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Investigation of Zoonotic *Cryptosporidium* and *Giardia intestinalis* Species and Genotypes in Cats (*Felis catus*)

Kedilerde (Felis catus) Zoonotik Cryptosporidium ve Giardia intestinalis Tür/Genotiplerinin Araştırılması

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ABSTRACT

Objective: *Giardia intestinalis* and *Cryptosporidium* spp. are important zoonotic protozoan parasites that infect humans and various animals. We investigated the occurrence of *G. intestinalis* and *Cryptosporidium* spp. infection in cats. To provide data on the zoonotic transmission dynamics of these parasites, genotypes of the detected isolates were investigated through DNA sequence characterization.

Methods: A total of 100 fecal samples were collected from cats between June and October 2020 in Kayseri and Samsun provinces. Fecal samples were examined by nested polymerase chain reaction (PCR), targeting the *β-giardin* gene of *G. intestinalis* and small subunit (SSU) *rRNA* gene of *Cryptosporidium* spp. All PCR products were sequenced for genotyping.

Results: Of the samples examined, *Giardia intestinalis* was determined in 8 samples (8.0%), whereas none of the samples were found positive for *Cryptosporidium* spp. Sequence analyses of the *β-giardin* PCR products indicated that all *G. intestinalis* isolates were classed into the zoonotic assemblage B.

Conclusion: This study adds to the current data on the molecular epidemiology of cryptosporidiosis and giardiasis in cats. The findings also highlight the potential risk of cats for public health concerning the zoonotic transmission dynamics of *G. intestinalis*.

Keywords: *Giardia intestinalis*, *Cryptosporidium* spp., cat, molecular characterization, genotyping

ÖZ

Amaç: *Giardia intestinalis* ve *Cryptosporidium* spp. insanları ve çeşitli hayvanları enfekte eden, önemli zoonotik protozoon parazitlerdendir. Bu çalışmanın amacı, kedilerde *G. intestinalis* ve *Cryptosporidium* spp. varlığını belirlemek ve zoonotik bulaşma dinamikleri açısından izolatların moleküler karakterizasyonunu yaparak genotiplerini ortaya çıkarmaktır.

Yöntemler: Kayseri ve Samsun illerinde, Haziran-Ekim 2020 tarihleri arasında toplam 100 kediden dışkı örneği toplanmıştır. Dışkı örnekleri, *G. intestinalis*'in *β-giardin* genini ve *Cryptosporidium* spp.'nin small subunit ribosomal RNA (SSU) *rRNA* genini hedefleyen nested polimeraz zincir reaksiyon (PZR) analizleri ile incelenmiştir. Tüm PCR ürünleri genotipleme için sekanslanmıştır.

Bulgular: İncelemesi yapılan örneklerin 8'inde (%8,0) *G. intestinalis* pozitifliği belirlenirken, *Cryptosporidium* spp. enfeksiyonuna rastlanmamıştır. *G. intestinalis* izolatlarının sekans analizleri sonucu zoonotik assemblage B içerisinde olduğu tespit edilmiştir.

Sonuç: Bu çalışma, kedilerde *Cryptosporidiosis* ve Giardiasis'in moleküler epidemiyolojisi üzerine mevcut bilgi birikimine katkı sağlamıştır. Ayrıca, elde edilen çıktılar *G. intestinalis*'in zoonotik bulaşma dinamikleri çerçevesinde kedilerin halk sağlığı açısından risk potansiyelini vurgulamıştır.

Anahtar Kelimeler: *Giardia intestinalis*, *Cryptosporidium* spp., kedi, moleküler karakterizasyon, genotipleme

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Türkiye PARAZİTOLOJİ Dergisi

INTRODUCTION

Giardia intestinalis and *Cryptosporidium* spp. are common and significant zoonotic protozoan parasites causing diarrhea and infect gastrointestinal tracts of humans and animals (1). These two pathogens can be transmitted to humans through the consumption of contaminated food or water and direct or indirect contact with infected animals or persons (2). Cystic stages of *G. intestinalis* and thick walled oocyts of *Cryptosporidium* spp. are highly resistant and can remain infectious for long periods in environments with suitable temperature and humidity conditions (3-5). The two pathogens are transmitted primarily through the fecal-oral route and have been responsible for waterborne outbreaks worldwide (5,6).

To date, 73 genotypes of 42 *Cryptosporidium* species have been identified in various hosts by molecular analyses (1,7). *Cryptosporidium hominis* and *C. parvum* are recognized as the most important species responsible for human infections (8). *Cryptosporidium felis* is the main species in cats but *C. hominis*, *C. parvum*, *C. muris*, and *C. ubiquitum* have also been occasionally detected (2,5,9,10).

Giardia intestinalis is a multi-complex species, with eight (A to H) distinct assemblages with different host specificities (5,11). Cats primarily tend to be infected with host-adapted assemblages with assemblage F. However, other assemblages, including assemblages A-E have also been identified in cats (12).

