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ASSESSING THE PREVALENCE OF *GIARDIA* INFECTION AND THE ASSOCIATED RISK FACTORS IN OWNED DOGS AND CATS, IN BUCHAREST'S URBAN AREA

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Abstract

Giardia is a ubiquitous organism that affects humans and animals, with potential to contaminate the water and food, raising a concern in public health interest due to its zoonotic risk. In order to estimate the parasitic pressure for the both animal and human health, it was evaluated the prevalence of Giardia infection in pets (owned dogs and cats) originated from the urban area of Bucharest. Therefore, within 4 months (May-August, 2016), a total number of 188 faecal samples from dogs and 79 fecal samples from cats were investigated. Animals were of different breeds or mixed and different ages (from 1 month up to 16 years for cats, and up to 18 years, for dogs, respectively). Faecal samples were processed by zinc sulphate 33% solution flotation, Lügol stained and microscopically examined for identification of Giardia cysts. Additionally, other parasitic stages (oocysts, eggs) were also registered. Overall, out of the total faecal samples of dogs and cats, 41.49% (95%CI: 34.36-48.89), and 34.18% (95% CI: 23.87-45.71), respectively, were positive for parasite infections. Giardia cysts were recorded in quite similar prevalence in dogs, of 21.28% (40/188) and cats, of 22.78% (18/79). A higher prevalence of Giardia infection was found in puppies (23.89%) and older dogs (30.00%), and kittens (26.42%), respectively, compared to the adults (15.38% in dogs and 14.29% in cats). Additional, other parasite infections were found, as follows: in dogs, Isospora spp. (12.23%), Ancylostoma caninum (5.85%), Toxocara canis (4.26%), Uncinaria stenocephala (0.53%), Toxascaris leonina (0.53%) and Dipylidium caninum (0.53%); while in cats, Toxocara cati (10.13%) and Isospora spp. (8.86%) infections were registered. The findings of the present study are of relevance for the both animal and public health, emphasizing potential high risks for parasite infection, including parasites with zoonotic potential.

Key words*:Giardia infection, dogs, cats, zoonotic risk, Bucharest, Romania.*

INTRODUCTION

Worldwide, dogs and cats as pets, or living around people play an important social role, contributing to the owners' wellbeing, to the emotional development of children, as well as for more complicated tasks such as utility animals for blind people, animal therapy, guard dogs, hunting dogs, military and police use (Nikolić et al., 2008; Martinez-Moreno et al., 2005; Mateus et al., 2014).

Parasites that infect companion animals are responsible for important zoonotic diseases worldwide. In humans, parasite species zoonotically transmitted can cause: larva migrans (*Toxocara* spp.), cutaneous larva migrans (*Ancylostoma* spp.), diarrhea and pruritus (*Dipylidium caninum*), hydatid disease (*Echinococcus* spp.), coenurosis (*Taeniidae*), mild to severe illness (fever, malaise, and lymphadenopathy to mental retardation, blindness, epilepsy in fetus) in case of *Toxoplasma gondii*, chronic malabsorptive and allergic manifestations (*Giardia duodenalis*), diarrhea (*Cryptosporidium* spp.) (Schantz, 2007; Mitrea, 2011).

From this point of view, infected animals can represent source of infection and potential risks for the public health due to their close proximity to humans. The risks are considered greater in urban areas, children communities (nurseries, kindergartens) or in urban areas with poor hygiene and ignorance (Mateus et al., 2014; P.A.H.O., 2003).

Giardia duodenalis (syn. *G. intestinalis* or *G. lamblia*) is a flagellate protozoan that can infect various mammalian hosts, including man, dogs and cats, and is considered a species complex.

Currently, based on genetic analyses, there are recognized at least seven assemblages (A-G). Out of these, dogs are infected primarily with Assemblages C and D, cats with Assemblage F, while humans are infected with Assemblages A and B (Monis et al., 2003; Thompson et al., 2008). However, assemblages A and B are considered to be of broad host specificity, as these have been reported also in several animal species, including cats and dogs, thus are potentially zoonotic (reviewed in Ballweber et al., 2010).

Oftenly, *Giardia* epidemics are transmitted through drinking water or recreational water, as well as directly from person to person, in communities and kindergartens (P.A.H.O., 2003; Cacciò et al., 2003; Carmena et al., 2007; Clayton, 2012).

