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Research Article

Prevalence of Gastrointestinal Parasites in Stray Cats of İzmir

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ABSTRACT

Gastrointestinal parasites of cats can affect animal health and welfare, as well as human health because of some zoonotic parasites including *Toxoplasma gondii*, *Cryptosporidium* spp., *Isospora* spp., *Blastocystis* sp., and *Toxocara* spp. Therefore, it is fairly important to investigate the presence of gastrointestinal parasites in stray cats in order to reveal the frequency of parasite diseases and to prevent the spread of parasitic diseases. A total of 465 feces samples were collected from Veterinary Clinics located in 5 different districts of İzmir. For microscopic examination, all feces samples were processed by centrifugation-sucrose flotation. In addition, cat feces with diarrhea were stained by the by Kinyoun acid-fast staining for the diagnosis of *Cryptosporidium* spp. As a result, 73 of 465 (15.6%) cats were found to be infected with at least one of the following parasites: *Blastocystis* sp., *Isospora* spp., *Cryptosporidium* spp., *Toxoplasma gondii*-like oocyte, *Toxocara* spp., *Hymenolepis* spp. and *Dipylidium caninum*. Among the studied stray cats, *Blastocystis* sp. was detected as the most prevalent protozoon parasite (10.5%) in stray cats. Overall, the results show that stray cats are a significant source for distribution of various parasite diseases to humans and animals in İzmir, Turkey.

Keywords: Blastocystis sp., Cryptosporidium spp., İzmir, stray cats, Toxocara spp.

İzmir İlinde Sokak Kedilerinde Gastrointestinal Parazitlerin Prevalansı

ÖZET

Kedilerde gastrointestinal parazitler hayvan sağlığını ve refahını etkilemesinin yanında *Toxoplasma gondii, Cryptosporidium spp., Isospora spp., Blastocystis sp., Toxocara spp*. gibi bazı zoonozlar nedeniyle insan sağlığını da etkilemektedir. Bu nedenle, sokak kedilerinde gastrointestinal parazitlerin varlığının araştırılması hastalıkların sıklığını ortaya çıkarmak ve yayılmasını önlemek için oldukça önemlidir. Bu çalışmada İzmir ilindeki sokak kedilerinde gastrointestinal parazitlerin sıklığının dışkı örneklerinin direkt mikroskobik incelenmesi ile belirlenmesi amaçlanmıştır. İzmir'in 5 farklı ilçesinde bulunan veteriner kliniklerine getirilen toplam 465 dışkı örneği toplanmıştır. Mikroskobik inceleme için, tüm dışkı örneklerine santrifüj-sükroz yüzdürme metodu uygulanmıştır. Ayrıca ishalli dışkı örnekleri *Cryptosporidium spp.* varlığının araştırılması amacıyla Kinyoun asit-fast boyama ile boyanmıştır. Sonuç olarak 465 kediden 73'ünün (%15,6*) Blastocystis sp., Isospora spp., Cryptosporidium spp., Toxoplasma* benzeri ookist*, Toxocara spp., Hymenolepis* spp. ve *Dipylidium caninum* türlerinden en az biri ile enfekte olduğu tespit edilmiştir. Sokak kedileri arasında *Blastocystis* sp. en yaygın protozoon paraziti (%10,5) olarak tespit edilmiştir. Sonuç olarak, elde edilen bulgular Türkiye'de İzmir ilindeki sokak kedilerinin çeşitli paraziter hastalıkların insan ve hayvanlara yayılması için önemli bir kaynak olabileceğini göstermektedir.

Anahtar Kelimeler: Blastocystis sp., Cryptosporidium spp., İzmir, sokak kedileri, Toxocara spp.

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Introduction

Humans initiated the cat's domestication process to conserve the grain stores from rodent when they began to cultivate crops and store grain (Clutton-Brock, 1999). Later, because of close contact with domestic cats that has been continued since long years, the relationship known as "human-animal bond" strengthen more and additional benefits such as companionship, socialization, mental health and physical well-being which contribute to development of positive mood in human have been noticed (Deplazes et al., 2011). These positive effects have caused that numerous humans or family living in developing and developed countries have or adopt a domestic cat. For example, it is stated that 34% of families located in United State have domestic cat and nearly half of cat owners consider cats to be family members (Dabritz and Conrad, 2010; Goldstein and Abrahamian, 2015). In addition, many adults or children contact with stray cat in order to feed, play or be friend with them. During this contact, some parasites known as zoonoses can be transmitted from cats to humans since these stray cats are not subjected to a regular anti-parasitic treatment or a routine vaccination program. At the same time, low socio-economic factors and education level, and poor hygiene practices are other important factors which facilitate the transmission of parasites from cats to humans.

