BF-1707
[1-6]

RESEARCH ARTICLE Indian Journal of Animal Research

Molecular Characterization and Zoonotic Significance of *Cryptosporidium* spp. and *Giardia duodenalis* in Asymptomatic Adult Stray Cats and Dogs in Turkey

Duygu Neval Sayın Ipek[1]

10.18805/IJAR.BF-1707

ABSTRACT

Background: *Cryptosporidium* species and *Giardia duodenalis* are important protozoan parasites due to their wide host range and potential as zoonotic diseases. These parasites, responsible for Cryptosporidiosis and Giardiasis, are significant health concerns and have garnered attention from both the public and veterinary fields. Pets, such as dogs and cats, are susceptible to these infections and play a crucial role in transmitting these protozoans to humans. This study was conducted to determine the prevalence, species, genotypes and zoonotic significance of *Cryptosporidium* and *Giardia* in asymptomatic adult stray cats and dogs.

Methods: Fecal samples from 75 adult stray animal, 42 dogs and 33 cats, were collected in Diyarbakır city, southeastern Anatolia, Turkey. Direct immunofluorescence test was used to confirm the presence of *G. duoenalis* cysts and *cryptosporidium* spp. oocysts in collected fecal samples. Microscopic analysis was used to count the number of cysts/oocysts per gram as specified by the manufacturer. Molecular confirmation was done with Polymerase chain reaction (PCR) using *Giardia* SSU-rDNA and *Cryptosporidium spp*. SSU rRNA gene. The secondary PCR products of all positive samples were sequenced in one directions on an automated sequencer. Nucleotide sequence analysis was performed by BLAST alignment using the National Center for Biotechnology Information database.

Result: The prevalence of *Cryptosporidium* was 7.14% in 42 cats and 9.09% in 33 dogs. The prevalence of *G. duodenalis* was 23.80% in cats and 54.54% in dogs. The average number of *Cryptosporidium spp.* oocysts per gram of cat and dog feces was 1866.3 and 1813.3, respectively. The average number of *G. duodenalis* cysts per gram of cat and dog feces was 1011.6 and 4786, respectively. All *Cryptosporidium* isolates were determined as *C. canis* in dogs and *C. felis* in cats. *C. canis* and *C. felis* isolates identified in the study, MT329018.1 and MN696800.1, AF159113.1 and KM977642.1 showed similarity with Genbank number, respectively. Assemblages A, E, C, D were found in dogs and assemblages A and E in cats in the result of the sequence of 130 bp gene fragments obtained from *G. duodenalis* isolates. This study sequence analysis of *G. duodenalis* cat isolates identified assemblages A (50%/10) and E (50%/10). In this study, assemblage E was the most common genotype isolated in dogs (38.8%), followed by Assemblage C (27.7%), Assemblage D (16.6%) and Assemblage A (15.5%). Assemblage A, which has zoonotic importance, was detected in cats and dogs, while assemblage E in cats and dogs was reported for the first time in Turkey.

Key words: Cat, *Cryptosporidium*, Dog, Giardia, PCR, Zoonotic.

INTRODUCTION

Gastrointestinal system diseases, particularly infections that cause diarrhea, are critical for cat and dog health. These infections include cryptosporidiosis and giardiasis caused by *Cryptosporidium spp*. and *Giardia duodenalis*, respectively, which are zoonotic and usually asymptomatic in cats and dogs. The diseases of cats and dogs, which are in close contact with humans and farm animals, are as important as zoonotic aspects of the health of these animals (Feng and Xiao, 2011; Xiao, 2010). Cysts and oocysts excreted with the host feces handle the spread of infection in humans and animals and the infection occurs through the consumption of contaminated water and food with cysts and oocysts (Heyworth, 2016; Ryan *et al.*, 2018).

There are around 40 different species of *Cryptosporidium* and over 21 of those have been linked to human infections. The majority of infections in dogs and cats are caused by *C. canis* and *C. felis*, respectively, according to molecular investigations (Ballweber *et al.*, 2010; Lucio-Forster *et al.*, 2010; Rossle and Latif, 2013; Ryan *et al.*, 2018) but *C. hominis*, *C. parvum*, *C. muris* and *C. ubiquitum* have sporadically been found in these species (Alves *et al.*, 2018; Feng *et al.*, 2018; Xiao, 2010).

[1]Department of Parasitology, Faculty of Veterinary Medicine, Dicle University, Diyarbakır-21000, Turkey.

Corresponding Author: Duygu Neval Sayın Ipek, Department of Parasitology, Faculty of Veterinary Medicine, Dicle University, Diyarbakır-21000, Turkey. Email: dneval.sayinipek@dicle.edu.tr

How to cite this article: Ipek, D.N.S. (2023). Molecular Characterization and Zoonotic Significance of *Cryptosporidium spp.* and *Giardia duodenalis* in Asymptomatic Adult Stray Cats and Dogs in Turkey. Indian Journal of Animal Research. DOI: 10.18805/IJAR.BF-1707.