Improving the knowledge of occurrence and prevalence of endoparasites in different areas and different communities contributes to improving animal health and to develop control measures, including for assessing the risks for the public health.

Therefore, the present study aimed to investigate the prevalence and the associated risk factors for *Giardia* infection in owned dogs and cats, as companion animals, originating from the urban area of Bucharest, in order to evaluate the potential risks for the both animal and public health.

MATERIALS AND METHODS

During of May - August 2016, a coprological study was carried out in 44 veterinary clinics, located in the six districts of Bucharest (South Eastern Romania) [44°26′7″N, 26°6′10″E] (Figure 1).

In the study were included 267 pets – companion animals: 188 dogs and 79 cats. Animals were assigned into different breed, age, and gender groups. For age criteria there were considered four categories: puppies and kittens under 6 months old, puppies and kittens between 6 and 12 months old, adults (between 1 and 8 years old) and older than 8 years.

Individual faecal samples were randomly collected from dogs and cats of all ages with or without intestinal symptoms.

The fecal samples were transported to the laboratory where they were examined immediately or stored at 4°C and examined within the next two days.

Figure 1. Veterinary clinics, number of samples and positive *Giardia* samples distribution, stratified by Bucharest's districts

All faecal samples were subjected for parasitological investigations by the flotation method, using a solution of zinc sulphate (ZnSO4) 33% and Lügol staining, and microscopically examined for the presence of *Giardia* cysts but also for worm eggs and protozoan oocysts.

Briefly described, for each sample, an amount of 2-3 g of faeces were mixed with 15 ml of 33% zinc sulphate solution, poured into a test tube, topped off with the zinc sulphate solution to form a convex meniscus and placed a coverslip for about 15 minutes.

A drop of Lügol's iodine was added to the slide, before placing the coverslip and after that the sample was examined by an optical miscroscope, using a 40x objective (Ionita and Mitrea, 2013).

Giardia cysts (occasionally trophozoites) were identified based on the morphological characteristics (Figure 2, Figure 3).

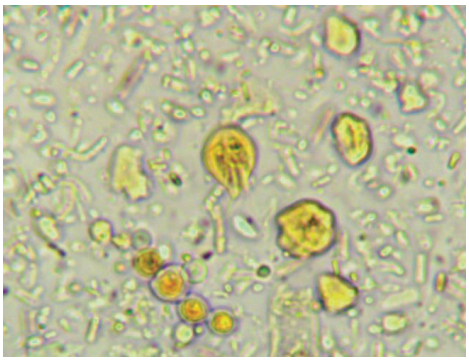

Figure2.*Giardia* trophozoite in dog faecal smear (Lügol staining; 40X)

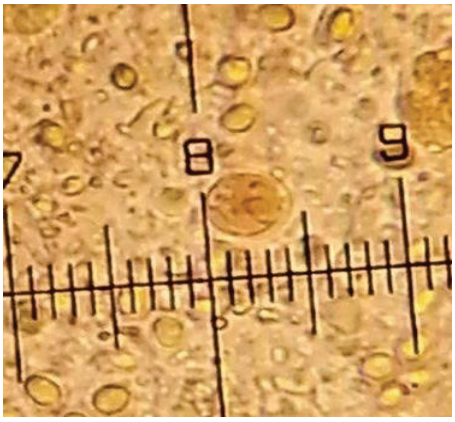

Figure 3.*Giardia* cyst in dog faecal smear (Lügol staining; 40X)

The statistical analysis was performed using Quantitative Parasitology 3.0 free software (Rozsa et al., 2000). 95% confidence interval (CI), *P*-values by chi-square test (for the differences of age class) were computed (Mitrea et al., 2013). Statistical significance was considered for $P \leq 0.05$.

RESULTS

For assessing the prevalence and the associated risk factors for *Giardia* and other parasite infections, we conducted a parasitological survey on 44 veterinary clinics in the six districts of Bucharest. The study comprised a total number of 267 pets – companion animals, including owned dogs (n=188) and cats (n=79) originating from the urban area of Bucharest.
Overall, of the total dogs and cats, 41.49% and 34.18%, respectively, were positive for at least one parasite infection. Detailed recorded data will be presented by host species, dogs and cats, respectively.

