

## Effects of repeat fenbendazole treatment in dairy calves with giardiosis on cyst excretion, clinical signs and production

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### Abstract

In this 90-day study, 60 male Holstein dairy calves were experimentally infected with *Giardia duodenalis*. Calves were randomly blocked by weight into treatment ( $N=30$ ) and placebo ( $N=30$ ) groups. Beginning on study Day 0, calves in the treatment group were administered an oral dose of 5 mg/kg of fenbendazole once daily for three consecutive days. Calves in the placebo group received a daily oral treatment of 5 ml of saline for 3 days. These treatments were repeated on Days 30 and 60 of the study. Fecal samples were collected from calves once per week and examined for the presence of *Giardia* cysts. Calves were monitored daily for clinical signs of intestinal disease and all episodes of diarrhea recorded. Calves were weighed once per week and total feed intake, on a dry matter basis, was calculated daily. Following each treatment, the number of calves shedding *Giardia* cysts in the fenbendazole group was reduced ( $p<0.001$ ) compared to the saline group. Also, calves in the fenbendazole group had fewer cysts ( $p<0.05$ ) detected in their feces following treatment compared with calves that received saline. Within 2 weeks post treatment, the number of infected animals and fecal *Giardia* cysts returned to placebo levels. This pattern of reinfection was consistent after every treatment period. Calves receiving fenbendazole had fewer total days with diarrhea ( $p<0.01$ ) and the average number of days each calf had diarrhea was reduced ( $p<0.05$ ), compared to the placebo group. There were no differences in mean body weight, average daily gain, or feed intake between the treatment or placebo groups. This study demonstrates that fenbendazole is an effective treatment for giardiosis, resulting in a clinical benefit and reducing the number of infective cysts shed

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by calves. However, this treatment regime had no impact on production parameters and reinfection occurred rapidly in these calves. © 2000 Elsevier Science B.V. All rights reserved.

**Keywords:** *Giardia*; Hexamitidae; Giardiasis; Fenbendazole; Diarrhea; Calves; Production

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## 1. Introduction

The intestinal protozoan *Giardia duodenalis* (= *G. intestinalis* = *G. lamblia*) is one of the most commonly identified pathogens of humans and animals throughout the world (Wolfe, 1992; Zajac, 1992; Barr and Bowman, 1994; Xiao, 1994a; Olson et al., 1997). Infections are associated with a decreased microvillus surface area, reduced intestinal enzyme activity, and increased intestinal transit (Buret et al., 1990b, 1991, 1992; Deselliers et al., 1997). These pathological changes result in a malabsorptive diarrhea, which is the major clinical sign of giardiasis. In addition to infections in humans and companion animals, many recent studies report *Giardia* infections in domestic livestock (Taylor et al., 1993; Xiao et al., 1993; United States Department of Agriculture, 1994; Xiao, 1994a; Xiao and Herd, 1994b; Olson et al., 1997; O'Handley et al., 1999a). In dairy calves, the parasite is highly prevalent with infection rates reported as high as 100% (Xiao and Herd, 1994b; O'Handley et al., 1999a). *Giardia* infections are often chronic in dairy calves and result in diarrhea (Xiao and Herd, 1994b; O'Handley et al., 1999a). The high prevalence of this parasite and its association with diarrhea in calves may be of concern to dairy producers. In a ruminant model *Giardia* infections resulted in decreased weight gain, impaired feed efficiency, and reduced carcass weight (Olson et al., 1995). Thus, giardiasis in dairy calves may have an impact in terms of animal health and economic losses to producers.

In addition to potential production losses, *Giardia* infections in food producing animals may serve as a possible source for human infections. Studies indicate giardiasis is a potential zoonosis (Buret et al., 1990a; Isaac-Renton et al., 1993; Ey et al., 1997; O'Handley et al., 1999b). *Giardia* isolates morphologically, phenotypically, and genotypically identical to human isolates were found in ruminants (Buret et al., 1990a; Archibald et al., 1991; Ey et al., 1997). Also, *Giardia* cysts were identified in agriculture effluent (Weniger et al., 1983; LeChevallier et al., 1991), and human outbreaks of giardiasis were attributed to pasture run-off contaminating drinking water (Weniger et al., 1983; Isaac-Renton et al., 1987; Gradus, 1989; LeChevallier et al., 1991). Therefore, infected livestock may act as an important reservoir for human infections, either through direct contact with infected animals, or through ingestion of *Giardia* cysts via contaminated drinking water.

