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Health benefits of orally administered anti-IL-10 antibody in milk-fed dairy calves

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ABSTRACT

The primary objective of this randomized controlled trial was to determine whether anti-IL-10 egg yolk antibodies fed upon arrival to a calf ranch would lower the prevalence of *Cryptosporidium parvum* shedding in naturally challenged preweaned dairy calves. The secondary objectives included measuring the effect of anti-IL-10 antibodies on calf health, performance, and shedding of less common diarrheal pathogens. A total of 133 calves, enrolled at 24 to 72 h of age, received a daily dose of 0.96 g of egg yolk powder with anti-IL-10 antibodies (MAB, n = 71) or without anti-IL-10 antibodies (MEP, n = 62) split between 2 feedings for the first 11 d on feed at a calf ranch. Daily health evaluations were completed for 15 d after arrival and on d 56. Digital weights were collected at enrollment and d 56, and hipometer weights were collected at enrollment and d 7 and 56. Packed cell volume and serum total protein concentration were measured at enrollment and on d 7 and 14. Fecal pH was measured at enrollment and on d 5 and 14, and fecal pathogen (*C. parvum*, coronavirus, rotavirus, and *Salmonella* spp.) shedding was assessed at d 5 and 14. Continuous outcomes were compared between groups using a Student's *t*-test or Wilcoxon rank sum test. Fecal pathogen shedding at d 14, respiratory disease at d 56, and antibiotic usage were compared using relative risk (RR) and chi-squared test. Fecal pH (median and interquartile range) on d 14 was 6.65 (6.39–6.99) and 6.52 (5.97–6.81) for MAB and MEP, respectively. On d 56, the risk of respiratory disease was lower for MAB compared with MEP (RR = 0.40; confidence interval = 0.16–0.99). The risk for antibiotic treatment was lower for MAB- compared with MEP-treated calves (RR = 0.38; confidence interval = 0.17–0.88). The risk of shedding rotavirus was higher in MAB (RR = 1.38; confidence interval = 1.10–1.81) calves. After multivariable analyses, hipometer weights (least squares means ± standard error) were 1.7 ± 0.8 kg greater on d 56 in MAB compared with MEP; however, ADG was 0.04 ± 0.02 kg/d lower in MAB calves. Total health score, diarrhea days, average respiratory score, packed cell volume, and serum total protein were not affected by feeding anti-IL-10 egg antibodies. In summary, feeding anti-IL-10 antibodies was associated with increased fecal pH, reduced risk of respiratory disease later in the preweaning period, and decreased antibiotic usage despite higher rotavirus infection. These findings might be associated with improved mucosal immunity, enhanced host defenses, or reduced susceptibility and warrant further investigation.

Key words: *Cryptosporidium parvum*, fecal pH, bovine respiratory disease, antibiotic alternative, neonatal calf diarrhea

INTRODUCTION

Neonatal calf diarrhea (**NCD**) and bovine respiratory disease (**BRD**) are the most common causes of morbidity and mortality in preweaned dairy calves (Windeyer et al., 2014; Cramer and Stanton, 2015). Although NCD and BRD may be independent events affecting calves before weaning, the risk of BRD is increased in calves that have had other disease problems such as NCD in the first 2-wk of life (Windeyer et al., 2014). Effective prevention, detection, and treatment are paramount for controlling NCD and, therefore, BRD, but the development of farm protocols is challenging because of the mixed array of causative organisms (viral, bacterial, or parasitic), the prevalence of mixed infections, and the potential role of nutrition. *Cryptosporidium parvum*, rotavirus, coronavirus, and *Salmonella* spp. are important enteropathogens of 5- to 14-d-old calves (McGuirk, 2008). Treatment is challenging because approved antimicrobial drugs have questionable efficacy (Smith, 2015) and antibiotic use may increase emergence of resistant bacteria (Berge et al., 2006). With few effective treatment and prevention options, passive transfer of the maternal antibody remains a mainstay in managing NCD. However, recent

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studies have provided evidence for the effectiveness of egg yolk–derived antibody protection against diarrhea (Yokoyama et al., 1998; Mine and Kovacs-Nolan, 2002; Vega et al., 2011; Diraviyam et al., 2014).

Egg yolk antibodies can target luminal contents or luminal receptors of specific pathogens, such as *Salmonella* Typhimurium and *Salmonella* Dublin; are not absorbed after gut closure (Vega et al., 2011); protect calves from disease (Yokoyama et al., 1998; Diraviyam et al., 2014); and pose no food safety concerns (Cook and Trott, 2010). Immunoglobulin Y (IgY), the major antibody in egg yolk, is functionally similar to mammalian IgG and economically feasible to produce in a noninvasive manner (Lee et al., 2012).