Submitted: 22-09-223 **Accepted:** 04-11-2023 **Online:** 28-11-2023

G. duodenalis has eight different genotypes with varying host ranges. Assemblages C to H have a limited host range, while assemblages A and B are zoonotic and can be found in humans and many animal species. The majority of assemblages C and D are found in canines, while the assemblages F are mostly found in cats. Assemblage E primarily affects cattle, sheep, goats and pigs, unlike assemblages C, D, E and F, which have been recorded in humans. (Ballweber *et al.*, 2010; Fantinatti *et al.*, 2016; Lucio-Forster *et al.*, 2010; Tungtrongchitr *et al.*, 2010; Vivancos *et al.*, 2018; Xu *et al.*, 2016; Zahedi *et al.*, 2017).

This study was conducted to determine the prevalence, species and genotypes and zoonotic significance of *Cryptosporidium* and *Giardia* and in asymptomatic adult stray cats and dogs in Diyarbakır, Turkey.

MATERIALS AND METHODS

Study areas and animal sources

Stool samples were collected from sterile gloves and swaps from the rectum of 42 cats and 33 dogs randomly selected to determine *Cryptosporidium* spp. and *Giardia duodenalis* in adult stray cats and dogs at in Diyarbakır city, southeastern Anatolia, Turkey.

Microscopic analysis

The Crypto/Giardia-Cel FITC Staining Kit (Cellabs Inc. Brookvale, Australia) was used to confirm the presence of *G. duodenalis* cysts and *Cryptosporium spp.* oocysts. 1g of fecal material was treated as directed by the manufacturer. A fluorescent microscope (Carl Zeiss Microscopy GmbH. Jena, Germany) was used to analyze each sample at 200x, 400x and 1000x magnifications. At a magnification of 200x, the oocytes and cysts discovered in each fecal sample were tallied and the number of oocysts per gram feces (opg) was calculated as follows:

$$\frac{\text{Number of cysts or oocysts}}{\text{Volume of sample examined (ml)}} \times \text{Weight of feces (g)}$$

DNA extraction

Total Genomic DNA was extracted from of each fresh 200 mg fecal sample with the direction of suggestion of kit by using ZR Fecal DNA MiniPrep kit (Zymo Research, Irvine, C.A.). Purified DNA samples (100 µL) were stored at -20°C.

Molecular detection of *cryptosporidium* spp.

To determine the species, all DNA extracts were submitted to a nested PCR procedure to amplify a fragment of the *Cryptosporidium spp*. SSU rRNA gene (830 bp). A PCR product of 1325 bp was amplified for the first PCR using the forward primer 5′-TTCTAGAGCTAATACATGCG-3′ and the reverse primer 5′-CCCTAATC CTTCGAAACAGGA-3′. The PCR reactions were performed in a total volume of 50 µL containing 10X PCR buffer, 3 mM $MgCl_2$, 0.2 mM each dNTP, 20 pmol of each primer and 2 U of Taq DNA polymerase under the following conditions: initial denaturation at 94°C for 3 min, 35 cycles at 94°C for 45 s, 55°C for 45 s and 72°C for 1 min and a final extension step at 72°C. For the second round of PCR, reactions were carried out using primers forward primer 5′-GGAAGGGTTGTATTTATTAGATAAAG-3′ and reverse primer 5′-AAGGAGTAAGGAACAACCTCCA-3′ (Xiao *et al.*, 1999). The nested PCR mixture and conditions were identical to the primary PCR except that a concentration of 1.5 mM $MgCl_2$ was used. The PCR products were subjected to electrophoresis in a 1.4% agarose gel and visualized by staining with ethidium bromide.

Molecular detection of giardia duodenalis

A nested PCR was utilized to amplify a 130 bp region of the SSU-rDNA gene using four primers (RH11, RH4, GiarF and GiarR) as previously described by Hopkins *et al.* (1997) and Read *et al.* (2002) (Hopkins *et al.*, 1997; Read, 2002). First and second PCR amplifications were performed in 25 µl volumes with the final mix containing, 2 µl Q solution, 10 pmol of each primer, 1.25 unit DNA polymerase, 0.2 mM of each dNTP, 2.5 mM $MgCl_2$, 10x PCR buffer and H_2O. First PCR reaction was heated to 96°C for 2 min followed by 35 cycles of 96°C for 4 s, 62°C for 30s and 72°C for 45s and one cycle of 72°C for 4 min and second PCR reaction was heated to 96°C for 5 min followed by 35 cycles of 96°C for 45 s, 55°C for 30 s and 72°C for 45 s and one cycle of 72°C for 7 min. The PCR products were subjected to electrophoresis in a 2% agarose gel and visualized by staining with ethidium bromide.

