

Efficacy of albendazole and paromomycin treatments against natural *Giardia* spp. infection in calves

ONUR KÖSE¹, REYDA KIYICI², MUSTAFA FURKAN PALA¹, RAMAZAN ADANIR¹,
BAYRAM KUDRET KARAAYVAZ², MEHMET KARACA³, HASAN ALTAN AKKAN³

¹Department of Parasitology, ³Department of Internal Diseases, Faculty of Veterinary Medicine, Burdur Mehmet Akif Ersoy University, Istiklal Campus, Burdur, Turkey

²Burdur Food Agriculture and Livestock Vocational School, Burdur Mehmet Akif Ersoy University, Istiklal Campus, Burdur, Turkey

Received 14.04.2023

Accepted 05.05.2023

Köse O., Kiyıcı R., Pala M. F., Adanır R., Karaayvaz B. K., Karaca M., Akkan H. A.

Efficacy of albendazole and paromomycin treatments against natural *Giardia* spp. infection in calves

Summary

The aim of the present study was to evaluate and compare the efficacy of albendazole and paromomycin treatments against natural *Giardia* spp. infection in calves. The calves were divided in two groups of six animals each with similar parasitic loads of *Giardia* spp. cysts. Albendazole (20 mg/kg) and paromomycin (50 mg/kg) were administered orally to group A and group P, respectively, for three consecutive days. The efficacies of drugs evaluated by analyzing the reduction in cyst excretion in native fecal examinations on days 0, 3, 7, 10, 14, 21 and 28. In addition, percentages of reduction for days 3, 7, 10, 14, 21 and 28 according to day 0 were evaluated. The geometric mean of fecal cyst excretion in both groups started to decrease from the 3rd day, in paromomycin group the decrease continued steadily throughout the examination days starting from the 3rd day, despite that in the albendazole group the cyst amount increased in the 7th day and then decreased again. Cyst excretion reached zero in all animals at the 21st and 28th days in group P and group A, respectively. The percentage of reduction started with a high rate of 47.72% on the third day and reached 100% on the 21st day in the paromomycin group. In the albendazole group, the percentage of reduction started with a rate of 35.29% on the third day and reached 100% on the 28th day. As a result, paromomycin was found to be more effective compared to albendazole in terms of both the regularity of the mean cyst reduction and the time it takes for the cyst excretion to reach zero.

Keywords: albendazole, calf, *Giardia*, paromomycin, treatment, efficacy

Giardiasis is a worldwide enteric infection of various animals and humans caused by flagellated protozoan parasite *Giardia* species (1, 20). The trophozoite form of the parasite attaches to the intestinal mucosa, leading to the decrease of the microvillus surface area, reduces intestinal enzyme activity, and increases intestinal transit (15). Therefore, these pathological changes may cause bloating, malabsorption, diarrhoea, weight loss and growth retardation (3, 4, 8). The invasion is of high importance among companion animals, livestock and, of course, humans. The disease is especially important in livestock animals because of it leading to the reduction of productivity. One of the most important aetiological agents responsible for calf diarrhoea is *Giardia intestinalis* (synonyms: *G. duodenalis*, *G. lamblia*) (13, 18). Invasion depends on many factors and can range

from asymptomatic to acute with gastroenterological symptoms. Although it may be asymptomatic in some cases depending on the patient's immune system and the severity of the infection, infected animals shed the cysts of the parasite with faeces for several weeks (25) and cause contamination of the environment such as water resources (8). Therefore treatment of infected animals is essential for control of the disease, prevention of environmental contamination, and avoiding economic losses.

Treatment of giardiasis is mainly based on drug administration for elimination of parasites and supportive applications for compensation of fluid and electrolyte loss because of diarrhea and malabsorption. Several drug options such as nitroimidazoles (metronidazole, tinidazole, dimetridazole, ipronidazole), nitazoxanide,

