ORIGINAL ARTICLE

Veterinary Research Forum. 2023; 14 (11) 595 - 599
doi: 10.30466/vrf.2023.1998950.3826
Journal Homepage: vrf.iranjournals.ir

Veterinary Research Forum

Prevalence and genotypes of *Giardia duodenalis* in shelter dogs of southeastern Türkiye

Burçak Aslan Çelik[1]*, Özgür Yaşar Çelik[2], Akın Koçhan[3], Adnan Ayan[4], Özlem Orunç Kılınç[5], Gürkan Akyıldız[6], Kıvanç İrak[7], Özge Oktay Ayan[8], Kerem Ercan[2]

[1] Department of Parasitology, Faculty of Veterinary Medicine, Siirt University, Siirt, Türkiye; [2] Department of Internal Medicine, Faculty of Veterinary Medicine, Siirt University, Siirt, Türkiye; [3] Department of Internal Medicine, Faculty of Veterinary Medicine, Dicle University, Diyarbakır, Türkiye; [4] Department of Genetics, Faculty of Veterinary Medicine, Van Yüzüncü Yıl University, Van, Türkiye; [5] Özalp Vocational School, Van Yüzüncü Yıl University, Van, Türkiye; [6] Department of Basic Health Sciences, Faculty of Health Sciences, Marmara University, İstanbul, Türkiye; [7] Department of Biochemistry, Faculty of Veterinary Medicine, Siirt University, Siirt, Türkiye; [8] Department of Parasitology, Van Yüzüncü Yıl University, Faculty of Medicine, Van, Türkiye.

Article Info

Article history:

Received: 25 March 2023
Accepted: 17 June 2023
Available online: 15 November 2023

Keywords:

Giardia duodenalis
Nested polymerase chain reaction
Shelter dog
Türkiye

Abstract

Giardia duodenalis is a protozoan parasite found in humans and several mammals. This parasite spreads worldwide and is generally recognized as a zoonotic agent being reported to be one of the most common causes of diarrhea in humans and animals. In this study, it was aimed to determine the prevalence and genotypes of *G. duodenalis* in shelter dogs in Diyarbakır province being located in the southeastern Anatolia region of Türkiye. Native-Lugol method and nested polymerase chain reaction analyses of 100 fecal samples showed a prevalence of 3.00 and 4.00%, respectively. The prevalence was higher in females and in those younger than 1 year. Sequence analysis revealed the presence of zoonotic assemblage B, assemblage D and assemblage E. The detection of zoonotic assemblage B in this study suggests that dogs may be a reservoir for human giardiasis. Further molecular research is needed to determine the genotype diversity of *Giardia* as well as its possible role in the transmission of this parasite to humans.

Introduction

Giardia spp. include six species characterized by diverse host ranges. Of these, *Giardia duodenalis* (synonyms: *Giardia lamblia, Giardia intestinalis*) is the only human-infective *Giardia* species, widespread worldwide, associated with diarrhea in humans and domestic and wild mammals.[1-6]

Giardia duodenalis is reported to have at least eight different genotypes (A - H) according to genetic characteristics and host range.[1,7-10] Of these, assemblages A and B; although seen in many mammals, are mainly associated with human infections.[6,8-10] The remaining assemblages (C - H) have a limited host spectrum and are considered host specific.[6,7,9] However, assemblages C, D, E and F have been reported to be isolated with low prevalence in humans.[6] Assemblages C and D occur in dogs,[2,7-10] assemblage E in ruminants,[1,8,9] assemblage F in cats,[7,9,10] assemblage G in mice and rats[1,9] and assemblage H in marine mammals.[1,6,7]

