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The prevalence of potentially zoonotic intestinal parasites in dogs and cats in Moscow, Russia

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Summary

Received October 7, 2022 This study was aimed to determine the prevalence of Toxocara canis/cati, Strongyloides stercora-Accepted March 6, 2023 lis, Giardia spp., and Cryptosporidium spp., which occur and are potentially zoonotic to humans in domestic dogs and cats in Moscow (Russia). The fecal flotation method and larvae detection by microscopy of a direct feces smear were performed to detect Toxocara, Giardia spp., and Cryptosporidium spp. The total parasitic prevalence in dogs was as follows: Giardia spp.: 10.2 % (226/2208), Cryptosporidium spp.: 2.7 % (60/2208), T. canis: 2 % (45/2208), S. stercoralis larvae: 1.1 % (25/2208). The younger animals under were infected more than those over 12 months of age (p<0.001). The prevalence rates were along these lines: Giardia spp. (18.2 %), Cryptosporidium spp. (5.7 %), T. canis (3 %), S. stercoralis larvae (2.3 %). The overall prevalence in cats was as follows: Giardia spp. - 5.2 % (71/1350), Cryptosporidium spp. - 4.8 % (65/1350), T. cati - 4.1 % (56/1350). Similarly to dogs, the infection rates were higher in cats under 12 months of age Giardia spp. (8.2 %), Cryptosporidium spp. (8.6 %), T. cati (7.5 %. Analysis of combined infections in dogs revealed the following combinations: Giardia spp. and Cryptosporidium spp. (35.5 %) larvae of S. stercoralis sp. and Giardia spp. (32.3 %), T.canis and Giardia spp. (22.6 %), T.canis and Cryptosporidium spp. (6.6 %), T.canis and S.stercoralis and (3.2 %), respectively. In cats, only two coinfections by Giardia spp. and Cryptosporidium spp. (58.3%), and T.cati with Giardia spp. (41.7%) were noticed. Further research is needed to study the spread of parasitic diseases in pet animals. The data will improve countermeasures to prevent these diseases' spread among animals and humans. Keywords: cat; dog; Toxocara canis/cati; Strongyloides stercoralis; Giardia spp.; Cryptosporidium spp.; infection prevalence; Russia

Introduction

Pets serve as companionship animals and provide emotional support for people worldwide (Luis Enrique *et al.*, 2018). Thus, becoming full members of a family, dogs and cats often relieve loneliness and bring joy to their owners (Ursache *et al.*, 2021; Gillespie & Bradbury, 2017; Luis Enrique *et al.*, 2018), and many pet

owners worldwide exist (Gillespie & Bradbury, 2017; Blanciardi *et al.*, 2004). For instance, in 2019, there were approximately 106.4 million cats as pets in Europe, and there were 4.2 million domestic dogs in Australia in 2017 (Ursache *et al.*, 2021; Gillespie & Bradbury, 2017). There is often a close relationship between humans and pets, especially in urban environments where the same space is shared (Ilic *et al.*, 2017).

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One important challenge is to prevent the spread of parasitic infections (Gillespie & Bradbury, 2017; Palmer *et al.*, 2008; Baneth *et al.*, 2016), where protozoans and helminths may cause gastrointestinal distress, resulting in diarrhea, vomiting, and loss of appetite (Bouzid *et al.*, 2015; Burgess *et al.*, 2017; Ursache *et al.*, 2021). Sometimes very intense infections lead to a dramatic deterioration of animal health, even with fatal outcomes (Ballweber *et al.*, 2010; Luis Enrique *et al.*, 2018). However, the intestinal parasites in dogs and cats proceed more often without clinical manifestation (Bilgic *et al.*, 2020; Moreira *et al.*, 2018; Li *et al.*, 2019; Mircean *et al.*, 2012; Stafford *et al.*, 2020); what represents an epidemiological risk because asymptomatic animals can be a source of infection to humans when potentially zoonotic parasites are involved (Bilgic *et al.*, 2020; Ilic *et al.*, 2016; Nguyen *et al.*, 2022).

