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Efficacy of Halofuginone Lactate against *Cryptosporidium parvum* in Calves

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The efficacy of halofuginone lactate against natural *Cryptosporidium parvum* infection in 150 neonatal market calves of a mixed Belgian breed was tested. The drug was administered orally in the milk replacer over a period of 3 to 14 days at doses ranging from 30 to 500 μg/kg of body weight. Over a period of 4 weeks, the animals were examined twice a week for shedding of *C. parvum* oocysts and were scored semiquantitatively for diarrhea. Weight gain was assessed after 2 and 4 weeks. Subclinical infections by rota-, corona-, and bovine picobirnaviruses were equally distributed in the different groups. In total, 93% of the unmedicated calves eliminated *C. parvum* within 10 days after arrival at the rearing unit and 62% of them showed diarrhea. Immediately after treatment with halofuginone was started, no more signs of *Cryptosporidium*-associated diarrhea were established. From the level of 60 μg/kg on, oocysts were no longer detected in 98% of animals 5 to 6 days after the start of treatment. Animals remained negative for at least 7 days after withdrawal of the drug. From 7 to 10 days after withdrawal, some animals excreted oocysts again. The number of shedders was closely linked with increasing doses of the drug, which indicates that lower doses do not interrupt infection completely and allow development of immunity. In this respect, a dose of 60 to 125 μg/kg over a period of 7 days seems most appropriate in practice. Toxic side effects were noticed only at 500 μg/kg.

Cryptosporidium parvum, a coccidian intestinal parasite, is the second most commonly identified infectious agent in outbreaks of diarrhea in neonatal calves (1, 7). The economic impact of cryptosporidiosis is regarded as comparable to that of rotavirus infection (9). The average annual loss due to bovine cryptosporidiosis in the United States was estimated to be at least $6.2 million in 1978 (9), but recently updated data suggest that this estimate is very low (7). Infected animals excrete huge numbers of oocysts. Mean total output of experimentally infected animals amounts to 2.5×10^{10} oocysts (2). Unlike oocysts of other coccidia, cryptosporidial oocysts are fully sporulated and ready to initiate infection upon excretion (4). These oocysts were reported to contaminate drinking water resources (12, 15). Since *C. parvum* can also infect human beings, contaminated drinking water may be a source of human cryptosporidiosis (6, 10, 15). Unfortunately, only ozone has been reported to be effective in disinfecting drinking water from *Cryptosporidium* spp. (10, 13).

In recent years, numerous cases of cryptosporidial infection in otherwise healthy people have been reported. The most common symptoms are profuse watery diarrhea and abdominal cramping. Immunodeficient people may get persistent infections (3). The principal modes of contamination are fecal-oral spread among human beings and animals and water-borne transmission. Therefore, any drug that limits fecal oocyst output and reduces clinical symptoms in calves would be welcome. Unfortunately, none of the 79 drugs tested against experimental cryptosporidiosis showed clear activity (7). Recently, Naciri and Yvoré reported that halofuginone lactate, a chemical variant of the anticoccidial halofuginone bromohydrate, stopped oocyst output in experimentally infected lambs (13). Therefore, we decided to study the activity of halofuginone lactate at different concentrations in naturally *C. parvum*-infected neonatal fattening calves.

MATERIALS AND METHODS

Animals and husbandry. A total of 150 male 3- to 6-day-old calves purchased from the local market were used in three experiments from late autumn 1989 to early spring 1990. In experiments 1 and 3, a red-and-white mixed Belgian breed was used, and a black-and-white mixed Belgian breed was used in experiment 2. Animals were not vaccinated, nor did they receive any vitamins, other nutritional supplements, or any type of medication during the experiments. Before the calves were admitted, the fattening unit was thoroughly cleaned and disinfected. In each experiment, 50 animals were allocated at random to individual wooden boxes measuring 0.55 by 1.75 m. Solid walls between the boxes prevented contact between the animals. The calves were separated from their feces by a wooden grid. The animals were fed a milk replacer four times a day. During the first 2 weeks after the arrival of the animals, the amount of replacer was kept restricted for them vis-à-vis ad libitum-fed animals to reduce the impact of possible neonatal diarrhea. All animals therefore took in the same amount of milk.

