

Original Research Paper

# Prevalence of *Giardia duodenalis* in Household and Shelter Cats in Almaty, South-Eastern Kazakhstan

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**Abstract:** *Giardia duodenalis* is a widely distributed protozoan parasite, that causes acute gastroenteritis in many animal species and humans all over the world. We aimed to carry out a cross-sectional study to determine the prevalence of *G. duodenalis* among cats in Almaty City, Kazakhstan. A total of 164 fecal samples from different origin cats (household, sheltered) in two veterinary clinics and three shelters of Almaty were collected taking into account animals' age, gender, housing type and consistency of feces. A study of each sample was performed using the zinc sulfate flotation method and Fastest<sup>®</sup>Crypto-Giardia (MEGACOR, Austria) Immunochromatographic (ICA) kit. In Almaty city, the prevalence of *Giardia* was significantly ( $p < 0.05$ ) higher in shelter cats (26.1%) than in pet animals (4.2%). The occurrence of the parasite was higher in young cats (18.8%) than in adults (8.3%). The presence of infection antigens was almost two times higher in diarrheal animals than in those with compact stool. *G. duodenalis* prevalence in males was slightly lower than in females. Further genetic research has to be performed to assess the zoonotic risk of the pathogen for regional public health.

**Keywords:** Almaty City, Cats, *Giardia duodenalis*, Prevalence

## Introduction

The cat was domesticated more than 9.5 thousand years ago in order to protect households from rodents (Driscoll *et al.*, 2007). During this period, the relationship between humans and cats has undergone a serious evolution and now these pets have become one of the most preferred companion animals. Cats, in addition to the initial function, provide owners with such benefits as psycho-emotional well-being, strengthening the bond between the family and community members and reducing the teenagers' time hanging after visual devices (Martins *et al.*, 2023; Zablan *et al.*, 2023; Charmaraman *et al.*, 2022; Koyasu *et al.*, 2023) and these pets become also a profit source for owners of valuable breeds. However, such type of relationship involves very close contact, excluding hygiene measures, when family members pet and carry cats in their arms, feed them raw meat and allow animals to perform such behavioral actions as sleeping together, licking owners' hands and faces, free walking and communicating with other pets, hunting rodents and small animals. These and other behavioral features pose a potential risk for owners since cats could be the transmitters of many

zoonoses, including giardiasis, an intestinal protozoa infection (Cai *et al.*, 2021; Mateo *et al.*, 2023).

The disease is by acute gastroenteritis in people and many animal species across the planet. Moreover, the infection can be asymptomatic in both humans and animals (Ballweber *et al.*, 2010). Wide distribution of the infection's main species, *Giardia duodenalis* (syn. *G. intestinalis*, *G. lamblia*), in people, is documented in the most developed and developing countries over the world. For example, *G. duodenalis* incidence was up to 7.6 and 5.5 cases per 100.000 people in the USA and the UK, respectively (Geurden *et al.*, 2008; Feng and Xiao, 2011). It is considered that these data are underestimated and annually up to  $2.8 \times 10^8$  new cases occur among people (Lane and Lloyd, 2002). In 2020, human giardiasis consisted of 99.4% of protozoal infections registered in Kazakhstan. The average annual national incidence of giardiasis was 6.95 cases per 100.000 people, of which children make up more than 39%. The greatest increase in the people giardiasis incidence has been noted in southern regions of the country (Ministry of Public Health RK, 2021).

*G. duodenalis* has eight genetic assemblages from which assemblages A and B mostly infect humans and

assemblage F infects cats. But often in addition to the assemblage F cats have also been infected with assemblages A, B, C, D and E (Pazol *et al.*, 2015; Geurden *et al.*, 2008; Barbosa *et al.*, 2023; Ryan *et al.*, 2021; Ramírez-Ocampo *et al.*, 2017). Although the sub-assemblage AI, confined to animal hosts, was mainly detected in cats (Mateo *et al.*, 2023; Zheng *et al.*, 2014; Saleh *et al.*, 2019), these pets were infected also with the sub-assemblage AII, which is mostly human-associated (Gil *et al.*, 2017; Agresti *et al.*, 2021; Guadano Procesi *et al.*, 2022). There were reported several cases of *G. duodenalis* assemblage in children (Silva *et al.*, 2022; Pipiková *et al.*, 2020; Sutthikomchai *et al.*, 2021). Thus, the pathogen assemblages' diversity determines the zoonotic risk of infected by *Giardia* cats to public health.