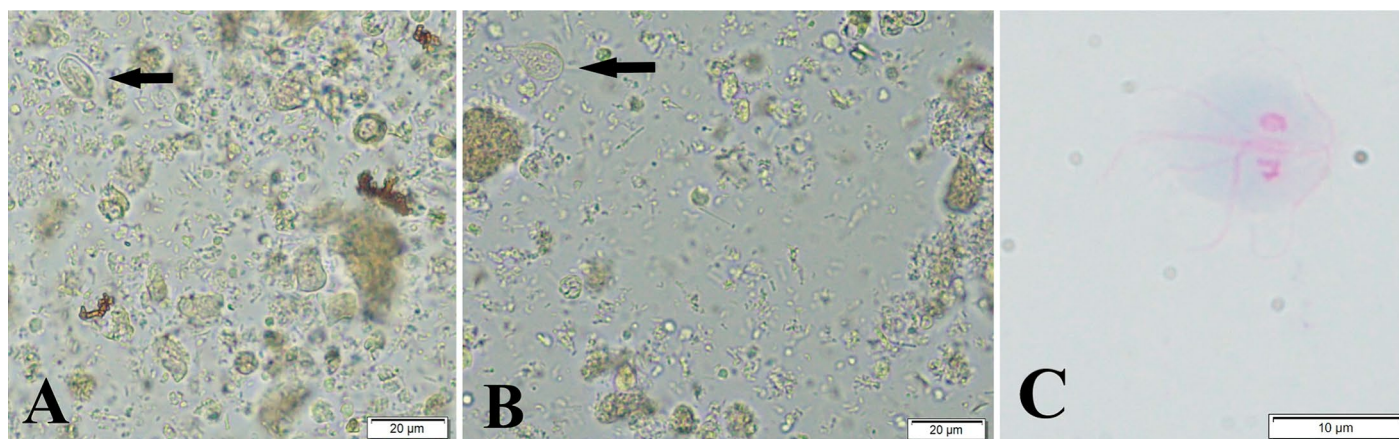


Fig. 1. *Giardia* spp. cyst (A) and trophozoite (B) forms in native fecal examination (40 × objective, scalebar 20 µm), trophozoite form (C) in Giemsa staining (100 × objective, scalebar 10 µm)

furazolidone, quinacrine, benzimidazoles (albendazole, mebendazole, fenbendazole), chloroquine, paromomycin and secnidazole can be used for treatment (9, 11, 23, 24). However, there are some limitations for use of some of these drugs such as quinacrine can easily cross the placenta, metronidazole and furazolidone are mutagenic (26), metronidazole may also cause anorexia and diarrhoea and they are not legal to use in food animals (19).

For the last few decades, studies are being carried out to find more effective and safe treatment options for *Giardia* spp. infections. Albendazole, a member of the benzimidazole group, has an inhibitor role for the attachment of *Giardia* spp. trophozoites to the intestinal mucosa. This inhibition provided by inhibiting the polymerization of tubulin to microtubules, which are major components of flagella, the median body, and the ventral disk of the parasite (6, 12, 21). Another drug paromomycin is an antibiotic in the amino glycoside group, which interferes with the 50S and 30S ribosomal subunits and cause misreading of mRNA codons, thus, protein synthesis of the *G. lamblia* is inhibited (7).

There are various studies about the treatment effectiveness of these drugs against giardiasis in veterinary practice. Xiao et al. (26) tested the efficacy of albendazole and fenbendazole against giardiasis in calves and reported that both drugs appear to be effective in suppressing cyst excretion. Geurden et al. (9) reported the efficacy and safety of paromomycin against experimentally *Giardia* spp. infection in calves and they emphasized the efficacy of paromomycin is comparable to the efficacy of both fenbendazole and albendazole treatments. In another study, the treatment efficacy of fenbendazole combined with environmental measures against natural giardiasis in calves was investigated and significant cyst reduction and clinical improvement were reported (10). Albay et al. (2) compared the therapeutic effect of 20 mg/kg albendazole, 50 mg/kg and 100 mg/kg paromomycin for 3 successive days in lambs naturally infected with giardiasis and found paromomycin to be more effective than albendazole.

In the present study the aim was to evaluate and compare the treatment efficacies of albendazole and paromomycin as noticed above (9) against natural *Giardia* spp. infection in calves.

Material and methods

The present study was approved by Burdur Mehmet Akif Ersoy University Animal Experiment Ethic Committee dated 16.02.2022 in accordance with decision number 862. In addition, written informed consent was obtained from the owners for the participation of their animals in the present study.

The materials of the present study consisted of 12 3-8 month old Holstein and Simental breed calves, obtained from a cattle farm in the Burdur province of Turkey. The calves were dehydrated and *Giardia* spp. cysts (Fig. 1) were detected in 12 calves in the native fecal examinations performed to reveal the etiology of diarrhea cases. In the period of a week, fecal samples were taken from each calf three times directly from the rectum using sterile plastic gloves with an interval of one day, stored at +4°C and examined for *Giardia* spp. cysts in the laboratory within 24 hours using the native examination method. The native examination method is briefly based on the principle of examining a drop (0.05 ml) taken from the homogeneous mixture obtained after 1 g of fecal sample treated with 1 ml distilled water. Calves with *Giardia* spp. cysts found in at least one or more of the three fecal samples of each animal were considered positive and included to the study.