Stray animals like cats or dogs are widespread in İzmir which is the third big city of Turkey in respect to population. According to records of Izmir Chamber of Veterinarians, there may be more than 500 thousand stray dogs and stray cats living on the streets of İzmir. These stray animals do not have very well living conditions because of inadequate medical care, even they reach to food which is left by animal lovers. Therefore, insufficient medical care offers negative influences for animal health as well as for human health because of zoonotic diseases. For instance, several types of endo-parasites, including protozoa, cestodes, trematodes and nematodes can infect stray cats and as a result of this, some clinical symptoms like diarrhea, vomiting, anemia, poor growth rate and rarely death can be monitored (Symeonidou et al., 2018). Furthermore, zoonotic diseases such as cryptosporidiosis, toxoplasmosis, giardiasis, blastocystosis, leishmaniasis, toxocariasis, opisthorchiasis, dipylidiasis, and echinococcosis are significant parasitic diseases that also infect humans (Baneth et al., 2016; Goldstein and Abrahamian, 2015).

The frequency of parasites in stray cats living in Turkey is not well known due to lack of sufficient number of studies performed in big cat groups. According to results of available studies, *T. gondii*, *Leishmania infantum* and *L. tropica*, *Toxocara* spp., *Ancylostoma spp*., *Joyeuxiella pasqualei*, *Hydatigera taeniaformis*, *Mesocestoides* spp., *Dipylidium caninum*, *Isospora* spp. and *Cryptosporidium* spp. have been reported in stray cats (Yaman et al., 2006; Can et al., 2014; Öge et al., 2014; Paşa et al., 2015; Can et al., 2016; Korkmaz et al., 2016).

In this study, we aimed to reveal the frequency of gastrointestinal parasites in 465 stray cats living in İzmir province. For this purpose, the presence of gastrointestinal parasites in feces samples collected from stray cats was investigated in concentrated samples by direct microscopy. Moreover, Kinyoun Acid–fast staining method was used for the diagnosis of *Cryptosporidium* spp. This study not only detected the frequency of gastrointestinal parasites but also revealed reservoir potential of stray cats in terms of zoonotic agents harbored.

Material and Methods

Study area and cat population

Between April and October in 2017, a total of 465 feces samples were collected from Veterinary Clinics located in 5 different districts (Çiğli, Karabağlar, Karşıyaka, Konak and Narlıdere) of İzmir. Among the 465 feces samples, 74 was from Çiğli, 25 from Karabağlar, 38 from Karşıyaka, 183 from Konak and 145 from Narlıdere. Of the 465 stray cats, 299 (64.35%) were female and the remaining 166 (35.65%) were male. The protocol for collecting feces from stray cats was declared as exempt according to the instructions and approval of the Institutional Animal Care and Use Committee (IACUC) of Ege University for animal ethical norms (Number: 109/2970).

Microscopic examination

Each feces sample was macroscopically examined to detect the possible presence of cestode proglottids and/or adult nematodes. For microscopic examination, all feces samples were processed by centrifugation-sucrose flotation method as described with minor modifications (Dubey et al., 1970). In brief, feces sample (~10 gr) was first transferred into the 50 ml tube filled with tap water and incubated for two hours at room temperature. Later, tap water was discarded, and 50 ml of sucrose solution (530 g sugar, 1-liter distilled water, 8 ml phenol) was added and emulsified. This mixture was then filtered with two layers of gauze and divided into 15 ml tubes and centrifuged at 400xg for 10 minutes. After centrifugation, approximately 0.5 ml supernatant from the top of each 15 ml tube was collected in another tube and the presence of parasites eggs or oocysts or cysts was investigated between slide-cover slide under light microscopy.

