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Research article

PRELIMINARY INVESTIGATION OF THE PREVALENCE AND GENOTYPE DISTRIBUTION OF *CRYPTOSPORIDIUM* SPP., AND *GIARDIA DUODENALIS* IN CATS IN SIIRT, TURKEY

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Cryptosporidium spp. and *Giardia duodenalis* are protozoan parasites found in humans and many animal species worldwide. The aim of this study was to determine the prevalence and genotypes of *Cryptosporidium* spp. and *Giardia duodenalis* in cats and to evaluate the zoonotic potential of these agents. The animal material of the study consisted of a total of 40 cats brought to the Veterinary Faculty. Fresh fecal samples taken from the cats were placed in individual sample containers. All samples were examined under the microscope by Kinyoun Acid Fast staining for *Cryptosporidium* spp. and by the native-Lugol method for *Giardia duodenalis*. Nested PCR and sequence analyses were then performed. As a result of microscopic and nested PCR analyses for *Cryptosporidium* spp., no positivity was found in any sample. The prevalence of *Giardia duodenalis* was 2.5% in both microscopic examination and nested PCR analyses. When the DNA sequences of the *β-Giardin* gene obtained in the study were compared with the database in NCBI Basic Local Alignment Search Tool, it was determined that one sample overlapped with Assemblage B samples. As a result of this study, the prevalence of *Cryptosporidium* spp. and *Giardia duodenalis* in cats was determined and the presence of Assemblage B was revealed. It is recommended that repetitive studies should be carried out as much as possible to determine the possible role of these parasites in the transmission of these parasites to humans.

Keywords: Nested PCR, Sequence, Phylogeny

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INTRODUCTION

Cryptosporidium spp. and *Giardia duodenalis* are protozoan parasites that are distributed worldwide, colonize the intestines of humans and domestic mammals, and cause clinical and subclinical infections in cats of all ages [1-3]. 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 [4].

To date, at least 26 *Cryptosporidium* species have been identified [1,3-5]. Among these species, cats are commonly infected with *C. felis* [5], however, *C. hominis*, *C. parvum, C. muris,* and *C. ubiquitum* are also reported [4]. Most cats infected with *Cryptosporidium* spp. are usually asymptomatic [5,6]. However, it can cause gastrointestinal problems, especially in young and newborn kittens [6,7]. Other common clinical signs of cryptosporidiosis in cats are anorexia and weight loss [5]. Eight species are responsible for the majority of human cryptosporidiosis cases [3]. Among these, *C. hominis* and *C. parvum* are recognized as the most important species infecting humans globally [1,3,4].

Giardia duodenalis, found in the small intestine of many vertebrates, is the only Giardia species infective for cats [8,9]. *G. duodenalis* has eight (A-H) genotypes with different host specificities [1,3]. Of these, assemblages A and B have been identified in many mammals, including humans, while the others (C-H) are more host-specific assemblages [1,10]. Cats are particularly infected with assemblage F [8]. However, assemblages A-E have also been described [3]. Infection is often subclinical in cats. Clinically, most infections occur in young animals and the most prominent symptom is diarrhea. Clinical signs are more likely to be seen in young animals in multi-cat households [9,10].

The aim of this study was to determine the prevalence and genotypes of *Cryptosporidium* spp. and *Giardia duodenalis* in cats and to evaluate the zoonotic potential of these agents.

MATERIAL AND METHODS

The study area and animal material

This study was carried out in Siirt province located in the Southeastern Anatolia Region of Turkey. The animal material of the study consisted of 40 cats brought to the Siirt University Faculty of Veterinary Medicine. Fresh fecal samples were placed in individual sample containers and the age and sex of the animals were recorded.

Microscopic examination

All samples were examined under the microscope by Kinyoun Acid Fast staining for *Cryptosporidium* spp. and by the native-Lugol method for *Giardia duodenalis*.

DNA extraction

All samples were subjected to DNA extraction using GeneMATRIX Stool DNA Purification Kit according to the manufacturer's protocol. The obtained DNAs were stored at -20°C until the next steps.

PCR amplification

Nested PCR was performed for *Cryptosporidium* spp. using primers described by Xiao et al. (2001). In the PCR step, 5'-TTCTAGAGCTAATACATGCG-3' and 5'- CCCATTTCCTTCGAAACAGGA-3' primers were used to amplify the 1325 bp gene region. In the nested PCR stage, primers 5'- GGAAGGGTTGTATTTATTTATTAGATAAAG-3' and 5'-AAGGAGTAAGGAACAACCTCCA-3' were used to amplify the 826-864 bp gene region.