The potential economic impact and possible zoonotic risk of *Giardia* may warrant treatment of the infection in domestic livestock. Drugs used to treat human cases of giardiasis are not approved for food producing animals due to their toxicity and unwanted side effects (Dow et al., 1989; Finch et al., 1992), but recent studies demonstrate that the anthelmintic benzimidazoles are effective against *Giardia* in vitro and in vivo (Morgan et al., 1993a; Xiao et al., 1996; O'Handley et al., 1997). Although some benzimidazoles may not be safe to administer to dairy cattle (Wetzel, 1985), fenbendazole, currently approved for use in cattle as an anthelmintic, is very safe and highly efficacious against *Giardia* in dairy calves (Muser and Paul, 1984; Xiao et al., 1996; O'Handley et al., 1997). Thus, fenbendazole is an

excellent candidate for use as a *Giardia* control agent in cattle. However, the efficacy of fenbendazole treatment for giardiasis in calves has yet to be evaluated under normal operating conditions. Furthermore, the benefits of treating calves for giardiasis with regard to animal performance, animal health, and reduced zoonotic risk have yet to be determined. In this study, the efficacy of repeat fenbendazole treatment for giardiasis in calves was examined, and the effects of treatment on animal health, performance, and number of infective cysts excreted by calves were studied.

## 2. Materials and Methods

### 2.1. Animals

For this 90-day study, 60 male dairy calves (1–2 weeks old) were obtained from various commercial dairy operations in the Fraser Valley region of British Columbia, Canada. Upon arrival (Day 10) calves were experimentally infected by oral administration of  $10^5$  *Giardia* cysts obtained from a naturally infected calf. After 7 days fecal samples were collected for three consecutive days (Days 3, 2, 1) from each calf and examined, as described further, to ensure successful establishment of *Giardia* infection. The presence of *Cryptosporidium*, *Eimeria*, and intestinal nematodes were also determined. Once infection with *Giardia* was established (Day 0), calves were weighed and randomly blocked by weight into treatment ( $N=30$ ) and placebo ( $N=30$ ) groups. All calves were housed individually in pens with concrete floors and solid wooden walls, which prevented contact between neighboring animals. The two groups were located on opposite sides of the alley to eliminate contact between treatment and control animals. The pens were cleaned daily by removing all of the bedding from within the pen and replacing it with fresh bedding, consisting of wood shavings. To further minimize contact, soiled bedding from each group was placed in separate gutters. All workers followed Agriculture and Agri-food Canada's recommended code of practice for the care and handling of dairy cattle (Agriculture Canada, 1990). All housing and procedures were conducted in accordance with the guidelines of the Canadian Council on Animal Care.

### 2.2. Treatment

Beginning on Day 0 (allocation day), calves in the treatment group were administered an oral dose of 5 mg/kg of fenbendazole (SafeGuard, Hoechst Roussel, Regina, Sask.), once daily for three consecutive days. This treatment regime was previously demonstrated to be effective against giardiasis in calves (O'Handley et al., 1997). Calves in the placebo group received a daily oral treatment of 5 ml of saline on the same 3 days. These treatments were repeated on Days 30 and 60 of the study.

### 2.3. Production measurements

Beginning at the time of allocation, calves were weighed on a weekly basis for 90 days to determine average body weight and average daily gain for each treatment group. Calves were

Table 1  
Ingredients and composition of concentrate fed to calves

Ingredients <sup>a</sup>	Percent of ration
Barley, rolled	76.73
Soybean meal	5.03
Molasses beet	3.14
Canola oil	3.77
Beet pulp	6.29
Trace-mineral salt <sup>b</sup>	5.03

<sup>a</sup> Monensin added at 36 g/tonne.

<sup>b</sup> Contains 92.6% NaCl; 1.1% Zn; 0.94% Mg; 0.32% Cu; 0.005% Co; 0.0044% Se; 0.0013% I; 5% Dynamate.

fed 4 l of a commercial milk replacer daily (Snowflakes, Nutrena Feeds, Winnipeg, Man.) until weaning. Calves had access to feed concentrate, containing monensin (Rumensin, Elanco Animal Health), continuously throughout the study (Table 1). Concentrate was provided to calves in both treatment groups ad libitum until weaning, after which, calves received 1.75% of their body weight in concentrate per day. Alfalfa hay was provided ad libitum throughout the study. Calves had unlimited access to water, via the use of individual nose pumps, as well as loose mineral. Total feed intake (milk replacer, concentrate, and hay), calculated on a dry matter basis, was measured daily for each calf for the duration of the study.

