CRYPTOSPORIDIUM INFECTION OF STRAY CATS IN MARDIN PROVINCE, SOUTHEASTERN ANATOLIA REGION, TURKEY

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ABSTRACT

Cryptosporidium spp. are one of the most common intestinal protozoan parasites in cats. This parasite can infect a wide range of hosts including humans and domestic animals. This aim of the study was to investigate the prevalence of Cryptosporidium spp. in stray cats of Mardin province, located in the Southeastern Anatolia region of Turkey, using microscopic and molecular methods. The result of the microscopic examination and Nested PCR, 2.5% (1/40) were positive. Sequence analysis revealed that the positive sample showed 100% similarity with Cryptosporidium felis. The result of this study indicated that Cryptosporidium spp. was detected for the first time in cats in Mardin province. Further studies are needfull to investigate in details the epidemiological status of cryptosporidiosis in domestic and stray cat populations in Mardin province of Turkey.

Keywords: *Cryptosporidium felis*, Microscopy and PCR diagnosis, Cat, Mardin, Turkey

INTRODUCTION

Some protozoan infections, hosted by cats and dogs, may cause heavy economic losses by targeting ruminants, while others may pose a risk to humans due to their zoonotic properties (Patton and Constable, 2022). *Cryptosporidium* spp. are one of the most common intestinal protozoan parasites in cats (Ito *et al*., 2017), and are capable of infecting a wide range of hosts, including humans and domestic animals (Fayer *et al*., 2000; Pavlasek and Ryan, 2007; Rambozzi *et al*., 2007; Samie *et al*., 2013; Meng *et al*., 2021; Köseoğlu *et al*., 2022).

Currently, there are 38 accepted *Cryptosporidium* spp. based on biological and molecular characterization (Pavlasek and Ryan, 2007; Yang *et al*., 2015; Sürsal *et al*., 2020). Although three *Cryptosporidium* spp. (*C. parvum, C. felis* and *C. muris*) have been reported in cats, only *C. felis* and *C. muris* were detected in naturally infected cats (Korkmaz *et al*., 2016; Patton and Constable, 2022). Although *C. hominis* and *C. parvum* are responsible for most human infections (Pavlasek and Ryan, 2007; Samie *et al*., 2013; Yang *et al*., 2015; Meng *et al*., 2021), *C. felis* is another important zoonotic pathogen causing disease in humans (Samie *et al*., 2013; Köseoğlu *et al*.,

2022). *C. felis* is the main *Cryptosporidium* species adapted to the feline host (Köseoğlu *et al*., 2022).

Transmission of infective oocysts occurs through direct contact with infected animals and humans or consumption of contaminated water and food (Sargent *et al*., 1998; de Oliveira Lemos *et al*., 2012; Yang *et al*., 2015; Köseoğlu *et al*., 2022). In addition, waterborne cryptosporidiosis has become a public health problem worldwide (Meng *et al*., 2021).

The infection can be self-limiting and even asymptomatic in humans and immunocompetent animals but can cause life-threatening severe diarrhea, abdominal pain, malabsorption, and weight loss in immunocompromised individuals (Sargent *et al*., 1998; Rambozzi *et al*., 2007; de Oliveira Lemos *et al*., 2012; Samie *et al*., 2013; Sürsal *et al*., 2020; Meng *et al*., 2021).

Microscopic staining methods (Ziehl-Neelsen, Kinyoun and Giemsa) (Orunç Kılınç *et al*., 2018; Sürsal *et al*., 2020; Köseoğlu *et al*., 2022), serological methods (IFAT, ELISA), and molecular methods (PCR) are used in the diagnosis of cryptosporidiosis (Orunç Kılınç *et al*., 2018; Sürsal *et al*., 2020; Köseoğlu *et al*., 2022; Patton and Constable, 2022). Molecular diagnostic methods are nowadays widely used in the specific diagnosis of cryptosporidiosis, identifying species, subspecies, or strains (Şimşek *et al*., 2012), and are considered to be more effective than staining methods (Orunç Kılınç *et al*., 2018).

Although there are studies determining the prevalence of *Cryptosporidium* spp. in cats from Turkey, studies investigating the subtyping of *Cryptosporidium* spp. are very limited. This study aimed to investigate the prevalence and subtype distributionof *Cryptosporidium* spp. in stray cats in Mardin province, Turkey.

