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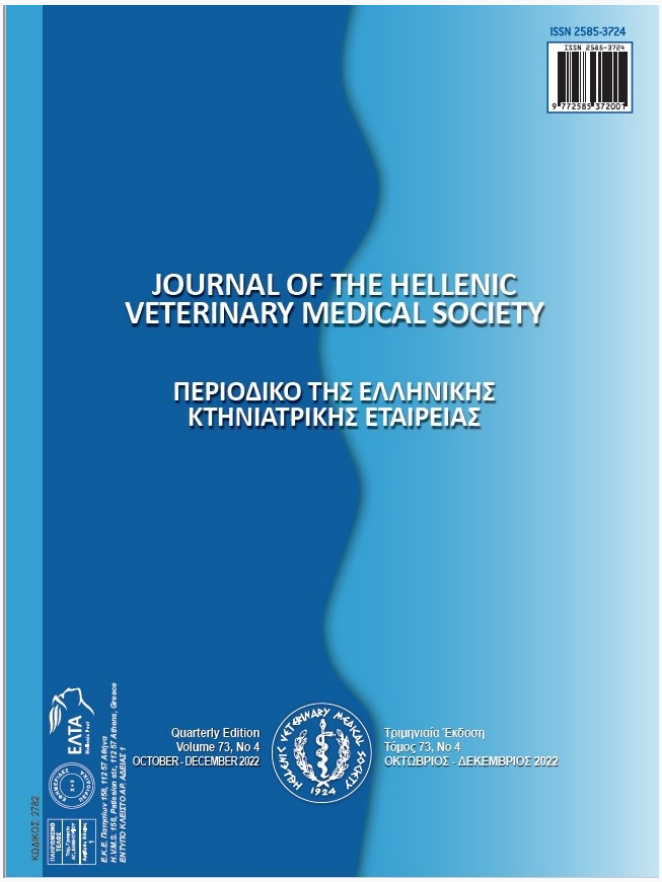

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Research article
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Comparison of the effects of buparvaquone and paromomycin on oocyst excretion and clinical parameters in diarrheal calves naturally infected with cryptosporidiosis

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ABSTRACT: *Cryptosporidium* spp. is an obligate intracellular parasite that infects a wide variety of hosts. Young calves are particularly susceptible to infection. In the present study, we investigated the effects of buparvaquone and paromomycin on oocyte shedding and clinical parameters in calves naturally infected with cryptosporidiosis. Twenty calves with naturally occurring cryptosporidiosis were divided into two groups (n=10, each). A single dose of 2.5 mg/kg buparvaquone was administered intramuscularly to one group, while the animals in the other group received 100 mg/kg/day paromomycin orally for seven days. On day 7, oocyst shedding ($p<0.001$) was reduced, and sucking reflex ($p=0.002$) and mental status ($p=0.034$) were improved in calves treated with buparvaquone. Our results suggest that applying a single dose of buparvaquone injection can be an excellent alternative to complex treatment methods applied to calves with cryptosporidiosis in the field regarding the economics and practicability.

Keywords: *cryptosporidiosis; calves; buparvaquone; diarrhea; paromomycin*

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INTRODUCTION

Cryptosporidiosis is one of the most important infections causing acute or chronic diarrhea in humans, as well as in domestic and wild animals (Senturk, 2019). Diarrhea of neonatal calves is a serious welfare problem and a significant cause of economic loss in the cattle industry because of high mortality, treatment costs, and poor growth of the affected animals (Pohjola, 1984; Bazeley, 2003; Cho et al, 2013; Ammar et al, 2014). Lymphocytic colitis and blood in the stool are seen in affected animals. In immunosuppressive calves, the agent alone can cause severe hypovolemic shock (Senturk et al, 2016).

Many drugs have been tried for the treatment of cryptosporidiosis, the most frequently used of these is paromomycin. Although several extensive studies have been done, no effective treatment has been found for cryptosporidiosis yet (Olias et al, 2018; Schneider et al, 2021). Paromomycin is an aminoglycoside-derived antibiotic and is one of the most commonly used agents in treating cryptosporidiosis. Intestinal absorption of paromomycin is low as reported by the manufacturer. It is considered a possible drug for giardia infections with proven efficacy (about 60% to 70% efficacy) in children. It and has beeen used for the treatment of giardiasis in pregnant women. Most of the paromomycin is excreted from the intestines without being metabolized in the body (Mineno & Avery, 2003).