Worldwide published research papers show that intestinal parasites in dogs and cats are widespread (Thompson, 2008; Mircean *et al.*, 2012; Liu *et al.*, 2014; Unterkofler *et al.*, 2022; Sweet *et al.*, 2020; Nguyen *et al.*, 2022; Abere *et al.*, 2013; Silva *et al.*, 2020). For example, Sweet *et al.* (2020) reported that the overall prevalence of cat intestinal parasites in the continental United States could range from 0.03 % to 33 %. Nguyen *et al.* (2022) reported that in Vietnam, infestation in dogs reaches 77.7 %.

lic health because it can cause human disease in types such as visceral toxocariasis, neuro-toxocariasis, ocular toxocariasis and latent toxocariasis (Ursache et al., 2021). The protozoa Cryptosporidium spp. and Giardia duodenalis parasitize in the gastrointestinal tract of humans and other vertebrates (Li et al., 1019). Among the approximately 40 Cryptosporidium species, C. hominis, C. parvum, C. meleagridis, C. canis, and C. felis are the most common in humans (Feng et al., 2018; de Oliveira et al., 2021). So, the potential role of domestic animals as a source of human infection with Giardia spp. is the most debated topic (Bouzid et al., 2015; Mircean et al., 2012; Mravcova et al., 2019; Thompson & Monis, 2004; Thompson et al., 2008). It has been confirmed that genotypes A and B affect many animal species and humans. while genotypes C and D are found in dogs and F in cats and are considered species-specific (Feng and Xiao, 2011; Uiterwijk et al., 2020; Jothikumar et al., 2021). According to the Companion Animal Parasite Council (CAPC), immunocompromised people should limit their exposure to Giardia-infected pets (CAPC guideline, 2019). S. stercoralis is an endemic parasite in tropical and subtropical regions, but recently there have been more confirmed reports of such infections in central and northern Europe, where humans and dogs became infected (Basso et al. 2019; Bourgoin et al. 2018; Cervone et al. 2016; Eydal & Skirnisson 2016; Liberato

Toxocara sp. that parasitizes dogs and cats poses a threat to pub-

Type of Infection		2018	2019	2020	2021	2018 – 2021	
Dogs (≤ 12) total		104	144	303	399	950	
Giardia spp.	N	24	30	55	64	173	
	%	23.08	20.83	18.15	16.04	18.21	
Cryptosporidium spp.	Ν	5	19	13	18	55	
	%	4.81	13.19	4.29	4.51	5.79	
T. canis	Ν	4	5	14	6	29	
	%	3.85	3.47	4.62	1.50	3.05	
S. stercoralis	Ν	3	3	7	9	22	
	%	2.88	2.08	2.31	2.26	2.32	
Dogs (> 12) total		215	285	324	434	1258	
Giardia spp.	N	14	12	11	16	53	
FF	%	6.51	4.21	3.40	3.69	4.21	
Cryptosporidium spp.	Ν	0	1	2	2	5	
<i>,</i> ,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,	%	0.00	0.35	0.62	0.46	0.40	
T. canis	Ν	3	6	3	4	16	
	%	1.40	2.11	0.93	0.92	1.27	
S. stercoralis	Ν	1	1	1	0	3	
	%	0.47	0.35	0.31	0.00	0.24	
Dogs total		319	429	627	833	2208	
Giardia spp.	N	38	42	66	80	226	
	%	11.91	9.79	10.53	9.60	10.24	
Cryptosporidium spp.	Ν	5	20	15	20	60	
	%	1.57	4.66	2.39	2.40	2.72	
T.canis	Ν	7	11	17	10	45	
-	%	2.19	2.56	2.71	1.20	2.04	
S. stercoralis	N	4	4	8	9	25	
	%	1.25	0.93	1.28	1.08	1.13	

Table 1. Prevalence of *Cryptosporidium* spp., *Giardia* spp., S. stercoralis and *T.canis* in dogs.

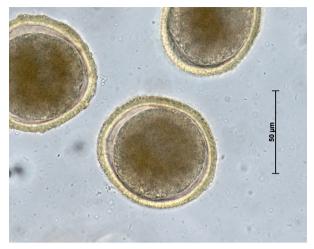


Fig.1. Toxocara cati eggs

et al. 2022; Jaleta *et al.* 2017; Raicevic *et al.* 2021; Unterkofler *et al.*, 2022). Recently, the scientific community has been interested in determining the role of helminth canine hosts (i.e., *S. stercoralis*) in the transmission of this infection to humans (Paradies *et al.*, 2017). Data on the epidemiology of canine strongyloidiasis are also limited at this time. Most likely due to the limitations in current diagnostic methods (Paradies *et al.*, 2017).

Humans are infected with zoonotic parasites by consuming contaminated food and water or by direct fecal-oral infection from infected animals (Luis Enrique *et al.*, 2018; Li *et al.*, 1019; Silva *et al.*, 2020). The occurrence of prolonged *Giardia* invasions or disseminated *S. stercoralis* or *Cryptosporidium* spp. infections are likely possible in immunocompromised individuals (Unterkofler *et al.*, 2022; Paradies *et al.*, 2017).