The calves were divided in groups randomly in order to obtain two groups of six animals each with similar parasitic loads of *Giardia* spp. cysts for day zero. All animals were treated for ethical reasons, therefore there was no control group. Albendazole administrated to group A at the dose of 20 mg/kg and paromomycin was administrated to group P at 50 mg/kg dose. Both drugs were administrated orally for three consecutive days.

The evaluation and comparison of treatment efficiencies of drugs was based on analyzing the reduction in cyst excretion in native fecal examinations. The fecal cyst excretion amounts of the animals in the groups on days 0 (before treatment), 3, 7, 10, 14, 21 and 28 were determined by counting 10 microscope fields on an 18 × 18 coverslip under

a 40 × magnification objective. The geometric mean (GM) of the fecal *Giardia* spp. cyst excretion of the groups were calculated by taking the average of the cyst amounts of the animals in the groups. Percentages of reduction for days 3, 7, 10, 14, 21 and 28 according to day zero were calculated via the following formula:

$$\% \text{ Reduction} = [(GM_{\text{before}} - GM_{\text{after}}) / GM_{\text{before}}] \times 100 \quad (10).$$

In addition, food and water samples given to animals were examined in terms of *Giardia* spp. cyst and trophozoites.

Statistical analysis was performed using Pearson chi-square test with the help of MiniTab16 Statistics program. The Chisquare test was used to compare the differences of reduction percentages compared to day zero in the geometric mean of cyst excretion on days 3, 7, 10, 14, 21 and 28 among the groups. Observed differences were considered to be statistically significant when $P < 0.05$.

Results and discussion

Clinically, paste-like yellowish-brown faeces and retardation in growth were detected in infected calves, but it was reported in the anamnesis that there was no decrease in feed and water consumption.

No cyst or trophozoites were detected as a result of food and water examination. No adverse affects of both drugs were observed during and post treatment period.

The geometric mean of the fecal *Giardia* spp. cyst excretion of groups before (D 0) and after treatment (D 3, 7, 10, 14, 21, 28) were given in Table 1.

The therapeutic efficacies of the drugs were evaluated according to the percentage of reduction in the amount of cysts output with the feces of the animals in both groups.

As seen in Figure 2, the geometric mean of cyst excretion in both groups were starting to decrease from the 3rd day, in the paromomycin group decrease continued regularly throughout the examination days starting from the 3rd day, despite that in the albendazole group cyst amount increased in the 7th day then decreased again. Cyst excretion reached zero in one calf at the 3rd day and in all animals at the 21st day in paromomycin group. However, cyst excretion reached to zero in two calves at the 10th day, in four calves at the 21st and in all animals at the 28th day in albendazole group.

In the paromomycine group, the percentage of reduction started with a high rate of 47.72% on the third day, there was no increase, even a slight decrease on the seventh day, then gradually increased in the following days and reached 100% on the 21st day. In the albendazole group, the percentage of reduction started with a rate of 35.29% on the third day; however, no reduction was observed on the seventh day. Then the percentage of reduction started with a high rate of 58.82% again on the 10th day, gradually increased in the following days and reached 100% on the 28th day. It was noted that the differences between the reduction rates of the groups on days 7 and 14 compared to day zero were significant ($P < 0.05$) as seen in Table 2.

Tab. 1. The geometric mean of the fecal *Giardia* spp. cyst excretion of groups on before and after treatment days

Treatment groups	Days						
	0	3	7	10	14	21	28
Albendazole (A)	17	11	17	7	6	0.6	0
Paromomycine (P)	22	11	10	4	1.3	0	0

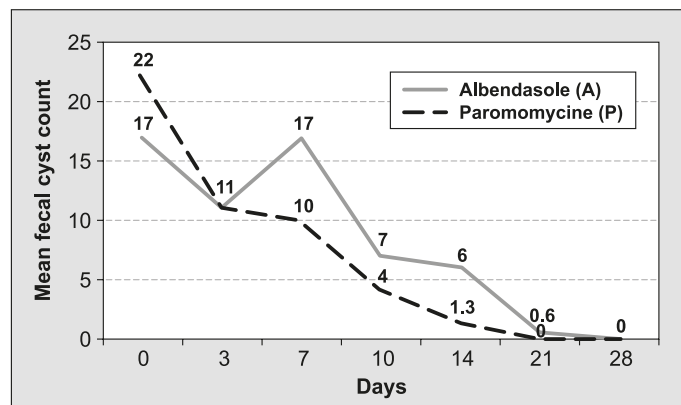


Fig. 2. The geometric mean of the fecal *Giardia* spp. cyst excretion of groups on before and after treatment days