Microscopic study,[7,11,12] indirect fluorescence antibody test (IFAT),[13] enzyme-linked immunosorbent assay (ELISA)[3,9,11,13] and polymerase chain reaction (PCR)[1,2,5,7,10,12-14] methods are used in the diagnosis of the disease. *Giardia* has two morphological forms including trophozoites and cysts being responsible for transmission.[15] Transmission of *G. duodenalis* occurs by fecal-oral ingestion of the contaminated food or water.[3,4,9,15] Reportedly, this parasite can cause growth and developmental retardation in children even in asymptomatic cases.[6] It is estimated that approximately 200 million people in Asia, Africa and Latin America have *Giardia* infection.[9]

In studies conducted around the world, the prevalence of *Giardia* was reported to be 31.33% in Brazil,[16] 11.20 - 15.50% in Korea,[1,15] 25.20 - 56.80% in Thailand,[10,13] 20.50% in Italy,[12] 1.90% in Poland,[4] 13.00 - 39.00% in Canada,[3,17] 4.50 - 11.00% in China,[2,14] 16.40 - 36.50% in Spain,[7,8] 75.55% in Iraq,[11] and 11.90 - 24.50% in Israel.[9,18] In Türkiye, the infection was first reported by Burgu[19] and the prevalence was recorded as 2.48 - 18.80%.[20-23]

***Correspondence:**

Burçak Aslan Çelik. PhD
Department of Parasitology, Faculty of Veterinary Medicine, Siirt University, Siirt, Türkiye
E-mail: burcakaslan@siirt.edu.tr

This study aimed to determine the prevalence and genotypes of *G. duodenalis* in shelter dogs in Diyarbakır province, Türkiye.

Materials and Methods

Study area and sample collection. Ethical clearance for the present study was obtained from the Dicle University Health Sciences Application and Research Centre, Diyarbakır, Türkiye (Approval Number: E-35582840-020). This study was carried out in Diyarbakır province (the main characteristics of its climate are high temperature and dryness) located in the southeastern Anatolia region of Türkiye (38° 02′ 33″ N, 40° 04′ 43″). The animals of the study consisted of 100 dogs of different breeds and sexes in Diyarbakır Municipality Animal Care and Rehabilitation Centre, Türkiye. The feces (non-diarrheic) were directly collected from the rectum of the dogs with disposable latex gloves and placed in individual sample containers. The sex and age (taken from the centre records) of the dogs were recorded and brought to the laboratory for examination.

Microscopic examination. The Nativ-Lugol technique was used to check all samples for the presence of *Giardia* cysts. A drop of saline solution was placed on one side of the clean slide and a drop of Lugol solution was placed on the other side. With the help of a plastic stick, rice grain sized pieces of faeces were taken from different parts of the faeces and homogenised on the slide. The coverslipped preparations were examined with the 40× objective of the microscope (Leica, Hamburg, Germany).[24]

DNA extraction. The DNA extraction was performed using GeneMATRIX Stool DNA Purification Kit (EURx, Gdańsk, Poland) according to the manufacturer's protocol. The obtained DNAs were stored at – 20.00 ˚C until further analysis.

Nested PCR. In the nested PCR analysis, the β-giardin gene region of 753 bp was amplified using the primers described by Cacciò *et al*.,[25] (G7 F 5'- AAGCCCGACGACGA CCTCACCCGCAGTGC-3' forward and G759R 5'- GAGG CCGCCCTGGATCTTCGAGACGAC-3' reverse). Nested PCR was then performed using the primers described by Lalle *et al.*,[26] (BG1F 5'- GAACGAGATCGAGGTCCG-3' forward and BG2R 5'-CTCGACGAGTTCGTGTGTT-3' reverse). In this study, the PCR product obtained in our previous study, being confirmed by sequence analysis as *G. duodenalis* assemblage B, was used as a positive control.[24] The PCR products obtained were stained with RedSafe™ Nucleic Acid Staining Solution (iNtRON Biotechnology Inc., Seoul, South Korea) and images were obtained on 1.50% agarose gel.

