Veterinary Parasitology 169 (2010) 18–23

Contents lists available at ScienceDirect

Veterinary Parasitology

journal homepage: www.elsevier.com/locate/vetpar

The effect of a fenbendazole treatment on cyst excretion and weight gain in calves experimentally infected with *Giardia duodenalis*

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ARTICLE INFO

Article history:
Received 21 August 2009
Received in revised form 1 December 2009
Accepted 16 December 2009

Keywords:
Giardia duodenalis
Calves
Experimental infection
Weight gain
Fenbendazole

ABSTRACT

A total of 28 Holstein-Friesian calves were experimentally infected with 10^5 *Giardia duodenalis* cysts. Eleven days later, all animals were allocated into two groups of 14 animals each, based on the average pre-treatment cyst counts. Treatment was randomly assigned to one of the two groups, and all animals in the treatment group received a daily oral dosage of 15 mg fenbendazole per kg bodyweight during 3 consecutive days. The calves in the control group received a placebo (water). From 3 days after treatment onwards, cyst excretion was determined three times a week during 4 consecutive weeks. The faecal consistency and general health were recorded on a daily basis, and all animals were weighed prior to treatment and weekly thereafter. At the end of the experimental period, there was a significant ($P < 0.001$) reduction (98%) of the cumulative cyst excretion. There were no significant differences in general health between both groups, but faecal consistency was significantly lower ($P < 0.002$) in the control group compared to the treatment group, although none of the animals displayed overt gastro-intestinal symptoms. Prior to treatment the weight did not differ between both experimental groups. At the end of the 4-week experimental period however, the animals in the treatment group gained on average 2.86 kg (=102 g per day) more than the animals in the control group ($P < 0.031$). This study demonstrates for the first time a significant difference in weight gain between fenbendazole treated and untreated calves experimentally infected with *G. duodenalis*, although additional data need to confirm the need for treatment in natural conditions.

1. Introduction

The intestinal protozoan parasite *Giardia duodenalis* (Syn. *G. intestinalis*, *G. lamblia*) occurs in a large number of hosts, including cattle. Although reported with considerable variation, recent studies confirm that worldwide *G. duodenalis* frequently infects calves (Geurden et al., 2009). Calves as young as 4 days can excrete cysts, but clinical symptoms are mainly observed in animals older than 1 month (Geurden et al., 2006a). Although the inconsistent correlation between cyst excretion and the onset of clinical symptoms has troubled the recognition of *G. duodenalis* as a pathogen, several studies did suggest the clinical and subclinical relevance of giardiasis in cattle. Similar to mice (Scott et al., 2000), infection with *G. duodenalis* in ruminants leads to villus atrophy, crypt hyperplasia and an increased number of intra-epithelial lymphocytes, mostly observed in the upper portion of the small intestine (Ruest et al., 1997; Koudela and Vitovec, 1998; O'Handley et al., 2001). As a result, *G. duodenalis* can cause an intermittent and pultaceous diarrhea both in experimentally infected (Ruest et al., 1997; Koudela and Vitovec,

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doi:10.1016/j.vetpar.2009.12.027

1998; O'Handley et al., 2001) and in naturally infected ruminants (StJean, 1987; O'Handley et al., 1999; Geurden et al., 2006a). The pathogenesis of giardiosis is however a combination of parasite and host factors, and the subsequent clinical symptoms may vary considerably. Infected calves therefore not consistently display overt clinical symptoms, but may still suffer from the subclinical impact of infection. Although field data suggested that a *G. duodenalis* infection may have a negative impact on growth performance in calves (StJean, 1987; Geurden et al., 2006a), confounding factors could not be excluded and the subclinical effect of giardiasis on growth was never experimentally confirmed in calves. In lambs, an experimental infection with *G. duodenalis* did lead to decreased weight gain and impaired feed efficiency (Olson et al., 1995). In a previous experimental infection trial in calves, treatment with 5 mg/kg fenbendazole during three consecutive days did not result in a better weight gain due to rapid re-infection of the animals (O'Handley et al., 2000). The objective of the present study was to re-evaluate the effect of a treatment with fenbendazole on cyst excretion, clinical symptoms and weight gain caused by a *G. duodenalis* infection in experimentally infected calves.