In the present study, the aim was to evaluate and compare the treatment effects of albendazole and paromomycine in natural *Giardia* spp. infections in calves as Geurden et al. (9) emphasized. According to the results; it is seen that paromomycin is more effective compared to albendazole in terms of both the regularity of the geometric mean of cyst reduction (cyst-suppressing effectiveness) and the time it takes for the cyst excretion to reach zero in the animals in the group. In a previous study (2), paromomycin was found to be more effective than albendazole in natural *Giardia* spp. infection in lambs in terms of both the time it takes for the cyst excretion to reach zero and cyst-suppressing effect. Results of both studies are similar and suggests that paromomycin is more effective (faster and better at cyst suppresssing) compared to albendazole in natural *Giardia* spp. infections. Paromomycin lead inhibition on protein synthesis and albendazole cause inhibition on attaching of trophozoites to the intestinal mucosa, therefore different mechanisms of drugs can be considered one of the most important reasons for the difference in treatment efficacies among the two drugs. On the other hand, the present study only represents results based on a native fecal examination method. Immunological tests for the

Tab. 2. Reduction percentages of days 3, 7, 10, 14, 21 and 28 compared to day zero in the geometric mean of fecal cyst excretion

Treatment groups	Days					
	0-3	0-7	0-10	0-14	0-21	0-28
Albendazole (A)	35.29%	0%	58.82%	64.70%	96.47%	100%
Paromomycine (P)	47.72%	46.36%	81.81%	94.09%	100%	100%
P-value	0.358	0.000	0.114	0.013		

diagnosis of *Giardia* specific antigens and real time pcr methods may give much more reliable results, therefore further studies are needed to better evaluate the efficiencies of these drugs.

There are several studies carried out to evaluate the anti-giardial effectiveness of various drugs. Efficiencies of albendazole and fenbendazole against *Giardia* spp. infection in calves were evaluated in a study (26) and both drugs were found to be effective in suppressing cyst excretion. However, it was reported that some animals in the albendazole group still had low *Giardia* spp. Cyst excretion after treatment, similarly in the fenbendazole group; although the cyst output was zero in all animals one week after treatment, a small amount of cyst output started in some calves. In another study, fenbendazole was found effective in the elimination of *Giardia* spp. infections in calves, but it was emphasized that duration of treatment, not dose, is the most important factor when treating calves for giardiasis with fenbendazole and repeat treatments may be required in reinfected animals (16). In a repeat treatment study, fenbendazole was administered to experimentally *Giardia* spp. infected calves and this treatment repeated on days 30 and 60 (15). As a result, it was reported that fenbendazole significantly reduced the number of the cysts; however, fecal cyst excretion returned to control levels within two weeks following treatment, for every treatment period (15). Three doses of (25, 50, 75 mg/kg) oral paromomycin were evaluated against experimentally *Giardia* spp. infection in calves in another study (9). It was reported that all animals, except one in the group treated with 75 mg/kg, were re-excreting cysts within 2 to 3 weeks after treatment and this efficacy and post treatment infection pattern was found to be similar to previous studies (15, 16, 26). Similarly, in the present study, although paromomycin and albendazole treatments leading to cyst excretion reached zero at day 21 and 28 respectively, it seems that cyst excretion and therefore environmental contamination lasted at least three more weeks after treatment for both two treatment groups. It has been stated that some *Giardia* spp. trophozoites may have survived treatment or animals may be reinfected from contaminated environment, hence this may lead to re-occurrence of cyst output for a while after treatment (9). The cyst form of *Giardia* spp. can stay an infective one up to seven and eleven weeks in feces, soil and water, respectively, at 4°C (17). In addition, even a small amount of cyst is sufficient to initiate an infection (5), and in ruminants the specific immune response to giardiasis does not develop very quickly (14, 27). Therefore, the results of the present study and previous studies mentioned above seem to indicate that mere treatment of infected animals is not enough for prevention and control of giardiasis; additional applications such as environmental disinfection are required, which is suggested by researchers (9, 15, 16, 26). To evaluate

