Effect of Halofuginone Lactate on the Occurrence of *Cryptosporidium parvum* and Growth of Neonatal Dairy Calves

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ABSTRACT

Thirty-one Holstein bull calves were purchased at birth from 3 dairy farms in Eastern Ontario. Each calf was assigned at random to oral treatment with either 5 mg of halofuginone lactate in 10.0 mL of aqueous carrier solution (Halocur, base comprised 10 mg of benzoic acid, 100 mg of lactic acid, and 0.3 mg of tartrazine) or 10 mL of placebo (Halocur base minus the active ingredient, halofuginone lactate) administered 15 to 30 min after morning milk feeding for the first 7 d of life. Intakes of milk, calf starter, and water, and fecal consistency score were recorded daily for 56 d. Calf weights were recorded weekly for 56 d. Fecal samples were taken from all calves at approximately 2, 7, 14, 21, and 28 d of age for isolation of *Cryptosporidium parvum* oocysts. Logistic and linear regression analyses were used to assess the effect of treatment on the incidence of diarrhea and *C. parvum* infection status. The odds of *C. parvum* shedding among calves in the halofuginone lactate-treated group was 70% lower than the odds of shedding among calves in the placebo group. In calves treated with halofuginone lactate, no oocyst shedding occurred until 2 wk of age, whereas 12.5% of calves in the placebo group began shedding oocysts during wk 1. From all ages of placebo-treated calves, 31 of 73 samples (42.5%) were positive for *C. parvum*, whereas only 15 of 67 samples (22.4%) from all ages of halofuginone lactate-treated calves tested positive. The largest number of *C. parvum*-positive samples occurred in the third week of life. There was a significant delay of 3.1 d in the incidence of diarrhea among calves treated with halofuginone lactate. Intake of milk and starter, body weight gains, and age at weaning were not significantly different between treatment groups.

(Key words: cryptosporidiosis, diarrhea, halofuginone lactate, neonatal calf)

Abbreviation key: HL = halofuginone lactate-treated, PC = placebo-treated.

INTRODUCTION

Cryptosporidium parvum is a coccidian agent of diarrheal disease affecting many mammals, including dairy calves (Anderson, 1998). The National Animal Health Monitoring System reported a *C. parvum* prevalence of 90% in US dairy farms, with peak prevalence in 1- to 3-wk-old animals (NAHMS, 1993). Of this age group, almost one-half of the calves were shedding oocysts (NAHMS, 1993). Calves infected by this common enteropathogen (Olson et al., 1997; O'Handley et al., 1999) may be asymptomatic or demonstrate mild to profuse diarrhea and dehydration (Fayer and Ungar, 1986). Cryptosporidiosis can lead to excessive fluid and electrolyte losses with subsequent metabolic acidosis, negative energy balance, and death (Constable, 2002). The current treatment goals involve correcting for fluid loss and balancing electrolytes.

Prevention of *C. parvum* infection and cryptosporidiosis relies on good management practices, as agent-specific preventives or therapies are not licensed in North America for use in domestic ruminants. Several compounds and vaccines have been evaluated for activity against *C. parvum* (Fayer et al., 1990; Perryman et al., 1999); however, to date no agents have been approved for the prevention or therapy of *C. parvum* infection in calves. Halofuginone, a synthetic quinazolinone, has been investigated for prophylactic effect against cryptosporidiosis in suckling calves (Lefay et al., 2001). That study showed evidence that halofuginone lactate (Halocur) significantly reduced oocyst shedding, and delayed onset and reduced severity of diarrhea in calves exposed to *C. parvum* infection. Halocur is a clear yellow, homogeneous solution that is administered orally to newborn calves daily for the first 7 d of life. The mechanism of action of this medication is unknown. However, halofuginone appears to reduce or halt shedding of oocysts, delay infection, and reduce the severity of cryptosporidiosis in calves (Yvoré and Naciri, 1989).