Sequence analysis

The secondary PCR products were sequenced in one directions on an automated sequencer (ABI PRISM 310 model, Perkin-Elmer, USA).

RESULTS AND DISCUSSION

The prevalence of *Cryptosporidium* was 7.14% (3/42) in 42 cats, whereas *G. duodenalis* was 23.80% (10/42). In 33 dogs, *Cryptosporidium* was found to be present in 9.09% (3/33) and *G. duodenalis* in 54.54% (18/33). The average number of *Cryptosporidium* oocysts per gram of cat and dog feces was 1866.3 and 1813.3, respectively. The average number of *G. duodenalis* cysts per gram of cat and dog feces was 1011.6 and 4786, respectively (Table 1).

As a result of the sequence of PCR products obtained from all positive samples, it was determined that *Cryptosporidium* was determined as *C. canis* in dogs and *C. felis* in cats. *C. canis* and *C. felis* isolates identified in the study, MT329018.1 and MN696800.1, AF159113.1 and KM977642.1 showed similarity with Genbank number, respectively. Assemblage A, E, C, D were found in dogs and assemblage A, E in cats in the result of the sequence of 130 bp gene fragments obtained from *G. duodenalis* isolates (Table 1).

Cryptosporidium species and *G. duodenalis*, which are the most important protozoan parasites transmitted by water in developed countries, are crucial for both human and animal health due to their wide host range and their expression as a zoonotic disease.

Table 1: Occurrence species and genotypes distributions of *Cryptosporidium* and *G. duodenalis* in stray dogs and cats.

Animals	*Cryptosporidium* spp.		*G. duodenalis*	
	No. of positives (%) SSU rRNA (no.)	Average number of oocysts	No of positives (%) SSU rRNA (no.)	Average number of cysts
Cats	7.14% (3/42) *C. felis* (3) 100%	1866.3	23.80% (10/42) Assemblage E(5) 50% Assemblage A(5) 50%	1011.6
Dogs	9.09% (3/33) *C. canis* (3) 100%	1813.3	54.54% (18/33) Assemblage E(7) 38.8% Assemblage C (5) 27.7% Assemblage D (3) 16.6% Assemlege A(1) 5.5% Unidendifed (2) 11.1%	4786

Studies on the number of *Cryptosporidium spp.* and *G. duodenalis* oocysts/cysts excreted by cats and dogs are limited (Enemark *et al.*, 2020; Kostopoulou *et al.*, 2017; Yang *et al.*, 2015). This study has shown that the average number of *Cryptosporidium* oocysts and *G. duodenalis* cysts per gram of cat feces was 1866.3 and 1011.6, respectively. The average number of *Cryptosporidium* oocysts is higher than that reported in Western Australia and Greece but lower than that reported in Danish cats *G. duodenalis* cyst numbers are lower than those reported in the Greece and much lower than those reported in the Australian and in the Danish. The average number of *Cryptosporidium* oocysts and *G. duodenalis* cysts per gram of dog feces were 1813.3 and 4.817.7 respectively. Our results are higher than that reported in Greece (Enemark *et al*., 2020; Kostopoulou *et al.*, 2017; Yang *et al.*, 2015). These differences are likely related mainly to differences in methodologies and the health status of the animals between studies. However, oocysts and cysts shed by asymptomatic dogs and cats show that they are an important risk factor for the spread of the disease.