#### 2.4. Parasite isolation and enumeration

Fecal samples were collected weekly from each calf for the duration of the study. Samples (1–5 g) were collected directly from the rectum of each calf and placed in pre-weighed centrifuge tubes containing 5 ml of 5% formaldehyde in phosphate buffered saline (PBS). Samples were shaken to disperse the feces, weighed, and stored at 4°C prior to examination.

Numbers of *Giardia* cysts and *Cryptosporidium* oocysts were determined using sucrose gradient centrifugation and immunofluorescence microscopy as previously described (O'Handley et al., 1999a). Beginning on Day 0, fecal floatations for *Eimeria* oocysts and nematode eggs were performed monthly using a commercially available fecal analysis kit (Fecalizer, Evsco Pharmaceuticals, Buena, NJ), utilizing a sodium nitrate solution (specific gravity, 1.2).

#### 2.5. Clinical signs

Clinical signs of intestinal disease were monitored for each calf and fecal consistency was recorded daily. The number of diarrhea episodes, defined as feces with a fluid consistency, and the duration of each episode were determined from these daily records. The mean number of diarrhea episodes, as well as the mean duration of each diarrhea episode, was then calculated for each treatment group.

## 2.6. Statistical analysis

Average daily gain, body weight, and average feed intake on a dry matter basis were compared between each treatment group using ANOVA and Newman-Keul's multiple comparison of means. Cyst count values for each group were compared using the same method after first being natural logarithmically transformed. The Fisher exact test was used to compare the number of animals infected and the number of days in which diarrhea and abnormal stool was observed for each group. All data are expressed as  $\pm$ SEM, and cyst counts are presented as geometric means. Analysis was performed using a statistical software package with a 95% confidence interval (Instat, Graphpad Inc., San Diego, CA).

## 3. Results

All 60 calves were confirmed positive for *Giardia* infection based on the pre-study fecal examinations. At the initiation of treatment (Day 0), 25 calves (83%) from each treatment group were shedding *Giardia* cysts. The geometric mean number of cysts per gram of feces on Day 0 was 871 for calves in the fenbendazole group and 982 for calves in the saline group. The number of calves infected with *Giardia* and the geometric mean number of *Giardia* cysts shed by calves differed between groups following each treatment (Fig. 1a and b). Seven days after treatment with fenbendazole the number of calves shedding *Giardia* cysts was reduced ( $p < 0.001$ ). Also, calves had fewer cysts ( $p < 0.05$ ) detected in their feces following treatment compared with calves that received saline. This reduction in the number of calves infected, and fecal cyst output, lasted a maximum of 2 weeks post treatment, after which the number of infected animals and fecal *Giardia* cysts returned to control levels. This pattern of reinfection was consistent after every treatment period.

Calves from both groups gained at a constant rate throughout the study, and the mean body weight from each group did not differ. There was no significant difference in body weight or average daily gain between the fenbendazole treated and saline treated groups (Table 2). Also, differences could not be detected between groups with respect to feed intake (Table 2).

Clinically, differences were observed between the treatment groups. The mean number of diarrhea episodes did not differ between the fenbendazole and saline treated groups. Calves in the fenbendazole treated group had an average of 0.83 ( $\pm 0.2$ ) episodes of diarrhea during the study, while calves in the saline treated group had 0.80 ( $\pm 0.2$ ) episodes of diarrhea. However, the duration of these diarrhea episodes differed significantly ( $p < 0.05$ ). Diarrhea occurring in the fenbendazole treated calves lasted an average of 4.12 ( $\pm 0.47$ ) days, while diarrhea occurring in the saline treated calves was an average duration of 6.21 ( $\pm 0.81$ ) days.