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through a proposed cryptosporidiostatic action on the sporozoite and merozoite stages of the parasite (Naciri et al., 1993). The work of Yvoré and Naciri (1989) was an initial study that demonstrated efficacy in a limited number of calves. This work was extended with the work of Lefay et al. (2001), which was conducted on farm with a larger number of suckling calves under European farm conditions. Because it was unclear whether the findings of these studies were applicable to dairy calves under North American conditions, the work reported here was carried out. Finally, as some research has suggested that halofuginone lactate administration may have negative effects on growth, feed intake, and feed efficiency (Committee for Veterinary Medicinal Products, 1999), an additional objective of this trial was to assess the effect of halofuginone lactate on calf performance.

MATERIALS AND METHODS

Research Facility and Animals

This trial was conducted at Kemptville College, University of Guelph, Kemptville (Ontario, Canada) from November 2002 to January 2003. The facility consisted of an open-air, graded site prepared with a gravel base covered with stone dust. Animals were cared for according to the guidelines of the Canadian Council on Animal Care (1993) and were housed individually in rectangular, polyethylene plastic hutches (Poly Square Calf Nursery, Agri Plastics, Grassie, Ontario, Canada) with straw bedding and chained outside access. Thirty-one Holstein bull calves were purchased at 1 d of age from 3 large local dairy farms. The calves were assessed as healthy with no current illness before enrollment and had an average BW of 45 kg (calves under 40 kg were not included in the trial). Calves received colostrum at the farm of origin; however, no additional medications or treatments were administered at the farm of origin. The calves were relocated using sealed crates (to prevent cold stress) to the Calf Research Facility, Kemptville College, at 1 d of age for the duration of the trial. One calf died early in the study and was replaced with a calf of similar health, BW, and age. Each calf was identified by a neck strap numbered from 1 to 30. Each calf was randomly assigned at birth to 1 of 2 treatment groups. Upon arrival, calves were placed individually in hutches and given 2 mL of vitamins E, A, and D intramuscularly (300, 100,000, and 10,000 IU/mL of vitamins E, A, and D, respectively; Servi Vet Inc., Montreal, Quebec, Canada) and 1 mL of Dystosel (3 mg/mL of selenium and 136 IU/mL of vitamin E; Pfizer, Kirkland, Ontario, Canada). Serum samples were collected upon arrival (1 d of age) and again at 7 d of age, and serum total solids were measured by total

solid refractometry (Leica Vet360, Richmond Hill, Ontario, Canada) to ensure that calves of both groups were similar overall in terms of passive immune transfer status. Upon arrival at the research facility, calves had free access to water and a commercial 18% CP nonmedicated calf starter (Shur-Gain, Stevensville, Ontario, Canada). A volume of 2.5 L of whole milk (nonmedicated) at body temperature was fed twice daily until each calf was at least 28 d old. Milk not consumed within 15 min was removed, and the volume was recorded. Each calf was weaned when they had consumed 750 g of 18% CP calf starter for 3 consecutive days. After weaning, free choice hay was offered.

Performance parameters, including milk and starter intake, BW gain, and age at weaning, were recorded for a period of 56 d. Calves were weighed by electric scale upon arrival at the research facility and every 7 d thereafter. Calves were not dehorned or castrated during this trial. Rectal temperatures and health treatments were recorded when calves displayed signs of illness, including reduced milk consumption, droopy ears, or dullness. Fecal consistency was recorded daily for each calf, using a score of 1 to 4 after the method described by Larson et al. (1977). Any calf with diarrhea (of fecal score 4) was treated with 45 mL/50 kg BW oral liquid sulfamethazine (Solution Sulfa 25%, MTC Pharmaceuticals, Cambridge, Ontario, Canada) beginning on the first day that a fecal score of 4 was observed; on each day thereafter, for 4 d, half the initial dosage was administered. Treatment was administered for 5 consecutive days in total. Oral electrolyte (Calf-Lyte II, Vetoquinol, Lavaltrie, Quebec, Canada) was given in cases of severe and continued diarrhea (fecal score of 3 or 4). To ensure that no bias existed with respect to additional health treatments given to diarrheic calves, the research staff were blinded to the treatment group assigned to individual calves.