MATERIALS AND METHODS

The Study Area: This study was conducted in Mardin province located in the Southeastern Anatolia Region of Turkey (37^0 17′ 52" N, 40^0 45′ 36" E) (Google Earth, 2023).

Animal and Sample Collection: The animal used for the study consisted of 40 cats from the Mardin Metropolitan Municipality Animal Rehabilitation Center between August – December 2022. Fresh fecal samples from the cats were placed in individual sample containers. All samples were brought to the laboratory following the cold chain rules

Microscopic Examination: All specimens brought to the laboratory were stained with Kinyoun's acid-fast and examined under a microscope (Leica, Switzerland) at x100 magnification. The fecal sediment thin smear was prepared and dried by air. Absolute methanol was used to fix the smear for one minute. The slide was then saturated for five minutes with Kinyoun's carbol fuchsin stain. The slide was washed with 50% ethanol for 3 – 5 seconds after staining, then with distilled water. Stained smear was counterstained for 1 minute with 1% methylene blue after being decoloured for 2 minutes with 1% sulphuric acid (Rekha *et al*., 2016).

Scanning Electron Micrography: The 0.5 g of fecal sample was diluted in 10 ml 1:5 ethanol/water solution and dropped onto a glass slide and then allowed to dry at room temperature.The dried samples were then coated with an Au sputter coater for 60 seconds to form a conductive layer on the surface. The coated sample was then placed on the sample holder and taken into the device for scanning electron microscope imaging (Ma *et al.,* 2021) The images were detected by the scattered electron detector (Sigma 300 Zeiss, Germany).

DNA Extraction: DNA extraction from fecal samples was performed using a commercial kit (GeneMATRIX STOOL DNA Purification Kit, EURx, Gdańsk, Poland). The extracted DNA was stored at -20°C for further use.

PCR Amplification: The method described by Xiao *et al.* (2001) was used to amplify the SSU rRNA gene region. In the PCR step, primers 5'-TTCTAGAGCTAATACATGCG-3' and 5'-CCCATTTC CTTCCTTCGAAACAGGA-3' were used to amplify

the 1325 bp gene region. In the nested PCR step, primers 5'-GGAAGGGTTGTATTTATTTA TTAGATAAAG-3' and 5'-AAGGAGTAAGGAACA ACCTCCA-3' were used to amplify the 826-864 bp gene region. PCR products were then run on agarose gel and images were obtained on a gel imaging device (Syngene Bioimaging System).

Sequence Analysis and Phylogeny: Pairwise sequence analysis of the positive PCR sample was performed by a commercial company (BM Labosis, Ankara, Turkey). The DNA sequences obtained were aligned using the BioEdit program and were prepared for analysis. The edited formats of the DNA sequences were compared with the data sets using the NCBI Basic Local Alignment Search Tool to identify assemblages.

Ethical Approval: Ethical clearance for the present study was obtained from the Siirt University Animal Experiments Local Ethics Committee with decision number of 2022/03/13.

RESULTS AND DISCUSSION

Microscopic examination revealed *Cryptosporidium* spp. oocysts in one sample (2.5%). Furthermore, nested PCR, specific 826-864 bp bands specific for *Cryptosporidium* spp. were obtained in one sample (2.5%). Scanning electron micrographs (SEMs) of the positive *Cryptosporidium* spp. oocytes were obtained (Figure 1). When the DNA sequences of the SSU rRNA gene of the sequenced sample were compared with the database in NCBI Basic Local Alignment Search Tool, it was determined that there was 100% similarity with *C. felis*. To create a phylogenetic tree, the data sets were aligned in the BioEdit program and a phylogenetic tree was created with 1000 bootstrap according to the model test and the model determined in the IQTREE web server program (Figure 2).

Cats and dogs are important companions in many households around the world, contributing to the physical, social and emotional development of children and the well-being of their owners (Robertson *et al.*, 2000). However, these animals are hosted to intestinal parasites that can cause infection in humans (McGlade *et al.*, 2003; Shukla *et al.*, 2006; Köseoğlu *et al.*, 2022).

Although zoonotic parasites can cause significant morbidity in all groups of the human population, they are of particular concern in vulnerable groups such as children, the elderly, and immunocompromised people (Robertson *et al.*, 2000; Shukla *et al.*, 2006).