On the other hand, buparvaquone (BPQ) is a hydroxynaphthoquinone group antiprotozoal efficient against bovine theileriosis (Wilkie et al, 1998) and temporarily effective against *Theileria equi* infections in horses (Baneth, 2018). The drug has also been used in combination with azithromycin to treat *Babesia vulpes* infection in dogs (Checa et al, 2017) and to treat *Sporothrix brasiliensis* infection in cats (Borba-Santos et al, 2021). To the best of our knowledge, there is only one study investigating the effect of buparvaquone against cryptosporidiosis, but that study solely evaluated oocyst shedding as a parameter (Alidadi et al, 2008).

This study aimed to evaluate the efficacy of BPQ on oocyst shedding in calves with cryptosporidiosis by comparing it with the commonly used paromomycin. This study was the first to investigate the effictiveness of BPQ on clinical parameters of oocyst scattering calves with cryptosporidiosis.

MATERIAL AND METHODS

The experimental design was approved by the Bursa Uludağ University Animal Experiments Local Ethics Committee (HADYEK) (2021-01/04).

The study material consisted of 20 Holstein calves of the same age (6-8 days, mean 7.4 days) and bred under similar conditions at Bursa Uludağ University Animal Health and Animal Production, Research and Application Center. Calves were divided into two groups (buparvaquone group [group B] and paromomycin [group P], each with 10 animals) with similar gender and age distribution.

Study design

The day for the onset of diarrhea was considered day 0, and the treatments were started. The animals were clinically examined and scored daily at 08:00, and stool samples were obtained at 08:00 on days 0, 3, and 7. The animals were scored for the sucking reflex (3=normal, 2=weak-diligent, 1=weak, 0=absent) (Şentürk, 1999), mental status (1=normal, 2=mild depression-weak sucking reflex-calf standing, 3=severe depression, no sucking reflex-calf in supine position) (Şentürk,1999), rectal temperature, respiratory rate, heart rate, and stool consistency (0 = normal; 1=half-shaped or pasty; 2=loose stool; 3=watery stool) (Wood et al., 2019).

Fecal samples were stored in sterile plastic containers, well suspended by adding the same amount of 2 % potassium dichromate (KCr_2O_7) solution, and stored at 4 °C (Igloo YA1178 digital display auto refrigerator) during the procedures, and were taken and brought to the laboratory. All of the samples were first examined using the carbol fuchsin staining method to reveal oocyst presence; one gram of stool was placed on a glass slide, and a drop of carbol fuchsin was added and mixed (Heine, 1982; Ahmed & Karanis, 2018). The smear was then dried, immersion oil was added, and the slide was covered with a coverslip (McCluskey et al., 1995). Slides were then examined under x400 magnification (Nikon Eclipse E100, Japan). Shedding at least $1.2x10^5$ oocysts per g stool was considered the inclusion criterion of the calves into the study (Xiao and Herd, 1994; Medema & Schijven, 2001). Accordingly, 50 µl of the stool samples, which were thoroughly mixed and homogenized, were taken with the help of a pipette on the slide, which was cleaned in an ether-alcohol mixture and degreased, and a thin stool smear was prepared with the edge of a coverslip by adding the same amount of carbol fuchsin. Immediately after this prepared smear was dried in the air, a drop of immersion oil was dropped, a coverslip was covered, and it was scanned for *Cryptosporidium* oocysts under the

microscope under x400 magnification. Microscopically, oocysts at on 10 sites were counted and averaged.

All animals were tested with a quick test kit (BOVID-5 Ag Bionote, Gyeonggi-do, Republic of Korea) and calves that were positive for cryptosporidiosis were included in the study, whereas calves with mixed infections were excluded. According to the data provided by the manufacturer, the sensitivity and specificity of the rapid test kit are 98.2% and 99.0%, respectively. In the present study, the diagnosis of cryptosporidiosis was made both by quick test kits and by having the number of oocytes (> $1.2x10^5$) in stool examination. No calf was treated with fluid support or antibiotics during the study.

Buparvaquone (Butalex®, MSD Animal Health, Germany) was administered intramuscularly (neck muscles) in a single dose of 2.5 mg/kg by measuring the calves' weights on day 0. Paromomycin (Gabbrocol®, Ceva, Italy) (100 mg/kg/day for seven days) was given orally to calves in group P.

Statistical analysis

Data entry was made using Microsoft Excel and transferred to SPSS v22 and MedCalc V19 software for statistical analysis. The normality tests of the data were performed with the Shapiro-Wilk method. Descriptive statistical outputs of the data were given as mean ± SE for normally distributed data and as median (min-max) for non-normally distributed data. Repeated measurement analysis of variance (ANOVA) of data that did not show normal distribution was performed by Friedman method. To reveal the statistical differences between the data, Mann-Whitney-U tests were also applied together with repeated measurement analyses. One-way ANOVA was used to compare day 0, day 3, and day 7. According to the results, Dunn's method, as one of the post-hoc tests, was applied. Pearson correlation and Spearman rank correlation tests were applied to determine the relationships between the parameters. A p-value of <0.05 was considered statistically significant in all statistical tests.

