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First Detection of *Giardia duodenalis* in Cats in Mardin Province

Burçak ASLAN ÇELİK [1, a]

[1] Siirt University,
Faculty of Veterinary Medicine,
Department of Parasitology,
Siirt, TÜRKİYE

[a] ORCİD: 0000-0002-0130-970X

Giardia duodenalis is one of the most common digestive protozoa in humans and animals worldwide. This study aimed to determine the prevalence of *Giardia duodenalis* in stray cats in Mardin province in the Southeastern Anatolia Region of Turkey by using microscopic and molecular methods. Fecal samples were taken from a total of 48 cats, 27 females, and 21 males. Analysis of the samples with native-Lugol and PCR methods revealed a prevalence of 8.33% and 16.67%, respectively (P<0.001). As a result of the study, a higher prevalence was found in females (18.52%) than in males (14.29%), in those under one year old (17.86%) than over one-year-old (15.00%), and in diarrheic cats (17.65%), than nondiarrheic cats (16.13) (P>0.05). As a result of this study, the first detection of *G.duodenalis* in Mardin cats was made. Further studies are needed to investigate the general epidemiological status of giardiasis in domestic and stray cat populations.

Key Words: *Giardia duodenalis, cats, PCR, Mardin, Türkiye*

Mardin İlindeki Kedilerde *Giardia duodenalis*'in İlk Tespiti

Giardia duodenalis, dünya çapında insanlarda ve hayvanlarda görülen en yaygın sindirim sistemi protozoonlarından biridir. Bu çalışmada Güneydoğu Anadolu Bölgesinde yer alan Mardin ili sokak kedilerinde *Giardia duodenalis* prevalansının mikroskobik ve moleküler olarak belirlenmesi amaçlandı. Çalışmada 27 dişi, 21 erkek olmak üzere toplam 48 kediden dışkı örnekleri alındı. Numunelerin nativ-Lugol ve PCR yöntemleriyle yapılan analizleri sonucunda sırasıyla %8.33 ve %16.67 prevalans tespit edildi (P<0.001). Çalışma sonucunda dişi kedilerde (%18.52), erkeklere göre (%14.29), bir yaş altı kedilerde (%17.86), bir yaş üstü kedilere göre(%15.00) ve ishalli kedilerde (%17.65) ishalli olmayanlara göre (%16.13) daha yüksek prevalans saptanmıştır (P>0.05). Bu çalışma sonucunda Mardin ili kedilerinde *G.duodenalisin* ilk tespiti yapıldı. Evcil ve sokak kedisi popülasyonlarında giardiasisin genel epidemiyolojik durumunu araştırmak için daha fazla çalışmaya ihtiyaç duyulmaktadır.

Anahtar Kelimeler: *Giardia duodenalis, kedi, PZR, Mardin, Türkiye*

Introduction

Giardia duodenalis is an enteric protozoan with flagella, found in the intestines of humans, domestic and wild animals (1-3). This parasite spreads worldwide and is generally recognized as a zoonotic agent (2, 4). *Giardia* spp. is reported to be one of the most common causes of diarrhea in humans and animals (2, 5). The genus *Giardia* includes many species that are often morphologically indistinguishable. Recognized species of this genus include *G. duodenalis*, *G. agilis*, *G. muris*, *G. microti*, *G. ardeae*, and *G. psittaci* (3) and the two recently described *G. peramelis* and *G. cricetidarum* (6). Only *G. duodenalis* species (syn. *G. intestinalis* or *G. lamblia*) have been identified in cats (2, 7).

There are two stages in the life cycle of *Giardia* species: trophozoite and cyst (infective stage) (3, 7, 8). Infection occurs by the fecal-oral route by ingestion of feces, fomites, or food contaminated with cysts (7, 9). The disease shows a diverse clinical course in humans, ranging from an asymptomatic state to an acute state. Diarrhea, vomiting, nausea, abdominal cramps, anorexia, and weight loss can be seen (10).

The disease may be asymptomatic in cats, or diarrhea may occur due to maldigestion, malabsorption, and increased motility (2, 8, 10, 11). Most infections in cats occur at an early age (2, 3). The chronic form of the disease is more common in immunocompromised cats and can continue for years (2, 11). Microscopic, serological and molecular methods are used in the diagnosis of the disease (1, 7, 9, 11). Since molecular techniques are more sensitive, they are more widely used in diagnosis (2, 7). In studies conducted around the world using the PCR method on cats, positivity rates of 3.9%, 6.1%, 8.18%, 9.2%, 13.1%, and 80% were reported in Poland, Italy, Japan, Spain, China, and Australia, respectively (2, 8, 12-15). In studies to figure out the prevalence of giardia on cats in Turkey, positivity has been reported between 4% and 50% (9, 16, 17).

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Correspondence
Yazışma Adresi

Burçak ASLAN ÇELİK
Siirt University,
Faculty of Veterinary Medicine,
Department of Parasitology
Siirt – TÜRKİYE

burcakaslan@siirt.edu.tr

The reports on the presence of *G. duodenalis* in cats in Turkey are quite limited. This study aimed to investigate the prevalence of *Giardia duodenalis* in stray cats in Mardin province of Turkey by microscopic and molecular methods.