DNA sequence analysis and phylogeny. Positive PCR samples were sequenced forward and reverse. The DNA sequences were individually checked, aligned and analyzed in BioEdit Sequence Alignment Editor (version 7.2.5; Tom Hall, Carlsbad, USA).[27] The edited formats of the DNA sequences were compared with the databases using the National Center for Biotechnology Information (NCBI) Basic Local Alignment Search Tool to determine the assemblages.[28] In addition, data sets were created using the *β-giardin* gene sequences obtained from the NCBI GenBank database and the DNA sequences were obtained as a result of the study. The data sets were aligned in the BioEdit program and the model test was performed using the maximum likelihood statistical method in the IQ-TREE program (version 1.6.12; http://www.iqtree.org). The phylogenetic tree was created with 1,000 bootstraps according to the Bayesian information criterion optimal model and it was shown which assemblages the study samples were related to.[29,30]

Statistical analysis. The data obtained in the study were analyzed using the SPSS Software (version 16.0; SPSS, Inc., Chicago, USA) program. The relationship between grouped variables was calculated using the Chi-square test. The difference was considered statistically significant when $p < 0.05$.

Results

Microscopic examination of all samples revealed 3.00% (3/100) *Giardia* spp. cysts (Fig. 1). Nested PCR analysis revealed specific bands of 511 bp in 4 (4.00%) of the samples (Fig. 2). The highest prevalence was found in females (4.55%) and in those younger than one year (5.88%, $p > 0.05$; Table 1). Sequence analysis revealed that two samples overlapped with assemblage E (99.78% and 100%), one sample overlapped 99.78% with assemblage D and one sample overlapped 100% with assemblage B. The phylogenetic tree shows the placement of the specimens (Fig. 3).

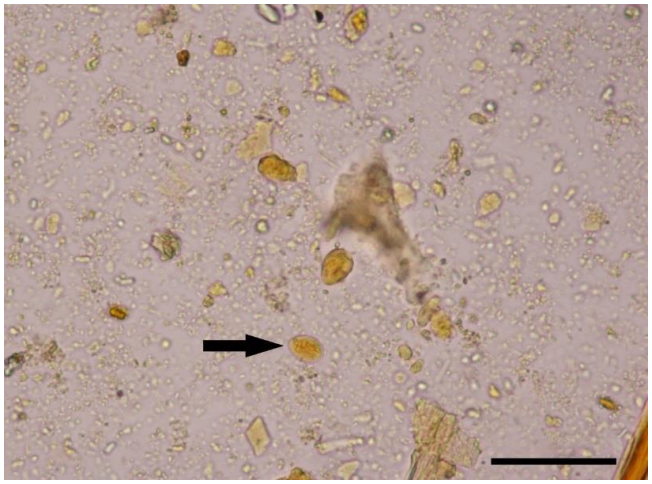

Fig. 1. Photomicrograph of the *Giardia duodenalis* cyst (arrow), (bar = 50 µm).

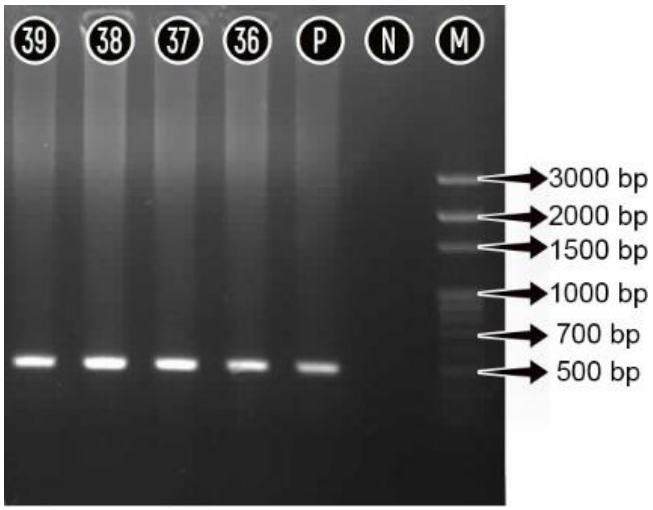

Fig. 2. Polymerase chain reaction products of *Giardia duodenalis*. Lane M: DNA marker (511 bp); Lane N: Negative control; Lane P: Positive control; Lanes 36 - 39: *G. duodenalis*.