Kinyoun acid -fast staining

Cat feces with (n:50) diarrhea were stained by Kinyoun acid-fast staining for *Cryptosporidium* spp. diagnosis as previously described (Turgay et al., 2012). Briefly, a drop of concentrated feces sample was smeared on a slide, air dried and fixed by methanol. Later, alkaline fuchsin was poured on the slides and incubated for 5 min. Next, the slides were washed with tap water, decolorized by 2.5% sulfuric acid for 1 minute and the slides were stained with 1% methylene blue for 1 min. Finally, slides were washed, air dried and examined under light microscopy using x1000 magnification.

Statistical analysis

Data obtained from this study were processed using PASW Statistics 18. A chi-square test was used to determine the significance between the positivity rates detected in different counties of İzmir and between male and female cats.

Results

Out of 465 feces samples, 73 were found to be infected with at least one parasite (15.6%) (Figure 1). Totally, 61 cats were infected with protozoon and 16 cats were infected with helminth. Among protozoon species, *Blastocystis* sp., *Isospora* spp. and *Cryptosporidium* spp. were identified. Additionally, *Toxoplasma gondii* like oocysts were identified in two feces samples (0.43%). Of the helminths 14 of them were *Toxocara* spp., one of them was *Hymenolepis* spp. and the other was *Dipylidium caninum*. *Blastocystis* sp. was the most identified parasite (10.5%). Percentage positivity value for *Toxocara* spp., *Isospora* spp., *Cryptosporidium* spp., Hymenolepis *spp.* and *D. caninum* were 3.0, 1.0, 1.0, 0.21 and 0.21, respectively. Also, co-infections were found in four cats. Co-infection with *Blastocystis* sp.-*Isospora* spp., *Blastocystis* sp.-*Toxocara* spp., *Blastocystis* sp.-*Cryptosporidium* spp. and *Cryptosporidium*