Prevalence of giardiasis in cats varies from 1.3-44.4% depending on origin strata and world regions (Pazol *et al.*, 2015; Mateo *et al.*, 2023; Barbosa *et al.*, 2023; Ramírez-Ocampo *et al.*, 2017; Saleh *et al.*, 2019; Gil *et al.*, 2017; Agresti *et al.*, 2021; Guadano Procesi *et al.*, 2022; Bouzid *et al.*, 2015; López-Arias *et al.*, 2019; Nagamori *et al.*, 2020). However, in Central Asia *Giardia* infection among companion animals has not been studied yet and information about this disease in cats in Kazakhstan presents interest for practicing veterinarians, public health services of the country and the scientific community to better understand the giardiasis at different epidemiological levels. Therefore, our study aimed to determine the prevalence of *G. duodenalis* among household and shelter cats in Almaty, Kazakhstan.

## Materials and Methods

### Sampling

Cross-sectional investigations were provided in Almaty City, located in the Southeast of the country, 43°15' n.L. and 76°54' e.L. The metropolis has a total area of 683.5 km<sup>2</sup>, a population of 2.5 million and a continental climate (average annual indicators: Temperature +10°C, precipitation 684 mm, humidity 62%).

A total of 164 fecal samples from different origin cats (household, sheltered) in two veterinary clinics and three shelters of Almaty were collected in special plastic cups (10-15 g) in 09 January-24 April 2024 (Table 1). During collection age, gender, housing type of cats and consistency of feces were recorded for each animal. Age was assessed through dental analysis and anamnesis information. Samples were stored at -20°C and processed within two to four days.

### Parasitological Studies

The investigation of samples was conducted at the Kadyrov Parasitology Laboratory, Seifullin Kazakh Agrotechnical Research University. A study of each fecal sample was performed using two (centrifugation-flotation and immunochromatography assay) laboratory methods.

**Table 1:** Sampling from cats in Almaty city

Age	Strata of origin				Total (n = 164)	
	Pets (n = 95)		Sheltered (n = 69)		♀	♂
	♀	♂	♀	♂		
<12 months	18	20	28	14	46	34
>1 year	30	27	13	14	43	41
Total	48	47	41	28	89	75

### Centrifugation-Flotation Technique

A fecal sample (1-5 g) in zinc sulfate solution (specific gravity = 1.18) was filtered through gauze and centrifuged with a coverslip in place for 10 min at 400× g. Then the coverslip was put down to a slide and examined microscopically for *G. duodenalis* cysts using a light microscope (Micros MC-20/Austria) at the magnifications 40× and 100× (Zajac *et al.*, 2002; Ndao, 2009; De Mendonça Uchôa *et al.*, 2018).

### Immunochromatography Assay (ICA) and the Test Interpretation

Samples were tested with an immunochromatographic assay using the commercial kit Fastest®Crypto-Giardia (MEGACOR, Austria) for qualitative detection of soluble *G. duodenalis* cyst and *Cryptosporidium* spp. oocyst antigens according to instructions of the manufacturer.

We put the required fecal sample volume (compact: 1 level spoon, pulpy: 2 level spoons and fluid or watery: 3 level spoons of feces) steadily into the sample tube with the buffer diluent. Then we closed the sample tube and rotated it to get the homogeneous mixture. Later, we placed the sample tube on a horizontal surface for 1-5 min. Then we introduced the dipstick vertically and with the arrows pointing downwards into the sample tube for at least 1 min. We removed the dipstick from the sample tube when the sample-buffer mixture reached the control line. After that, we placed the dipstick on a flat and horizontal surface for incubation and read the test result after 5 min. We considered a negative result if only the green control line appeared (Fig. 1) and a positive for *G. duodenalis* result when a red test line appeared (Fig. 2).

### Statistical Analysis

A Fisher analysis was performed to assess the associations between the *Giardia* occurrence and such variables as age, gender, the origin of cats and fecal consistency using r-4.4.0 for Windows. Statistically significant associations were considered when p<0.05.



**Fig. 1:** Negative sample of Fastest®Crypto-Giardia in household cats by ICA, 2024



**Fig. 2:** Positive sample of Fastest®Crypto-Giardia in household cats by ICA, 2024

## Results

The fecal sample examination results by two methods included in this study are shown in Table 2.