Dogs

In dogs, fecal samples were collected from 188 animals (107 males; 81 females), with age ranging from one month to 18 years (mean 2.23 years, SD=3.21), of which: 78 were puppies under 6 months old (43 males; 35 females), 35 were puppies between 6 months and 12 months (19 males; 16 females), 65 adults (40 males; 25 females) and 10 were old animals (5 males; 5 females). The majority of dogs (n=146) were pure-breed (36 different breeds) and the rest (n=42) were mixed-bred (Figure 4).

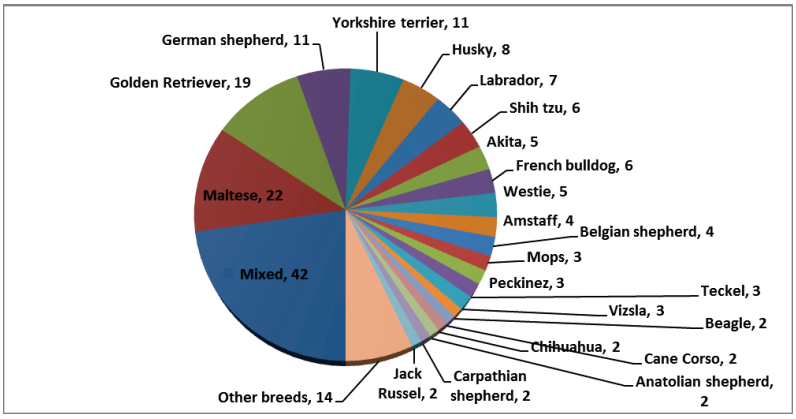

Figure 4. Dog breeds included in study

Of the total dogs, 105 (55.85%) displayed clinical signs such as vomiting, soft faeces, fetid diarrhea, and/or presence of mucus in stool. Eighty-three dogs (44.15%) were considered as clinically healthy on the time of presentation.

All dogs originated from the studied area and had access to urban green areas/parks in the municipality of Bucharest.

Overall, out of the total 188 dog faecal samples investigated, 78 (41.49%; 95%CI: 34.36 - 48.89) were found positive for at least one intestinal parasite species. 66 samples (35.11%) were positive for one parasite species and in 12 samples (6.38%), were detected mixed infections. Of the positive subjects, 60 dogs showed digestive clinical signs (60/78; 76.92%).

Giardia cysts were found in 40 of the 188 samples (21.28%) (Table 1).

Table 1. Prevalence of *Giardia* cysts in faecal samples of owned dogs, stratified by age and gender

	Positive sample (%) / Samples by age and sex								Total
Age	1month≤6months		>6months ≤ 1year		>1 year ≤ 8 years		>8 years		
Sex	M	F	M	F	M	F	M	F	
Dogs	12/43	8/35	5/19	2/16	3/40	7/25	2/5	1/5	40/188
Dogs Total	20/78 25.64%		7/35 20.00%		10/65 15.38%		3/10 30.00 %		21.28 %
	27/113 (23.89%)				13/75 (17.33%)				
	P = 0.282								

Six dogs were found co-infected with other parasites, as follows: *Isospora* spp. (in 2 samples), *Ancylostoma caninum* (in 3 samples) and *Trichuris vulpis* (in one sample) (Table 2).

The rate of infection, according to the age groups was higher in old dogs (30.00%), followed by puppies under one year old (23.89%), and adults (15.38%) (Figure 5).

When comparison was made between infected dogs <1 year old (and dogs >1 year old, a higher prevalence was found in the first age group (27/113; 23.89%) than for the second group (13/75; 17.33%), but no statistically significant (*P*=0.282).

Males (n=107) and females (n=81) had close value prevalences 20.56% and 22.22% (*P*=0.783).

Figure 5.Prevalence of*Giardia* cysts in faecal samples of owned dogs, according to age and gender category

Regarding the breeds, *Giardia* infection was found most frequently in Golden Retriever (n=8), mixed (n=8), Belgian Shepherd (n=3) and Maltese Bichon (n=3). Other affected breeds were Amstaff, Husky and Westie, with 2 samples each, and Akita, Beagle, Chihuahua, Anatolian Shepherd, German Shepherd, Corgi, Argentinian Mastiff, Jack Russel, Kangal, Labrador, Malinois and Yorkshire Terrier with one sample each.

Of the 40 positive subjects for *Giardia* infection, 35 displayed digestive clinical signs (ranging from soft faeces, diarrhea, flatulence to blood drops in faeces). It is noteworthy that five animals positive for *Giardia* showed noclinical digestive signs. They were from all age categories: 2 smaller than 6 months, one puppy between 6 and 1 year old, one adult and one old dog.