For *Giardia* duodenalis the 753 bp β-giardin gene region was amplified using the primers described by Cacciò et al. (2002) (G7 F5'-AAGCCCGACGACGACCTCACCCGCAGTGC-3' forward and G759R 5'- GAGGCCGCCCTGGATCTTCGAGACGAC-3' reverse). Nested PCR was then performed using the primers described by Lalle et al. (2005) (BG1F 5'- GAACGAGATCGAGGTCCG-3' forward and BG2R 5'-CTCGACGAGTTCGTGTGTT-3' reverse).

The PCR products obtained were stained with RedSafe™ Nucleic Acid Staining Solution and images were obtained on 1.5% agarose gel.

DNA sequence analysis and phylogeny

One PCR-positive sample for Giardia was sequenced forward and reverse. The DNA sequences obtained were checked, aligned, and analyzed in BioEdit software [14]. The edited formats of the DNA sequences were compared with the databases using the NCBI Basic Local Alignment Search Tool to determine the assemblages [15]. In addition, a phylogenetic tree was constructed with the data set created using the β-giardin gene sequences obtained from the NCBI GenBank database and the DNA sequences obtained as a result of the study, and it was shown which assemblages the study samples were related to. In order to create the phylogenetic tree, the data sets were aligned in BioEdit program and model test was performed using Maximum Likelihood statistical method in IQTREE program and phylogenetic tree was created with 1000 bootstrap according to BIC optimal model [16, 17].

Ethical approval

This study was approved by Siirt University Animal Experiments Local Ethics Committee (Approval numbers 2022/02/11, and 2022/03/14).

RESULTS

As a result of microscopic examination and nested PCR analyses for *Cryptosporidium* spp. were negative in all samples. However, in both microscopic examination and PCR analysis of all samples examined for *Giardia* spp., positivity was detected in 1 (2.5%) sample. When the DNA sequences of the *β-giardin* gene obtained in the study were compared with the database in NCBI Basic Local Alignment Search Tool, it was observed that one sample overlapped with Assemblage B samples. As can be seen in the phylogenetic tree, this sample is located in the assemblage B clade (Figure 1).

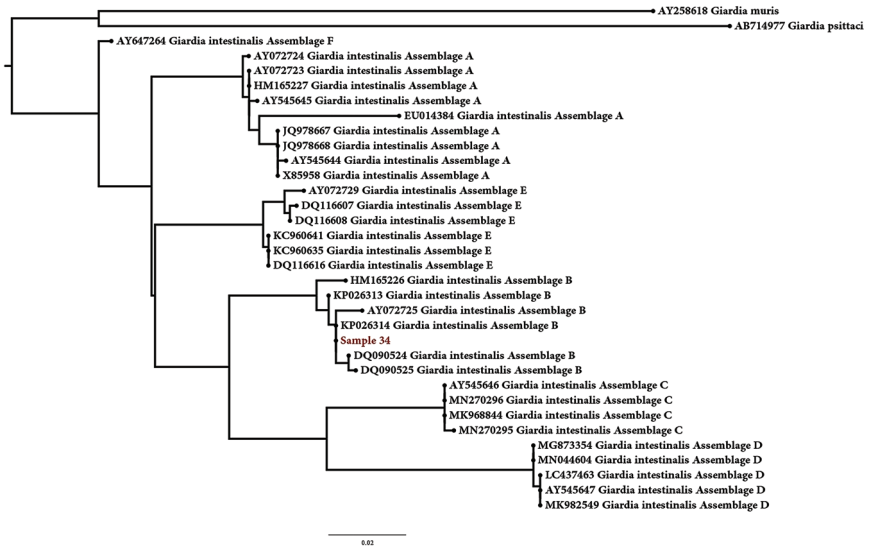

Figure 1. Phylogenetic relationships of *Giardia duodenalis* isolates, using Maximum Likelihood method analysis based on β-*giardin* gene region. Numbers at the nodes represent the Bootstrap values (1000 bootstrap). *Giardia psitacci* and *Giardia muris* were used as an outgroup.

DISCUSSION

Today, the number of domestic cats in many regions of the world is estimated to be around half a billion [18]. Since these cats may be reservoirs for *Cryptosporidium* spp. and *Giardia duodenalis*, it is very important to know the prevalence of these agents in cats.