Out of the 164 samples analyzed, 8 samples on the zinc sulfate flotation method and 22 samples when the immunochromatographic kit was used were positive for *G. duodenalis* out of the 164 samples analyzed.

Based on the immunochromatography assay technique, antigens of *G. duodenalis* were detected by the kit in 13.4% of samples.

A statistically significant association for *G. duodenalis* infection was determined only for the origin strata of cats (Table 3).

**Table 2:** Efficacy of laboratory methods for diagnostics of *G. duodenalis*

Age of cats	Number of samples	Diagnostic technique			
		Centrifugation-flotation		ICA	
		Positive	%	Positive	%
<12 months	80	6	7.5	15	18.8
>1 year	84	2	2.4	7	9.3
Total	164	8	4.9	22	13.4

**Table 3:** Prevalence of *Giardia duodenalis* in the cat population of Almaty city by origin, age, fecal consistency and sex of animals

	Positive	Negative	Prevalence (%)	P-value	Odds ratio	95% CI
Total (164)	22	142	13.4			
Animals' origin						
Pet cats	4	91	4.2	9.772		[2.4340; 33.959]
Shelter cats	18	51	26.1	$\cdot 10^{-5}$	7.927	
Age						
<12 months	15	65	18.8	0.066	0.396	[0.1284; 1.1079]
>12 months	7	77	8.3			
Fecal consistency						
Diarrheal	5	20	20	0.337	0.559	[0.1714; 2.1589]
Normal	17	122	12.2			
Gender						
♂	9	66	12.0	0.654	1.252	[0.4612; 3.5491]
♀	13	76	15.4			

So, the occurrence of *Giardia* was significantly ( $p < 0.05$ ) and several times higher in shelter cats than in pet animals. Regarding age, the prevalence was higher in young cats (18.8%) than in adults (8.3%). For fecal consistency, the presence of *G. duodenalis* was almost two times higher in diarrheal animals with pulpy and watery stool than those with compact stool. Gender did not have a significant association too, as the prevalence in males was slightly lower than in females.

## Discussion

This cross-sectional surveillance is the pilot report on giardiasis prevalence in household and shelter cats in the Central Asian region, particularly in Almaty city. Results obtained highlighted the zoonotic potential of household and shelter-housing cats for *G. duodenalis* transmission in the city environment of eastern and southern Kazakhstan.

Giardiasis is characterized by such distinctive features as a low number and intermittent excretion of cysts in fecal samples as well as an asymptomatic clinic of the disease, which significantly complicates the pathogen finding. Therefore, the parasite is not detected in all hosts and it is considered that the infection prevalence is underestimated all over the world (Ignatius *et al.*, 2012; Pazol *et al.*, 2015).

For the giardiasis diagnosis, some techniques were elaborated. In feces of animal and human patients *G. duodenalis* cysts and trophozoites are identified by microscopic examination; specific antigens of the parasite can be recognized by ELISA, immunochromatographic assay and DNA fragments of the pathogen are detected by PCR (Babaei *et al.*, 2011; Mosallanejad *et al.*, 2010; De Mendonça Uchôa *et al.*, 2018;

Veyna-Salazar *et al.*, 2023). Choosing the available methodologies for parasite diagnostics depends on the presence of needed equipment and chemicals, experienced laboratory practitioners and financial and other resources (Ndao, 2009).

This survey assessed the performance of conventional microscopy using the zinc sulfate flotation technique and the modern immunochromatographic test for the diagnosis of *G. duodenalis* infection in fecal samples from cats. The study revealed that the overall giardiasis occurrence was 4.9 and 13.4% by the flotation technique and ICA respectively. So, the commercial immunochromatography kit was more efficient than the classical microscopy and performed well in the detection of *Giardia* in fecal samples. Analogic results have been reported in most publications for assessment of the laboratory tests in the diagnosis of this parasite in stool of different species over the years (Garcia and Garcia, 2006; Papini and Cardini, 2006; Mosallanejad *et al.*, 2010).

The prevalence of *G. duodenalis* among cats in Almaty was 13.4% and similar to the overall occurrence reported worldwide (12%) in a meta-analysis of more than 68 epidemiological surveillance of cats in different countries and geographical regions (Bouزيد *et al.*, 2015). Moreover, shelter animals' infection level in Almaty was 26.1% and analogical to that of cats from Serbia, Colombia and Mexico but lower than in Brazil (Aleksandra *et al.*, 2002; López-Arias *et al.*, 2019; Veyna-Salazar, 2023).