Although the prevalence and molecular characterization of feline *Giardia* and *Cryptosporidium* infections have been investigated worldwide (5,13-17), limited data is available on the presence of these protozoans in cats and their genotypes in Turkey (18,19-31). Thus, we aimed to investigate *Cryptosporidium* spp. and *G. intestinalis* in cats in Kayseri and Samsun provinces and identify the genotypes of the detected isolates for evaluating the zoonotic potential of these pathogens.

METHODS

Collection of Fecal Samples

A total of 100 fresh fecal specimens were collected from cats admitted to the Veterinary Education, Research and Practice Hospital for their routine health examinations in Samsun and Kayseri provinces between June and October 2020. The feces were collected in universal plastic tubes using sterile disposable gloves by the owner after defecation and placed into plastic bags. The bags were marked with age, breed, and geographic region of collection. All samples were transported to the laboratory and preserved at -20 °C until used for the genomic DNA extraction.

Genomic DNA Extraction and Nested PCR Amplification of SSU rRNA and *β-giardin* Gene

Total genomic DNA was extracted from 200 mg of the individual fecal sample using QIAamp® DNA Stool Mini Kit (Qiagen, Germany). The feces for DNA extraction were primarily subjected to five freezing with liquid nitrogen/thawing (with boiling water) to ensure efficient lysis of oocysts/cysts before extraction protocol. Qubit Nanodrop spectrophotometer (Thermo Fisher Scientific, USA) was used to measure DNA concentration. The extracted genomic DNA was stored at -20 °C until used for the PCR analyses.

The presence of *Cryptosporidium* spp. and *G. intestinalis* were screened by nested PCR analyses targeting a 850 bp and ~530 bp fragment of small subunit (SSU) *rRNA* gene and of the *β-giardin* gene, respectively (20,21). The primers and cycling conditions of both SSU *rRNA* and *β-giardin* genes were performed as previously described (20,21). Positive (genomic DNA of bovine *C. parvum* and *G. intestinalis* positive fecal samples) and negative (ddH_2O) controls were included in each PCR run. The secondary PCR products were visualized on 1.5% agarose gel electrophoresis with GelRed™ gel stain (Biotium, Inc., Hayward, CA, USA).

Sequencing and Phylogenetic Analysis

All PCR positive products were purified using the High Pure PCR Product Purification Kit (Roche Diagnostics GmbH, Germany). The purified products were sequenced in both directions by the same PCR primers in a Genetic Analyser ABI 3130 XL (Macrogen, Amsterdam, the Netherlands). For the remove to low quality reads from the raw sequence, the DNA sequences obtained were trimmed and then edited using Geneious Prime 2020.0.3 software (https://www.geneious.com). Sequences were aligned with reference sequences downloaded from GenBank to determine the *Cryptosporidium* species identity and *G. intestinalis* genotypes.

Phylogenetic analyses based on the *β-giardin* data set of *G. intestinalis* were performed using the maximum likelihood method (ML) with 1.000 replicates bootstrap values, using in mega 7 software (22,23).

Statistical Analysis

Statistical analysis has not been made in this study.

RESULTS

Occurence of *Cryptosporidium* and *G. intestinalis* in Cats

No samples were found as positive for *Cryptosporidium* spp. *G. intestinalis* was detected in 8 (8.0%) out of 100 fecal samples by nested PCR analyses of *β-giardin* gene region.

Giardia intestinalis Assemblage

A 490 bp fragment of the *β-giardin* gene was successfully obtained from all positive isolates. The assemblage and distrubution of *G. intestinalis* in the cats are presented in Table 1. The BLASTn (Basic Local Alignment Search Tool) analyses of the sequences showed that all isolates were genetically identical (100%) to each other. According to the *β-giardin* sequence analysis, all of the *G. intestinalis* isolates were identified as zoonotic assemblages B. All nucleotide sequences obtained in this study were deposited in GenBank database under accession numbers MW727284-86.

Phylogenetic Analysis

The ML tree is shown in Figure 1 with bootstrap values. Based on the phylogenetic analysis, all sequences from the present study were clustered within assemblage B comprising species mostly (or only) infecting humans and several other mammals such as livestocks, dogs and cats. The phylogenetic tree indicated that all obtained sequences clustered with human and cat *G. intestinalis* assemblage B sequences which is distant from the other *G. intestinalis* assemblages (Figure 1). Our sequences exhibited 100% homology with isolates reported from humans in Turkey (MT166367), China (KY696836), Mozambique (MT332804),

Table 1. The assemblage identification and distrubution of *G. intestinalis* in fecal samples in cats

Breeds and numbers of cats	Number of positive samples	Mean age of the cats (months)	*G. intestinalis* assemblage
British Shorthair (2)	-	4 (3-5)	
Mackerel (25)	2	7 (7-12)	Assemblage B
Mix (32)	2	5 (2-12)	Assemblage B
Tabby (27)	1	7 (4-12)	Assemblage B
Birman (1)	-	4	
Persian (1)	1	6	Assemblage B
Siamese (2)	1	8 (5-10)	Assemblage B
Tuxedo (10)	1	10 (5-12)	Assemblage B
Total (100)	**8**		