Our work aimed to examine the current situation regarding the prevalence of potentially zoonotic intestinal parasites: *Cryptosporidium* spp. and *Giardia* sp., *S. stercoralis*, and *Toxocara* sp. in domestic dogs and cats in urban environments, and to establish the frequency of coinfections.

Materials and Methods

Two thousand two hundred eight dogs' fecal samples were examined in 2018 – 2021. Sampling included 950 animals aged 1 to 12 months and 1258 over 12 months. Meanwhile, one thousand three hundred fifty cats' fecal samples were examined, where 531 were under the age of 12 months, and 819 were over 12 months of age. The information about the age and likelihood of the animals visiting the outdoor environment was obtained from the animal owners. All examined dogs were kept in apartments and had daily outdoor exposure. Cats were kept solely indoors. In the case of *S. stercoralis* larvae detection, anamnesis regarding the state of the animal and the defecation type was collected from the pet owners. Finally, all data from the owners were processed after receiving their written or verbal consent. Collected fecal samples were submitted to the «Pasteur» laboratory in Moscow for endoparasite testing. Samples were sent to the laboratory diagnosis when infection symptoms were present or during the therapy outcome control or routine monitoring. The fecal flotation method using a zinc sulfate solution with a density of 1.24, as described by Zajac A (2012), for detecting intestinal parasites was performed. For larvae detection, direct feces smear microscopy was performed. The microscopy was performed with a Lomo microscope at 100X and 400X magnifications (Joint-stock company Lomo, Russia).

Ethical Approval and Informed Consent

The study protocol was reviewed and approved by the scientific and methodological commission of VNIIP - a branch of the Federal State Budget Scientific Institution "Federal Scientific Center VIEV" (Protocol No. 1 dated January 19, 2018). The procedures used in this study are in line with the principles of the Declaration of Helsinki and the European Convention for the Protection of vertebrate animals used for experimental and other scientific purposes. Written informed consent was obtained from the owners for the participation of their animals in this study.

Statistics

Data analysis was performed using the statistical package SPSS version 26.0. We assessed the statistical significance of observed differences in the degree of infestation in animals of two age groups using the Chi-square criterion, with a threshold set at 0.05 (p-value).



Fig.2. Larva of Strongyloides sp. in dog, stage L1.

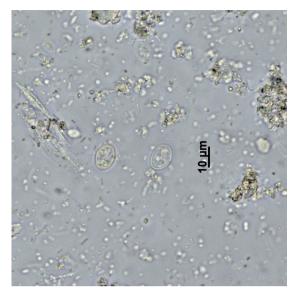


Fig.3 Cysts of Giardia spp.

Results

The results showed that *Giardia* spp. cysts (Fig. 3) were detected the most frequently (Table1). The overall prevalence was 10.24 %, with a width of 18.2 % in dogs under 12 months. The infection rates with other parasites were as follows: *Cryptosporidium* spp. - 5.79 %, *T. canis* - 3 %, larvae of *S. stercoralis* (Fig.2) - 2.3 %. The infestation rates in dogs older than 12 months were like this: *Giardia* spp. - 4.21 %, *Cryptosporidium* spp. - 0.4 %, *T. canis* - 1.27 %, and *S. stercoralis* larvae - 0.24 %. Animals under 12

months of age were infected more than those over 12 months of age (p<0.001).

The infection rates in cats were found in this manner (Table 2). *Giardia* spp. - 8.2 %, *Cryptosporidium* spp. - 8.6 %, *T. cati* (Fig.1) - 7.5 %. Cats older than 12 months showed the following prevalences: *Giardia* spp. - 3.3 %, *Cryptosporidium* spp. (Fig.4) - 2.3 %, *T. cati* - 2.3 %. The study revealed that in cats under one year of age, *Cryptosporidium* spp. and *Giardia* spp. were detected at the same level; meanwhile, the *T. cati* eggs were present to a lesser extent. In cats under 12 months of age (p < 0.001), the prevalence was higher when compared with older than 12 months cats.

In the analysis of combined infections in dogs, we observed the following (Table 3). Coinfections of *Giardia* spp. and *Cryptosporidium* spp. (35.5 %), larvae of *S. stercoralis* and *Giardia* spp. (32.3 %), *T. canis* and *Giardia* spp. (22.6 %). The combination of *T. canis* and *Cryptosporidium* spp. (6.6 %) or *T. canis* and *S. stercoralis* (3.2 %) in dogs were observed much less frequently. In cats, only two coinfections were found and were caused by *Giardia* spp. and *Cryptosporidium* spp. (58.3 %) or *T. cati* and *Giardia* spp. (41,7 %).

