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Original article

A SURVEY OF THE PREVALENCE AND GENOTYPES OF *CRYPTOSPORIDIUM* SPP. AND *GIARDIA DUODENALIS* IN SHELTER DOGS IN BATMAN, TURKEY

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Summary

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Cryptosporidium spp. and *Giardia duodenalis* are opportunistic zoonotic protozoan parasites related to diarrhea in humans and many mammals. This study aimed to determine the prevalence and genotypes of *Cryptosporidium* spp. and *Giardia duodenalis* in shelter dogs in Batman province. The animal material of the study consisted of 100 dogs of different breeds and sexes. Fresh fecal samples taken from the dogs were examined under the microscope by Kinyoun Acid Fast staining for *Cryptosporidium* spp. and by the native-Lugol method for *Giardia*. DNA extraction, nested PCR analysis, and sequence analysis were then performed. As a result of the analyses, all samples were negative for *Cryptosporidium* spp., while *Giardia duodenalis* was positive in 2% (2/100) of two female dogs less than one-year-old. Sequence analyses of PCR-positive samples showed that the samples overlapped with assemblage C and D samples. Although these results show that shelter dogs in Batman province do not carry a risk for humans in terms of *Cryptosporidium* spp. and *Giardia duodenalis*, it is recommended that repeated faecal examinations should be carried out as much as possible to determine the possible role of these parasites in human transmission.

Key words: assemblage, Batman (Turkey), *Cryptosporidium* spp., *Giardia duodenalis,* molecular analysis

INTRODUCTION

Cryptosporidium spp. and *Giardia duodenalis* are opportunistic zoonotic protozoan parasites associated with diarrhoea in humans and many mammals (Huber *et al.*, 2005; Uehlinger *et al.*, 2013; Tangtrongsup *et al.*, 2020).

Giardia duodenalis has eight different assemblages (A-H) according to its genetic characteristics (Dado *et al.*, 2012; Adell-Aledón *et al.*, 2018; Kim *et al.*, 2019; Tangtrongsup *et al.*, 2020). Of these; Assemblages A and B are seen in many mammals but are mainly associated with human infections (Zhang *et al.*, 2012; Salant *et al.*, 2020). Assemblages C and D are seen in dogs (Adell-Aledón *et al.*, 2018; Salant *et al.*, 2020), assemblage E in ruminants (Dado *et al.*, 2012; Kim *et al.*, 2019), assemblage F in cats (Adell-Aledón *et al.*, 2018; Tangtrongsup *et al.*, 2020), assemblage G in mice, rats (Kim *et al.*, 2019; Salant *et al.*, 2020), and assemblage H in cetaceans (Zhang *et al.*, 2012; Kim *et al.*, 2019).

Today, many *Cryptosporidium* species have been identified and most of these species have host adaptation (Jian *et al.*, 2014; Tangtrongsup *et al.*, 2017; Alves *et al.*, 2018; Gharieb *et al.*, 2018). Although *C. canis* is the most common species in dogs, *C. muris*, *C. meleagridis,* and *C. parvum* have also been detected (Jian *et al.*, 2014; Tangtrongsup *et al.*, 2017). Among these, *C. parvum* is recognised as a zoonotic species infecting a wide range of mammals (Gharieb *et al.*, 2018; Ranjbar *et al.*, 2018). Contact with animals has been identified as an important route of transmission in human cryptosporidiosis epidemiology (Li *et al.*, 2021). This study aimed to investigate the prevalence of *Cryptosporidium* spp. and *Giardia duodenalis* in shelter dogs in Batman province by microscopic and molecular methods and to determine their genotypes.

MATERIALS AND METHODS

Ethical approval

This study was approved by Siirt University Animal Experiments Local Ethics Committee with numbers 2022/01/03 and 2022/01/04.

Study area and animal material

This study was carried out on a total of 100 dogs of different breeds and sexes in Batman Municipality Animal Treatment Care and Rehabilitation Centre.

