef calf milk replacer, Land O’Lakes Animal Milk Products Co., Shoreview, MN) over a 2-wk period (step 1: 60% whole milk and 40% milk replacer; step 2: 75% milk replacer and 25% whole milk; and step 3: 100% milk replacer). Calves remained on milk replacer for approximately 45 d. Calves had ad libitum access to water and medicated calf starter (20% CP, 7% crude fiber, and 2.25% crude fat) 3 d postbirth. Calves were weaned when they were consuming 1.81 kg of starter ration for 5 consecutive days (approximately 60 d of age).

Once weaned, calves were fed once daily in the morning and gradually transitioned to an alfalfa steam-flaked, corn-based growing diet (62.38% alfalfa hay, 15.60% steam-flaked corn, 4% cane molasses, and 18.02% premix). Diets were formulated to meet or exceed all nutrient requirements for growing Holstein bull calves (NRC, 1989). Once transitioned to the growing diet, calves were fed twice daily at 0700 and 1600 h in amounts adequate to allow ad libitum access to feed throughout the day, and orts were weighed and recorded daily. When all calves were weaned and acclimated to the basal growing diet, calves were moved from the calf huts into individual pens (2.0 × 13.0 m) equipped with an automatic water fountain and a concrete feed bunk. All calves were fed the growing diet until the youngest calf was 3 mo of age. When the youngest calf was 3 mo of age, the initiation of the experiment began (Figure 1).

This study was conducted during the summer and early fall. Upon initiation of the experiment, individual BW was obtained on 2 consecutive days and calves were blocked by BW and age and assigned to 1 of 3 treatment groups. Treatments consisted of 1) dewormed 2 wk before vaccination (DPV), 2) dewormed at the time of vaccination (DV), and 3) control, vaccinated but not dewormed (CONT). Individual feed intake and health status were recorded daily. Calves were determined to be morbid if rectal temperatures exceeded 39.7°C and were treated as prescribed by the attending veterinarian. Fecal samples, rectal temperatures, and a jugular blood sample (10 mL, collected in a nonheparinized Vacutainer tube; Becton Dickinson Co.) were obtained from each calf weekly.
All calves (average age = 130 ± 22 d.) were orally inoculated 5 times with 23,571 infective larvae of brown stomach worms (*Ostertagia ostertagi*) and 23,571 intestinal worms (*Cooperia* spp.) on d 1, 7, 10, 14, and 18 of the study for a total infective larvae dose of 235,710 per calf. Immediately before and at each parasite inoculation, a fecal sample was collected per rectum.

**Deworming Phase**

On d 21 (3 wk post-initial parasite inoculation), DPV calves were dewormed orally with a 10% fenbendazole suspension (Safe-Guard, Intervet, Millsboro, DE) at 5 mg/kg of BW 2 wk before vaccination. On d 35, DV calves were dewormed and all calves were vaccinated subcutaneously with 2 mL of a modified-live virus respiratory vaccine containing IBRV, BVDV-1, BVDV-2, PI-3, and BRSV (Vista 5SQ, Intervet-Schering Plough Animal Health, and De Soto, KS).

**Postvaccination Phase**

Daily and weekly feed intake and health observations were recorded, and samples were obtained postvaccination, as described previously. On d 88 (53 d postvaccination), all calves were challenged intranasally with 4 mL of a solution containing $1.8 \times 10^7$ cell culture infectious doses of the Cooper strain of bovine herpesvirus-1 (*BHV-1*) via nebulization (2 mL/nostril). Blood samples (10 mL) were obtained from all calves on d 0, 1, 3, 4, 6, 8, 10, and 12 d postinoculation, and rectal temperatures were obtained every morning before feeding. Fourteen days post-*BHV-1* challenge, all calves were euthanized, and necropsies were performed for a separate experiment (data not presented).

**Analytical Procedures**

**Blood Preparation.** Blood was stored on ice, transported to the laboratory, and stored in a refrigerator at 5°C for 12 h to allow clot formation. Whole blood was then centrifuged at 1,200 × *g* for 25 min at room temperature. The serum was harvested and stored in polyethylene tubes (12 × 75 mm) at −70°C. Serum was analyzed for IBRV, BVDV-1, BVDV-2, and PI-3 serum-neutralizing (SN) antibodies using a microtiter serum neutralization format (Carbrey et al., 1971). After heat inactivation at 56°C for 30 min, 2-fold serial dilutions of serum were made in triplicate wells in a 96-well microtiter plate for each of the viruses. One hundred 50% tissue culture infectious dose (TCID$_{50}$) of IBRV, BVDV-1, BVDV-2, or PI-3 was added to duplicate columns of wells. The third column of diluted serum served as the serum control.
The microtiter plates with virus and serum samples were incubated for 1 h at 37°C. A suspension of Madin-Darby bovine kidney (MDBK) cells at a concentration of $1 \times 10^5$ cells/50 μL were added to each well containing BHV-1 virus, and bovine turbinate (BT) cells were added to each well for BVDV-1, BVDV-2, or PI-3. The microtiter plates were incubated at 37°C for an additional 3 d, and then cells were examined for cytopathic effects of each of the test viruses using an inverted light microscope. The reciprocal of the highest dilution at which the test virus was completely neutralized was recorded as the SN titers for each virus and sample.