The *Cryptosporidium* species in cats was first described in 1979 and genetically characterized in 1998 (Sargent *et al.*, 1998; Pavlasek and Ryan, 2007; Rambozzi *et al.*, 2007). Since then, several studies on cryptosporidiosis in cats have been conducted worldwide.

In studies conducted on felin cryptosporidiosis around the world, the prevalence was reported 8.2% in Scotland (Mtambo *et al.*, 1991), 5.9% in the Czech Republic (Svobodova *et al.*, 1995), 1.2%-10 in Australia (Sargent *et al.*, 1998; McGlade *et al.*, 2003; Yang *et al.*, 2015), 13% in Colombia (Santín *et al.*, 2006), 7.3% in Niger (Shukla *et al.*, 2006), 24.5% in Italy (Rambozzi *et al.*, 2007), 2.5% in Thailand (Koompapong *et al.*, 2014), 2 - 12.7% in Japan (Yoshiuchi *et al.*, 2010; Ito *et al.*, 2017), 8.33% in Brazil (de Oliveira Lemos *et al.*, 2012), 5.26% in Germany (Sotiriadou *et al.*, 2013), and 3.84% in China (Li *et al.*, 2015).

Turkey is a country where protozoans that affect cats and dogs can easily continue their life cycle in terms of its climate and geographical features (Ceylan *et al.*, 2021). Studies on the detection of *Cryptosporidium* spp. in cats in Turkey are vast, with a prevalence of 1.7 – 10.44% (Korkmaz *et al.*, 2016; Orunç Kılınç *et al.*, 2018; Karakavuk *et al.*, 2021; Karakuş and Denizhan, 2021; Köseoğlu *et al.*, 2022) reported.

In this study, microscopic and Nested-PCR methods revealed a prevalence of 2.5%. This prevalence was higher than in previous studies (Sargent *et al.*, 1998; Ito *et al.*, 2017; Karakavuk *et al.*, 2021), similar to some studies (Koompapong *et al.*, 2014; Li *et al.*, 2015; Korkmaz *et al.*, 2016; Köseoğlu *et al.*, 2022), but lower than other studies (Mtambo *et al.*, 1991; Svobodova *et al.*, 1995; McGlade *et al.*, 2003; Santín *et al.*, 2006; Shukla *et al.*, 2006; Rambozzi *et al.*, 2007).