Table 1. Prevalence of *Giardia duodenalis* infection in examined dogs (n) according to the sex and age.

Variables	Examined dogs	Infected dogs (%)	*p*-value
Sex			
Female	44	2 (4.55)	0.805
Male	56	2 (3.57)	
Age (year)			
≤ 1	34	2 (5.88)	0.491
> 1	66	2 (3.03)	
Total	100	4 (4.00)	

Discussion

Dogs are important companions in many homes around the world, contributing to the physical, social and emotional development of children and the well-being of their owners.[31] However, dogs are recognized as natural reservoirs of several zoonotic parasitic infections.[7] This increases the risk of human exposure to zoonotic parasites.[31] Therefore, it is important to determine the prevalence of infectious agents with zoonotic potential having the risk of transmission to humans and other animals.

It has been reported that IFAT has the highest sensitivity and specificity for *Giardia* detection and is considered as a gold standard test.[32] However, in the study conducted by Traub *et al*.,[13] it was reported that more prevalence was detected by PCR method, being similar to this study.

The prevalence determined in this study was higher than some previous reports,[4,23] similar to some of them[2,19] and lower than others.[1,3,9,10,12,16,17] The reasons for the difference between the studies may be due to the factors affecting the prevalence of the parasite, such as the age of the dogs, living conditions, animal density, nutritional and immune status and diagnostic methods.[12]

Giardia cysts may endure conditions with high humidity, low temperature, little sunshine exposure and low salinity for months.[10] The location where this study was carried out is located in the hot and dry region of Türkiye. The main characteristics of its climate are high temperature and dryness.[33] This explains the low prevalence rate obtained in this study.