**Fecal Analysis.** Approximately 100 g of fresh fecal matter was placed in an individual plastic bag, labeled, and placed on ice. The samples were refrigerated until analyzed. Samples were shipped to an independent laboratory (Animal Production Consulting, Lincoln, NE) for analysis. The modified Wisconsin sugar flotation technique (Cox and Todd, 1962) was utilized to examine each individual fecal sample. A 3-g base sample was used for analysis. An egg- per-gram count was determined by multiplying the total count by 150 and then dividing that number by 454.

**Cytokine Immunosorbent Assay.** All serum analyses were assayed in duplicate. Serum concentrations of pro-inflammatory cytokines TNF-α, IL-4, IL-6, and IFN-γ were determined per the manufacturer’s protocol using a bovine-specific custom-developed multiplex ELISA that was commercially validated for bovine cytokines (SearchLight, Pierce Biotechnology Inc., Rockford, IL). For all cytokines, as determined from running replicates of pooled serum on all plates, the intraassay CV was less than 10% and the interassay CV was less than 20%. The minimum detectable concentrations in this multiplex ELISA for TNF-α, IL-4, IL-6, and IFN-γ were 0.5, 2.6, 3.3, and 0.1 pg/mL, respectively.

**Statistical Analysis.** Statistical analyses of data were performed for a completely randomized block design using the MIXED procedure (SAS Inst. Inc., Cary, NC). Calf was considered the experimental unit. Initial BW was used as a blocking factor. Where appropriate, repeated measures analyses were used. The model for ADG, DMI, eggs per gram, and temperature contained treatment, day, and all possible interactions. Each phase (growing, parasite inoculation, postvaccination, and post-IBRV challenge) was analyzed independently. When treatment × day interactions were significant ($P < 0.05$), the effect of treatment was analyzed for each day. Logarithmic transformations were applied to all titer values.

**RESULTS AND DISCUSSION**

**Performance**

Performance was similar across all treatments. No BW, DMI, or ADG ($P > 0.05$) differences among groups were shown to exist during the duration of this trial. These results were in contrast with previous studies in which feed intake was reduced in calves infected with *O. ostertagi* larvae (Horak and Clark, 1964; Fox et al., 2002). Earlier work reported BW loss in cattle infected with internal parasites (Anderson et al., 1965; Wiggins and Gibbs, 1990) and greater ADG in cattle treated with deworming agents (Flack et al., 1967). A possible reason for the conflicting results between experiments may be due to the differences in the environmental conditions in which the cattle were reared. In previously cited experiments, cattle were raised in groups, whereas cattle in this experiment were reared in individual pens. Additionally, although the parasite inoculation loads were similar between this study and earlier studies, the duration of days spent inoculating calves differed. Total infective larvae inoculations were given as a single dose in earlier studies (Horak and Clark, 1964; Fox et al., 2002), and parasite inoculation was carried out over several different days with smaller daily doses in this study. The reason for the multiple parasite inoculations was to maximize parasite establishment in the gut and to help to overcome any gut immune responses that may occur during inoculation.

During period 3 (postvaccination phase), CONT calves had greater ($P = 0.04$) rectal temperatures (Figure 2) compared with DPV and DV treatments on d 49 and had greater rectal temperatures ($P < 0.01$) throughout the period. During period 4 (IBRV challenge phase), the CONT group had greater ($P < 0.01$) rectal temperatures on each sampling day except d 90 compared with the DPV and DV treatments. Determining bovine parasitic disease can be challenging, and the measurement of fever (pyrexia) via rectal temperature has been shown to be a viable
way to detect infection (Magona et al., 2009). Reinhardt et al. (2006) reported that heifers treated with a combination fenbendazole (Safeguard, Intervet Inc., Millsboro, DE) oral drench (5 mg of fenbendazole/kg of BW) and an ivermectin (Ivomec, Merial, Duluth, GA) pour-on (500 μg of ivermectin/kg of BW) tended to have reduced morbidity rates (as determined by rectal temperature) compared with heifers given an ivermectin pour-on alone.

By the beginning of period 2 (approximately 14 d after initial infestation), parasite eggs were detected in feces (Figure 3). By design, CONT animals had greater ($P < 0.01$) fecal egg counts for periods 2 and 3. By period 4, the fecal egg counts in the CONT group had decreased and no significant differences were detected. The decrease in fecal egg count over time has been associated with the development of effective immunity to the parasites by the host (Gordon, 1948). After a parasite inoculation, T and B lymphocytes react to parasite antigens and help to protect the gastrointestinal tract from the parasites (Claerebout and Verdruysse, 2000). Cytokines play a major role in controlling parasitic infection and can help in the expulsion of adult worms. Immunity against $O. ostertagi$ in ruminants is unique as a reduction of worm fecundity has been shown to occur in calves, possibly regulated by the local IgA response. Thus, fecal egg counts would only be reduced after a prolonged period of host-parasite contact (Claerebout and Verdruysse, 2000).