Additionally, were also found other parasites, as follows: *Isospora* spp. (12.23%), *Ancylostoma caninum* (5.85%), *Toxocara canis* (4.26%), *Uncinaria stenocephala* (0.53%), *Toxascaris leonina* (0.53%) and *Dipylidium caninum* (0.53%) (Table 2).

Among these, were found 4 types of associations: *Isospora* spp. and *Toxocara canis*, *Ancylostoma caninum* and *Toxocara canis* associations were found each one in 2 samples; *Isospora* spp. and *Ancylostoma caninum*, *Trichuris vulpis* and *Toxascaris leonina* associations were found in one sample each (Table 2).

Table 2. *Giardia* infection prevalence and other intestinal parasites, including co-infections, in owned dog originating from urban area of Bucharest; presence (+/-) of clinical signs

Parasite species and/or associations	Puppies (≤1 year)		Adult (>1 ≤8 yrs)		Old (>8years)		Total		Clinical signs	
	No.	%	No.	%	No.	%	No.	%	+	-
Giardia duodenalis total	27	23.89	10	15.38	3	30	40	21.28	35	5
G. duodenalis as single parasite	26	23.01	6	9.23	2	20	34	18.09	29	5
G. duodenalis and *Isospora* spp.	1	0.88	1	1.54	0	0	2	1.06	2	0
G. duodenalis and *A. caninum*	0	0	3	4.62	0	0	3	1.60	3	0
G. duodenalis and *T. vulpis*	0	0	0	0	1	10	1	0.53	1	0
Isospora spp	18	15.93	0	0	0	0	18	9.57	15	3
Isospora spp. with *T. canis*	2	1.77	0	0	0	0	2	1.06	2	0
Isospora spp. with *A. caninum*	1	0.88	0	0	0	0	1	0.53	1	0
Ancylostoma caninum	2	1.77	3	4.62	0	0	5	2.66	4	1
A. caninum and *T. canis*	2	1.77	0	0	0	0	2	1.06	0	2
Toxocara canis	3	2.65	1	1.54	0	0	4	2.13	2	2
Trichuris vulpis	1	0.88	2	3.08	0	0	3	1.60	0	3
T. vulpis with *T. leonina*	1	0.88	0	0	0	0	1	0.53	1	0
Uncinaria stenocephala	0	0	1	1.54	0	0	1	0.53	0	1
Dipylidium caninum	0	0	0	0	1	10	1	0.53	0	1

Cats:

Cats were predominantly of common breed cats (n=60), the rest 19 belonging to 8 breeds as shown in Figure 6. There were 47 males and 32 females, with age varying between one month and 16 years (mean 2.26 years; SD = 3.24). Digestive signs were registered only in 34 of the subjects and ranged from soft stools to vomit, vomit and stools with parasitic elements, stools with streaks of blood.

Out of the 79 investigated cats, 27 were positive for parasite infections (34.18%; 95% CI: 23.87 - 45.71). In 22 (27.85%) samples, one single parasite species was detected, while in five (6.33%) were found mixed infections: 4 (5.06%) with two species and 1 (1.27%) for three parasite species. Out of the 27 positive cats for at least one parasite, 17 (62.96%) manifested clinical signs.

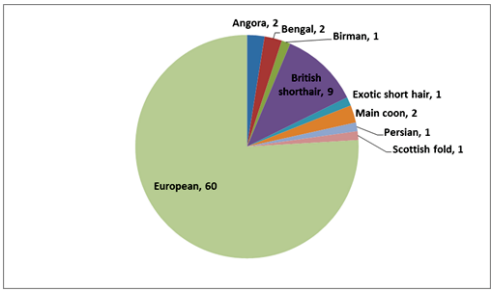

Figure 6.Cat breeds included in the study

Giardia cysts were found in 18 samples (22.78%) (Table 3).

Out of these, there were found mixed infections with *Isospora* spp. (in one sample), with *Toxocara cati* (in 2 samples) and with both (*Isospora* spp. and *T. cati*) in one sample (Table 4).

Higher prevalence was found in kittens under one year old (26.42%) compared to adults (14.29%) (Figure 7). No race susceptibility was noted. Females had higher prevalence (28.13%) than males (19.15%), but with no statistical significance $P = 0.35$.

It should be noted that 6 cats with positive samples for *G. duodenalis* showed no clinical digestive signs. They were 5 kittens under 6 months old and one cat of 2 years old.