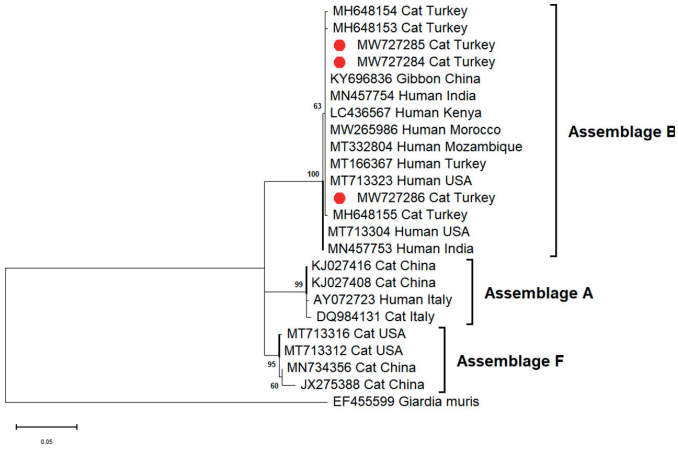

Figure 1. Phylogenetic relationships among *G. intestinalis* isolates in GenBank based on the *β-giardin* gene. *Giardia muris* (EF455599) was used as an out group. Isolates involved in the data set are given with GenBank accession number, host, and country. Isolates characterized in this study are indicated with red circle characters. The scale bar represents 0.05 substitutions per nucleotide position

USA (MT713323), and Kenya (LC436567). The sequences also showed 99.78% identity with the cat isolates reported from Turkey (MH648153-55).

DISCUSSION

Although there have been many molecular epidemiological studies on the prevalence of *Cryptosporidium* and *G. intestinalis* infections in water sources and various animal hosts in Turkey (24-30), there are limited molecular survey on detecting genotypes and the presence of these parasites in cats (18,19,31). Therefore, the present study is contributed to molecular data on determining genotype distribution, and presence of zoonotic protozoans.

The prevalence of *Cryptosporidium* spp. infection in cats has been reported in the range between 0% and 29.4% in the world and these prevalence differences have been related to studied cat populations as well as different diagnostic techniques (5,12). However, the prevalence of this protozoon in cats with the coprological examination and molecular techniques has been reported from 1% to 13% in Turkey (18,31-33). In the present study, none of the cats were determined positive for *Cryptosporidium* spp. A similar finding was also reported in a study in Italy (34). However, some authors reported that the absence of cryptosporidiosis in cats has led to sporadic character of the diseases (34,35).

Giardia intestinalis infection in cats has been reported in many countries. We found similar results with the previous studies in Denmark (7.0%) and Spain (9.2%) (12,34). However, our results were lower than previously reported in studies from Australia (10.1%), Thailand (12.1%), Germany (10.5%), and Brasil (11.1%) (5,10,36,37) and higher than the findings of studies from China (3.6%) and Italy (6.1%) (2,34). One recent study reported the molecular detection of *G. intestinalis* in cats with diarrhea in the Central Anatolia Region of Turkey with 68.6% prevalence. Infection rate determined in the present study was lower than previous report (68.6%) in Turkey (19).

The role of pet animals as an important source of human giardiasis was extensively discussed. Some researchers showed that pet animals play an important role as reservoirs of zoonotic assemblage and transmission from these animals to humans (38,39). Cats are primarily infected with feline-specific *G. intestinalis* assemblage F (12). However, the assemblages A, B, C, D, and, E have also been occasionally reported in cats (12). In this study, we determined the presence of potentially human-pathogenic assemblages B, with sequence analyses of *β-giardin* gene in the cats. This result is consistent with the previous reports in cats from different regions around the world (40-44). To date, there has been only one report on *G. intestinalis* in cats in Turkey (19) indicating the presence of assemblage B which is consistent with the findigs of current study. In addition, assemblage B is dominant in human, dog, and drinking/environmental waters in Turkey (26,45-47). Host-adapted *G. intestinalis* assemblage F has been also reported in humans. Thus, cats might be a potential reservoir for human giardiasis (48,49).

CONCLUSION

This study provides important data on molecular characterization of *G. intestinalis* in cats in Turkey indicating the occurrence of zoonotic assemblage B. Further studies are necessary to determine the molecular epidemiology of *Cryptosporidium* spp. and *G. intestinalis* in cats with large-scale sampling in the different geographic regions of Turkey and to reveal potential risk factors for public health.

* Ethics

Ethics Committee Approval: As fecal material was obtained by the owner after defecating animals, there was no need to ethical approval. There are no human patients in the study.

Informed Consent: A consent form was completed by all participants.

Peer-review: Externally and internally peer-reviewed.

* Authorship Contributions

Concept: Z.Ö., D.P., G.Z.P., A.Y., Design: Z.Ö., G.Z.P., A.Y., Ö.D., A.İ., Data Collection or Processing: D.P., Z.Ö., G.Y., G.Z.P., Analysis or Interpretation: Z.Ö., A.Y., G.Y., N.D.K., A.Ç., Literature Search: Z.Ö., A.Y., Ö.D., A.İ., G.Y., N.D.K., Writing: Z.Ö., Ö.D., A.Y., D.P., G.Z.P.

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