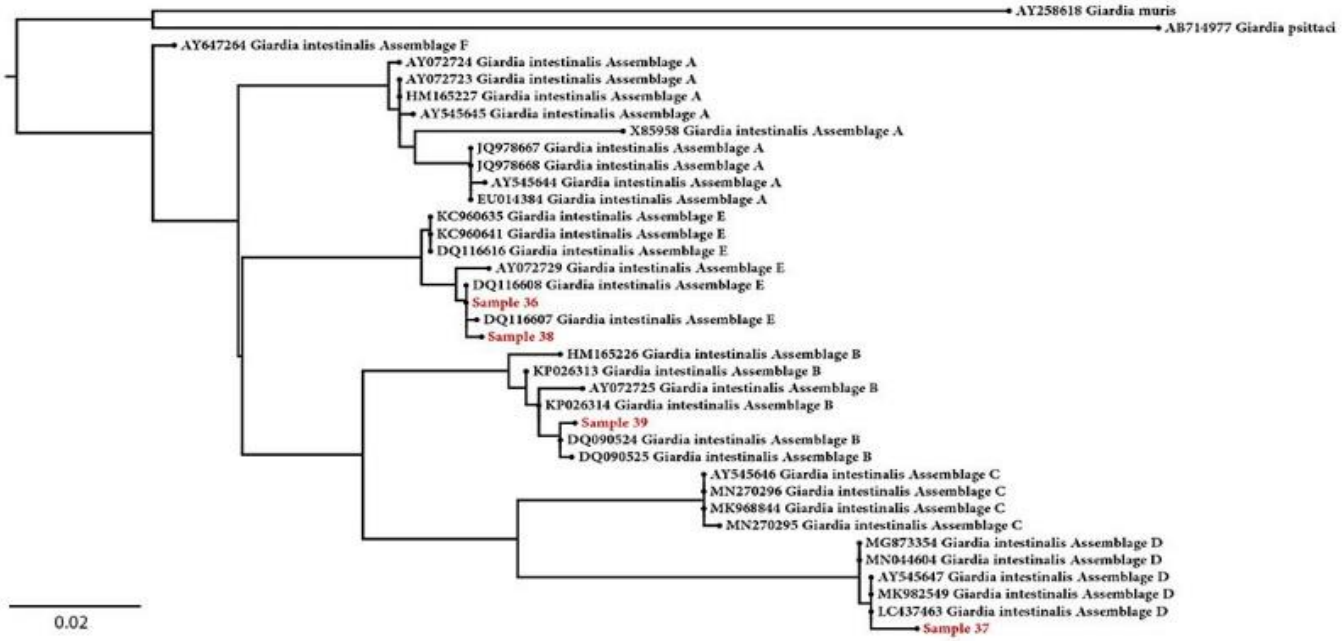

Fig. 3. Phylogenetic relationships of *Giardia duodenalis* isolates using maximum likelihood method analysis based on *β-giardin* gene region. Numbers at the nodes represent the bootstrap values (1,000 bootstrap). *Giardia psitacci* and *Giardia muris* were used as outgroup.

Assemblages C and D have been reported as host-specific genotypes in dogs.[4,5,22] Also, assemblage A,[2,7,17,34] assemblage B,[7,17] assemblage C,[1,4,9,10,14,17,34] assemblage D[1,4,9,10,17,34] and assemblage E[8,14] were reported in dogs. In this study, one of the 4 positive samples was zoonotic assemblage B, one was dog-specific assemblage D and two were assemblage E, being interestingly seen especially in ruminants. The reason for the occurrence of assemblage E may be due to the fact that these dogs lived in rural areas with dense farm animal populations.[35]

While some studies have reported higher prevalence in female dogs,[7,10,11,14,16] others reported in males.[1,2,15,18,22] In this study, a higher prevalence was found in female dogs. This result is similar to the former findings of the researchers.[7,10,11,14,16] The reason for the higher prevalence in females may be due to the decreased immunity of these animals during certain periods of their physiological cycle.[36]

In several studies, it was reported that a higher prevalence was detected in young animals compared to the adult ones.[10,11,14,16,18] In this study, similar to the previous findings, a higher prevalence was found in animals younger than one year (5.88%) in comparison with animals older than one year(3.03%). This may be due to the fact that the immune system is not developed in young animals and they cannot form an effective immune response to eliminate the infection.[11,37]

The detection of assemblages D and E as well as zoonotic assemblage B in this study suggests that dogs may be a source of giardiasis in humans. Therefore, it is important that those working with dogs, including veterinarians and shelter workers, be aware of this potential risk and take appropriate precautions to prevent infection. Further molecular epidemiological research is also needed to determine the genotype diversity of *Giardia* in dogs as well as its possible role in the transmission of this parasite to humans.

Acknowledgments

The authors are grateful for support of the Preclinical Science Department of Faculty of Veterinary Medicine, Siirt, Türkiye.

Conflicts of interest

The authors state no conflict of interest.

