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Efficacy of Azithromycin Dihydrate in Treatment of Cryptosporidiosis in Naturally Infected Dairy Calves

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The objective of this study was to evaluate the therapeutic efficacy of azithromycin treatment of cryptosporidiosis in naturally infected calves under field conditions. Fifty Holstein calves with cryptosporidiosis infection were divided into 5 groups: 1 group (10 calves) was unmedicated and served as the control group and was given distilled water only, whereas the other groups (10 animals per group) were medicated orally with azithromycin at the doses of 500 (group 1), 1,000 (group 2), 1,500 (group 3), and 2,000 mg (group 4) PO once daily for 7 days. The animals were examined clinically and fecal samples were collected on the 1st (inclusion day), 7th, 14th, and 21st days of the study. Drug efficacy was assessed by evaluating diarrhea, oocyst shedding, and weight gains from days 1 to 21 (4 assessments). Significant differences were observed in reductions of oocyst shedding ($P < .05$) and the fecal diarrhea incidence ($P < .05$) in groups 3 and 4 when compared with groups 1 and 2 and the control group. Weight gain of medicated calves was significantly higher than that of the unmedicated calves throughout the study ($P < .05$). The drug significantly suppressed oocyst shedding and resulted in significant improvements in clinical signs. Therefore, this suppression may have significant effect on the reduction of environmental contamination by cryptosporidial oocysts. From the economic point view, authors suggest that the most effective dose of azithromycin for the treatment of cryptosporidiosis in calves should be at 1,500 mg/d for 7 days.

Key words: Azithromycin; Cattle; Pathogen; Protozoan; Therapy.

Cryptosporidium parvum is commonly associated with diarrhea in neonatal ruminants.[1,2] Infected animals excrete large numbers of resistant oocysts that are fully sporulated and capable of initiating infection. Oocysts may be transmitted by direct host-to-host contact or by contaminated food or water supplies.[3,4] *C parvum* infection in calves has become a major economic concern for producers. The economic impact of cryptosporidiosis is attributable, not only to the resulting deaths, but also to the slower growth of affected animals and expenditure on veterinary assistance.[5]

More than 100 therapeutic and prophylactic agents, including those with anticoccidial activity, have been tested for efficacy against cryptosporidiosis in human beings and domestic animals, but no cure for cryptosporidiosis exists at this time.[6–8] Anticryptosporidial activity has been reported for lasalocid in calves and halofuginone in both calves and lambs, although the effective dose was too close to the toxic concentration.[6,9,10] Allicin, a sulfur containing a component of garlic, which is available as an additive to milk substitutes was shown, in a randomized controlled trial, not to alter the duration of diarrhea due to *C parvum* or to enhance weight gain.[11] Paromomycin, a poorly absorbed aminoglycoside antibiotic, is a well tolerated and easily administered agent for the management of human cryptosporidiosis. Despite encouraging anecdotal data, the drug does not appear to be uniformly effective in oocyst eradication or resolution of signs.[12] Halofuginone lactate was useful for treating 1-day-old calves when administered at least 60 μg/kg for 7 days.[6,10] This agent is able to prevent clinical signs, but calves begin shedding oocysts soon after the withdrawal of the drug. Unfortunately, halofuginone is not commercially available in the most countries.

Azithromycin, a macrolide antibiotic, has been used in cryptosporidiosis both in immunocompromised animals and humans, with variable efficacy.[13,14] A subclass of macrolide antibiotics derived from erythromycin, azithromycin differs chemically from erythromycin with a methyl-substituted nitrogen atom incorporated into the lactone ring. The drug is rapidly absorbed from the gastrointestinal tract, and although the absorption of the drug is incomplete, it exceeds that of erythromycin.[15] Azithromycin is highly effective in a dose-dependent manner in the prevention and the treatment of experimental cryptosporidial infection in the ileum of immunocompromised rats.[13]

Based on these data, the current study was carried out to investigate the efficacy of azithromycin for the therapy of cryptosporidiosis in naturally infected calves under field conditions.