Discussion

Our study was focused on the research of helminths and protozoan prevalence where *T. canis/cati, S. stercoralis, Giardia* spp., and *Cryptosporidium* spp. in domestic dogs and cats living represent a potential health threat to humans.

Our study has shown that *Giardia* spp. cysts were found most frequently in domestic dogs, especially in young animals up to 12 months old (18.2 %), while the other parasites from the studied

Type of Infection		2018	2019	2020	2021	2018 - 2021	
Cats (≤ 12) total		80	98	159	194	531	
Giardia spp.	N	10	7	15	12	44	
	%	12.50	7.14	9.43	6.19	8.29	
Cryptosporidium spp.	Ν	7	10	16	13	46	
, , , , , , , , , , , , , , , , , , , ,	%	8.75	10.20	10.06	6.70	8.66	
T. cati	Ν	4	5	17	14	40	
	%	5.00	5.10	10.69	7.22	7.53	
Cats (> 12) total		134	198	234	253	819	
Giardia spp.	Ν	2	4	10	11	27	
	%	1.49	2.02	4.27	4.35	3.30	
Cryptosporidium spp.	Ν	1	4	9	5	19	
,	%	0.75	2.02	3.85	1.98	2.32	
T. cati	Ν	1	3	8	5	19	
	%	0.75	1.52	3.42	1.58	2.32	
Cats total		214	296	393	447	1350	
Giardia spp	N	12	11	25	23	71	
	%	5.61	3.72	6.36	5.15	5.26	
Cryptosporidium spp.	Ν	8	14	25	18	65	
	%	3.74	4.73	6.36	4.03	4.81	
T. cati	Ν	5	8	25	18	56	
	%	2.34	2.70	6.36	4.03	4.15	

Table 2. Prevalence of Cryptosporidium spp., Giardia spp. and T.cati in cats.

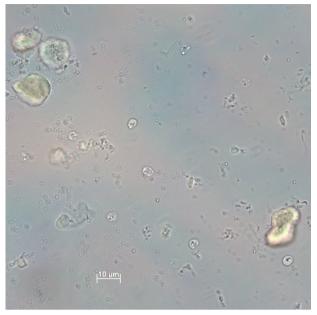


Fig.4. Oocysts of Cryptosporidium sp. in cat.

group were detected less often. Many researchers reported similar results (Bouzid et al., 2015; Geurden et al., 2008; Hussein et al., 2017; Liu et al., 2014; Agresti et al., 2022; Piekara-Stepinska et al., 2021). For example, Bouzid et al. (2015) reported that the overall prevalence of Giardia spp. in dogs and cats is about 15.2 % and 12 %, respectively. Mircea et al. (2012) showed that the prevalence of Giardia spp. in domestic dogs is up to 4.8 %. According to Li et al. (2019), Giardia spp. is found in up to 6.9 % of dogs and 9.4 % of cats. We studied domestic animals, so the prevalence of Giardia spp. and Cryptosporidium was lower than in animals living in the shelters. In shelter dogs, Adeell-Aledon et al. (2018) reported Giardia spp. infestation up to 40.4 %. Tangtrongsup et al. (2020) described the prevalence of Giardia spp. up to 25.5 % in dogs and 27.3 % in cats. Cryptosporidium in dogs was up to 7.6 % and 12.1 % in cats. Silva (2020) reported a 28 % prevalence of Giardia spp. in stray dogs and only 6.2 % in household animals. Despite the high levels of Giardia spp. in dogs, we noticed a gradual

decrease in infestation intensity during the 2019 - 2021 period. This may be due to the growing awareness of veterinarians and pet owners about the spread of giardiasis. When symptoms of

giardiasis are present, tests are prescribed to exclude them, or anthelmintics against giardiasis are administered for preventive and treatment purposes.

We detected T. canis in dogs and cats to a lesser extent than Giardia spp. and Cryptosporidium spp. (3 % in dogs under 12 months and 7.5 % in cats). Still, the prevalence remained practically at the same level during the observation period. Our data differ significantly from the results of other researchers, where Genchi et al. (2021) reported a 25.6 % infection rate of Toxocara in cats, while Luis Enrique et al. (2018) reported a 25.3 % detection rate of T. canis in dogs. This is likely related to the region and category of animals studied. Many researchers have reported a higher prevalence of parasites in animals in countries with hot climates and abundant rainfall. (Li et al., 2019; Liberato et al., 2022; Umar et al., 2017). We believe that preventive deworming prescribed by veterinarians affects the rates of T. canis infestation. Thus the infection rate remains low but stable. As Zanzani et al. (2014) and Silva et al. (2020) noted, introducing preventive deworming can significantly reduce the rate and risk of infection and invasion transmission.