RESULTS

Oocyte shedding decreased after both BPQ and paromomycin administrations. The decrease in buparvaquone group was more significant than in paromomycin group and almost no oocysts were detected on day 7 in buparvaquone group ($p<0.001$) (Table 1). In paromomycin group, shedding decreased in time, but the difference between day 3 and day 7 could not be substantiated statistically (Table 2).

Sucking reflex and stool score were better on day 3 and day 7 than before treatment (day 0) in group B. No difference was observed in group P regarding these parameters. In the comparison of the groups, no difference was observed regarding the stool score, whereas BPQ had a more significant effect on the mental state and sucking reflex than paromomycin (Table 3) ($p<0.05$). There was no difference between the groups or day-wise in terms of fever, respiratory frequency, and heart rate (Table 3) ($p>0.05$).

DISCUSSION

Various drug trials have been conducted in the treatment of cryptosporidiosis. Drugs including paro-

Table 1. Buparvaquoneand Paromomycin group oocyst numbers in ($x10^6$/g stool)

	$x10^6$/g stool oocyst numbers		
Groups	Day 0	Day 3	Day 7
Buparvaquone	3765 (1020-8640)	232 ± 70.83	2 (0-20)
Paromomycin	4204 ± 835.72	482 ± 121.13	386 ± 122.96
P value	0.571	0.092	< 0.001

The P-value in the table represents the differences between the groups.

Table 2. Post-hoc test of the mean oocyst counts of the buparvaquone (B) and paromoycin (P) groups on days 0, 3, and 7 of their measurements. Mean differences, standard errors, and P values are presented

(Post-Hoc)		Diff of Ranks	Standard Error (SE)	P value
B day 0	B day 3	10.50	3.32	0.003
	B day 7	19.50	6.16	0.002
B day 3	B day 7	9.00	2.84	0.01
	(Post-Hoc)	Diff of Ranks	Standard Error (SE)	P value
P day 0	P day 3	3722	5.35	0.002
	P day 7	3818	5.49	0.002
P day 3	P day 7	96	0.13	0.454

Table 3. Stool score, mental status, sucking reflex, temperature, pulsation, and respiration 0th, 3rd and 7th day measurements of buparvaquone (B) and paromomycin (P) groups on daily basis

	Buparvaquone (B) group			Paromomycin (P) group			P Value		
	Day0	Day3	Day7	Day0	Day3	Day7	Day0	Day3	Day7
Stool Score	1.70[a] (1-3)	0.7[b] (0-1)	0.3[b] (0-1)	1.30 (1-3)	1.30± 0.36	0.8 (0-3)	0.278	0.268	0.501
Mental Status	1.50 (1-2)	1(1-1)	1.40 (1-1)	1.20 (1-2)	1.60 (1-2)	1.40 (1-2)	0.185	0.005	0.034
Sucking Reflex	2[c] (1-3)	3[d] (3-3)	3[d] (3-3)	2.3 (1-3)	2 (1-3)	2 (1-3)	0.349	0.006	0.002
T(°C)	38.58±0.06	38.76±0.07	38.62±0.11	38.49±0.09	38.60±0.13	38.65±0.09	0.450	0.471	0.841
R(/m)	28±1.578	26.80±1.2	26±1.22	27.20±1.86	28.80±4.07	36.80±6.07	0.747	0.556	0.150
P(/m)	112.80±4.98	114.80±3.58	119.20±5.65	117.20±5.40	122.40±9.60	121.20±4.95	0.557	0.789	0.7930

a-b: $p<0.001$ c-d: $p=0.008$

momycin, toltrazuril, halofuginone, decoquinate, azithromycin, some feed additive and tylosin are used for prophylaxis and treatment of cryptosporidiosis (Paraud & Chartier, 2012; Aydogdu et al, 2018; Yagci et al, 2017; Vélez et al, 2019). Paramomycin is one of the most efficient drugs used against *Cryptosporidium* spp. infection. The aminoglycoside paromomycin has been found effective in treatment of calves with cryptosporidiosis when administered at a dose of 100 mg/kg/day for seven days (Aydogdu et al 2018). Aminoglycosides are generally known for their nephrotoxic and ototoxic effects. However, the aminoglycosides such as paramomycin and neomycin are water-soluble highly polar cations that are poorly absorbed from the gastrointestinal tract; less than 1 percent of an oral. Therefore, the paramomycin has no toxic effect throughout the body, when administered orally (Steven, 1998; Heit and Riviere, 2001). It has been shown that paromomycin does not cause toxicity in calves, lambs and kids experimentally or naturally infected with cryptosporidiosis (Fayer and Ellis, 1993; Mancassola et al, 1995; Viu et al, 2000). Even if the intestinal mucosa is destroyed, it acts on the protozoan and ensures the treatment of the infection. Therefore, paromomycin was given orally and possible systemic side effects were not observed in the study (Şentürk, 2019).