Material and Methods

Research and Ethical Approval: Ethical approval for this study was obtained from the Siirt University Local Ethics Committee for Animal Experiments (Decision number: 20210542).

The Study Area and Animal Material: This study was carried out in Mardin province, located in the Southeastern Anatolia Region of Turkey (37^0 17' 52" N, 40^0 45' 36" E). The animal material of the study consisted of a total of 48 cats, 21 male, and 27 female, in the Mardin Metropolitan Municipality Stray Animal Rehabilitation Center. The cats have not been treated for giardiasis. Fresh fecal samples from cats were placed in individual specimen containers. Sex, age, and fecal type information were recorded. All samples were transferred to the parasitology laboratory of the veterinary faculty within the iceboxes.

Microscopic Examination: One drop of physiological saline solution and one drop of lugol solution were placed in different areas on the slide. One or two gr stool samples were taken by the plastic stick and mixed first with physiological saline and then with lugol solution. Covered with a coverslip, specimens were examined under a microscope (Leica, Hamburg, Germany) at x40 magnification.

Genomic DNA Extraction: Genomic DNA (gDNA) extraction from the samples was performed by using the GeneMATRIX Stool DNA Purification Kit (Gdansk, Poland) according to the manufacturer protocol and the obtained gDNAs were stored at -20°C until further analysis.

Nested-PCR Reaction: In the first PCR, a 753 bp β-giardin gene fragment was amplified by using the published primers (G7 F5'-AAGCCCGACGACCTCACCCGCAGTGC-3' and G759R 5'-GAGGCCCGCCCTGGATCTTCGAGACGAC-3') (18). The PCR mix was inculded 6 pmol of forward and reverse primers, 4 µl of 5x FIREPol® Master Mix (7.5 mM $MgCl_2$, Solis BioDyne, Estonia) 1.6 µl of gDNA and 13.2 µl Nuclease Free Water. The reaction consisted of pre-denaturation for 5 minutes at 95°C, followed by each cycle of denaturation (1 min at 95°C), annealing (1 min at 60°C), and extension (1 min 30 seconds at 72°C), 35 cycles and a final extension of 7 minutes at 72°C. Subsequently, using the following primers (BG1F 5'-GAACGAGATCGAGGTCCG-3' and BG2R 5'-CTCGACGAGTTCGTGTT-3') a nested-PCR reaction was performed. (19). For this aim, 6 pmol of forward and reverse primer in 20 µL of mastermix, 4 µl of 5x FIREPol® Master Mix (7.5 mM $MgCl_2$, Solis BioDyne, Estonia) 1 µl of PCR product and 13.8 µl Nuclease Free Water were used. The reaction was carried out followed by pre-denaturation at 95°C for 5 minutes, with each cycle consisting of denaturation (1 min at 95°C), annealing (1 min at 55°C) and extension (1 min at 72°C), 35 cycles and at 72°C with a final extension of 7 minutes. PCR products were stained with RedSafe™ Nucleic Acid Staining Solution and images were obtained on 1.5% agarose gel.

Statistical Analysis: The studied cats were divided based on sex (female, male), age (≤1, >1), fecal type (diarrheic and non-diarrheic), and technique (Microscopy, PCR) into two groups. Age was estimated by the dental formulary. The data obtained in the study were analyzed using the SPSS V16.0 (IBM, Chicago, IL, USA) program. The chi-square test was used to compare differences in infection rates among the investigated groups. The differences were considered significant at P≤ 0.05 (9).

Results

The rates of *G. duodenalis* infection in cats according to sex, age, fecal type, and the technique used are shown in Table 1. As a result of the study, a higher prevalence was found in female cats (18.52%) than males (14.29%), in under one year old (17.86%) than over one-year-old (15.00%), and in diarrheic cats (17.65%), than nondiarrheic cats (16.13) (P>0.05). When the methods used in diagnosis are evaluated; while positivity was detected in 4 (8.33%) samples in microscopic examination (Figure 1), specific bands of 511 bp were obtained in 8 (16.67%) samples by PCR analysis (Figure 2). The difference between the two methods was found to be statistically significant (P<0.001).

Table 1. Infection rates of *G. duodenalis* in cats according to sex, age, fecal type, and used technique

Variable	Number of cats (n)	Positive		P
		(n)	(%)	
Sex				
Female	27	5	18.52	0.696
Male	21	3	14.29	
Age (year)				
≤ 1	28	5	17.86	0.793
>1	20	3	15.00	
Fecal Type				
Diarrheic	17	3	17.65	0.893
Non-diarrheic	31	5	16.13	
Technique				
Microscopy	48	4	8.33	0.000
PCR	48	8	16.67	