Material and Methods

Animals

The study was conducted on a 200-cow farm located in the city of Diyarbakır (located in southeastern Turkey). In this farm, cryptosporidiosis infection was endemic and repeated outbreaks occurred during the last calving periods. Twenty-eight male and 22 female Holstein neonatal calves (mean body weight [BW] of 45 kg; age 1–28 days, mean age 10 days) naturally infected with *C parvum* were used in the study. These calves were selected for the study because all were diagnosed as shedding oocysts. Throughout the experiment, the calves were maintained in the same pen under field conditions; they were allowed to suckle their mothers and did not receive any type of medication or nutritional supplements.

Experimental Design

Drug efficacy was assessed by evaluating the presence of diarrhea, oocyst shedding, and weight gains. Calves were divided into 5 groups:

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Table 1. Oocyst count (10^5 per gram feces) in control calves and calves administered azithromycin.

Group[a]	Day 1	Day 7	Day 14	Day 21
Control	106[e] (45–139)	186[d] (66–105)	248[bc] (108–364)	268[b] (134–396)
Group 1	112[b] (24-296)	34[d] (17–52)	88[c] (30–104)	128[b] (62–196)
Group 2	118[b] (84–316)	15[d] (14–30)	67[c] (48–164)	104[b] (74–246)
Group 3	106[b] (32–168)	3[e] (0.08–54)	44[d] (18–92)	66[c] (18.4–68.1)
Group 4	117[b] (67–206)	0.05[e] (0–9)	29[d] (17–67)	36[d] (19–40.5)

[a] Group 1, calves treated with azithromycin at a dose of 500 mg per day from days 1 to 7; group 2, calves treated with azithromycin at a dose of 1000 mg per day from days 1 to 7; group 3, calves treated with azithromycin at a dose of 1500 mg per day from days 1 to 7; group 4, calves treated with azithromycin at a dose of 1500 mg per day from days 1 to 7.

[b–e] The superscript upper key letters were statically significant ($P < .05$). Data are geometric means with ranges in parentheses.

(i) 10 calves were unmedicated and kept as the control (placebo) group (given distilled water only) and (ii) 4 groups, 10 animals each, were orally medicated with single doses of azithromycin dihydrate[a] at doses of 500 (group 1), 1,000 (group 2), 1,500 (group 3), or 2,000 mg (group 4) for 7 consecutive days, respectively. Animals were monitored throughout the study and closely examined at 1-week intervals for 4 weeks.

Collection of Samples and Detection of Oocysts

Fresh fecal samples, approximately 10–20 g, were taken directly from the rectum by digital evacuation on 2 occasions at 12-h intervals from all calves on days 1, 7, 14, and 21. Specimens were placed into sterile plastic containers, refrigerated at 4°C and analyzed within 24 hours. Fecal smears were examined under a microscope. Heine's negative stain[16] was used to identify *C parvum* oocysts. Oocyst numbers were scored semiquantitatively according to the average number of oocysts in 10 randomly selected microscopic fields at 400× magnification. Oocyst counts were categorized as follows: 0 (no oocysts), 1 (0–1 oocysts per field), 2 (1–5 oocysts), 3 (5–10 oocysts), and 4 (more than 10 oocysts per field). Furthermore, the number of oocysts per gram of feces was calculated using a previously described method.[17]

Clinical Variables

The animals were clinically examined by the same investigators (BE and HP) after treatment on days 1 (inclusion day), 7, 14, and 21 for general condition (fatness and alertness), feeding behavior, fecal appearance (liquid, soft, solid, or bloody), and dehydration state. Each variable was scored using a scale of 0, normal; 1, medium; 2, mediocre; and 3, poor according to Naciri at al.[18] When fecal score exceeded 3 or a calf exhibited other signs of disease, oral electrolyte therapy[b] was initiated without discontinuing the suckling.

Measurement of BW

Measurement of girth circumference was used to predict body weight. A nonelastic measuring tape[c] was placed just behind the front legs and behind the shoulders of the heifer. The tape was pulled snug and the circumference recorded.[19]

Statistical Analyses

The statistical analyses were performed either by paired *t*-test or by analysis of variance (ANOVA) with repeated measures, the computer software SPSS for Windows, Version 10.0, 1999. Upon detection of significant increases by ANOVA, post hoc pairwise comparisons were conducted using Tukey's test. The significance level was set at $P < .05$.