this need, Geurden et al. (10) combined fenbendazole administration with environmental measures such as removal of bedding, thorough cleaning and ammonia 10% disinfection or relocation of the treated animals to a *Giardia* spp. free environment. It was reported that these protocols lead to a significant reduction of the cyst output at least 4 weeks after treatment, a low environmental infection pressure and preventing clinical symptoms for at least 4 months post treatment period (10). There are also studies reporting that a single dose of secnidazole results in a short-term and effective cyst reduction. Efficacy of 30 mg/kg single dose secnidazole in calves naturally infected with *G. duodenalis* was evaluated in a study (22) and it was reported that geometric mean of cyst excretion revealed 100% reduction on the 7th and 10th days. However, it seems that cyst excretion lasted at least one more week after treatment. In another study (23), 10 mg/kg oral secnidazole was concluded to be 100% effective in eliminating the cyst excretion in natural *Giardia* spp. infection in calves. However, as a result of this study (23), fecal samples of most treated animals (83.3%) remained free of cysts, therefore it is understood that some of the treated animals (16.7%) continued cyst excretion and environmental contamination.

Although each drug has different degrees of effectiveness, it can be concluded according to the results of the mentioned studies (9, 10, 15, 16, 26) that environmental hygiene applications should not be ignored in addition to the treatment of infected animals in order to prevent contamination.

References

1. Adam R. D.: Biology of *Giardia lamblia*. Clin. Microbiol. Rev. 2001, 14 (3), 447-475, doi: 10.1128/CMR.14.3.447-475.2001.
2. Albay M. K., Sahinduran S., Adanir R., Yukari B. A., Kose O.: Efficacy of albendazole and two different doses of paromomycin for treatment of naturally occurring *Giardia* infection in lambs. Kafkas Univ. Vet. Fak. Derg. 2011, 17 (6), 1021-1024, doi: 10.9775/kvfd.2011.5040.
3. Buret A., Gall D. G., Olson M. E.: Growth activities of enzymes in the small intestine and ultra-structure of microvillus border in gerbils infected with *Giardia duodenalis*. Parasitol. Res. 1991, 77, 109-114, doi: 10.1007/BF00935423.
4. Buret A., Scott K. G., Chin A. C.: Giardiasis pathophysiology and pathogenesis. [in:] Olson B. E., Olson M. E., Wallis P. M. (eds): *Giardia: The Cosmopolitan Parasit*, 1st ed., CAB International, Wallingford UK 2002, p. 109-125.
5. Caccio S. M., Thompson R. C. A., McLauchlin J., Smith H. V.: Unravelling *Cryptosporidium* and *Giardia* epidemiology. Trends Parasitol. 2005, 21, 430-437.
6. Chavez B., Cedillo-Rivera R., Martinez-Palomo A.: *Giardia lamblia*: ultra-structural study of the in vitro effect of benzimidazoles. J. Protozool. 1992, 39 (4), 510-515, doi: 10.1111/j.1550-7408.1992.tb04841.x.
7. Edlind T. D.: Susceptibility of *Giardia lamblia* to aminoglycoside protein synthesis inhibitors: correlation with rRNA structure. Antimicrob. Agents Chemother. 1989, 33 (4), 484-488, doi: 10.1128/AAC.33.4.484.
8. Feng Y., Xiao L.: Zoonotic potential and molecular epidemiology of *Giardia* species and giardiasis. Clin. Microbiol. Rev. 2011, 24 (1), 110-140, doi: 10.1128/CMR.00033-10.
9. Geurden T., Claerebout E., Dursin L., Deflandre A., Bernay F., Kaltsatos V., Vercruysse J.: The efficacy of an oral treatment with paromomycin against an experimental infection with *Giardia* in calves. Vet. Parasitol. 2006, 18, 135 (3-4), 241-247, doi: 10.1016/j.vetpar.2005.09.006.
10. Geurden T., Vercruysse J., Claerebout E.: Field testing of a fenbendazole treatment combined with hygienic and management measures against a natural *Giardia* infection in calves. Vet. Parasitol. 2006, 142 (3-4), 367-371, doi: 10.1016/j.vetpar.2006.07.019.