Treatment Solutions and Administration

Halofuginone lactate, which is manufactured in a solution of 0.5 mg/mL (Halocur, Intervet Canada, Whitby, Ontario, Canada) was obtained with Experimental Study Certificate #20025 under the authority of Health Canada, Veterinary Drugs Directorate. Animals were treated orally with either 10.0 mL of Halocur (**HL**) or 10 mL of placebo (**PC**) administered daily by syringe 15 to 30 min after the morning milk feeding for the first 7 d of life, the recommended dose (at 0.1 mg/kg) regimen for a 50-kg calf. The placebo solution comprised all the constituents of Halocur minus the active ingredient (halofuginone lactate). Specifically, the placebo solution was manufactured by dissolution of 1.00 g of benzoic acid, 10.00 g of lactic acid, and 0.03 g of tartra-

zine (E 102) in 1 L of distilled water. Treatment and placebo products were prepackaged into seven 12-mL syringes (Becton Dickinson, Mississauga, Ontario, Canada) per calf at the Ontario Veterinary College and shipped to Kemptville College with labels showing only calf ID and day of administration. The 2 solutions were very similar in appearance and personnel were blinded as to which solution they were administering.

The first administration of halofuginone lactate or placebo was administered on the farm of origin before relocation of the calves to the research station. Administration entailed placing the syringe into the far back of the mouth between the jaw and tongue and then discharging the syringe to ensure that the entire dose was swallowed. Daily oral administration of treatment or placebo continued for 7 d.

Fecal Sample Analysis

Fecal consistency scores were recorded once daily, to 28 d of age, as follows: 1 = normal; 2 = soft, does not hold form; 3 = runny, spreads easily; 4 = devoid of solid matter (Larson et al., 1977). Diarrhea was defined as a fecal score of 3 or 4. Any calf not passing feces on a particular day was scored as an average of the fecal score the day before and day after the event. Five fecal samples per calf were taken during d 2 to 6, 7 to 13, 14 to 20, 21 to 27, and 28 to 32 d of age, respectively. These samples were refrigerated at 4°C and examined at the University of Guelph laboratory for the presence of *C. parvum* oocysts using a standardized sucrose flotation method. Briefly, 1 g of feces was mixed with 3 mL of sucrose solution (specific gravity 1.32). One drop of the mixture was then placed on a microscope slide, covered with a 22 × 22 mm coverslip (Fisher Scientific, Pittsburgh, PA), and examined at 400× magnification using a light microscope (Leica, Opti Tech Scientific, Inc., Scarborough, Ontario, Canada). *Cryptosporidium parvum* oocysts were identified as spherical, pink-tinged, refractile structures approximately 4 × 5 μm in size. Oocysts were quantified by enumerating the number of oocysts in 10 fields and multiplying the average number per field by the number of fields in the coverslip area to evaluate potential associations between intensity of *C. parvum* shedding and treatment with halofuginone lactate during the first month of life.

Statistical Analyses

Performance data were analyzed by week for 2 to 28 and 2 to 56 d of age using the PROC Mixed procedures in SAS (SAS Institute, 1999–2001), with treatment as class. The PROC FREQ procedures were used to test for all treatment comparisons that would provide evi-

Table 1. Effect of treatment with halofuginone lactate (HL) or placebo (PC) on proportion of calves with diarrhea (fecal score 3 or 4).

Day of age ¹	Treatment		Total of 31 calves, no. (%)	P ²
	HL, of 15 calves, no. (%)	PC, of 16 calves, no. (%)		
7	5 (33.3)	5 (31.3)	10 (32.3)	0.9
8	4 (26.7)	9 (56.3)	13 (41.9)	0.1
9	5 (33.3)	12 (75.0)	17 (54.8)	0.02
10	5 (33.3)	12 (75.0)	17 (54.8)	0.02
11	7 (46.7)	11 (68.8)	18 (58.1)	0.21
12	10 (66.7)	8 (50.0)	18 (58.1)	0.35
13	8 (53.3)	8 (50.0)	16 (51.6)	0.85
14	5 (33.3)	5 (31.3)	10 (32.3)	0.9
15	4 (26.7)	3 (18.8)	7 (22.6)	0.6
16	6 (40.0)	3 (18.8)	9 (31.0)	0.18
17	6 (40.0)	3 (18.8)	9 (31.0)	0.18
18	6 (40.0)	3 (18.8)	9 (31.0)	0.18
19	3 (20.0)	2 (12.5)	5 (17.2)	0.56
20	2 (13.3)	1 (6.3)	3 (10.3)	0.5
21	1 (6.7)	2 (12.5)	3 (10.3)	0.58