There are studies on the prevalence of *Cryptosporidium* and *G. duodenalis* in dogs and cats in many countries. The prevalence of *G. duodenalis* have been reported in dogs 6.3% and cats 2.0% in Australia (Palmer *et al.*, 2008), in dogs 25.2% and cats 20.5% in Greece (Kostopoulou *et al.*, 2017), in dogs 33% and cats 9.2% in Spain (Gil *et al.*, 2017), in dogs 64.0% and cats 87.0% in Canada (McDowall *et al.*, 2011), in dogs 19.6% in Brazil (Fava *et al.*, 2016; Wang *et al.*, 2021), in dogs 4.5-26.2% and cats 1.2-13.1% in China (Wang *et al.*, 2021), in dogs 16.2% in Saudi Arabia (Malki, 2021), in dogs 16.4-18.8% and cats 8-29.4% in Turkey (Çelik, 2022; Önder *et al.*, 2021; Sursal *et al*., 2020). In our study, *G. duodenalis* was found in asymptomatic cats and dogs at a rate of 23.8% and 54.54%, respectively and the rate detected in dogs is higher than in cats, similar to many studies conducted worldwide (Gil *et al.*, 2017; Kostopoulou *et al.*, 2017; Palmer *et al.*, 2008; Wang *et al.*, 2021). While the rate we found in cats is higher than the rates reported in Australia, Spain, Brazil, China, Saudi Arabia and some cities in Turkey (Mardin, Samsun and Kayseri), it is similar to the rate reported in Greece (Çelik, 2022; Fava *et al.*, 2016; Gil *et al.*, 2017; Kostopoulou *et al.*, 2017; Malki, 2021; Önder *et al.*, 2021; Palmer *et al.*, 2008; Wang *et al.*, 2021). On the other hand, it was lower than the rate reported in asymptomatic cats in the Central Anatolian Region of Turkey and Canada (McDowall *et al.*, 2011; Sursal *et al.*, 2020). The rate detected in dogs in our study is the highest reported in Turkey. It is higher than the studies conducted in Australia, Spain, Brazil and China in the world and lower than the rate reported by McDowall *et al.* in Canada (Fava *et al.*, 2016; Gil *et al.*, 2017; McDowall *et al.*, 2011; Palmer *et al.*, 2008; Wang *et al.*, 2021). These differences in the rates reported in the studies are due to factors such as the diagnosis method used in the study, geographical area, feeding areas, age, the health status of the animals, population density, ownership, or not. However, the molecular prevalence of *Giardia duodonalis* varies considerably depending on the method used.

The prevalence of Cryptosporidium has been reported 3.9% in dogs (Yoshiuchi *et al.*, 2010) and 1.4%- 12.7% in cats in Japan (Ito *et al.*, 2016; Yoshiuchi *et al.*, 2010), 5.4-5.5% in dogs and 8.8% in cats in Spain (de Lucio *et al.*, 2017; Gil *et al.*, 2017), 1.7%-6% in dogs and 0.6-5.6% in cats in China (Cao *et al.*, 2022; Xu *et al.*, 2016; Wang *et al.*, 2021), 0.6% in dogs in Iran (Homayouni *et al.*, 2019), 1.6% in dogs in USA (Wang *et al.*, 2012), 0.2-1.7% in dogs in İtaly (Paoletti *et al.*, 2015; Simonato *et al.*, 2017), 4.2% in dogs and 8.2% in cats in Brazil (Alves *et al.*, 2018), 5.9% in dogs and 6.8% in cats in Greece (Kostopoulou *et al.*, 2017). The prevalence of Cryptosporidium has been reported to be 2.1%-5% in cats and 0.5-15.78% in dogs, in Turkey (Köseoğlu *et al.*, 2022; Kilinç *et al.*, 2018; Korkmaz *et al.*, 2016; Denizhan and Karakuş, 2019; Ağaoğlu *et al.*, 2022; Çelik *et al.*, 2023; Görkem and Ulutaş, 2022; Ünal and Gokpinar, 2020). In our study, the molecular prevalence of cryptosporidium in cats was 7.14%. While this rate is higher than those reported in Japan, China, Greece and Turkey, (Cao *et al.*, 2022; Ito *et al.,* 2016;.Kostopoulou *et al.*, 2017; Wang *et al.*, 2012; Xu *et al.*, 2016; Köseoğlu *et al.*, 2022; Kilinç *et al.*, 2018; Korkmaz *et al.*, 2016) it is lower than those reported in Spain and Brazil (Denizhan *and* Karakuş, 2019; Alves *et al.*, 2018; de Lucio *et al.*, 2017). In the present study, the prevalence of cryptosporidium in dogs (9.09%) is higher than in Japan, China, Iran, USA, Italy, Greece, Sivas and Ankara provinces, Turkey (Yoshiuchi *et al.*, 2010;

Ağaoğlu *et al.*, 2022; Cao *et al.*, 2022; Xu *et al.*, 2016; Wang *et al.*, 2021; Ünal and Gokpinar, 2020; Paoletti *et al.*, 2015; Simonato *et al.*, 2017; Homayouni *et al.*, 2019). By contrast, higher infection rates were reported in dogs in Brazil and Van and Ege provinces, Turkey (Görkem and Ulutaş, 2022; Alves *et al.*, 2018) These different rates reported in studies conducted in various parts of the world may be due to geographic area, feeding sites, animal health status and population densities of the animals.