As a cause of NCD, protozoal infections due to *C. parvum* are particularly challenging because of the high prevalence of the organism, environmental persistence, and public health concerns (Nydam and Mohammed, 2005). In particular, *C. parvum* causes enterocyte changes resulting in diarrhea, dehydration, and abnormal gut fermentation in the large bowel, producing fecal acidity (Sato, 2010; Wyatt et al., 2010).

Recovery from *C. parvum* depends largely on the cytotoxic activities involved in a T helper 1 response (Weaver et al., 2000; Wyatt et al., 2010). More specifically, in calves recovering from *C. parvum* infection, IFN-gamma and IgG_2 have been documented, signifying a T helper 1 response (Wyatt et al., 2001).

Research has shown that the protective T helper 1 immune response is suppressed by IL-10, an anti-inflammatory cytokine produced by intraepithelial lymphocytes of calves infected with *C. parvum* (Wyatt et al., 2002). Interleukin-10 knockout mice are more resistant to *C. parvum* infection (Campbell et al., 2002), suggesting that Il-10 induction may prolong *C. parvum* infection and slow recovery. Provision of an oral antibody to IL-10 to broiler chickens infected with the coccidian parasite, *Eimeria* spp., eliminated growth depression (Sand et al., 2016). Therefore, the primary objective of this study was to determine whether anti-IL-10 egg yolk antibodies fed upon arrival to a calf ranch would lower the fecal shedding of *C. parvum* in preweaned dairy calves after natural exposure. Our secondary objectives included measuring the effect of anti-IL-10 antibody on calf health, performance, fecal acidity, and shedding of less common diarrheal pathogens.

MATERIALS AND METHODS

Animals and Experimental Groups

This study was performed on a single calf-raising facility in southwest Wisconsin. A total of 134 calves from 12 source dairies were enrolled on 5 d over a 2 wk period (July 29, 2014, through August 7, 2014) as they arrived at a calf-raising operation. Calves were 24 to 72 h of age on the day of enrollment and had been administered colostrum at their home farms. Male and female Holstein and Jersey calves were included. A random number generator was used on each enrollment day to randomize calves into 3 color-coded groups to receive a daily dose of 0.96 g of egg yolk powder with anti-IL-10 antibodies (**MAB**: green and purple) or without anti-IL-10 antibodies (**MEP**: orange). Color-coded zip ties were attached to each outdoor hutch to ensure all calves received the correct dose. All calf feeders, health screeners, and farm personnel were blinded to the color code. This study was approved by the Research Animal Resource Center and the Animal Care and Use Committees in the School of Veterinary Medicine at the University of Wisconsin–Madison (Protocol: V01637).

Calf Housing and Feeding Management

Upon arrival calves were housed in indoor, individual pens with straw bedding in a naturally ventilated barn for 1 to 4 d. Once calves were nursing well from a bottle, calves were moved to an individual, outdoor hutch, where they remained for the duration of the study. Calves were fed pasteurized waste milk by nipple bottle. Calves were fed 2 L twice per day at 12-h intervals while in indoor housing. Once in the outdoor hutch, calves were fed 2 L 3 times a day until 21 d, when they were fed 3 L twice daily until 49 d. At 49 d calves were fed 3 L once daily until d 56, when they were weaned. Water and an 18% calf starter texturized feed was offered upon arrival to the outdoor calf hutch.

Formulation of Anti-IL-10 Antibodies

Single Comb White Leghorn laying hens raised for life in cages with raised wire were injected (100 μg of conjugate per chicken) with bovine IL-10-bovine gamma globulin-vmpqaenh conjugate emulsified with Freund's Complete Adjuvant at 1:1 vol/vol (Thermo Fisher Scientific Inc., Waltham, MA) for a total volume of 1 mL per chicken. The protein sequence VMPQAENH was used to conjugate to the carrier protein bovine gamma globulin. Sequence VMPQAENH is the portion of the IL-10 sequence that was used to make the antibody to IL-10 for use in dairy calves. This protein sequence was made at GenScript (Piscataway, NJ). Hens injected with adjuvant and bovine gamma globulin only (no peptide conjugate) were used for making control antibodies. Chickens were injected 1 wk later using the antigens described above. Eggs were collected beginning 3 wk after the initial injection for a period of 8 wk.