References

1. Kim HY, Lee H, Lee SH, et al. Multilocus genotyping and risk factor analysis of *Giardia duodenalis* in dogs in Korea. Acta tropica 2019; 199: 105113. doi: 10.1016/j.actatropica.2019.105113.
2. Li J, Zhang P, Wang P, et al. Genotype identification and prevalence of *Giardia duodenalis* in pet dogs of Guangzhou, Southern China. Vet Parasitol 2012; 188(3-4): 368-371.
3. Olson ME, Leonard NJ, Strout J. Prevalence and diagnosis of Giardia infection in dogs and cats using a fecal antigen test and fecal smear. Can Vet J 2010; 51(6): 640-642.
4. Solarczyk P, Majewska AC. A survey of the prevalence and genotypes of *Giardia duodenalis* infecting household and sheltered dogs. Parasitol Res 2010; 106(5): 1015-1019.
5. Uiterwijk M, Nijsse R, Kooyman FNJ, et al. Comparing four diagnostic tests for *Giardia duodenalis* in dogs using latent class analysis. Parasit Vectors 2018; 11(1): 439. doi: 10.1186/s13071-018-3014-2.
6. Zhang W, Zhang X, Wang R, et al. Genetic characterizations of *Giardia duodenalis* in sheep and goats in Heilongjiang Province, China and possibility of zoonotic transmission. PLoS Negl Trop Dis 2012; 6(9): e1826. doi: 10.1371/journal.pntd.0001826.
7. Adell-Aledón M, Köster PC, de Lucio A, et al. Occurrence and molecular epidemiology of *Giardia duodenalis* infection in dog populations in eastern Spain. BMC Vet Res 2018; 14(1): 26. doi: 10.1186/s12917-018-1353-z.
8. Dado D, Montoya A, Blanco MA, et al. Prevalence and genotypes of *Giardia duodenalis* from dogs in Spain: possible zoonotic transmission and public health importance. Parasitol Res 2012; 111(6): 2419-2422.
9. Salant H, Kuzi S, Navarro D, et al. Prevalence and molecular characterization of *Giardia duodenalis* in dogs in Israel. Comp Immunol Microbiol Infect Dis 2020; 73: 101548. doi: 10.1016/j.cimid.2020.101548.
10. Tangtrongsup S, Scorza AV, Reif JS, et al. Seasonal distributions and other risk factors for *Giardia duodenalis* and *Cryptosporidium* spp. infections in dogs and cats in Chiang Mai, Thailand. Prev Vet Med 2020; 174: 104820. doi: 10.1016/j.prevetmed.2019.104820.
11. Naser A, Wadood IA. Detection of Giardia infection in dogs of Basrah City. Bas Vet Res 2017; 16(2): 159-171.
12. Scaramozzino P, Di Cave D, Berrilli F, et al. A study of the prevalence and genotypes of *Giardia duodenalis* infecting kennelled dogs. Vet J 2009; 182(2): 231-234.
13. Traub RJ, Inpankaew T, Reid SA, et al. Transmission cycles of *Giardia duodenalis* in dogs and humans in Temple communities in Bangkok - - a critical evaluation of its prevalence using three diagnostic tests in the field in the absence of a gold standard. Acta Trop 2009; 111(2): 125-132.
14. Li W, Li Y, Song M, et al. Prevalence and genetic characteristics of *Cryptosporidium*, *Enterocytozoon bieneusi* and *Giardia duodenalis* in cats and dogs in Heilongjiang province, China. Vet Parasitol 2015; 208(3-4): 125-134.
15. Liu J, Lee SE, Song KH. Prevalence of canine giardiosis in South Korea. Res Vet Sci 2008; 84(3): 416-418.