In studies to determine the prevalence of Cryptosporidiosis in the world; The prevalence was reported as 32% in South Africa [19], 5.26% in Germany [2], 9.9% in Australia [3], 1.4% in Japan [7], 2.3% in China [20], and 0-5% in Turkey [4, 6, 21]. In this study, no positivity was found as a result of microscopic examination and nested PCR analyses. This result is similar to the findings of Paoletti et al. (2011) and Önder et al. (2021). The absence of *Cryptosporidium* spp. in the cats examined in this study may be due to the sporadic character of this disease.

In the studies conducted to determine the prevalence of Giardiasis in the world; The prevalence was reported as 3.33% in Iran [9], 10.1%-80% in Australia [3,10], 5.8% in China [22], 6.1% in Italy [1], 9.2% in Spain [8], 10.52% in Germany [2], 3.9% in Poland [23], and 2.12%-68.6% in Turkey [4,18,24-26]. In this study, a prevalence of 2.5% was determined as a result of microscopic examination and nested PCR analysis. This result is similar to the findings of the studies conducted by Korkmaz et al. (2016) and Pan et al. (2018)

Although feline-specific assemblage F has been reported to be seen in cats, assemblage A [1,2,8], assemblage B [4,26], assemblage D [27] and assemblage F [1,20,27] have also been reported. One positive sample obtained as a result of this study was determined to be zoonotic assemblage B, and this result coincides with the findings of Sursal et al. (2020) and Önder et al. (2021).

Mosallanejad et al. (2010) and Gil et al. (2017) found a higher prevalence in males by gender and in those under one year of age by age. The fact that one positive sample obtained in this study was a male cat under one year of age supports the findings of the researchers.

CONCLUSION

As a result of this study, the prevalence of *Cryptosporidium* spp. and *Giardia duodenalis* in cats in Siirt was determined and the presence of assemblage B was revealed. The detection of the presence of assemblage B indicates that cats may pose a risk to human health. It is recommended that repetitive studies should be carried out as much as possible to determine the possible role of these parasites in the transmission of these parasites to humans.

Authors' contributions

BAÇ and ÖYÇ designed the study and drafted the manuscript. ÖYÇ, KE and ÖOA were responsible for the sampling. AA and ÖOK performed laboratory molecular analyses, GA responsible for the phylogenetic analysis. All authors read and approved the final manuscript.

Declaration of conflicting interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Statement of Informed Consent

The owner understood procedure and agrees that results related to investigation or treatment of their companion animals, could be published in Scientific Journal Acta Veterinaria-Beograd.

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PRELIMINARNO ISTRAŽIVANJE PREVALENCIJE I DISTRIBUCIJE GENOTIPA *CRIPTOSPORIDIUM* SPP. I *GIARDIA DUODENALIS* KOD MAČAKA U SIIRTU, TURSKA

Burçak ASLAN ÇELİK, Özgür Yaşar ÇELİK, Adnan AYAN, Gürkan AKYILDIZ, Özlem ORUNÇ KILINÇ, Özge OKTAY AYAN, Kerem ERCAN

Criptosporidium spp. i *Giardia duodenalis* su protozoitski paraziti koji se nalaze kod ljudi i mnogih životinjskih vrsta širom sveta. Cilj ovog istraživanja bio je da se utvrdi prevalencija i genotipovi *Criptosporidium* spp. i *Giardia duodenalis* kod mačaka, kao i procena zoonotskog potencijala ovih agenasa. Životinjski materijal činilo je ukupno 40 mačaka ispitanih na Veterinarskom fakultetu. Sveži uzorci fekalija uzeti od mačaka stavljeni su u pojedinačne kontejnere za uzorke. Svi uzorci su ispitani pod mikroskopom pomoću *Kinioun Acid Fast* bojenja za *Criptosporidium* spp. i nativna Lugol metoda za *Giardia duodenalis*. Zatim su izvršene nest- PCR i analize sekvence. Kao rezultat mikroskopskih

i PCR analiza za *Criptosporidium* spp., nije pronađena nikakva pozitivnost ni u jednom uzorku. Prevalencija *Giardia duodenalis* bila je 2,5% i u mikroskopskom pregledu i u PCR analizi. Kada su sekvence DNK gena b-giardin dobijene u studiji upoređene sa bazom podataka u *NCBI Basic Local Alignment Search Tool*, utvrđeno je da se jedan uzorak preklapa sa uzorcima skupa B. Kao rezultat ove studije, određena je prevalencija *Criptosporidium* spp. i *Giardia duodenalis* kod mačaka i otkriveno je prisustvo skupa B. Preporučuje se da se dalja istraživanja sprovode što češće kako bi se utvrdila moguća uloga ovih parazita u prenošenju na ljude.