Drug. Halofuginone lactate was supplied as a 2.97% (wt/vol) concentrate (lot C006; Hoechst Ag, Frankfurt, Germany). A working solution was prepared daily by diluting the concentrate in tap water. Treatment always started the day the animals arrived.

Experimental design. At arrival, the 50 calves were divided into groups of 12 to 17 animals with a mean body weight of 46.6 kg (coefficient of variation = 4 to 12%). Only healthy calves were used. They were allocated alternately to the

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different boxes. In each experiment, one group was not medicated and the other groups received the daily dose of the drug in the first ration of milk replacer. The different treatments used are listed in Table 1. Individual weight was recorded at arrival and 14 and 28 days later. Animals were observed daily. Over a period of 4 weeks, fresh fecal material was collected twice a week by rectal sampling. The consistency of feces was assessed as pastose, semiliquid, or liquid. After *C. parvum* was scored and oocyst output was evaluated, 3 g of fecal material was suspended in 15 ml of phosphate-buffered saline (pH 7.2) and spun down at 650 × *g* for 15 min. The supernatant was stored at −30°C and checked later for the presence of picobirnavirus and rotavirus by polyacrylamide gel electrophoresis (17) and coronavirus by enzyme-linked immunosorbent assay (18). The sediment was tested for salmonellae by standard bacteriological procedures.

Parasitology. Before the oocysts were counted, fecal smears were made on microscopic slides and stained with carbol fuchsin (8). The rates of infection of the samples were evaluated semiquantitatively at a magnification of ×500 with a Leitz Laborlux 12 microscope as follows: 0, no oocysts; 1, fewer than 5 oocysts per microscopic field; 2, between 5 and 25 oocysts per field; and 3, more than 25 oocysts per field. For each sample, the complete surface of the slide was examined. Subsequently, the oocysts were counted in a modified Neubauer hemacytometer after 0.1 ml of a 10% (wt/vol) suspension of feces in phosphate-buffered saline (pH 7.2) was mixed with 0.9 ml of malachite green (malachite green, 0.16 g; sodium dodecyl sulfate, 0.1 g; 100 ml of distilled water).

Statistics. The mean oocyst output was tested by the nonparametric Wilcoxon Mann-Whitney *U* test, and the numbers of shedders and animals showing diarrhea were analyzed by the χ^2 test. Weight gain was evaluated by analysis of variance (16).

RESULTS

The kinetics of elimination of *C. parvum* oocysts were very similar in unmedicated animals in all three experiments (Fig. 1). During the first experiment, a close relationship between enumeration of oocysts and semiquantitative scoring was established. Consequently, animals were examined only by the latter method in trials 2 and 3. In total, 93% of 42 unmedicated animals excreted huge numbers of oocysts within 9 days after arrival at the farm. Five days after arrival, 57% of them already shed oocysts. Maximal oocyst output values of 7 to 8 $\log_{10}$ per g of feces were detected 9 to 13 days after arrival. Twenty days after arrival of the animals, oocysts were no longer found in any of the unmedicated calves. A total of 62% of the animals suffering from cryptosporidiosis showed diarrhea, which was liquid in 38% of the animals and sometimes associated with mucus. Diarrhea occurred mostly at the beginning of oocyst output and lasted for about 3 days. The possibility that diarrhea was not detected in some animals is not excluded, since fecal consistency was assessed only twice a week. Other clinical signs, such as anorexia and weight loss, due to *C. parvum* were difficult to assess, since feed intake was restricted during the first 2 weeks after arrival at the farm.

Experiment 1. In experiment 1, two groups of calves were medicated with 500 μg of halofuginone lactate per kg of body weight, one group on days 1, 2, and 3 and the other on days 1, 4, and 7. The third group was not medicated. The calves accepted the milk replacer containing halofuginone without any problem. However, 24 h after administration the animals became depressed and anorectic. Calves medicated on days 1, 2, and 3 lost 2,400 g of weight in 2 weeks, whereas calves medicated on days 1, 4, and 7 lost 1,500 g ($P < 0.001$). This was followed by incomplete compensatory growth during the next 2 weeks (Table 1): 28 days after the trial was started, calves medicated on days 1, 2, and 3 still showed significantly lower weight gain than unmedicated animals ($P < 0.05$).