11. *Morch K., Hanevik K.*: Giardiasis treatment: an update with a focus on refractory disease. *Curr. Opin. Infect. Dis.* 2020, 33 (5), 355-364, doi: 10.1097/QCO.0000000000000668.
12. *Morgan U. M., Reynoldson J. A., Thompson R. C.*: Activities of several benzimidazoles and tubulin inhibitors against *Giardia* spp. in vitro. *Antimicrob. Agents Chemother.* 1993, 37 (2), 328-331, doi: 10.1128/AAC.37.2.328.
13. *O'Handley R. M., Buret A. G., McAllister T. A., Jelinski M., Olson M. E.*: Giardiasis in dairy calves: effects of fenbendazole treatment on intestinal structure and function. *Int. J. Parasitol.* 2001, 31 (1), 73-79, doi:10.1016/s0020-7519(00)00148-x.
14. *O'Handley R. M., Ceri H., Anette C., Olson M. E.*: Passive immunity and serological immune response in dairy calves associated with natural *Giardia duodenalis* infection. *Vet. Parasitol.* 2003, 113, 89-98, doi: 10.1016/s0304-4017(03)00059-1.
15. *O'Handley R. M., Cockwill C., Jelinski M., McAllister T. A., Olson M. E.*: Effects of repeat fenbendazole treatment in dairy calves with giardiasis on cyst excretion, clinical signs and production. *Vet. Parasitol.* 2000, 89 (3), 209-218.
16. *O'Handley R. M., Olson M. E., McAllister T. A., Morck D. W., Jelinski M., Royan G., Cheng K. J.*: Efficacy of fenbendazole for treatment of giardiasis in calves. *Am. J. Vet. Res.* 1997, 58 (4), 384-388.
17. *Olson M. E., Goh J., Phillips M., Guselle N., McAllister T. A.*: *Giardia* cyst and *Cryptosporidium* oocyst survival in water, soil, and cattle feces. *J. Environ. Qual.* 1999, 28, 1991-1996.
18. *Olson M. E., O'Handley R. M., Ralston B. J., McAllister T. A., Thompson R. C. A.*: Update on *Cryptosporidium* and *Giardia* infections in cattle. *Trends Parasitol.* 2004, 20, 185-191, doi: 10.1016/j.pt.2004.01.015.
19. *Plumb D. C.*: *Veterinary Drug Handbook*. 6th ed., Iowa State University Press, Ames 2008.
20. *Radostits O. M., Gay C. C., Hinchcliff K. W., Constable P. D.*: *Veterinary Medicine: A Textbook of Diseases of Cattle, Sheep, Pigs, Goats and Horses*. 10th ed., W. B. Saunders Company Ltd. London 2008.
21. *Reynoldson J. A., Thompson R. C., Horton R. J.*: Albendazole as a future anti-giardial agent. *Parasitol. Today (Personal ed.)*, 1992, 8 (12), 412-414, doi: 10.1016/0169-4758(92)90193-6.
22. *Toros G., Ural K.*: Single dose secnidazole treatment efficiency against naturally occurring *Giardia duodenalis* infection in calves. *Revista MVZ Córdoba*, 2018, 23 (2), 6660-6670, doi: 10.21897/rmvz.1340.
23. *Volpato A., Fortuoso B. F., Campigotto G., Glombowsky P., Bottari N. B., Lopes L. S., Da Silva A. S.*: Secnidazole for control of giardiasis in dairy calves. *Exp. Parasitol.* 2018, 189, 16-18, doi: 10.1016/j.exppara.2018.04.008.
24. *Watkins R. R., Eckmann L.*: Treatment of giardiasis: current status and future directions. *Curr. Infect. Dis. Rep.* 2014, 16 (2), 396, doi: 10.1007/s11908-014-0396-y.
25. *Xiao L., Herd R. P.*: Infection pattern of *Cryptosporidium* and *Giardia* in calves. *Vet. Parasitol.* 1994, 55 (3), 257-262, doi: 10.1016/0304-4017(93)00645-f.
26. *Xiao L., Saeed K., Herd R. P.*: Efficacy of albendazole and fenbendazole against *Giardia* infection in cattle. *Vet. Parasitol.* 1996, 61 (1-2), 165-170, doi: 10.1016/0304-4017(95)00802-0.
27. *Yanke S. J., Ceri H., McAllister T. A., Morck D. W., Olson M. E.*: Serum immune response to *Giardia duodenalis* in experimentally infected lambs. *Vet. Parasitol.* 1998, 75, 9-19, doi: 10.1016/s0304-4017(97)00187-8.

Corresponding author: Onur Köse, DVM, PhD, Asist. Prof., Burdur Mehmet Akif Ersoy University, Faculty of Veterinary Medicine, Department of Parasitology, İstiklal Campus, 15030, Burdur, Türkiye; e-mail: onurkose@mehmetakif.edu.tr