In recent years, with the increasing use of molecular methods in studies on giardia in cats and dogs, information about the zoonotic potentials of species and genotypes related to these genera has increased. Research has shown that the most prevalent genotype present in cats is F assemblages, followed by assemblages A. In recent investigations, only a few samples showed the presence of Assemblages B, C and D (Cai *et al.*, 2021; Gil *et al.*, 2017; Palmer *et al.*, 2008). Assemble E has been reported in a limited number of studies. (Read *et al.*, 2004). The results of studies conducted in cats in Turkey showed that all the isolates were identified as *G. duodenalis* assemblage B (Önder *et al.*, 2021; Sursal *et al.*, 2020). This study sequence analysis of G. duodenalis cat isolates identified assemblages A (%50 /10) and E (%50/10). The presence of zoonotic assemblage A in our study is consistent with many studies reported in cat worldwide (Cacciò *et al.*, 2008; Enemark *et al.*, 2020; Hoopes *et al.*, 2015; McDowall *et al.*, 2011; Papini *et al.*, 2007; Read *et al.*, 2004). However, assemblage A with zoonotic importance in cats in Turkey is reported for the first time in this study. Our study found genotype assemblage E that was previously not reported in cats in Turkey.

The most common genotypes in dogs are C and D, followed by the zoonotic genotype A, B ,and E (Cai *et al.*, 2021; Fantinatti *et al.*, 2016; Zahedi *et al.*, 2017). In addition, assemblages B and E have also been reported (Adell-Aledón *et al.*, 2018; Cai *et al.*, 2021; Dado *et al.*, 2012; Uehlinger *et al.*, 2013) It has been reported that Assemblages A, B, C and D have been detected in dogs in Turkey (Çelık *et al.*, 2022; Gultekin *et al.*, 2017) In this study, assemblage E was the most common genotype isolated in dogs (38.8%), followed by Assemblage C (27.7%), Assemblage D (16.6%) and Assemblage A (15.5%). Although the studies reporting the presence of Assemblages E in dogs are limited, there is yet to be a study reporting Assemblages E in dogs in Turkey. (Dado *et al.*, 2012) In our research, it is thought that the most common subspecies of assemblage E, written for the first time in dogs in Turkey, is due to the contact of stray dogs with rural areas where farm animals are located. The assemblages C, D and A that were found in our study match the subspecies that have been previously identified in dogs.

Cryptosporidium canis and *C. felis* are host-adapted to dogs and cats, respectively. However, they are also among the five most common *Cryptosporidium* spp. that can infect humans (Xiao and Feng, 2008; Li *et al.*, 2021). In Our study, sequence analysis of *Cryptosporidium spp*. isolates revealed that the positive samples were all identified as *C. canis* in dogs and *C. felis* in cats. Our results are consistent with the studies reported in our country and in the world (Gil *et al.*, 2017; Homayouni *et al.*, 2019; Kostopoulou *et al.*, 2017; Köseoğlu *et al.*, 2022; Li *et al.*, 2019; Yoshiuchi *et al.*, 2010).

CONCLUSION

In conclusion, the relatively high prevalence of giardiasis in dogs and cats, the presence of potential zoonotic subgenotypes A and E and the zoonotic characteristics of cryptosporidium species detected in dogs and cats indicate the importance of treatment and preventive measures. However, further studies in human and animal populations living in this region will be useful to determine the zoonotic epidemiology of *G. duodenalis*.

Funding

This work was supported by the authors' previous project (Tubitak. 18.002). Financial support was provided by the Dicle University Scientific Research Projects.

Declaration of interest

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

REFERENCES

Adell-Aledón, M., Köster, P.C., de Lucio, A., Puente, P., Hernández-de-Mingo, M., Sánchez-Thevenet, P., Dea-Ayuela, M.A. and Carmena, D. (2018). Occurrence and molecular epidemiology of Giardia duodenalis infection in dog populations in eastern Spain. BMC Veterinary Research. 14: 1-11.

Ağaoğlu, Z.T., Başbuğ, O. and Özpinar, N. (2022). Sivas ilindeki köpeklerde *cryptosporidium* spp.'nin prevalansý. Fýrat Üniversitesi Sağlık Bilimleri Veteriner Dergisi. 36(3): 224-228.

Alves, M.E.M., Martins, F.D.C., Bräunig, P., Pivoto, F.L., Sangioni, L.A. and Vogel, F.S.F.(2018). Molecular detection of *cryptosporidium* spp. and the occurrence of intestinal parasites in fecal samples of naturally infected dogs and cats. Parasitology Research. 117: 3033-3038.

Ballweber, L.R., Xiao, L., Bowman, D.D., Kahn, G. and Cama, V.A. (2010). Giardiasis in dogs and cats: Update on epidemiology and public health significance. Trends in Parasitology. 26(4): 180-189.

Cacciò, S., Beck, R., Lalle, M., Marinculic, A. and Pozio, E. (2008). Multilocus genotyping of *Giardia duodenalis* reveals striking differences between assemblages A and B. International Journal for Parasitology. 38(13): 1523-1531.

Cai, W., Ryan, U., Xiao, L. and Feng, Y. (2021). Zoonotic giardiasis: An update. Parasitology Research. 1-20.