Sample collection and examination

Fresh faecal samples taken with disposable gloves were placed in individual sample containers and the age and sex of the animals were recorded. The samples were then brought to the laboratory for analysis. All samples were examined under a microscope by Kinyoun Acid Fast staining for *Cryptosporidium* spp. and by the native-Lugol method for *Giardia*.

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 further analysis.

Nested PCR reaction

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 step, primers 5'- GGAAGGGTTGTATTTAT TTATTAGATAAAG-3' and 5'-AAG GAGTAAGGAACAACCTCCA-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'- AAGCCCGACGAC GACCTCACCCGCAGTGC-3' forward and G759R 5'- GAGGCCGCCCTGG ATCTTCGAGACGAC-3' reverse). Nested PCR was then performed using the primers described by Lalle *et al.* (2005) (BG1F 5'- GAACGAGATCGAGGT CCG-3' forward and BG2R 5'-CTC GACGAGTTCGTGTGTT-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

Two *Giardia*-positive PCR samples were sequenced forward and reverse. The DNA sequences obtained were controlled, aligned, and analysed in BioEdit software. The edited formats of the DNA sequences were compared with the datasets using NCBI Basic Local Alignment Search Tool to determine the assemblages. Datasets were aligned in the BioEdit program and the model test was performed using the Maximum Likelihood statistical method in the IQTREE program and the phylogenetic tree was constructed with 1000 bootstrap according to BIC optimal model.

RESULTS

Microscopic examination and nested PCR analyses revealed that all samples were negative for *Cryptosporidium* spp. As a result of analyses for *Giardia duodenalis*, 2% (2/100) positivity was detected in two female dogs less than one-year-old. When the DNA sequences of the β-giardin gene obtained in the study were compared with the database in the NCBI Basic Local Alignment Search Tool, it was observed that the samples overlapped with assemblage C and D samples (Table 1). The placement of the samples is shown in the phylogenetic tree (Fig. 1).

DISCUSSION

Dogs in many households around the world are hosts of some zoonotic parasitic agents, including *Cryptosporidium* and *Giardia* species (Robertson *et al.*, 2000; Adell-Aledón *et al.*, 2018).

In the studies conducted to determine the prevalence of cryptosporidiosis in the world; 3.3% in Italy (Giangaspero *et al.*, 2006), 3.8% in China (Jian *et al.*, 2014), 4.1% in Spain (Gil *et al.*, 2017), 31.2% in Thailand (Tangtrongsup *et al.*, 2020), and 43.9% in Turkey (Ayan *et al.*, 2020) were reported. In this study, all samples were negative for *Cryptosporidium* spp. as a

Table 1. Comparison of results of the study samples generated using the NCBI Basic Local Alignment Search Tool

Samples	Access codes of the most similar sample	Assemblage	Similarity ratio
4	MK982549, MK982252, LC437463	D	99.59%
17	MK968844, MN270295, MN270296	C	99.57%