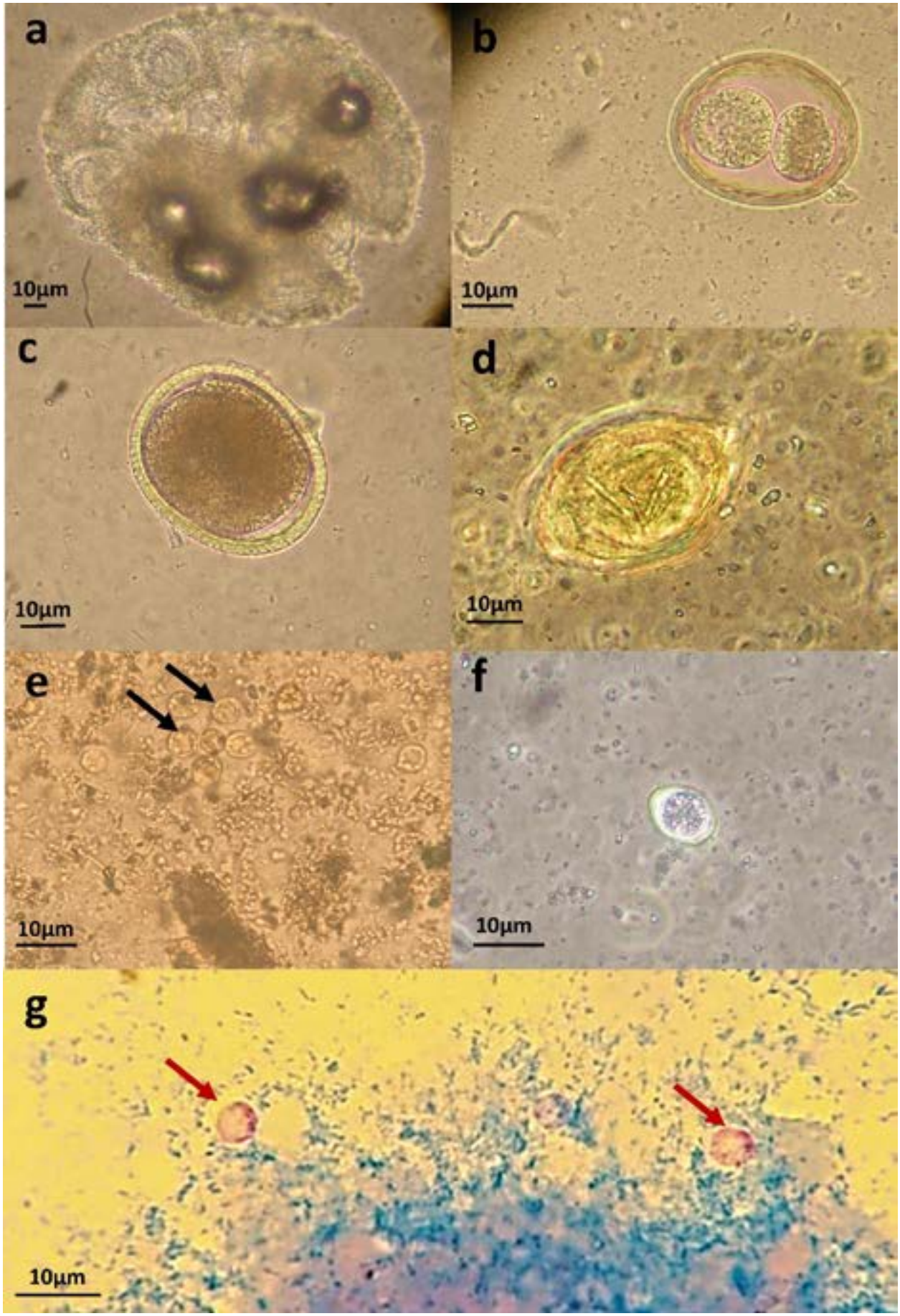

Figure 1. a) Egg packet of *Dipylidium caninum,* **b)** *Isospora spp.* oocyst*,* **c)** *Toxocara* spp. egg**, d)** *Hymenolepis* spp. egg*,* **e)** *Blastocystis* spp. *cysts*, **f)** *T. gondii*-like oocyst, unsporulated**, g)** *Cryptosporidium* spp. oocysts.