¹Incidences of diarrhea were low before d 7 and after d 21, and therefore, these data are not shown.

² χ^2 probability of a difference between halofuginone-treated (HL) and placebo-treated (PC) groups.

dence of difference in distribution of fecal score by day, due to treatment. Treatment comparisons were further scrutinized using LSMeans with the Tukey-Kramer adjustment (SAS Institute, 1999–2001). Logistic and linear regression analysis modeling were used to assess the effect of halofuginone lactate treatment on the incidence of *C. parvum* shedding and fecal score, respectively. Complete table analysis (PROC FREQ of SAS), with χ^2 and Fisher's Exact test, were used to test for difference in proportions of diarrhea by day, due to treatment.

RESULTS

Mean serum total solid results for HL and PC groups at the time of enrollment were 5.6 ± 0.6 and 6.0 ± 0.6 g/dL, respectively. These values are not significantly different, which demonstrated that the calves were randomly assigned to treatment groups with no bias toward immunity status. The mean BW for both the HL and the PC groups at the time of enrollment was 45.4 ± 1.8 kg.

Most calves experienced scours (fecal score of 3 or 4) at some time during the trial (Table 1). Between d 7 and 21 of age, the daily proportion of diarrheic calves (fecal score 3 or 4) was significantly lower ($P < 0.05$) in the HL group at d 9 and 10 of age than in the PC group (Table 1). The effects of halofuginone lactate treatment on various performance parameters from 2 to 28 d of age are presented in Table 2. There were no differences ($P > 0.05$) in daily BW gains, milk intake, starter con-

Table 2. Effect of halofuginone lactate on performance of dairy calves from 2 to 28 d of age.

Treatment ¹	Average daily gain (g/d)	Starter intake (g/d)	Water intake (L/d)	Milk intake (L/d)	Average time to fecal score 3 or 4 (d)	Days to weaning
HL	416	192.4	0.13	4.39	14.7 ^b	37.5
PC	278	184.0	0.11	4.24	11.6 ^a	38.6
SEM	82	39.5	0.01	0.14	0.54	1.72

^{a,b}Values within columns with different superscript letters are significantly different at $P < 0.05$.

¹HL = Halofuginone lactate-treated calves; PC = placebo-treated calves.

sumption, water consumption, or days to weaning between treatment groups (Table 2). However, PC calves started scouring 3.1 d earlier ($P < 0.05$) than HL calves (Table 2).

Performance was also evaluated by week and over the complete 56-d trial period, and no differences ($P > 0.05$) were apparent. Overall, 27 calves were treated orally with sulfamethazine. Furthermore, one calf from each treatment group died because of complications from diarrhea. There was no significant ($P > 0.05$) difference between the HL and PC groups in sulfamethazine use when the numbers of daily treatments were compared between groups.

Mean group *C. parvum* oocyst counts at the various sampling times are summarized in Table 3. Among the PC calves, the highest total number of oocysts was recovered from samples taken between d 7 and 13, with very low numbers shed by d 28 to 32. In contrast, no HL calves were found to be shedding oocysts before d 14, and the highest intensity of shedding occurred in these calves in the third week of life (d 14 to 20). With respect to the risk of shedding, the largest percentage of positive samples from both groups occurred among the samples taken on d 14 to 20 of life (Table 4). Overall, 31 of 73 samples (42.5%) from PC calves were positive for *C. parvum*, whereas only 15 of 67 samples (22.4%) from HL calves tested positive. A Z-test of these 2 proportions showed a highly significant difference ($P < 0.01$). Logistic regression of *C. parvum* status (positive or negative) on group (PC or HL) and sample number