The occurrence of giardiasis differs in all studied categories such as origin strata (pet and shelter animals), age, gender and fecal consistency of cats. A significant association of *G. duodenalis* prevalence was seen regarding their origin, pets were infected for more than six times lower than shelter animals. Similar levels of infection from 2.3% in owned cats to 22.4% in shelter ones are reported in Greece and Germany respectively (Symeonidou *et al.*, 2018; Cirak and Bauer, 2004).

In this study, age was not significantly associated with giardiasis statistically, but young cats (<a year) were more than two times higher infected with the parasite than older ones (>a year). These data are correspondent to the modern opinion that *G. duodenalis* has a higher risk for invasion by younger cats of <6 or <12 months of age (De Santis-Kerr *et al.*, 2006; Epe *et al.*, 2010; Bouزيد *et al.*, 2015; Sauda *et al.*, 2019; Nagamori *et al.*, 2020). The result obtained suggests that the cats' age could be a risk factor for infection with giardia due to the weak development of young animals' immunity (Aleksandra *et al.*, 2002). The lower occurrence of giardiasis in mature cats, seen in this study, may be attributed to developing the humoral immunity with age. Therefore, it was proposed that infected kittens with their behavior habit like biting and licking objects could be a powerful source of environment contamination and infestation of other animals and owners with parasitic cysts and trophozoites (Veyna-Salazar *et al.*, 2023).

The gender of investigated cats was not a statistically significant factor too ( $p>0.05$ ) and prevalence was 12% for males and 15.4% for females, similar to previously published data (Mosallanejad *et al.*, 2010; Mircean *et al.*, 2011).

Diarrhea or pasty stool were reported as the major clinical signs of *Giardia* infestation (Hill *et al.*, 2000; Read *et al.*, 2002) but subclinical and asymptomatic forms of the disease are also common (Thompson *et al.*, 2000). In this study, it was found that fecal consistency was a real factor for detecting giardiasis because the difference in the proportion of infected and noninfected cats among diarrhoeal animals was strong and consisted of 20 and 12% respectively. These results fit with other surveillances where animals with diarrhea were infected by *G. duodenalis* more commonly than cats with firm stools (Mekaru *et al.*, 2007; Sursal *et al.*, 2020).

The research demonstrated the potential role of cats in the transmission of *Giardia* to people and contamination of the environment with cysts or trophozoites of this pathogen. In this study, the *G. duodenalis* prevalence in cats of different origin strata was indicated in Almaty, the Southeast of Kazakhstan. In the next step, we have to perform further studies in various areas to survey the overall epidemiological status of giardiasis in household, shelter and stray cat populations; to study *G. duodenalis* genetic types to assess the zoonotic risk of the pathogen from public health position.

## Conclusion

The performance of the Fastest® Crypto-Giardia ICA kit exhibited higher sensitivity compared to the zinc sulfate flotation technique for the diagnosis of *G. duodenalis* in feces. In Almaty, the prevalence of giardia was significantly higher in shelter cats (26.1%) than in pet animals (4.2%). The occurrence of the parasite was higher in young cats (18.8%) than in adults (8.3%). The presence of infection antigens was almost two times higher in diarrheal animals than in those with compact stool. *G. duodenalis* prevalence in males was slightly lower than in females. Further genetic research has to be performed to assess the zoonotic risk of the pathogen for regional public health.

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## Author's Contributions

**Lyudmila Lider:** Coordinated the collection of samples, performed data analysis, and drafted the manuscript.

**Altay Ussenbayev:** Analyzed the experimental data and contributed to drafting the manuscript.

**Vladimir Kiyan:** Conducted a critical review for intellectual content and contributed to drafting the manuscript.

**Dariyash Kurenkeyeva:** Provided statistical analysis for the study.

**Dinara Seitkamzina and Botakoz Akmambayeva:** Collected and studied samples using the flotation method.

**Rabiga Uakhit and Ainur Smagulova:** Collected and studied samples using the immunochromatographic method.

**Igor Sytnik:** Designed the study and drafted the manuscript.

## Ethics

The research methodology received approval from the Bioethics Commission of the Faculty of Veterinary and Animal Husbandry Technology at Seifullin Kazakh Agrotechnical Research University (Meeting Protocol No. 4, January 5, 2024). It adheres to the profes.

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