16. Huber F, Bomfim TC, Gomes RS. Comparison between natural infection by *Cryptosporidium* sp., *Giardia* sp. in dogs in two living situations in the West Zone of the municipality of Rio de Janeiro. Vet Parasitol 2005; 130(1-2): 69-72.
17. Uehlinger FD, Greenwood SJ, McClure JT, et al. Zoonotic potential of *Giardia duodenalis* and *Cryptosporidium* spp. and prevalence of intestinal parasites in young dogs from different populations on Prince Edward Island, Canada. Vet Parasitol 2013; 196(3-4): 509-514.
18. Kuzi S, Argentaro SE, Baneth G. Prevalence of *Giardia duodenalis* infection, co-morbidities and associated risk factors in dogs admitted to a veterinary teaching hospital in Israel. Comp Immunol Microbiol Infect Dis 2020; 68: 101401.doi: 10.1016/j.cimid.2019.101401.
19. Burgu A. Distribution of *Giardia canis* in dogs in Ankara and its importance for public health [Turkish].Ankara Üniv Vet Fak Derg 1979; 26(03-04): 184-194.
20. Denizhan V, Karakuş A. Prevalence of gastrointestinal protozoon in stray dogs in the Van Province [Turkish]. Dicle Üniv Vet Fak Derg 2019; 12(1): 25-29.
21. Dumanlı N. A survey of the incidence of protozoer agents in dogs in Elazığ vicinity [Turkish]. Ankara Üniv Vet Fak Derg 1984; 31(3): 383-387.
22. Gultekin M, Ural K, Aysul N, et al. Prevalence and molecular characterization of *Giardia duodenalis* in dogs in Aydin, Turkey. Int J Environ Health Res 2017; 27(3): 161-168.
23. Uslu U, Ceylan C, Ceylan O, et al. Gastrointestinal protozoa and helminths detected in 2-6 months old puppies in Konya [Turkish]. Dicle Üniv Vet Fak Derg 2022; 15(2): 74-78.
24. Çelik BA, Çelik ÖY, Ayan A, et al. Molecular prevalence of *Giardia duodenalis* and subtype distribution (assemblage E and B) in calves in Siirt, Turkey. Egypt J Vet Sci 2023; 54(3): 457-463.
25. Cacciò SM, De Giacomo M, Pozio E. Sequence analysis of the beta-giardin gene and development of a polymerase chain reaction–restriction fragment length polymorphism assay to genotype *Giardia duodenalis* cysts from human faecal samples. Int J Parasitol 2002; 32(8): 1023-1030.
26. Lalle M, Pozio E, Capelli G, et al. Genetic heterogeneity at the beta-giardin locus among human and animal isolates of *Giardia duodenalis* and identification of potentially zoonotic subgenotypes. Int J Parasitol 2005; 35(2): 207-213.
27. Hall TA. BioEdit: A user-friendly biological sequence alignment editor and analysis program for windows 95/98/nt. Nucleic Acids Symp Ser 1999; 41: 95-98.
28. Altschul SF, Gish W, Miller W, et al. Basic local alignment search tool. J Mol Biol 1990; 215(3): 403-410.
29. Minh BQ, Nguyen MA, von Haeseler A. Ultrafast approximation for phylogenetic bootstrap. Mol Biol Evol 2013; 30(5): 1188-1195.
30. Trifinopoulos J, Nguyen LT, von Haeseler A, et al. W-IQ-TREE: a fast online phylogenetic tool for maximum likelihood analysis. Nucleic Acids Res 2016; 44(W1): W232-W235. doi: 10.1093/nar/gkw256.
31. Robertson ID, Irwin PJ, Lymbery AJ, et al. The role of companion animals in the emergence of parasitic zoonoses. Int J Parasitol 2000; 30(12-13): 1369-1377.
32. Tangtrongsup S, Scorza V. Update on the diagnosis and management of Giardia spp infections in dogs and cats. Top Companion Anim Med 2010; 25(3): 155-162.
33. Gedik GZ. Climatic design: an analysis of the old houses of Diyarbakir in the southeast region of Turkey. Archit Sci Rev 2004; 47(2): 145-154.
34. Leonhard S, Pfister K, Beelitz P, et al. The molecular characterisation of Giardia from dogs in southern Germany. Vet Parasitol 2007; 150(1-2): 33-38.
35. Harvey TV, Carvalho JPDS, Aquino MCC, et al. Giardiasis in children and dogs, and the first report of assemblage E in dogs from northeastern Brazil. Rev Bras Parasitol Vet 2023; 32(1): e012222. doi: 10.1590/S1984-29612023010.
36. Öner G, Ulutaş B. Prevalence of Cryptosporidium spp. in dogs in the Aegean Region. Anim Health Prod Hyg 2022; 11(1): 26-31.
37. Mundim MJ, Rosa LA, Hortêncio SM, et al. Prevalence of *Giardia duodenalis* and *Cryptosporidium* spp. in dogs from different living conditions in Uberlândia, Brazil. Vet Parasitol 2007; 144(3-4): 356-359.