produced odds ratios and statistics that are summarized in Table 5. This revealed that the odds of shedding *C. parvum* for calves in the HL group was 0.3 times the odds of shedding for calves in the PC group ($P < 0.01$). The odds of a calf shedding *C. parvum* oocysts was higher for all samples from d 8 to 32 than for samples from d 2 to 7, irrespective of treatment group. Regressing a log (y+1) transformation of oocyst count on group and sample number gave the coefficients and statistics shown in Table 6. This indicated that, controlling for sample number, HL calves shed significantly fewer oocysts than PC calves.

To assess the effect of halofuginone lactate on the onset of shedding of *C. parvum* oocysts, a new variable was created to indicate the first day when a sample taken from each calf tested positive for *C. parvum*. Fecal samples from some calves in the PC group tested positive for oocysts in the first week of life, whereas no shedding occurred until 2 wk of age in calves given halofuginone lactate treatment. This indicated a delay in the onset of shedding of *C. parvum* oocysts in the calves treated with halofuginone lactate.

DISCUSSION

In this study, there was no apparent difference in acceptance of halofuginone lactate (Halocur) or placebo by the calves. It was clear that both the treatment and the placebo were palatable, as indicated by the calves' acceptance and sucking during administration.

Although the risk of scouring over the course of the study was similar between treatment groups, it would appear that treatment with halofuginone lactate delayed the onset of scours compared with calves receiving the placebo. Uga et al. (2000) suggested that calves shedding *C. parvum* oocysts had a significantly higher rate of diarrhea, implicating *C. parvum* as the agent of diarrhea in that work. Thus, in our study, the delay in scour incidence (fecal score of 3 or 4) after birth may be due to a delay in *C. parvum* infection. In a clinical trial conducted in France, suckling calves that received halofuginone lactate had a 44% reduction in the occur-

Table 3. Effect of treatment on mean numbers of *Cryptosporidium parvum* oocysts detected in fecal samples at various sampling times in placebo (PC)- and halofuginone (HL)-treated groups.

Sample number/time	Mean no. of oocysts per gram of fecal slurry	
	PC	HL
1 (d 2 to 6)	25,625	0
2 (d 7 to 13)	1,066,500	0
3 (d 14 to 20)	446,625	669,375
4 (d 21 to 27)	15,250	76,500
5 (d 28 to 32)	500	250

Table 4. Effect of halofuginone lactate on distribution of *Cryptosporidium parvum* positive and negative fecal samples from calves at various times of sampling in placebo (PC)- and halofuginone (HL)-treated groups.

Sample number/time	PC sample status		PC % positive	HL sample status		HL % positive
	Negative	Positive		Negative	Positive	
1 (d 2 to 6)	14	2	12.5	14	0	0
2 (d 7 to 13)	5	13	72.2	16	0	0
3 (d 14 to 20)	4	11	73.3	7	8	53.3
4 (d 21 to 27)	12	3	20.0	6	6	50.0
5 (d 28 to 32)	7	2	22.2	9	1	10.0
Total	42	31	42.5	52	15	22.4

rence of diarrhea over 3 wk (Lefay et al., 2001). In contrast, our study did not show a reduction in the incidence of diarrhea in the calves over the first 4 wk of data collection. This may be associated with different management practices between dairy calves and suckling beef calves. However, the small sample size used suggests that a larger clinical trial under similar conditions is warranted before definite conclusions can be made.

The odds of shedding *C. parvum* oocysts appeared to be highest in all the calves between d 14 and 20; however, the high odds ratio associated with this age group (Table 5) may be an artifact of the relatively small number of observations. The logistic model indicated a significant beneficial effect of halofuginone lactate on shedding for the age group represented in the trial (2 to 32 d old), with halofuginone lactate reducing the odds of an animal shedding *C. parvum* by 70%. Similarly, suckling calves that received halofuginone lactate using the same treatment regimen as described here during the aforementioned clinical trial in France, shed 44% fewer oocysts than calves in the control group (Lefay et al., 2001).