2. Materials and methods

2.1. Animals and housing

Based on variability of weight gains and differences between treatment groups in a previous trial (Geurden et al., 2006b), a sample size of 14 animals in each experimental group was calculated. Hence, male Holstein calves aged 3–5 weeks were purchased from commercial dairy farms and screened upon arrival for the presence of Bovine Viral Diarrhea antigen in blood samples and for the presence of *Giardia* cysts and *Cryptosporidium* oocysts, *Eimeria* spp. oocysts and nematode eggs in faecal samples, as described below. None of the calves had access to pasture prior to purchase or at any moment thereafter. Only healthy calves negative for the above-mentioned pathogens were included in the study. The animals were housed per experimental group, each at one side of the stable separated from each other by a 1.5 m high wall. Within each experimental group, animals were housed in pairs. The housing consisted of large pens with concrete floors and walls, which prevented contact between neighbouring calves. Prior to the start of the study, the housing was cleaned and disinfected with a product containing quaternary ammonium to eliminate any existing environmental contamination. During the entire experimental period straw bedding was added to the pens on a daily basis. Furthermore, strict measures were taken to prevent cross-contamination between both experimental groups: the passage between both groups was only possible via one door. Separate overalls and boots were used to enter both compartments of the stable, and the boots were disinfected after each usage. Furthermore, each calf had his own milk bucket and milk was prepared and provided per group. All calves in the study received the same commercial milk replacer (Spraystart-Z® from Aveve; 6l per calf/day) and the same calf starter (Startflakes extra® from Aveve: 250 g/calf/day: composition in terms of percentage of the dry matter: 15.75% of crude protein and 3.20% fat), to ensure equivalent diets. Water and hay was provided ad libitum throughout the experiment.

This study was conducted in accordance with the VICH Guidelines for Good Clinical Practice and the E.U. Animal Welfare Directives, and ethical approval to conduct this study was obtained from the Ethical Committee of the Faculty of Veterinary Medicine, Ghent University.

2.2. Infection

Prior to infection all animals were screened again to confirm their *Giardia* negative status. For the infection, *Giardia* cysts were purified from the faeces of a naturally infected, 10 weeks old Holstein calf. These cysts were confirmed by PCR (Geurden et al., 2008) to be a mixed infection of *G. duodenalis* assemblage A and E. The cysts in the inoculum were enumerated based on multiple counts using the Merifluor immuno-fluorescence assay (IFA) and diluted in distilled water.

Eleven days before the start of the treatment (D11), all calves were orally inoculated twice with a 12 h interval, with a total infection dose of 10^5 *Giardia* cysts. At that time all calves were between 6 and 8 weeks of age. From 7 days after infection (D4) until D-1, faeces were collected daily from all calves to confirm the presence of cysts and to calculate the average pre-treatment cyst count for each animal. On D1 calves were blocked by descending order of the average pre-treatment cyst excretion and within each block calves were randomly assigned to either the treatment group or the control group (14 calves/group).

2.3. Treatment

The calves in the treatment group received an oral treatment with fenbendazole (Panacur® 10% suspension; Intervet-Schering Plough Animal Health) at a dose rate of 15 mg/kg bodyweight (BW) during 3 consecutive days (D0–D2). The occurrence of adverse events was monitored 1, 2, 3, 12, 24 and 48 h after treatment. The control group received a placebo (water).

2.4. Assessment of clinical effect and efficacy of treatment

2.4.1. Detection of G. duodenalis cysts

A commercial, direct immunofluorescence assay (IFA: MERIFLUOR *Cryptosporidium*/*Giardia* kit; Meridian Diagnostics Inc., Cincinnati, Ohio) was used in a previously described protocol for the quantitative detection of *G. duodenalis* cysts (Geurden et al., 2006a). This method allows the simultaneous detection of *Cryptosporidium* and *Giardia*. Furthermore, the presence of *Eimeria* spp. oocysts and nematode eggs was examined once every week during the entire experimental period, by sedimentation flotation followed by identification.