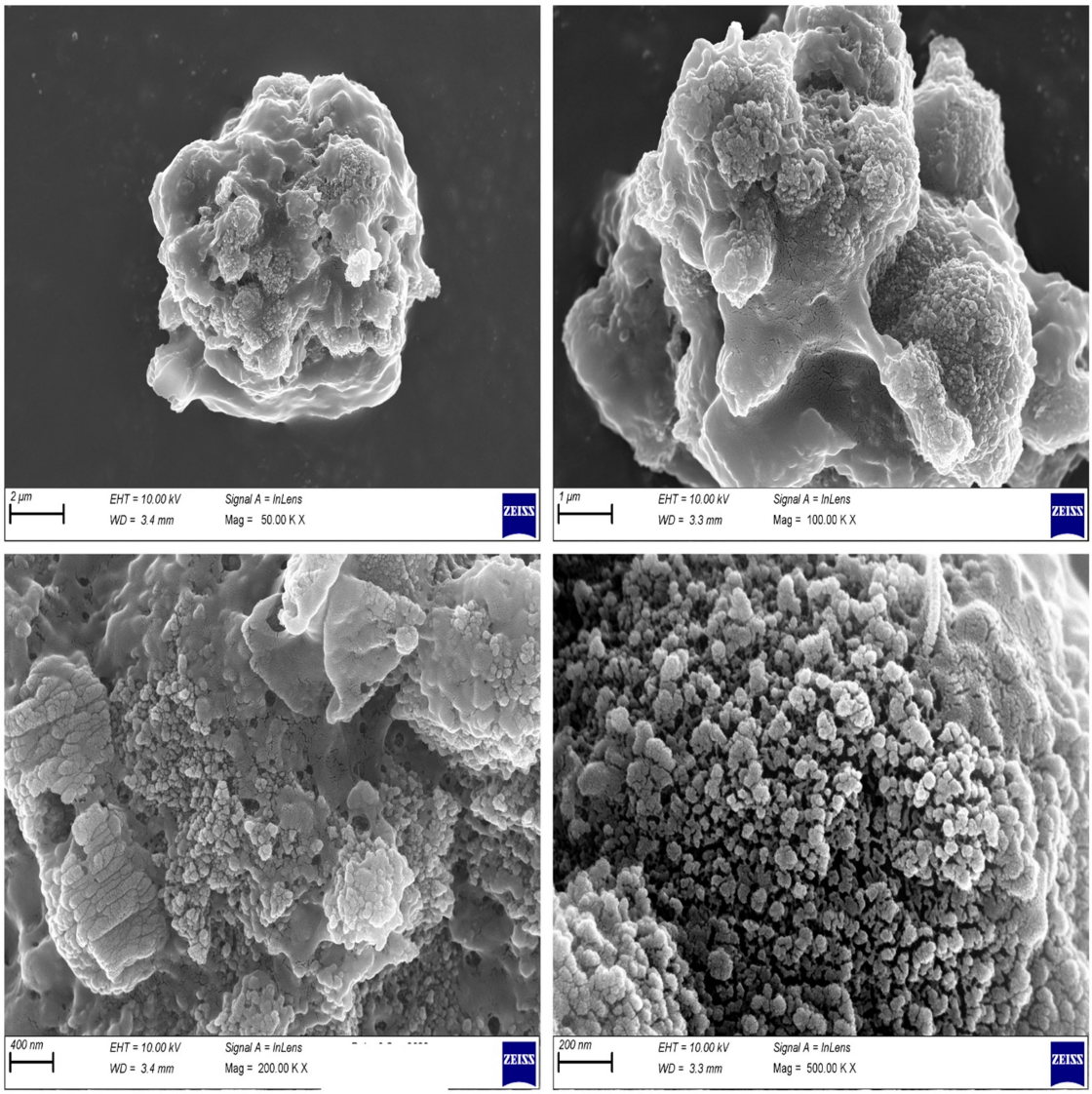

Figure 1: Scanning electron micrographs (SEMs) of *Cryptosporidium* spp. oocyst

Figure 2: Phylogenetic relationships of *Cryptosporidium* spp. isolates, using Maximum Likelihood Method analysis based on SSU rRNA gene region. Numbers at the nodes represent the Bootstrap values (1000 replicates). *Eimeria tenella* and *Toxoplasma gondii* were used as an out-group

The reasons for the differences between the studies include geographical conditions, sample size, and diagnostic methods used.

It has been reported that *C. felis* (Santín *et al.*, 2006; Yoshiuchi *et al.*, 2010; Koompapong *et al.*, 2014; Li *et al.*, 2015; Yang *et al.*, 2015; Ito *et al.*, 2017; Sürsal *et al.*, 2020; Tangtrongsup *et al.*, 2020; Köseoğlu *et al.*, 2022), *C. muris* (Santín *et al.*, 2006; Pavlasek and Ryan, 2007; Yang *et al.*, 2015), *C. ryanae* (Yang *et al.*, 2015), and *C. parvum* (Sotiriadou *et al.*, 2013; Li *et al.*, 2015; Tangtrongsup *et al.*, 2020) were detected in studies conducted on cats. In this study, similar to the findings of the researchers (Santín *et al.*, 2006; Yoshiuchi *et al.*, 2010; Koompapong *et al.*, 2014; Li *et al.*, 2015; Yang *et al.*, 2015; Ito *et al.*, 2017; Sürsal *et al.*, 2020; Tangtrongsup *et al.*, 2020; Köseoğlu *et al.*, 2022), *C. felis*, which has zoonotic potential, was detected.

Conclusion: Cats, especially stray cats, are hosts to various parasites, some of which are zoonotic, without showing clinical signs. One of these parasites is cryptosporidium species. In this study, *C. felis* was detected in stray cats in

Mardin province. Although *C. felis*, a feline-specific pathogenic species, carries relatively less zoonotic risk for public health compared to *C. parvum*, the potential role of stray cats in the transmission of *Cryptosporidium* spp. should not be ignored considering the close relationship between humans and cats. It is recommended that cats should be routinely examined for parasites and cats found to have parasites should be treated immediately further studies are needed to investigate the general epidemiologic status of cryptosporidiosis in domestic and stray cat populations.

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