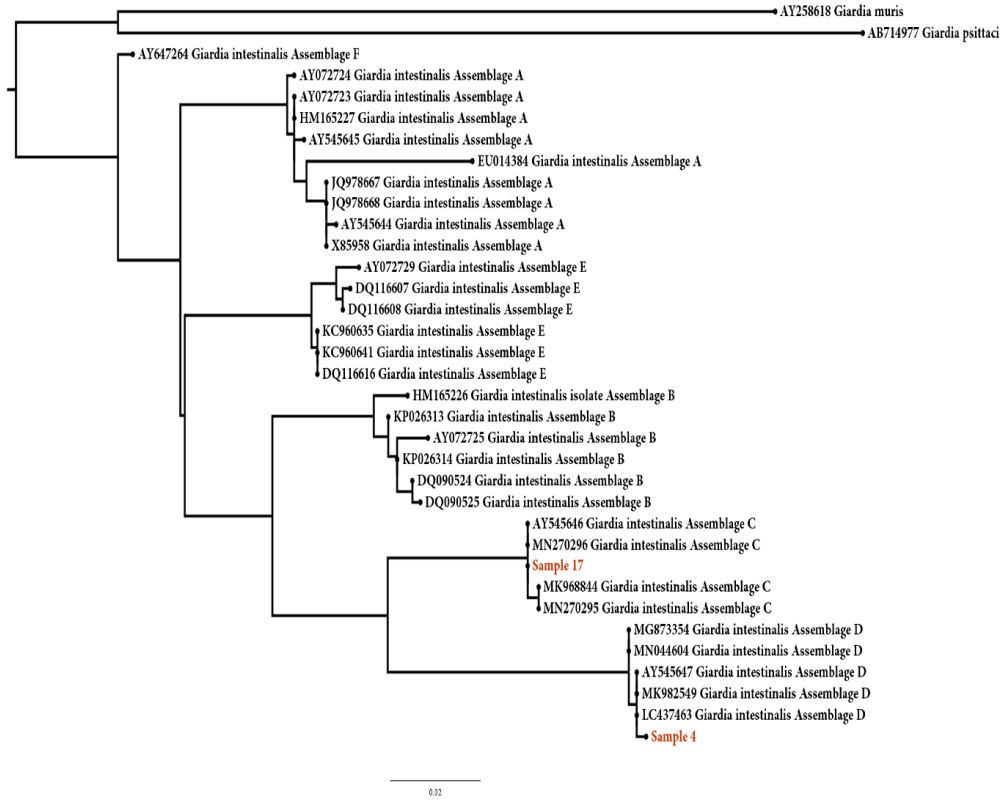

Fig. 1. Phylogenetic relationships of *Giardia duodenalis* isolates, using Maximum Likelihood method analysis based on *β-giardin* gene region. Numbers at the nodes represent the Bootstrap values (1000 bootstrap). *Giardia psitacci* and *Giardia muris* were used as an outgroup.

result of both microscopic and nested PCR analyses, which may be because only one faecal examination was performed on each dog.

In the studies conducted to determine the prevalence of *Giardia* in the world; prevalence rates of 1.9% in Poland (Solarczyk & Majewska, 2010), 20.5% in Italy (Scaramozzino *et al.*, 2009), 75.55% in Iraq (Naser & Wadood, 2017), 31.33% in Brazil (Huber *et al.*, 2005), and 2.48% –18.8% in Turkey (Gultekin *et al.*, 2017; Uslu *et al.*, 2022) were reported. In this study, a prevalence of 2% was determined as a result of microscopic and nested PCR analyses. These results are similar to the findings of Solarczyk & Majewska (2010) and Uslu *et al.* (2022).

Studies have reported that especially host-specific assemblages C and D are seen in dogs (Solarczyk & Majewska, 2010; Gultekin *et al.*, 2017). In addition, assemblages A, B, and E have also been reported (Leonhard *et al.*, 2007; Dado *et al.*, 2012; Uehlinger *et al.*, 2013; Li *et al.*, 2015; Adell-Aledón *et al.*, 2018). As a result of this study, it was determined that the two positive samples were host-specific assemblages C and D. This result is similar to the findings of Solarczyk & Majewska (2010) and Gultekin *et al.* (2017).

Studies by Li *et al.* (2015), Naser & Wadood (2017), and Adell-Aledón *et al.* (2018) reported a higher prevalence in females than males and studies by Huber *et al.* (2005), Naser & Wadood (2017) and Tangtrongsup *et al.* (2020) reported a higher prevalence in dogs younger than one year of age than in dogs older than one year of age. The fact that the two positive samples obtained in this study were detected in two female dogs under one year of age supports the researchers.

CONCLUSION

As a result of this study, *Cryptosporidium* spp. was not found in shelter dogs in Batman province, but the presence of non-zoonotic *Giardia duodenalis* assemblages C and D was detected in two dogs. Although these results show that shelter dogs in Batman province do not carry a risk for humans in terms of *Cryptosporidium* spp. and *Giardia duodenalis*, it is recommended that repeated faecal examinations should be carried out as much as possible to determine the possible role of these parasites in human transmission.

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