2.4.2. Clinical criteria

Data on faecal consistency and general health were recorded during the entire duration of the experimental period on a daily basis. The faecal consistency was scored as (1) normal (normal formed calf pats), (2) decreased

consistency (faecal pats losing their normal form), (3) pasty (faeces are wet but do not run on a flat surface), (4) pasty to watery (faeces are wet and start to run on a flat surface) and (5) watery (faeces are wet and run on a flat surface). Watery faeces were considered as severe diarrhoea, whereas pasty was considered as mild diarrhoea. The general health was described as either normal or abnormal and did not include data on faecal consistency. In the case of an abnormal health observation, a description of the abnormality of the general health was recorded. Faecal scoring was performed by a third person not involved in the experiment.

2.4.3. Weight gain

The weight of the animals was recorded on a weekly basis during the entire experimental period (D1; D7; D14; D21 and D28), using a cattle-weighing scale with a calibration of 1 kg. The weight gain per animal and per group was calculated at each time point. The weighing was performed by a third person not involved in the experiment.

2.4.4. Efficacy criteria and statistical analysis

The efficacy of fenbendazole was evaluated based on the reduction in cumulative cyst excretion (cyst per gram of faeces or CPG), which was calculated for each animal as follows: the cyst output between each pair of sampling days was calculated using the trapezium rule, i.e. the average CPG of two sequential sampling days was multiplied by the number of days in between the sampling days. The cumulative CPG from the start of the experiment to each sampling day for each animal was then calculated by adding together the period cyst counts. The cumulative CPG is thus a measure of the total cyst output during the experimental period, which is of importance to estimate the capacity for environmental contamination.

The efficacy of fenbendazole on faecal cyst excretion at each sampling date was calculated using the Henderson Tilton formula including the cyst counts of the treated (T) and control (C) animals (Henderson and Tilton, 1955):

$$100 \times \left[1 - \frac{\mathrm{Ta} \times \mathrm{Cb}}{\mathrm{Tb} \times \mathrm{Ca}}\right]$$

Tb was the arithmetic mean number of cysts counted in the treated group before treatment, Ta was the arithmetic mean number of cysts counted after treatment, Cb was the arithmetic mean number counted from the control animals before treatment, and Ca was the arithmetic mean number counted from the control group after treatment. The arithmetic mean weight gain and diarrhea score was calculated for each animal and for each group and compared between groups using a Mann–Whitney *U*-test. Probability (P) values <0.05 were considered to indicate significant differences.

3. Results

During the 4-week experimental period, no *Cryptosporidium* oocysts or nematode eggs were detected in any of the faecal samples. In several animals oocysts from non-pathogenic *Eimeria* spp. were detected on multiple samplings days, although not consistently in the same animals or in the same group.

3.1. Giardia cyst excretion

The cyst excretion in all animals and the reduction in cyst excretion in the treated group at each sampling day are presented in Table 1. The average pre-treatment cyst excretion did not significantly differ between both groups, and in both groups animals with high, moderate and low excretion were included. In the treatment group, all but three of the animals remained negative until D14, with only minor excretion (CPG ≤ 150) of each of those three calves on one sampling day. From D14 onwards, the cyst excretion increased and by the end of the experimental period (D28) all but three animals in the treatment group were re-excreting cysts. The reduction in cyst excretion was consistently high (>91%) until D17 and decreased afterwards. Overall, treatment with fenbendazole resulted in a significant ($P < 0.001$) reduction (98%) of the *Giardia* cumulative cyst excretion over the 4-week experimental period.

3.2. General health and faecal consistency

None of the animals displayed any adverse reaction during or following treatment with fenbendazole. There was no significant difference in general health between the fenbendazole treated group and the control group, and no general health deviations were recorded at any point during the experimental period in either group. Furthermore, all animals maintained their appetite and finished the daily portions of the diet that was offered. Although none of the animals displayed overt diarrhea, the average faecal consistency over the 4-week experimental period was significantly lower ($P < 0.002$) in the control group (an average of 1.90) compared to the fenbendazole treated group (average of 1.58).

3.3. Effect of treatment on weight and weight gain

The weight was recorded on a weekly basis during the 4-week experimental period. At the start of the trial the mean weight was not significantly different in the fenbendazole treated group (62.1 ± 1.5 kg) compared to the control group (61.7 ± 1.8 kg). On average, the animals in the fenbendazole treated group gained 2.86 kg more ($P < 0.031$) during the 4-week experimental period compared to the control animals, which corresponds to a difference of 102 g daily weight gain. On a weekly basis, weights were significantly ($P < 0.05$) different on D7, D21 and D28, but not on D14 (Table 2).