Cao, Y., Fang, C., Deng, J., Yu, F., Ma, D., Chuai, L., Wang, T., Qi, M. and Li, J. (2022). Molecular characterization of *Cryptosporidium* spp. and Giardia duodenalis in pet dogs in Xinjiang, China. Parasitology Research. 121(5): 1429-1435.

Çelik, B.A. (2022). First Detection of *Giardia duodenalis* in Cats in Mardin Province. Age (year). 1(28): 5.

Çelik, B.A., Yilmaz, R., Çiftçi, T., Çelik, Ö., Ayan, A. and Orunç, Ö.A. (2022). Survey of the prevalence and genotypes of *cryptosporidium* spp. and giardia duodenalis in shelter dogs in Batman, Turkey. Bulgarian Journal of Veterinary Medicine. DOI: 10.15547/bjvm.2023-0042

Çelik, Ö. Y., Koçhan, A., Çelik, B.A., Ayan, A., Akyildiz, G., Kilinç, Ö.O., Ercan, K., Baldaz, V. and Ayan, Ö.O. (2023). *Cryptosporidium* spp. in Dogs-Prevalence and Genotype Distribution. Acta Scientiae Veterinariae, 51.

Dado, D., Montoya, A., Blanco, M.A., Miró, G., Saugar, J.M., Bailo, B. and Fuentes, I. (2012). Prevalence and genotypes of Giardia duodenalis from dogs in Spain: possible zoonotic transmission and public health importance. Parasitology Research. 111: 2419-2422.

de Lucio, A., Bailo, B., Aguilera, M., Cardona, G.A., Fernández-Crespo, J.C. and Carmena, D. (2017). No molecular epidemiological evidence supporting household transmission of zoonotic *Giardia duodenalis* and *Cryptosporidium* spp. from pet dogs and cats in the province of Álava, Northern Spain. Acta Tropica. 170: 48-56.

Denizhan, V. and Karakuş, A. (2019). Van Ýlindeki Sokak Köpeklerinde Gastrointestinal Protozoonlarýn Prevalansý. Dicle Üniversitesi Veteriner Fakültesi Dergisi. 12(1): 25-29.

Enemark, H.L., Starostka, T.P., Larsen, B., Takeuchi-Storm, N. and Thamsborg, S.M. (2020). *Giardia* and *Cryptosporidium* infections in Danish cats: risk factors and zoonotic potential. Parasitology research. 119: 2275-2286.

Fantinatti, M., Bello, A.R., Fernandes, O. and Da-Cruz, A.M. (2016). Identification of giardia lamblia assemblage E in humans points to a new anthropozoonotic cycle. The Journal of Infectious Diseases. 214(8): 1256-1259.

Fava, N.M., Soares, R.M., Scalia, L.A., da Cunha, M.J.R., Faria, E.S. and Cury, M.C. (2016). Molecular typing of canine *Giardia duodenalis* isolates from Minas Gerais, Brazil. Experimental Parasitology. 161: 1-5.

Feng, Y., Ryan, U.M. and Xiao, L. (2018). Genetic diversity and population structure of *Cryptosporidium*. Trends in parasitology. 34(11): 997-1011.

Feng, Y. and Xiao, L. (2011). Zoonotic potential and molecular epidemiology of *Giardia* species and giardiasis. Clinical Microbiology Reviews. 24(1): 110-140.

Gil, H., Cano, L., de Lucio, A., Bailo, B., de Mingo, M.H., Cardona, G.A., Fernández-Basterra, J.A., Aramburu-Aguirre, J., López-Molina, N. and Carmena, D. (2017). Detection and molecular diversity of *Giardia duodenalis* and *Cryptosporidium* spp. in sheltered dogs and cats in Northern Spain. Infection, Genetics and Evolution. 50: 62-69.

Görkem, Ö. and ULUTAŞ, B. (2022). Prevalence of *cryptosporidium* spp. in dogs in the aegean region. Animal Health Production and Hygiene. 11(1): 26-31.

Gultekin, M., Ural, K., Aysul, N., Ayan, A., Balikci, C. and Akyildiz, G. (2017). Prevalence and molecular characterization of *Giardia duodenalis* in dogs in Aydin, Turkey. International Journal of Environmental Health Research. 27(3): 161-168.

Heyworth, M.F. (2016). *Giardia duodena*lis genetic assemblages and hosts. Parasite. 23.

Homayouni, M.M., Razavi, S.M. and Asadpour, M. (2019). Prevalence and molecular characterization of *Cryptosporidium* spp. and *Giardia intestinalis* in household dogs and catsfrom Shiraz, Southwestern Iran. Veterinaria Italiana. 55(4): 311-318.