Results

Animals

Five calves (50%) from the control group and 5 calves (12.5%) from treated groups (3 from group 1 and 2 from group 2) excreting large numbers of oocysts died during oocyst shedding (score >3) between days 2 and 6 although oral electrolyte therapy was administered. There were no deaths between days 1 and 21 in groups 3 and 4, but 2 calves from group 3 and 3 calves from group 4 were sold on day 11 by the owner. Therefore, these calves were discounted for the trial after 6–11 days of the study.

Oocyst Shedding

Calves in all groups excreted large numbers of cryptosporidial oocysts on day 1 (inclusion day) (Table 1). On day 7, control-group calves excreted large numbers of cryptosporidial oocysts (mean score, 3), whereas oocysts were not detected in 85% of medicated calves. In contrast with calves treated with azithromycin at a dose of 500 or 1,000 mg/d, animals treated with azithromycin at 1,500 or 2,000 mg/d had a significant reduction in oocyst shedding ($P < 0.05$) (Table 1).

After cessation of the drug (the 7th day of the study), calves were monitored on days 14 and 21 to observe the efficacy of the drug. Azithromycin at 1,500 and 2,000 mg/d were the most effective doses in reduction of oocyst count when compared with azithromycin at 500 and 1,000 mg/d. Oocyst shedding in calves in groups 3 and 4 was low (Table 1).

Diarrhea Status and Clinical Score

Of the 50 calves examined, 15 (30%) had liquid feces, 30 (60%) had soft feces, and 5 (10%) had bloody feces. *C parvum* oocysts were detected in all 50 calves. Medication with azithromycin reduced but did not completely prevent clinical signs, although the percentage of diarrheic calves in all medicated groups declined by the end of the treatment. Four control calves (80%) continued showing persistent diarrhea on day 7, whereas 30 treatment calves (85%) were asymptomatic. Differences in the percentage of diarrheic calves between medicated and control groups were statistically significant ($P < .05$). Diarrhea disappeared in groups 3 and 4 by day 7, and no clinical signs were noted

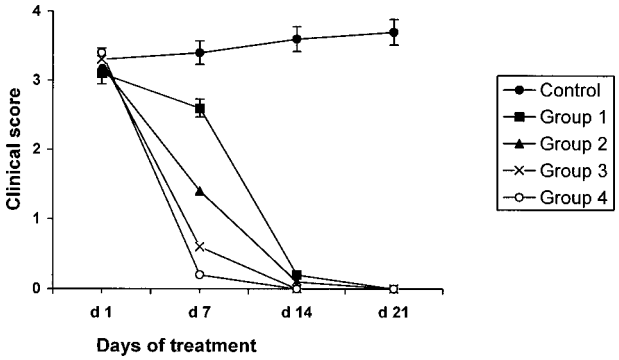

Fig 1. Clinical scores in calves with cryptosporidiosis administered placebo (control) or azithromycin.

until the end of the experiment, whereas 3 calves, 2 from group 1 and 1 from group 2, had diarrhea.

Clinical scores during the study were greater in control calves ($P < .05$) than in calves treated with azithromycin. However, total clinical scores were greater on days 7, 14, and 21 in group 1 and 2 than in groups 3 and 4 (Fig 1). Throughout the observation period, all therapy with azithromycin were effective, but over the complete period of clinical observation, azithromycin therapy at 1,500 and 2,000 mg/d for 7 consecutive days was most effective in the clinical recovery from cryptosporidiosis.

Weight Gains

The body weight gain of calves medicated with azithromycin was greater than that of control calves throughout the study and statistically significant differences were seen between days 1 and 21 ($P < .05$). By contrast, the growth rate of calves in groups 3 and 4 was impaired in comparison with the other treatment groups ($P < .05$) (Table 2).