Treatment with 500 μg of halofuginone lactate per kg caused a significant rise in the number of animals with liquid feces ($P < 0.05$). After the drug was withdrawn, mean diarrhea scores followed the same pattern as in unmedicated controls. Two calves medicated on days 1, 2, and 3 died. Mortality of both calves was associated with enteric bovine viral diarrhea infection, while PI3 virus was detected in the lungs of one calf and IBR virus was detected in the other calf. All surviving animals were examined twice a week for other bacterial and viral infections. No significant differences among groups were recorded, except for rotavirus in calves medicated on days 1, 4, and 7 (Table 1).

All 16 unmedicated calves acquired cryptosporidial infection, and 5 of them showed liquid diarrhea (Table 1). Medication with 500 μg of halofuginone lactate per kg prevented oocyst excretion and associated diarrhea completely 5 days after treatment was started ($P < 0.001$). Oocysts reappeared 9 to 10 days after the drug was withdrawn, and 4 of 34 calves showed liquid diarrhea again. Nevertheless, fewer calves medicated on days 1, 4, and 7 reexcreted oocysts ($P < 0.05$) or showed diarrhea in comparison with the number of calves medicated on days 1, 2, and 3.

Experiment 2. In experiment 2, two groups of calves were medicated with 250 or 125 μg of halofuginone lactate per kg on days 1, 4, and 7, one group received 60 μg/kg continuously over a period of 2 weeks, and one group was unmedicated. No mortality, depression, or anorexia was noted in any group. The drug did not influence weight gain negatively, nor did it induce diarrhea. Bacteriological and virological examination of all animals confirmed that viral infections were equally distributed among the groups (Table 1). Twelve of 13 unmedicated calves became infected by *C. parvum*, and 5 of them showed liquid diarrhea. Medication with 60 to 250 μg of halofuginone lactate per kg prevented oocyst excretion and associated diarrhea ($P < 0.001$). However, oocysts reappeared 7 to 10 days after withdrawal of the drug. The number of reexcreting animals decreased as lower doses were administered. In only the 250-μg group did one reexcreting animal show liquid diarrhea.

Experiment 3. In experiment 3, two groups of calves received 125 or 60 μg of halofuginone lactate per kg for 1 week, one group received 30 μg/kg for 2 weeks, and one group was unmedicated. The experiment confirmed the former trial completely. The drug had no negative influence on zootechnical performance, and *C. parvum* was the main pathogen detected (Table 1). Eleven of 13 unmedicated calves excreted oocysts, and 6 of them showed liquid diarrhea. Medication with 60 or 125 μg of halofuginone lactate per kg prevented oocyst excretion and associated diarrhea after 6 days of treatment ($P < 0.001$), but 3 of 12 and 6 of 12 medicated calves, respectively, again eliminated oocysts 7 to 21 days after withdrawal of the drug. Only one of these animals (60-μg group) showed liquid diarrhea. Oocyst numbers ranged between 0.13×10^6 and 1.25×10^6/g of feces in the 60-μg/kg group and between 0.25×10^6 and 7×10^6/g in the 125-μg/kg group. In the group medicated with

TABLE 1. Influence of halofuginone lactate on natural infection with *C. parvum*[a,b,c]