In this study, there was no significant difference in sulfamethazine treatment required for neonatal calf

diarrhea between the HL and PC groups. It is conceivable that this population of calves was infected with other enteropathogens, necessitating antibiotic administration irrespective of *C. parvum* infection status and treatment group.

Finally, intake of milk and starter, BW gains, and age at weaning were not different between treatments. These findings alleviate concerns reported in one European dossier of a reduction in performance on the seventh day of 7 consecutive treatments, at 3 times the test daily dose (0.3 mg/kg halofuginone base), as determined by BW and milk consumption (Committee for Veterinary Medicinal Products, 1999). In addition, the European dossier reported a significant increase in BW in the halofuginone-treated group at label daily dose (0.1 mg/kg halofuginone base; Committee for Veterinary Medicinal Products, 1999).

CONCLUSIONS

The odds of shedding *C. parvum* was reduced by 70% for neonatal dairy calves treated orally by syringe with 5 mg of halofuginone lactate in 10 mL for the first 7 consecutive days of life compared with the placebo-treated group. In addition, there was a delay in shed-

Table 5. Effect of halofuginone lactate on risk of *Cryptosporidium parvum* infection, controlling for sample number (odds ratios and statistics of logistic regression model). A value of $P > 0.05$ associated with the likelihood ratio test was the criterion for removal of a variable from the model.

Predictor variable ¹	Odds ratio	z	P	95% confidence interval for odds ratio
Halofuginone-treated group	0.30 ^a	-3.2	0	0.2, 0.6
Sample number 2	9.62 ^b	2.95	0	2.1, 43.4
Sample number 3	30.5 ^b	3.47	0	4.4, 210.5
Sample number 4	7.41 ^b	2.34	0.02	1.4, 39.5
Sample number 5	2.89 ^b	0.97	0.33	0.3, 24.6

^aOdds ratio comparing risk of oocyst shedding in halofuginone-treated group with risk of shedding in placebo-treated group.

^bOdds ratio comparing risk of oocyst shedding at second to fifth sampling with risk of shedding at first sampling.

¹Sample numbers (times): 1 (d 2 to 6), 2 (d 7 to 13), 3 (d 14 to 20), 4 (d 21 to 27), and 5 (d 28 to 32).

Table 6. Effect of halofuginone lactate on intensity of shedding of *Cryptosporidium parvum* oocysts, controlling for sample number (coefficients and associated statistics of least squares regression model¹). A value of $P > 0.05$ was used as the criterion for removal of a variable from the model. Outcome variable was log (number of oocysts per drop of slurry + 1).

Predictor variable ²	Regression coefficient	t	P	95% confidence interval for coefficient
Halofuginone-treated group	-1.50 ^a	-3.65	0	-2.3, -0.7
Sample number 2	2.67 ^b	3.5	0	1.1, 4.2
Sample number 3	3.87 ^b	4.58	0	2.1, 5.6
Sample number 4	1.50 ^b	2.28	0.03	0.2, 2.8
Sample number 5	0.16 ^b	0.4	0.69	-0.7, 1.0
Intercept	2.54	3.5	0	1.1, 4.0

^aCoefficient indicating decrease in intensity of oocyst shedding by halofuginone-treated calves compared with placebo-treated calves.

^bCoefficient indicating increase in intensity of oocyst shedding at second to fifth sampling compared with intensity of shedding at first sampling.

¹Regression with robust standard errors (adjusted for within-calf clustering): Number of observations = 140; $F(5, 30) = 17.47$; $P > F = 0.00$; $R^2 = 0.23$; number of clusters = 30; root mean square error = 3.06.

²Sample numbers (times): 1 (d 2 to 6), 2 (d 7 to 13), 3 (d 14 to 20), 4 (d 21 to 27), and 5 (d 28 to 32).

ding of *C. parvum*, as well as a delay of 3.1 d in the onset of diarrhea in calves treated with halofuginone lactate. There was no impact of halofuginone lactate treatment on milk and starter intake, BW gain, and days to weaning in treated calves compared with control (placebo-treated) calves.

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