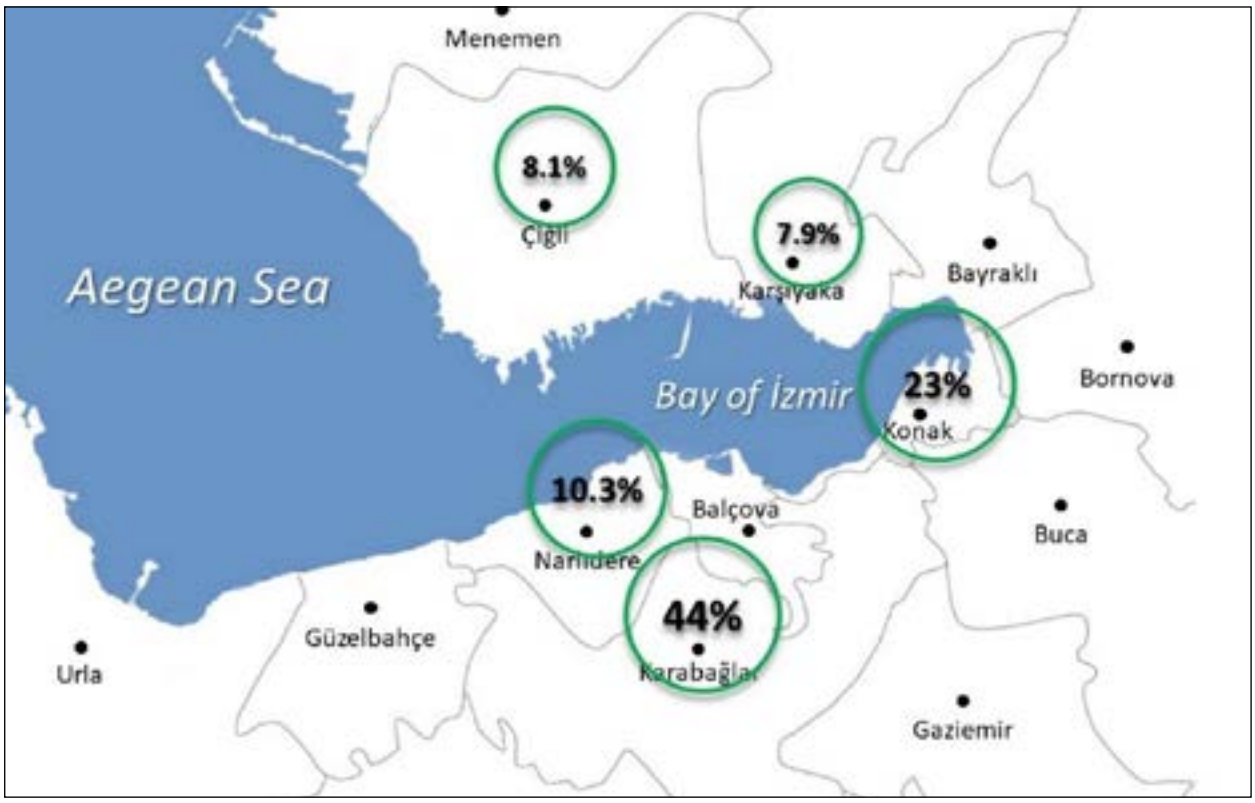

Figure 2. Percentage distribution of positive samples

spp.-*Toxocara* spp, were detected. Karabağlar and Konak had the highest percentage positivity values when compared to other districts (*P<0.05*) (Figure 2). Statistically significant difference was not found when the percentage positivity values were compared among female and male cats (female: 15.38%, male: 14.46%; *P>0.05*). The identified parasites species in each district of İzmir was given in detail in Table 1.

is required to be investigated in specified periods as well as positive animals should be treated with specific drugs.

In this study, we investigated the prevalence of gastrointestinal parasites in a big stray cat group living in İzmir which is third big city of Turkey and found that 15.6% of feces samples were infected with at least one parasite. In Turkey, a study conducted

Table Legends:

Table1. Numbers of the parasite species detected in five different districts of İzmir

			Districts		
Parasite Species	Çiğli	Karabağlar	Karşıyaka	Konak	Narlıdere
Blastocystis sp.	6	5	-	25	13
Toxocara spp.	-	1	1	10	2
Isospora spp.	-	1	-	4	-
Cryptosporidium spp.	-	4	1	-	-
Toxoplasma gondii-like oocysts	-	-	-	2	-
Hymenolepis spp.	-	-	-	1	-
D. caninum	-	-	1	-	-
Total	6	11	3	42	15

* In Narlıdere, co-infection with *Blastocystis* sp.-*Isospora* spp., was detected in one cat. In Konak, co-infection with *Blastocystis* sp.-*Toxocara* spp., *Blastocystis* sp.-*Cryptosporidium* spp. and *Cryptosporidium* spp.-*Toxocara* spp., were detected in three different cats.

Discussion

Considering the importance of one health approach, studies focusing the diagnosis of infectious agents in stray animals are crucial and a lot of studies are being performed in this field worldwide. Although there is numerous stray animal such as dog and cat in Turkey, these animals do not take sufficient medical care, and this threatens animal and human health. Therefore, the presence of infectious agents in these animals

in Kırıkkale province reported that 47% (n=100) of feces samples collected from cats were parasite positive using microscopy. *Isospora spp., Toxocara spp., Joyeuxiella spp., Cryptosporidium spp.,* and hookworm were detected in positive cats. (Korkmaz et al., 2016). In another study performed in Hatay, %87,5 (n=8) of dead cat necroscopy and feces samples was found to be positive for parasites using microscopy. *Toxocara cati, Joyeuxiella pasqualei, Hydatigera taeniaformis, Mesocestoides sp. and Dipylidium caninum* were found in study (Yaman et al.,

2006). It is known that positivity value can change depending on number of samples tested, methods used, geographical region and cat lifestyle.

In our study, *Blastocystis* sp. was detected as the most prevalent protozoon parasite (10.5%) in stray cats although it has not been reported previously in cats of Turkey. In a study from Australia, among the 52 feces samples collected from cats, 67.3% was reported to be *Blastocytis* sp. positive by microscopic examination (Duda et al., 1998). In a different study conducted in US Pacific Northwest, it was found that positivity of *Blastocystis* sp. was 11.65% in 105 feces samples belonging to shelter-resident felines using nested PCR targeting the SSU rDNA fragment. Also, same study reported that none of client-owned cats was positive for *Blastocystis* sp. (Ruaux and Stang, 2014). *Blastocystis* sp. is also accepted as the most common eukaryotic organism detected in gastrointestinal tract of humans (Lepczyńska et al., 2017) and molecular epidemiological investigations have showed that some sub-types of *Blastocystis* sp. play role in zoonotic transmission (Lee et al., 2012; Cian et al., 2017; Greige et al., 2018). Parallel to this, previously conducted studies reported that the frequency of *Blastocystis* sp. was prevalent in humans who live in İzmir province. Results obtained from these studies showed that prevalence of *Blastocystis* sp. was 4.96% in 2005 and increased to 32.33% in 2012 (Değirmenci et al., 2007; Turgay et al., 2012). All these findings indicate that stray cats can be a source of *Blastocystis* sp. transmission to humans in İzmir.