^{NS}: Non-significant, ***: P<0.00

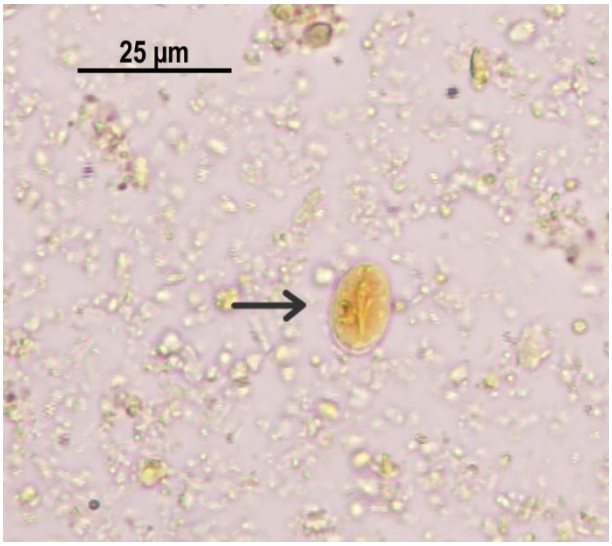

Figure 1. *G. duodenalis* cyst in stool samples, x40 magnification

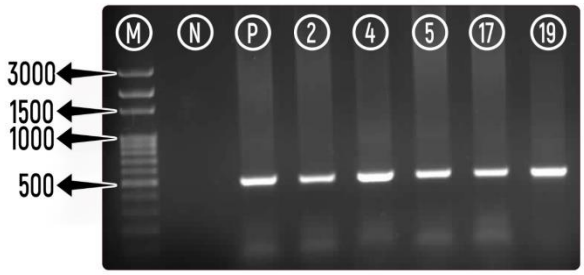

Figure 2. PCR amplification products of some selected *G. duodenalis* samples. Lanes M: Marker, N: Negative control, P: positive control, Lanes 2,4,5,17,19 represent *G. duodenalis* positive samples (511 bp)

Discussion

Giardia duodenalis is commonly seen in cats (9). Despite the many advantages of having a pet, close contact between humans and cats and dogs can cause zoonotic diseases (6, 8). The prevalence of infection varies depending on the age of the animals, clinical condition, shelter conditions, geographical region and diagnostic methods used (11).

In the world, different results have been reported in studies to determine the prevalence of *G. duodenalis* in cats. Respectively, 5% and 80% by microscopic and PCR methods in Australia (2), 13.1% by PCR method in China (15), 8.18% in Japan (14), in Italy 4.4% and 6.1% by IFAT and PCR method respectively (13), 9.2% by PCR method in Spain (12), 3.9% by PCR method in Poland (8) and a prevalence of 2% and 3.33%, respectively were reported in Iran by microscopic and immunochromatography assay methods (7).

In Turkey, studies to determine the prevalence of *Giardia* spp. in cats are very limited. Some studies report its presence at the rate of 4% as *G. cati* (16) in Ankara, and 8% as *G. intestinalis* in a study including the Samsun and Kayseri provinces (17). In another study in the Central Anatolia Region where microscopic examination, rapid immunochromatographic test, and PCR methods were used, the prevalence of *G. duodenalis* was reported to be 37.3%, 50% and 29.4%, respectively (9).

Microscopic, serological and molecular methods are used in the diagnosis of the disease (1, 7, 9, 11), among which is reported that the PCR method is more sensitive (2, 7). In this study, microscopic examination and PCR methods were used for diagnosis. As a result of the study, 8.33% positivity was detected by microscopic examination and 16.67% by PCR method. The results of this study are similar to the results of the studies carried out by Önder et al. (17) and Xu et al. (15). However, it is nonetheless lower than the results of the study by McGlade et al. (2), while it was higher than the ones carried out by Sursal et al. (9), Suzuki et al. (14), Paoletti et al. (13) and Gil et al. (12). Differences between studies may be caused by different geographical conditions, the diagnostic procedure used, the age of the animals and the population.

While some researchers (7, 12, 20) found more positivity in males, some (6, 9) reported more positivity in females. In this study, compatible with the findings of Sursal et al. (9) and Procesi et al. (6), higher positivity was detected in females.

In the study by Li et al. (20) a statistically significant difference between the sexes was reported, while other researchers (6, 7, 9, 12) stated no significant difference. In this study, no statistically significant difference was found between the sexes, as well. (P>0.05).

Procesi et al. (6) reported a higher prevalence in those older than one year and stated that this difference was statistically significant. On the other hand, some other researchers (7, 8, 12, 14, 15) show that the prevalence is higher in those under the age of one, and there is no statistically significant difference between age groups. In this study, a higher prevalence was found in cats under one year of age, as well (P>0.05). The low prevalence in cats over one year old may be due to the development of humoral immunity with advancing age.

In a study by Li et al. (20), more positivity was found in the non-diarrheic group than in the diarrheic group (P>0.05). On the other hand, in a study by Mosallanejad et al. (7), more positivity was found in the diarrheic group and a statistically significant difference was reported. In this study, more positivity was detected in the diarrheic group (17.65%) than in the non-diarrheic group (16.13%), but no statistically significant difference was detected.

As a result, the prevalence of *G. duodenalis* in stray cats in Mardin province was revealed in this study. The results show that cats can be a potential reservoir for human infection and demonstrate a potential risk to public health. Further studies are needed to investigate the general epidemiological status of giardiasis in domestic and stray cat populations.

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