*Cryptosporidium parvum* and *Eimeria* sp. were the only other parasites to be observed in the feces of calves during this study. *Cryptosporidium parvum* was only observed in fecal samples collected during the first 3 weeks of the study, and *Eimeria* oocysts were only observed in fecal samples collected on Day 0 of the study. The number of calves shedding *Cryptosporidium* oocysts and the number of oocysts shed by calves did not differ between each group. On Days 7, 14, and 21 of the study four (13%), six (20%), and two (7%) calves respectively shed *Cryptosporidium* oocysts following fenbendazole treatment,

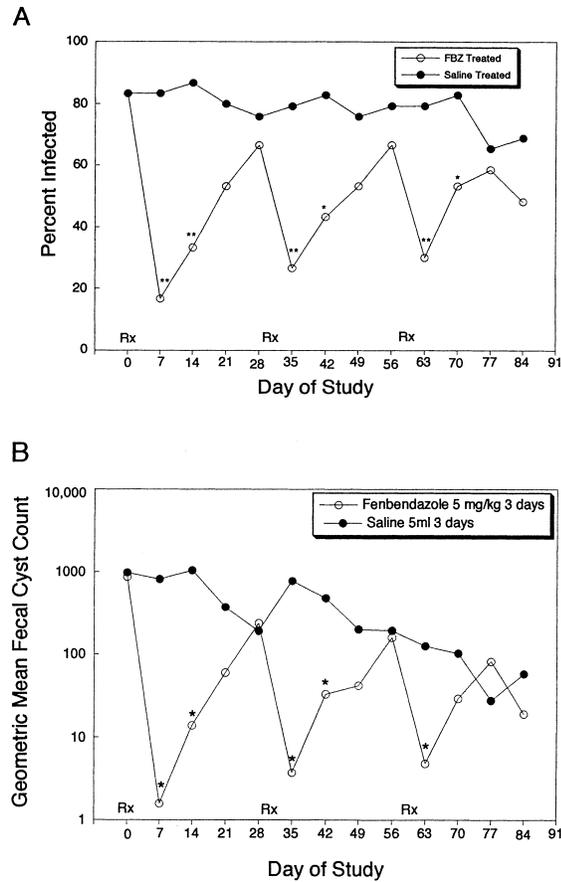


Fig. 1. Percent of calves shedding fecal *Giardia* cysts (A) and geometric mean number of *Giardia* cysts shed per gram of feces (B) per day of study following treatment with fenbendazole or saline. Rx indicates initiation of treatment. \* Indicates significantly different ( $p < 0.05$ ) from saline group. \*\* Indicates significantly different ( $p < 0.001$ ) from saline group.

and two (7%), four (13%), and one (3%) calves shed oocysts following treatment with saline. With respect to *Eimeria*, eight (27%) calves in the fenbendazole treatment group shed oocysts while 8 (27%) calves shed oocysts in the saline treatment group.

Table 2  
Effect of fenbendazole treatment for giardiasis on dairy calf performance

Treatment group	Mean initial weight (kg)	Mean final weight (kg)	Average daily gain (kg)	Mean feed intake (kg) <sup>a</sup>
Fenbendazole	54.5±2.1	132.3±4.9	0.862±0.03	214.01±11.1
Saline	51.8±2.0	132.6±5.3	0.898±0.04	216.86±11.6

<sup>a</sup> Feed intake on a dry matter basis (milk replacer, feed concentrate, and alfalfa hay).

#### 4. Discussion

Previous studies have demonstrated that fenbendazole, a broad-spectrum anthelmintic, was highly effective in eliminating *Giardia* infections in dairy calves (Xiao et al., 1996; O'Handley et al., 1997). A ruminant *Giardia* isolate was found to be susceptible to fenbendazole at a concentration of 0.024 µg/ml in vitro (O'Handley et al., 1997), and a daily oral dose of 5 mg/kg administered for 3 days completely eliminated excretion of *Giardia* cysts in infected calves (O'Handley et al., 1997). In addition, *Giardia* resistance to benzimidazoles has yet to be documented after continuous exposure to sublethal concentrations for up to 6 months (Morgan et al., 1993b; O'Handley et al., 1997). Fenbendazole is currently approved for treatment of helminth infections in food and companion animals (Lacey, 1990) and it is safe and well tolerated by cattle. In fact, cattle administered 400 times the label dose fail to show any signs of toxicity (Muser and Paul, 1984).