Expt and no. of calves	Medication		Daily wt gain (g) (mean ± SD)		No. of calves				Mean oocyst output (log_{10}) during the following days after end of treatment:		Mean oocyst score during the following days after end of treatment:		No. of animals shedding[d]:		
					With liquid diarrhea[e]		Shedding oocysts during the following days after end of treatment:								
	μg/kg	Days	Days 0–14	Days 14–28	Period 1	Period 2	0–7	7–21	0–7	7–21	0–7	7–21	Coronavirus	Picobirnavirus	Rotavirus
1															
16	0		39 ± 92[a]	388 ± 127[a]	5[a]	0[a]	16[a]	8[a,b]	6.89[a]	0.95[a]	1.81	0.23	6[a]	16[a]	1[a]
17	500	1–3	−184 ± 110[c]	459 ± 116[a]	0[c]	3[a]	0[c]	12[a]	0.00[c]	2.27[c]	0.00	0.63	9[a]	17[a]	3[a]
17	500	1, 4, 7	−118 ± 191[c]	445 ± 151[a]	0[c]	1[a]	0[c]	5[b,c]	0.00[c]	0.85[a]	0.00	0.25	9[a]	17[a]	9[c]
2															
13	0		−171 ± 220[a]	560 ± 61[a]	5[a]	0[a]	12[a]	6[a,b]	ND[f]	ND	1.35	0.13	11[a]	3[a]	5[a]
12	250	1, 4, 7	−143 ± 160[a]	534 ± 117[a]	0[c]	1[a]	0[c]	8[a]	ND	ND	0.00	0.33	12[a]	3[a]	7[a]
12	125	1, 4, 7	−19 ± 226[a]	535 ± 64[a]	0[c]	0[a]	0[c]	6[a,b]	ND	ND	0.00	0.21	11[a]	3[a]	8[a]
12	60	1–14	133 ± 139[a]	539 ± 115[a]	0[c]	0[a]	1[c]	3[b,c]	ND	ND	0.00	0.003	12[a]	4[a]	5[a]
3															
13	0		−3 ± 176[a]	395 ± 721[a]	6[a]	0[a]	9[a]	0[a]	ND	ND	0.82	0.00	ND	ND	3[a]
12	125	1–7	−8 ± 950[a]	419 ± 68[a]	0[c]	0[a]	1[c]	6[c]	ND	ND	0.03	0.27	ND	ND	2[a]
12	60	1–7	−1 ± 96[a]	351 ± 40[a]	0[c]	1[a]	0[c]	3[a,c]	ND	ND	0.00	0.10	ND	ND	5[a]
12	30	1–14	−20 ± 61[a]	331 ± 69[a]	0[c]	0[a]	1[c]	1[a]	ND	ND	0.08	0.04	ND	ND	5[a]

[a,b,c] Values with different superscripts are significantly different ($P \leq 0.05$).
[d] No animal shed salmonellae.
[e] Period 1: experiment 1, days 9 to 16; experiments 2 and 3, days 7 to 13. Period 2: experiment 1, days 16 to 26; experiments 2 and 3, days 20 to 27.
[f] ND, Not determined.

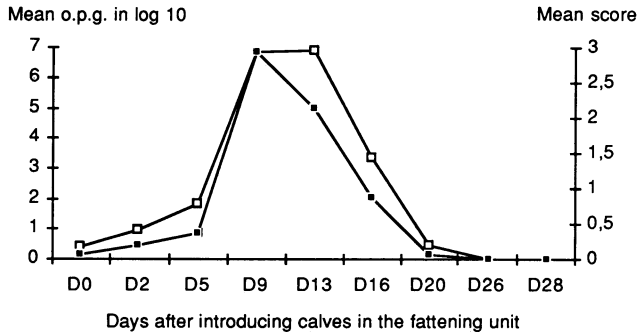

FIG. 1. Experiment 1: relationship between oocyst outputs (□) and fecal oocyst scores (■) of unmedicated calves after natural infection with *C. parvum*. o.p.g., Number of oocysts per gram.

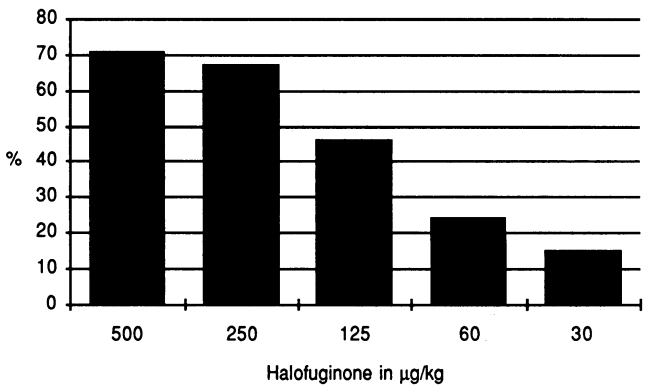

FIG. 2. Influence of dose of halofuginone lactate on percentage of *C. parvum*-reexcreting animals.

30 μg/kg over a period of 14 days, all calves became negative on day 6 of medication, but during the following 8 days of medication 0.125×10^6 to 2×10^6 oocysts per g of feces were detected in 3 of 13 animals. After the drug was withdrawn, only two animals reexcreted oocysts.