4. Discussion

In the present study, calves were experimentally infected with 10^5 *G. duodenalis* cysts, which is a similar infection dose as used in previous experimental infection trials (O'Handley et al., 2000; Geurden et al., 2006b). All calves were excreting cysts within 11 days after the infection, although different excretion levels were

Table 1
The *Giardia duodenalis* cyst excretion in the treated and control group over the 4-week experimental period. The % reduction in cyst excretion at each sampling day is presented (D = day post-treatment; treated = treatment group; control = control group; mean = arithmetic mean).

No.	Group	D0	D3	D5	D7	D10	D12	D14	D17	D19	D21	D24	D26	D28
8185	Treated	50	0	0	0	0	0	0	0	100	3450	0	14,000	5800
2498	Treated	417	0	0	0	0	0	0	1150	0	0	250	11,850	12,650
2095	Treated	838	0	0	0	0	0	0	0	0	0	0	0	0
1332	Treated	1425	0	0	0	0	0	50	100	0	50	1000	400	450
4716	Treated	2033	0	0	0	0	0	0	0	0	0	200	50	400
7285	Treated	3484	0	0	0	0	0	0	0	0	0	0	50,450	9550
2497	Treated	18,250	0	0	0	0	0	11,600	800	600	2100	3900	1400	7500
4750	Treated	30,300	0	0	0	0	0	0	7600	5500	200	0	2000	450
7684	Treated	80,267	0	0	50	0	0	0	7000	36,550	62,400	3500	15,900	59,200
7664	Treated	13	0	0	50	0	0	0	0	100	0	0	0	0
8863	Treated	25	0	0	0	0	0	0	0	0	100	3300	200	5350
4085	Treated	2700	0	0	0	0	0	0	100	34,600	22,800	2300	78,000	96,150
77	Treated	13,967	0	0	0	0	150	0	50	3100	70,750	2850	6100	11,900
9554	Treated	38,267	0	0	0	0	0	0	0	0	0	0	0	0
Mean		13,717	0	0	7	0	11	832	1200	5754	11,561	1236	12,882	14,957
3188	Control	17	200	0	0	0	100	15,900	6500	12,150	3250	8150	8250	10,400
9389	Control	67	0	0	50	1300	100	1150	13,800	17,900	12,400	12,650	9150	14,950
8862	Control	767	0	50	450	99,600	1300	5300	50,000	43,800	89,100	50,850	62,600	74,200
7964	Control	1167	100	0	50	50	0	50	700	0	0	0	28,150	2050
7683	Control	1617	250	4800	7150	1100	1800	4950	15,000	2700	2600	4250	2250	15,650
2097	Control	2800	0	0	0	0	2400	8650	15,300	ND	1300	7600	100	700
4752	Control	5700	0	0	50	300	1600	10,950	13,200	0	350	6800	7400	17,300
8372	Control	21,333	0	0	250	33,000	1100	1250	130,200	28,350	13,500	79,700	6250	9550
4086	Control	30,450	0	0	0	300	100	400	50	700	600	1100	350	200
1779	Control	86,933	0	150	2000	1700	29,400	17,250	22,000	119,100	4350	1600	86,100	84,450
1611	Control	25	0	350	150	50	0	0	5900	3250	29,000	22,200	8500	21,250
6036	Control	25	15,200	0	400	9050	550	19,000	31,400	8100	20,000	16,150	36,500	41,500
2096	Control	7817	0	0	0	800	500	7100	2700	550	16,000	115,300	43,300	47,350
8637	Control	18,733	0	0	100	550	5400	33,350	45,900	4250	700	156,300	1300	166,900
Mean		12,675	1125	382	761	10,557	3168	8950	25,189	18,527	13,796	34,475	21,443	36,175
Reduction			100%	100%	99.1%	100%	99.7%	91.4%	95.4%	71.3%	22.6%	96.7%	44.5%	61.8%

Table 2
The average weight and average weight gain (arithmetic mean) recorded at the end of each week, and over the 4-week experimental period (total) (D = day post-treatment; treatment = treatment group; control = control group).