**Immune Variables**

All calves in all treatment groups developed SN titers for IBRV, BVDV-1, BVDV-2, and PI-3 by d 15 post-vaccination (Figure 4). Additionally, DV animals had increased ($P = 0.02$) titers for BVDV-1 during period 3 on d 66, 73, and 79. During period 1, the CONT calves had greater ($P < 0.02$) titers for PI-3. The effects of an $O. ostertagi$ infection can cause interference in the ability of the infected calf to produce an adequate immunological response to vaccination.

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**Figure 3.** Average fecal egg counts (FEC; SE = 7.97) of parasite-inoculated calves ($n = 33$) dewormed before vaccination (DPV), dewormed at vaccination (DV), or never dewormed but vaccinated (CONT) by period where periods were broken down as follows: 1) period of parasite inoculation, 2) period beginning postdeworming of DPV, 3) period directly preceding vaccination of all treatments, and 4) the 2 wk postslaughter.

**Figure 4.** Titer responses (log-transformed) for infectious bovine rhinotracheitis virus (IBRV; 3.13 ± 0.16), bovine viral diarrhea virus genotype (BVDV)-1 (4.55 ± 0.29), BVDV-2 (4.90 ± 0.26), and parainfluenza-3 (PI-3; 4.92 ± 0.24) for parasite inoculated calves ($n = 33$), dewormed before vaccination (DPV), dewormed at vaccination (DV), or never dewormed but vaccinated (CONT) by period where periods were broken down as follows: 1) period of parasite inoculation, 2) period beginning postdeworming of DPV, 3) period directly preceding vaccination of all treatments, and 4) the 2 wk postslaughter. There were no overall effects of treatment ($P > 0.05$).
Th-1 immune response may decrease the ability of a vaccine. In a study conducted by Su et al. (2006), a gastrointestinal infection downregulated Th-1 cytokine response for IL-2 and IFN-γ concentrations such that concentrations of TNF-α were 344.49 ± 460.76, 1,837.13 ± 460.76, and 665.62 ± 460.76 pg/mL for the DPV, DV, and CONT calves, respectively.

Animals with parasitic infections have altered cell-mediated immune responses to nonparasitic antigens such as vaccines (Kullberg et al., 1992). Helminth infections can cause suppression of the immune response of the host to vaccine components, thus inhibiting the efficacy of the vaccine. In a study conducted by Su et al. (2006), a gastrointestinal nematode-malaria model was used to determine if concurrent helminth parasitic infections impair the effects of vaccine-induced protective immunity against malaria. A nematode infection indeed suppressed the helper T cell-1 (Th-1) associated immune responses to immunization and reduced the protective efficacy of the vaccine. It was concluded that deworming would be an effective strategy for improving vaccine efficacy.

Alterations in immune responses to foreign nonparasitic antigens have been observed in murine models infected with parasites. In a study conducted by Kullberg et al. (1992), the effect of a Schistosoma mansoni infection downregulated Th-1 cytokine response for IL-2 and IFN-γ compared with immunized uninfected controls. A downregulated Th-1-dependent immunity could lead to an increased susceptibility to infection as well as altered immune responses to vaccinations (Kullberg et al., 1992). Fasciola hepatica has been shown to cause an upregulation of Th-2 (helper T cell-2) immune response specifically associated with an increase in the cytokine IL-4, which inhibits certain Th-1 responses to foreign antigens (Flynn et al., 2007). Inhibition of the Th-1 immune response may decrease the ability of an animal to respond to an intracellular pathogen such as Mycobacterium bovis (Flynn et al., 2007).

These prior studies suggest that specific parasite infections have significant effects on the immunological ability of calves to properly combat a viral infection, which could have implications regarding vaccine efficacy because parasitic infection could influence the ability of Th-1 and Th-2 immunity, leading to an upregulated cytokine response to infection.

Overall, these data indicated that deworming colostrum-deprived Holstein bull calves 2 wk before, or at the time of vaccination, reduced parasite burden and rectal temperature response after an IBRV challenge. However, timing of deworming relative to vaccination had no impact on SN titers to viral vaccine components or cytokine response to IBRV challenge. Further studies are needed to gain a more fundamental understanding of the relationship between parasite burden and vaccine efficacy in regard to the immunological associations and economic implications for cattle production.

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### Table 1. Influence of deworming calves before or at vaccination on cytokine concentrations

| Trait       | Treatment | Linear | Quadratic |
|-------------|-----------|--------|-----------|
| IL-4, pg/mL | DPV       | 2.07   | 3.76      | 0.05 |
| IL-6, pg/mL | DV        | 171.09 | 139.15    | 33.60 |
| TNF-α, pg/mL| CONT      | 1,837.13 | 665.62 | 460.76 |
| IFN-γ, pg/mL|           | 87.49  | 89.52     | 30.96 |

1TNF-α = tumor necrosis factor-α; IFN-γ = interferon-γ.
2DPV = calves dewormed 2 wk before vaccination; DV = calves dewormed at vaccination; CONT = calves were vaccinated but never dewormed after parasite inoculation.
3P-values were considered significant at P < 0.05.
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