Discussion

In the present study, all calves showed profuse watery diarrhea and were positive for cryptosporidial oocysts on day 1. This high incidence may be attributed to poor hygiene and, more importantly, that the calves remained with their mothers until day 7 after birth. Asymptomatic carriers intermittently shed oocysts and are responsible for the diarrhea in calves born during early calvings,[20–22] and cows that are clinically healthy may excrete between 750,000 and 720 million oocysts in a day, thus contributing to the contamination of the newborn calf's environment.[23]

The mean age of calves used in this study was 10 days, and all of the calves on day 1 excreted oocysts, and diarrhea was detected at the time of their inclusion. A study carried out in central Spain indicated that, in calves less than 30 days of age with diarrhea, the prevalence of infection varied considerably according to the age of the animals. The lowest prevalence was found in calves at the extremes of the age range (43.8% in calves <7 days old and 6.9% in calves between 22 and 30 days old); in contrast, the prevalence in calves of 8–14 and 15–21 days of age rose to 71.9 and 63.2%, respectively.[24]

To date, drugs, such as halofuginone lactate, paromomycin, and decoquinate, have been demonstrated to be partially effective in the prevention and treatment of cryptosporidiosis in ruminants by preventing or decreasing oocyst excretion and the severity of diarrhea when administered during periods ranging between 3 and 21 days and even up to 8 weeks.[6,10,17] In their retrospective studies, the treatment of natural cryptosporidiosis infections in calves and in lambs, Villacorta et al[6] and Causapé[25] found that withdrawal of the drug halofuginone lactate caused reappearance of oocysts in the feces, indicating reinfection. However, most of the animals remained free of clinical signs of infection.

In the present study, we examined the fecal samples collected weekly until day 21 and did not detect any signs of reoccurrence in animals treated with azithromycin at the doses of 1,500 and 2,000 mg/d for 7 consecutive days. Similar results were obtained by Viu et al.[26] in a field trial on the therapeutic efficacy of paromomycin on natural *C parvum* infections in lambs. However, paromomycin should be used cautiously because it may have toxic effects.[27]

The economic losses due to cryptosporidial infections of neonatal calves are related to diarrhea: dehydration, growth retardation, and, to a lesser extent, mortality.[28] Although the drug did not suppress cryptosporidiosis completely, it gave significant improvements by reducing the clinical symptoms, the intensity of infection, and the fecal output of oocysts. Therefore, this suppression may have a significant effect on the reduction of environmental contamination by cryptosporidial oocysts.

From an economic point of view, we suggest that the most effective dose of azithromycin for the treatment of cryptosporidiosis should be 1,500 mg/d for 7 days. In some countries, this drug seems to be highly expensive (ie, United States); however, it is relatively cheap in comparison with other routine drugs used in the treatment of cryptosporidiosis in most countries, including Turkey.

Table 2. Estimated body weights (mean ± SD) of calves naturally infected by *Cryptosporidium parvum* after administration of placebo or azithromycin.

	Weight (kg)				
Day of Study	Control	Group 1	Group 2	Group 3	Group 4
1	45.1 ± 2.4	46.4 ± 1.8	46.2 ± 1.6	45.7 ± 4.0	46.0 ± 3.0
7	46.0 ± 2.2^{d}	48.2 ± 2.8^{c}	49.4 ± 2.0^{c}	53.4 ± 2.2^{b}	57.1 ± 3.6^{a}
14	45.4 ± 3.0^{d}	52.1 ± 2.4^{c}	53.7 ± 3.4^{c}	57.4 ± 3.8^{ab}	60.2 ± 4.8^{a}
21	44.1 ± 3.2^{d}	56.4 ± 3.0^{c}	57.4 ± 4.3^{c}	63.0 ± 4.2^{ab}	67.3 ± 5.0^{a}

$^{a–d}$ Superscript letters indicate statistical significance.

Footnotes

[a] ZITROMAX Suspension®, Pfizer, İstanbul, Turkey
[b] LECTATE Powder®, Pfizer, İstanbul, Turkey
[c] Combi weight measuring tape®, VE-BO, Marslev, Denmark

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