While there are treatment protocols followed on certain days in all these studies in which used drugs against cryptosporidiosis, this creates a follow-up difficulty for businesses and veterinarians. This study provides a practical solution to the industry by delivering better recovery with a single injection. Our study determined that a single dose application of buparvaquone significantly reduced oocyte scattering compared to seven days of paromomycin application. Administering a single dose may provide a practical advantage in field conditions in addition to the positive effect of buparvaquone on clinical signs and oocyte scattering.

Paromomycin was not given to the calves parenterally in this study, it was given orally and possible systemic side effects were not observed in all these studies. While there are treatment protocols followed on certain days in all these studies, this creates a follow-up difficulty for businesses and veterinarians. This study provides a practical solution to the industry by delivering better recovery with a single injection. Our study determined that a single dose application of buparvaquone significantly reduced oocyte scattering compared to seven days of paromomycin application. Administering a single dose may provide a practical advantage in field conditions in addition to the positive effect of buparvaquone on clinical signs and oocyte scattering.

Although there are no comprehensive studies in the literature regarding the use of buparvaquone in *C. parvum* infections, Alidadi et al. (2008) investigated the effect of the buparvaquone on oocyst density in calves with cryptosporidiosis and found that buparvaquone significantly reduced oocyst scattering. But these researchers applied isotonic saline to the calves in the control group and did not evaluate the clinical improvement. In the present study, BPQ was compared with paromomycin, one of the most widely used products in preventing and treating cryptosporidiosis. We found that BPQ decreased oocyte scatter more than paromomycin, and also provided significant improvements in mental depression and sucking reflex, which are indicative of clinical improvement in cryptosporidiosis-infected diarrheal calves (Table 3).

Buparvaquone is an antiprotozoal drug used mainly in infections such as theileriosis and babesiosis in cattle and sheep. Buparvaquone causes the lysis of lymphocytes and erythrocytes invaded by *Theileria* schizonts and *Babesia* pyroplasma forms. Although its mechanism of action has not been fully elucidated, it is thought that the drug prevents the mitochondrial electron transport of the protozoon and the pyrimidine synthesis (Sherman, 1998; Gebru et al., 2006) which is necessary for the development and reproduction of protozoa (Hacilarlioglu, 2013). Buparvaquone is thought to have similar effects with atovaquone, which is used in the treatment of malaria and *Toxoplasma gondii* infection (Mehlhorn 2008). A transmission electron microscopic study, in which the effect of BPQ on oxidative phosphorylation in the mitochondria of *E. multilocularis* cells was measured in mice, revealed that treatment with BPQ prematurely disrupted parasitic mitochondria (Rufener et al., 2018). In studies to explore the survival pathways of infected cells (Guergnon et al., 2003), parasites exposed to the BPQ terminate their proliferation after the death of macroschizonts and trigger apoptosis of host cells within a few days (Rashid et al., 2019, Ram et al., 2021). A research conducted to compare the intestinal permeability of BPQ used for the treatment of *Leishmania donovani* infections with other drugs emphasized that the active substance that remained in the tissues for the longest time was BPQ (Venkatesh et al., 2007). In the present study, there were significant positive improvements in sucking reflexes of calves with diarrhea after BPQ administration at 3rd and 7th days compared to before treatment. These effects may be related to possible antiprotozoal effects of BPQ against *Cryptosporidium* spp. (McHardy et al, 1985, Singh et al, 1993). Additionally, treating cryptosporidiosis in a 40 kg calf using paromomycin with a seven-day administration schedule costs approximately 18.58 dollars, whereas a single dose buparvaquone is 0.89 dollars. Therefore, buparvaquone was found more economical and practical than paromomycin.

CONCLUSIONS

In the present study, it was determined that a single dose of buparvaquone administration in calves with cryptosporidiosis diarrhea significantly reduced oocyte scattering and clinical severity of diarrhea and accelerated clinical recovery. In addition, in this study we compared buparvaquone with paromomycin, a preparation very commonly used to treat cryptosporidiosis in calves, and provided better clinical progress with BPQ. These results suggest that BPQ is valuable and promising for the treatment of cryptosporidiosis.

CONFLICT OF INTEREST STATEMENT

The authors declare that they have no conflicts of interest.

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