DISCUSSION

The three experiments clearly demonstrate that *C. parvum* is involved with enteric problems in neonatal calves: 38% of *C. parvum*-infected calves showed liquid diarrhea, while medicated (≤250 μg/kg) animals were unaffected. Within 9 days after arrival on the farm, 93% of the unmedicated market calves excreted huge numbers of oocysts. Since 57% of them already shed oocysts 5 days after arrival and since the prepatent period amounts to 4 to 5 days, we may assume that most animals acquired infection during transit to the rearing unit and that the remaining ones acquired infection in the rearing unit itself. As mentioned in many other reports (1), some animals showed mixed infections of *C. parvum* with corona-, picobirna-, and/or rotaviruses. In any event, these agents were equally distributed over the different experimental groups and did not influence the results significantly.

The excretion kinetics of oocysts in unmedicated calves coincided fairly well with those described by Blewett (2), and the data confirmed a good correlation between oocyst counts and subjective assessment of oocyst numbers from stained smears (Fig. 1). Twenty days after arrival of the calves, oocysts were no longer detected in any of the unmedicated animals, which indicates that they developed resistance to reinfection.

As a result of the good hygienic conditions in the fattening unit, no severe pathology, e.g., salmonellosis, enterotoxigenic *Escherichia coli*, or severe virus infections, was found in the course of the trial. This explains the low level of mortality (1.3%). Most researchers agree that problems with diarrhea in neonatal calves are complex. Reduction or elimination of any of the pathogens involved in this complex is a step forward in reducing economic losses due to diarrhea, unfavorable feed conversion, and mortality in calves. This report clearly indicates that administration of halofuginone lactate stops *C. parvum*-associated diarrhea and oocyst output at doses between 60 and 250 μg/kg. Since the intake of milk replacer was kept restricted by the breeder during the first 2 weeks after arrival of the animals, it was difficult to assess reduction of other clinical signs, such as anorexia and weight loss, due to *C. parvum*.

At 500 μg/kg, treatment was associated with toxic side effects, such as liquid feces, anorexia, and weight loss (Table 1). Similar findings were reported by Naciri and Yvoré (13), who warned to use extreme caution in treating calves with 500 μg of halofuginone per kg and to calculate drug doses very carefully. Yet our trials showed that toxic side effects disappeared at 250 μg/kg and less, with preservation of anti-*C. parvum* activity. Indeed, 5 to 6 days after treatment with 60 to 250 μg was started, no oocysts were detected in 106 of 108 medicated animals (98%). Since Current and Haynes (5) showed the life cycle to be completed within 72 h, the latter finding could indicate that the drug does not interrupt the life cycle but only prevents reinfection of the gut by sporozoites or recycling merozoites of the first generation. Since 30 μg/kg was not able to prevent oocyst output completely during medication, the active dose of halofuginone lactate should be between 60 and 250 μg/kg.

Seven to 10 days after the drug was withdrawn, oocysts appeared again in the feces, indicating reinfection. However, most of the reinfected animals remained free of clinical signs. Figure 2 shows that the number of oocyst-reexcreting animals was closely linked with the dose administered. This indicates that lower doses do not completely prevent development of the parasite and allow development of immunity during medication. The schedule of treatment, either interrupted or continuous over a period of 7 or 14 days, had no distinct influence on the degree of reinfection. We propose a 1-week treatment, since continuous medication is more practical to breeders.

The practical dose has to be between 60 and 100 μg/kg to guarantee a security factor of about 5. A dose of 60 μg/kg seems most appropriate to prevent or treat cryptosporidiosis because it allows development of immunity during medication, limits the number of animals reexcreting oocysts after medication, and consequently reduces soiling of drinking water resources. This is important, since in recent years different water-borne outbreaks of cryptosporidiosis in human beings have been reported (6, 10, 15). Since only ozone treatment is able to disinfect drinking water from cryptosporidial oocysts (11, 14), contamination of surface water by intensive cattle and sheep raising should be avoided as much as possible. On the other hand, 60 μg/kg could enhance development of resistance to the drug. So, before determining a definite dosage, laboratory resistance studies should be done.

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