	Average weight				
Day	D0	D7	D14	D21	D28
Treatment	62.1	69.8	75.9	79.0	85.4
Control	61.7	65.2	73.2	77.5	82.1
	Average weight gain				
Week	1	2	3	4	Total
Treatment	7.71	6.07	3.14	6.36	23.29
Control	3.50	8.00	4.29	4.64	20.43

observed. In order to evaluate the benefit of a treatment on clinical and subclinical effect of a specific infection, it is important to eliminate concurrent infections. In a previous experimental infection trial with *Giardia* (Olson et al., 1995), lambs were infected through laparatomy and direct injection of cultured trophozoites into the duodenum. As this approach is somewhat cumbersome, and as the abdominal surgery and consequent recovery might influence the observations on clinical and subclinical impact of the *G. duodenalis* infection, a different approach was used in the present study: all calves were infected with cysts obtained from a single source, and a *G. duodenalis* targeted treatment was applied to one of the experimental groups. Furthermore, the most common intestinal infections in calves of that age category, *Cryptosporidium*, *Eimeria* spp., BVD virus and nematode eggs, were monitored and ruled out as potential pathogens in the present study.

The results of the present study confirm the high efficacy of an oral treatment with fenbendazole at 15 mg/kg/day during 3 consecutive days (Xiao et al., 1996; Geurden et al., 2006a). Although cyst excretion was observed in most animals from D14 after the start of the treatment onwards, the treatment significantly reduced the cumulative cyst excretion by 98% for at least 28 days. The fenbendazole treatment resulted in a significantly higher faecal consistency compared to the control group, although there were no overt gastrointestinal symptoms in either group. No other health deviations were recorded throughout the experiment, illustrating the vagueness or absence of overt clinical symptoms caused by giardiasis in calves. Previous studies reported variable symptoms associated with infection, both in experimental and natural conditions (StJean, 1987; Ruest et al., 1997; Koudela and Vitovec, 1998; O'Handley et al., 2000, 2001; Geurden et al., 2006a). The absence of obvious clinical symptoms together with the intermittent cyst excretion often troubles the diagnosis of giardiasis, and confirms the necessity of frequent sampling to diagnose infection and to evaluate the effect of treatment.

The most striking observation of the present study is the 2.86 kg higher weight gain in the fenbendazole treated group compared to the control group after 4 weeks. Although in a comparable infection trial (O'Handley et al., 2000) no differences in weight gain were recorded at a treatment dosage of 5 mg fenbendazole/kg during three consecutive days, other studies indicated that natural and experimental *Giardia* infections can affect weight gain (Geurden et al., 2006a,b). The major difference in weight gain between treated and untreated animals was observed in the first week of the experiment, and weight gains leveled out to some extent from then onwards, as in the O'Handley et al. (2000) experiment which lasted for 3 months. This pattern in weight gain difference might be due to the elimination of the *G. duodenalis* infection in the first week and re-infection from the second week onwards in the treated animals, combined with the onset of immunity development in the control animals. Alternatively, the large difference in weight gain observed in the first week after treatment might be due to an underlying effect of the fenbendazole treatment, although a growth-promoting effect of fenbendazole in calves has never been described.

Compared to experimental infections with pathogenic *Eimeria* spp. (Bangoura and Daugschies, 2007; Deniz et al., 2009; Mundt et al., 2005), the benefit of treatment against *G. duodenalis* on daily weight gain is somewhat less. Furthermore, *G. duodenalis* infections rarely lead to mortality in calves, in contrast to coccidiosis. It is hence difficult to extrapolate the current findings and to estimate the economic impact of a *G. duodenalis* infection on calf rearing in natural conditions. The current findings on the benefit of fenbendazole treatment in experimentally infected calves, the high prevalence of giardiosis in calves up to 6 months (Geurden et al., 2009) and reports of impaired health in naturally infected calves (StJean, 1987; Geurden et al., 2006a) suggest that *G. duodenalis* has a potential impact on calf husbandry, but longitudinal studies on commercial farms are needed to confirm and further quantify the benefit of treatment.

Acknowledgements

The authors would like to thank Dr. Lydia Bommelé and co-workers of the Ghent University experimental farm for their constructive collaboration during this trial, and Intervet-Shering-Plough Animal Health for funding the study.

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