Hoopes, J., Hill, J.E., Polley, L., Fernando, C., Wagner, B., Schurer, J. and Jenkins, E. (2015). Enteric parasites of free-roaming, owned and rural cats in prairie regions of Canada. The Canadian Veterinary Journal. 56(5): 495.

Hopkins, R.M., Meloni, B.P., Groth, D.M., Wetherall, J.D., Reynoldson, J.A. and Thompson, R.A. (1997). Ribosomal RNA sequencing reveals differences between the genotypes of Giardia isolates recovered from humans and dogs living in the same locality. The Journal of Parasitology. 44-51.

Ito, Y., Itoh, N., Kimura, Y. and Kanai, K. (2016). Molecular detection and characterization of *Cryptosporidium* spp. among breeding cattery cats in Japan. Parasitology Research. 115: 2121-2123.

Kilinç, Ö.O., Yilmaz, A.B., Göz, Y., Özkan, C. and Denizhan, V. (2018). Determination of *Cryptosporidium* spp. in Van cats by nested PCR1. Med. Weter. 74(7): 456-459.

Korkmaz, U. F., Gökpınar, S. and Yıldız, K. (2016). Kedilerde bağırsak parazitlerinin yaygınlığı ve halk sağlığı bakımından önemi.

Kostopoulou, D., Claerebout, E., Arvanitis, D., Ligda, P., Voutzourakis, N., Casaert, S. and Sotiraki, S. (2017). Abundance, zoonotic potential and risk factors of intestinal parasitism amongst dog and cat populations: The scenario of Crete, Greece. Parasites and vectors, 10, 1-12.

Köseoğlu, A.E., Can, H., Karakavuk, M., Güvendi, M., Değirmenci Döşkaya, A., Manyatsi, P.B., Döşkaya, M., Gürüz, A.Y. and Ün, C. (2022). Molecular prevalence and subtyping of *cryptosporidium* spp. in fecal samples collected from stray cats in İzmir, Turkey. BMC Veterinary Research. 18(1): 89.

Li, J., Dan, X., Zhu, K., Li, N., Guo, Y., Zheng, Z., Feng, Y. and Xiao, L. (2019). Genetic characterization of *Cryptosporidium* spp. and *Giardia duodenalis* in dogs and cats in Guangdong, China. Parasites and Vectors. 12: 1-9.

Li, J., Ryan, U., Guo, Y., Feng, Y. and Xiao, L. (2021). Advances in molecular epidemiology of cryptosporidiosis in dogs and cats. International Journal for Parasitology. 51(10): 787-795.

Lucio-Forster, A., Griffiths, J.K., Cama, V. A., Xiao, L. and Bowman, D.D. (2010). Minimal zoonotic risk of cryptosporidiosis from pet dogs and cats. Trends in Parasitology. 26(4): 174-179.

Malki Al S. Jamila (2021). Prevalence of Intestinal Parasite Infections in Stray Dogs in Taif Region, KSA. Indian Journal of Animal Research. 55(12): 1491-1497 . doi: 10.18805/IJAR.BF-1421.

McDowall, R.M., Peregrine, A.S., Leonard, E.K., Lacombe, C., Lake, M., Rebelo, A.R. and Cai, H.Y. (2011). Evaluation of the zoonotic potential of *Giardia duodenalis* in fecal samples from dogs and cats in Ontario. The Canadian Veterinary Journal. 52(12): 1329.

Önder, Z., Yetişmiş, G., Pekmezci, D., Kökçü, N.D., Zafer, G., Pekmezci, A.Ç., Düzlü, Ö., İnci, A. and Yıldırım, A. (2021). Investigation of Zoonotic Cryptosporidium and *Giardia intestinalis* Species and Genotypes in Cats (*Felis catus*). Turkiye Parazitol Derg. 45(4): 252-256.

Palmer, C.S., Traub, R.J., Robertson, I.D., Devlin, G., Rees, R. and Thompson, R.A. (2008). Determining the zoonotic significance of Giardia and Cryptosporidium in Australian dogs and cats. Veterinary Parasitology. 154(1-2): 142-147.

Paoletti, B., Traversa, D., Iorio, R., De Berardinis, A., Bartolini, R., Salini, R. and Di Cesare, A. (2015). Zoonotic parasites in feces and fur of stray and private dogs from Italy. Parasitology Research. 114: 2135-2141.

Papini, R., Cardini, G., Paoletti, B. and Giangaspero, A. (2007). Detection of *Giardia* assemblage A in cats in Florence, Italy. Parasitology Research. 100(3): 653-656.

Read, C. (2002). Correlation between genotype of *Giardia duodenalis* and diarrhoea. Int J Parasitol. 32: 229-231.