In our study, *Toxocara* spp. was the second prevalent parasite in stray cats of İzmir and was found in 3.0% of (14/465) feces samples. In Turkey, it has been stated that the prevalence of *T. cati* varied from 27.6 to 47.2% in cats (Burgu et al., 1985; Doğanay, 1992; Doğanay and Öge 1993; Öge et al., 2014). A study conducted in Ankara reported that *Toxocara* eggs were found in 13% of feces samples collected from owned cats (Öge et al., 2014). Another study found that the prevalence of *Toxocara* spp. was 48.9% in cats in Kırıkkale (Korkmaz et al., 2016). Worldwide, it has been reported that prevalence of *T. cati* was within range of 8% to 91% in cats (Overgaauw , 1997; Öge and Öge, 2000; Macpherson, 2005; Bowman, 2009; Öge et al., 2014). Although obtained results show different prevalence values, all of them indicate that cats are significant source for the transmission of *Toxocara* spp. to humans.

Cats are definitive host for some apicomplexan parasite such as *Toxoplasma gondii* which can infect all mammalian, including humans as well as birds and cause important clinical cases and economic losses. In this study, three different apicomplexan parasite species were detected in 12 feces samples. Among apicomplexan parasites, *Isospora* spp. and *Cryptosporidium* spp. were detected in five of feces samples tested while *T. gondii*-like oocysts were detected in only two feces samples. In Turkey, the presence of *Cryptosporidium* spp. and *Isospora* spp. has been reported in previously conducted studies on cats. Accordingly, a study showed that *Isospora* spp. and *Cryptosporidium* spp. positivity was found to be 65.9% and 2.12%, respectively (Korkmaz et al., 2016). In two different studies, *Isospora* spp. positivity was reported to be 2.8% and 43% in stray cats (Burgu et al., 1985; Doğanay, 1992). In a different study performed in Turkey, prevalence of *Cryptosporidium* spp. was found to be 13% in kittens (Goz et al., 2005). *T. gondii* oocyst positivity in cat feces samples is lower when compared to antibody positivity because of short term oocyst shedding period. According to this, many studies reported that *T. gondii* oocysts were not detected in seropositive cats (Miró et al., 2014; Dubey et al., 2007; Qian et al., 2012). In our study, *T. gondii* like oocysts positivity was found to be 0.43% in stray cats. For the diagnosis of *T. gondii* infection, further techniques like PCR are needed to be applied to these two positive samples because *T. gondii* oocysts cannot be differentiated from oocysts of *Hammondia hammondi* and *Besnoitia* spp. by microscopic techniques (Dabritz et al., 2007). This condition also demonstrates the importance of molecular tests used in diagnosis of *T. gondii* infection.

In this study, *Hymenolepis* spp. and *D. caninum* were detected in stray cats. Prevalence of these helminths was lower than others. In a study conducted in Elazığ, *D. caninum* positivity in cats was 33% while *Hymenolepis* spp. has not been detected (Altaş et al., 1999). In another study, *D. caninum* positivity in cats was 12.5% and *Hymenolepis* spp. has not been detected (Yaman et al., 2006). In a different study, both *Hymenolepis* spp. and *D. caninum* have not been detected in feces of cats (Korkmaz et al., 2016).

Conclusions

In conclusion, the accumulated data show that stray cats are a significant source for the distribution of various parasites in nature. Therefore, these stray cats contacting with humans should be routinely checked for parasites and cats with parasitic diseases should be treated immediately. By this way, the quality of life of cats can be improved and, transmission of zoonotic parasites to humans can be prevented.

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Conflict of Interest

The authors declare that they have no conflicts of interest.

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