Efficacy of oral immunization with *Cryptosporidium parvum* in a colostrum-deprived neonatal calf challenge model

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**Introduction**

A major cause of reduced performance and death in neonatal dairy calves is diarrhea, regardless of cause. *Cryptosporidium parvum* is a protozoal pathogen that is frequently associated with diarrhea in neonatal dairy calves. Virtually all dairy calves that are raised on commercial operations are infected with *C. parvum* by 3 weeks of age. Conventional colostrum does not contain protective levels of antibodies against *C. parvum*, and therapeutic agents with efficacy for prevention of *C. parvum* oocyst excretion or reduction of clinical severity of diarrhea are lacking. The objective of this study was to determine the efficacy of an inactivated *C. parvum* preparation as an oral immunogen in a colostrum-deprived calf challenge model.

**Materials and Methods**

The study was conducted at a research facility in central Iowa. Clean-caught, colostrum-deprived calves were immediately removed from the dam at birth and transferred to individual isolated housing. All calves (n=44) without abnormal clinical signs were enrolled in the study. Calves were weighed at birth and at 10, 24, and 31 days of age. From each calf, a fecal sample was collected daily through 31 days of age. Fecal consistency was scored twice daily at feeding on a descriptive graduated scale of 1 to 6. All calves were fed 2 quarts of commercial milk replacer twice daily with approximately 8 hours between the morning and afternoon feedings. Calves that refused to voluntarily consume the entire 2 quarts of a feeding were fed the remainder with an esophageal feeder. During the study period, calves did not have access to supplemental water or solid feed and were not administered electrolytes, antimicrobials, or other therapeutics. Calves were blocked into pairs on the basis of birth order and then randomly assigned to each treatment. At 10 days of age, all calves were orally inoculated with 20,000 *C. parvum* oocysts that were obtained from stock purified calf passage harvest. Collected fecal samples were assayed with a fluorescent antibody technique to determine the titer of *C. parvum* oocysts. Calves that died during the study were necropsied to determine cause of death. Outcomes of interest evaluated included incidence of clinical diarrhea, extent of oocyst shedding, weight gain, and mortality rate.

**Results**

Prior to *C. parvum* challenge inoculation, oocysts were not detected in the feces of any calf, and mean daily fecal consistency score and average daily gain (ADG) did not vary significantly between treatment groups. On days 4 through 7 after challenge inoculation, calves fed the *C. parvum* test article had significantly (P<0.05) lower mean fecal consistency scores than did calves fed the placebo. During the 21-day observation period immediately following challenge inoculation, all 22 calves fed the placebo treatment had at least 1 fecal consistency score ≥ 3, whereas only 15 of 22 (68%) of calves fed the *C. parvum* test article had at least 1 fecal consistency score ≥ 3. The morbidity score was ≥ 3 for 28% and 12% of calf days evaluated for the calves in the placebo and *C. parvum* test article treatment groups, respectively, and on days 2 through 7 after challenge inoculation the mean morbidity score for the *C. parvum* treatment group was significantly (P<0.05) lower than that for the placebo group. Incidence of oocyst fecal shedding was significantly (P<0.05) lower for the *C. parvum* treatment group than that for the placebo group on days 5 through 10 after challenge inoculation, and mean fecal oocyst titer was significantly (P<0.05) less for the *C. parvum* treatment group compared with that for the placebo group on days 4 through 8 after challenge inoculation. During the 14 days immediately after challenge inoculation, ADG was significantly (P<0.05) higher for the *C. parvum* treatment group, compared with the ADG for the placebo group. During the 21-day observation period following challenge inoculation, none of the calves in the *C. parvum* treatment group died, whereas 3 of 22 calves in the placebo group died.

**Significance**

Repeated oral administration of an inactivated *C. parvum* preparation to neonatal dairy calves resulted in significant improvements in fecal consistency and ADG,
and reduced fecal oocyst shedding and mortality rate, compared with those for calves that were administered a placebo. Studies are warranted to determine whether the inactivated C. parvum preparation will have similar efficacy in the field. If it does, the immediate short-term benefits on calf health would include decreased morbidity because of clinical diarrhea, decreased mortality rate, and increased ADG. The reduced oocyst shedding could have long-term benefits on calf health by decreasing the amount of environmental contamination. Additionally, disease severity and costs associated with treatment of diarrhea (labor and supportive treatment) for calves co-infected with rotavirus or coronavirus could be substantially reduced if C. parvum infection is controlled.