Read, C.M., Monis, P. T. and Thompson, R. A. (2004). Discrimination of all genotypes of Giardia duodenalis at the glutamate dehydrogenase locus using PCR-RFLP. Infection, Genetics and Evolution. 4(2): 125-130.

Rossle, N. F. and Latif, B. (2013). *Cryptosporidiosis* as threatening health problem: A review. Asian Pacific journal of tropical biomedicine. 3(11): 916-924.

Ryan, U., Hijjawi, N. and Xiao, L. (2018). Foodborne cryptosporidiosis. International Journal for Parasitology. 48(1): 1-12.

Simonato, G., Frangipane di Regalbono, A., Cassini, R., Traversa, D., Tessarin, C., Di Cesare, A. and Pietrobelli, M. (2017). Molecular detection of *Giardia duodenalis* and *Cryptosporidium* spp. in canine faecal samples contaminating public areas in Northern Italy. Parasitology Research. 116: 3411-3418.

Sursal, N., Simsek, E. and Yildiz, K. (2020). Feline Giardiasis in Turkey: Prevalence and genetic and haplotype diversity of Giardia duodenalis based on the β-Giardin gene sequence in symptomatic cats. The Journal of Parasitology. 106(5): 699-706.

Tungtrongchitr, A., Sookrung, N., Indrawattana, N., Kwangsi, S., Ongrotchanakun, J. and Chaicumpa, W. (2010). *Giardia intestinalis* in Thailand: identification of genotypes. Journal of Health, Population and Nutrition. 28(1): 42.

Uehlinger, F.D., Greenwood, S.J., McClure, J.T., Conboy, G., O'Handley, R. and Barkema, H.W. (2013). Zoonotic potential of *Giardia duodenalis* and *Cryptosporidium* spp. and prevalence of intestinal parasites in young dogs from different populations on Prince Edward Island, Canada. Veterinary Parasitology. 196(3-4): 509-514.

Ünal, G.G. and Gokpinar, S. (2020). Prevalence of intestinal parasites in dogs and its importance in terms of public health. International Journal of Veterinary and Animal Research (IJVAR). 3(3): 64-68.

Vivancos, V., González-Alvarez, I., Bermejo, M. and Gonzalez-Alvarez, M. (2018). Giardiasis: Characteristics, pathogenesis and new insights about treatment. Current Topics in Medicinal Chemistry. 18(15): 1287-1303.

Wang, A., Ruch-Gallie, R., Scorza, V., Lin, P. and Lappin, M.R. (2012). Prevalence of *Giardia* and *Cryptosporidium* species in dog park attending dogs compared to non-dog park attending dogs in one region of Colorado. Veterinary parasitology, 184(2-4): 335-340.

Wang, Y.G., Zou, Y., Yu, Z.Z., Chen, D., Gui, B.Z., Yang, J.F., Zhu, X.Q., Liu, G.H. and Zou, F.C. (2021). Molecular Investigation of zoonotic intestinal protozoa in pet dogs and cats in Yunnan Province, southwestern China. Pathogens. 10(9), 1107.

Xiao, L. (2010). Molecular epidemiology of cryptosporidiosis: An update. Experimental parasitology. 124(1): 80-89.

Xiao, L. and Feng, Y. (2008). Zoonotic cryptosporidiosis. FEMS Immunology and Medical Microbiology. 52(3): 309-323.

Xiao, L., Morgan, U.M., Limor, J., Escalante, A., Arrowood, M., Shulaw, W., Thompson, R., Fayer, R. and Lal, A.A. (1999). Genetic diversity within *Cryptosporidium parvum* and related Cryptosporidium species. Applied and Environmental Microbiology. 65(8): 3386-3391.

Xu, H., Jin, Y., Wu, W., Li, P., Wang, L., Li, N., Feng, Y. and Xiao, L. (2016). Genotypes of *Cryptosporidium* spp., Enterocytozoon bieneusi and Giardia duodenalis in dogs and cats in Shanghai, China. Parasites and Vectors. 9(1): 1-9.

Yang, R., Ying, J.L.J., Monis, P. and Ryan, U. (2015). Molecular characterisation of *Cryptosporidium* and Giardia in cats (*Felis catus*) in Western Australia. Experimental Parasitology. 155: 13-18.

Yoshiuchi, R., Matsubayashi, M., Kimata, I., Furuya, M., Tani, H. and Sasai, K. (2010). Survey and molecular characterization of *Cryptosporidium* and *Giardia* spp. in owned companion animal, dogs and cats, in Japan. *V*eterinary Parasitology. 174(3-4): 313-316.

Zahedi, A., Field, D. and Ryan, U. (2017). Molecular typing of *Giardia duodenalis* in humans in Queensland-first report of Assemblage E. Parasitology. 144(9): 1154-1161.