The results of this study demonstrated that fenbendazole administered orally at 5 mg/kg once daily for 3 days can significantly reduce the number of *Giardia* cysts in the feces of calves under normal operating conditions. Thus, treatment may have an environmental benefit by reducing the number of infective cysts passed by calves to the environment. However, fecal cyst excretion returned to control levels within 2 weeks following treatment. Despite daily cleaning of pens, it is likely the environment in which the calves were housed was contaminated with infective *Giardia* cysts leading to rapid reinfection. In an earlier study, fenbendazole administered at 5 mg/kg once daily for 3 days eliminated cyst excretion in calves for 28 days when pens were washed daily with a quaternary ammonium disinfectant (O'Handley et al., 1997). However, in the same study, reinfection occurred in 60% of calves administered 10 mg/kg of fenbendazole for 3 days and in 20% of calves administered 20 mg/kg of fenbendazole for 3 days despite daily disinfecting of pens. Contamination of dairy facilities by infected calves and the resistant nature of *Giardia* cysts must therefore be considered when treating calves for giardiasis.

In a previous study, fenbendazole administered to calves at the same dose and for the same duration proved to be 100% efficacious against *Giardia* (O'Handley et al., 1997). In this study, 100% efficacy was not observed with regard to the number of calves shedding fecal cysts. Following each treatment 17, 26, and 30% of calves respectively were still shedding cysts. However, geometric mean cyst counts were very low following the first fenbendazole treatment (1.6 cysts per gram of feces) and only increased slightly following the second and third treatments (3.7 and 4.8 cysts per gram of feces, respectively). Fecal samples were examined for *Giardia* cysts 7, 5, and 3 days after the initiation of treatments due to the weekly interval at which samples were collected. It is likely that *Giardia* cysts remain in the fecal stream for a few days following treatment, therefore, the increased percentage of calves shedding cysts is likely due to the shorter period of time between initiation of treatment and sample collection.

Despite rapid reinfection in these calves, treating giardiasis with fenbendazole significantly reduced the duration of diarrhea episodes. Intestinal nematodes were not observed in this study, and although *Cryptosporidium* and *Eimeria* were observed during the first 3 weeks of the study, fenbendazole has no effect against these two parasites (Fayer and Fetterer, 1995). Previously, *Giardia* was identified as the sole pathogen in many episodes of diarrhea in dairy calves (O'Handley et al., 1999a), and symptomatic improvement was

observed in another study when calves were treated for giardiasis using metronidazole (Xiao et al., 1993). Fenbendazole is not known to exhibit bactericidal effects, therefore the results of this study suggest that elimination of *Giardia* from calves using fenbendazole provides a clinical benefit.

Although fenbendazole treatment resulted in a significant reduction in cyst excretion, and a clinical benefit, there was no difference between the fenbendazole treated and saline treated calves with respect to production parameters. Reinfection occurred rapidly after calves were treated with fenbendazole. As a result, cyst excretion levels in fenbendazole treated calves returned to the same level as saline treated calves within 2 weeks after each treatment. The prepatent period for *Giardia* is between 7 and 10 days (Olson et al., 1995), therefore, it is possible that trophozoite colonization of calves' intestinal tracts occurred within a week after treatment. Although the length of time calves spent free of infection was sufficient to reduce clinical signs, calves may not have had sufficient time to completely recover from the intestinal pathology associated with giardiasis. In order to prevent reinfection from occurring it appears that a different treatment regime must be employed. It was recently demonstrated that duration of treatment, not dose, is the most important factor when treating calves for giardiasis with fenbendazole (O'Handley et al., 1997). It is possible that a continuous, low dose treatment would prevent calves from becoming reinfected with *Giardia* and result in improved performance. However, this treatment method would have to be studied further with regard to efficacy and the potential for developing fenbendazole resistant *Giardia*.

Studies show *Giardia* is highly prevalent in dairy calves, is an etiologic agent of diarrhea in calves, and has a negative impact on growth and performance in ruminants (Xiao et al., 1993; Xiao and Herd, 1994b; Olson et al., 1995, 1997; O'Handley et al., 1999a). Some studies also suggest that giardiasis may be a zoonosis (Buret et al., 1990a; Isaac-Renton et al., 1993; Ey et al., 1997; O'Handley et al., 1999b). If so, domestic livestock may act as an important source for human outbreaks of giardiasis (Craun, 1986). Thus, treatment of *Giardia* infections in calves may be warranted. In this study, fenbendazole was effective at significantly reducing the number of *Giardia* cysts shed by calves. Fenbendazole treatment also had a clinical benefit by reducing the number of days calves had diarrhea. Although treatment in this study did not improve calf performance, it can be concluded that treating giardiasis in calves with fenbendazole is beneficial from an animal health and environmental standpoint.

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