Li et al. (2019), Sweet et al. (2021), Genchi et al. (2021), Uiterwijk et al. (2019), and other authors reported that the age of animals, especially if they younger (under 12 months) is a significant infection risk factor. It is valid not only for the Giardia but also for the other parasite species with more pronounced clinical manifestations (Sweet et al., 2020; 2021; Luis Enrique et al., 2018; Silva et al., 2020; Liberato et al., 2022). Our studies are aligned with those data and show that in dogs and cats, T. canis/cati, S. stercoralis larvae (in dogs), Giardia spp., and Cryptosporidium spp. are more frequent in younger animals. In general, parasites detected in older dogs and cats occurred less frequently. The high level of infection in young animals may be due to the immaturity of their immune systems. In addition, in the case of Toxocara canis, the transplacental and transmammary routes of transmission promote its occurrence in puppies (Palmer et al., 2008; Gharekhani, 2014). The detection of S. stercoralis larvae in puppies is of particular interest. Despite the low prevalence of these parasites in dogs, their presence cannot be left unattended. When S. stercoralis larvae are detected, an unformed mucous stool is observed. According to the owners, some puppies were active, but some had prolonged diarrhea associated with loss of appetite and depression. Although

Table 3	Combination	mixed	infections	in	doas	and	cats
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Type of co-infection	Type of animal					
51 	Cats		Dog	js		
	Ν	%	N	%		
Giardia spp +Cryptosporidium spp.	7	58.3	11	35.5		
S. stercoralis + Giardia spp	-	-	10	32.3		
T.canis + Cryptosporidium spp.	-	-	2	6.5		
T. cati/canis + Giardia spp	5	41.7	7	22.6		
T. canis + S. stercoralis						
Total	12	100	31	100		

the endemicity of the disease has been reported, we should consider its recent spread into temperate areas. (Liberato *et al.*, 2022; Unterkofler *et al.*, 2022). However, the limited reports of its prevalence may indicate the lack of feasible detection in dogs. It is due to the unsuitable diagnostic methods used for this parasite laboratory analysis (Unterkofler *et al.*, 2022; Paradies *et al.*, 2017; Umur *et al.*, 2017).

Detection of combined infestations plays a significant role when studying the prevalence of intestinal parasites. In our study, combined infections of two different parasites were observed most frequently in dogs than in cats. This observation in dogs can probably be explained by contact with the external environment, whereas the cats included in this study lived only in the apartments (Kostopoulou *et al.*, 2017; Ursache *et al.*, 2021, Genchi *et al.*, 2021; Rojekittikhun *et al.*, 2014).

It is quite common to observe intestinal parasites in animals without clinical manifestation. We should remember that even asymptomatic parasite carriage can lead to various intestinal pathologies. (Bilgic *et al.*, 2020; Luis Enrique *et al.*, 2018; Uiterwijk *et al.*, 2019; Stafford *et al.*, 2020; Liberato *et al.*, 2022; Moreira *et al.*, 2008). Asymptomatic carrier animals excrete protozoan cysts, larvae, and helminth eggs with feces for a long time. Consequently, they become a source of infestation for healthy animals and cause risks for environmental contamination (Bilgic *et al.*, 2020).

Current discoveries in the biology, genetics, and taxonomy of *Giardia* spp., *Cryptosporidium* spp., and *Strongyloides* sp. isolates obtained from different hosts and their molecular similarities reflect the zoonotic potential of these parasites. (Unterkofler *et al.*, 2022; Li *et al.* 2019; Bilgic *et al.*, 2020; Bahramdoost *et al.*, 2021; Agresti *et al.*, 2022).

Our results show the necessity for ongoing surveillance of the prevalence of pet intestinal parasites sharing the same environment as humans. Our research will further study parasites' genetic identity to understand their zoonotic potential. It will lead to the better implementation of preventive measures against the spread of parasites and improvement of the epizootic situation in the urban environment.

Conflict of Interest

The authors declare that they did not have any conflict of interest in conducting this study. Moreover, the authors do not have any potential conflict of interest pertaining to this submission to Helminthologia.

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