Other parasite species were recorded, as follows: *Toxocara cati* (10.13%) and *Isospora* spp. (8.86%)

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Table 3. Prevalence of *Giardia* cysts in faecal samples of owned cats, stratified by age and gender category

	Positive sample (%) / Samples by age and gender								Total
Age	1month ≤=6months		>6months <= 1year		>1 year ≤8 years		>8 years		
Sex	M	F	M	F	M	F	M	F	
Cats	6/23	7/16	1/7	0/7	1/13	2/8	1/4	0/1	
Cats Total	13/39 (33.33%)		1/14 (7.14%)		3/21 (14.29%)		1/5 (20.00%)		18/79 22.78%
	13/39 (33.33%)		5/40 (12.50%)						
	P = 0.027								

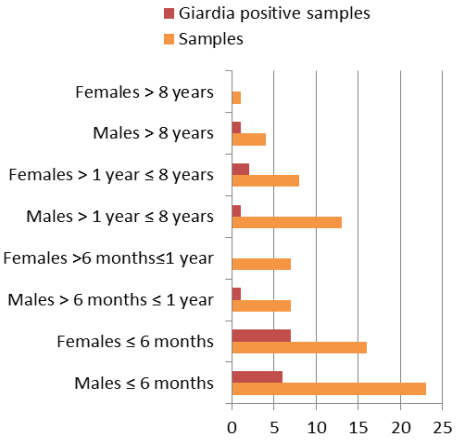

Figure 7. Prevalence of *Giardia* cysts in faecal samples of owned cats, according to age and gender category

Table 4. Prevalence of *Giardia* infections and other parasitic associations found in cats; presence (+/-) of clinical signs

Parasite species and/or associations	Kittens ≤6 months		Kittens >6mos≤1 yr.		Adult cats >1 ≤8years		Old cats >8 years		Total		Clinical signs	
	No.	%	No.	%	No	%	No.	%	No.	%	+	-
Giardia duodenalis total	13	33.33	1	7.14	3	14.29	1	20.00	18	22.78	12	6
G. duodenalis as single parasite	10	25.64	1	7.14	2	9.52	1	20.00	14	17.72	9	5
G. duodenalis and *Toxocara cati*	2	5.13	0	0	0	0	0	0	2	2.53	1	1
G. duodenalis and *Isospora* spp.	0	0	0	0	1	4.76	0	0	1	1.27	1	0
G. duodenalis, *Isospora* spp. and *Toxocara cati*	1	2.56	0	0	0	0	0	0	1	1.27	1	0
Isospora spp.	3	7.69	1	7.14	0	0	0	0	4	5.06	2	2
Toxocara cati	4	10.26	0	0	0	0	0	0	4	5.06	3	1
Isospora spp. *and Toxocara cati*	1	2.56	0	0	0	0	0	0	1	1.27	0	1

DISCUSSION

Here we present a study assessing the prevalence of *Giardia duodenalis* in owned dogs and cats from Bucharest area. The rate of *Giardia* infection was recorded in quite similar prevalence for the both, dogs (21.28%) and cats (22.78%).

The resemblance is maintained even within age groups: 23.89% and 26.42% in puppies respectively kittens, 15.38% in adult dogs and respectively 14.29% in adult cats and 30.00% in old dogs and 20.00% old cats.

The age is considered a risk factor; in this study, subjects under 6 months old had registered higher prevalence values than average: puppies 25.64% (average was 21.28%), kittens 33.33% (average = 22.78%). In case of the kittens ≤ 6 months old (13/39; 33.33%) compared with cats > 6 months old (5/40; 12.5%) a statistical significance was noted (*P*= 0.027) highlighting that age is a risk factor for *Giardia* infection in kittens under 6 months.

Dogs under one year old were having a prevalence of 23.89%, and dogs older than one year having 17.33%. The same can be observed for cats aged under one year (26.42%) and those over one year old (15.38%), but not statistical significant (*P*=0.303; *P*=0.394, respectively).

The mean prevalence values found in this study (of approximately 22%) is higher when compare with results reported from similar studies in Romania, for instance of 4.8% in clinically healthy household dogs from urban

areas in Romania (Mircean et al., 2012). This can be explained by the fact that the subjects of this study were animals brought to the veterinary clinics with various health problems, which can result in a higher prevalence than a simple screening. However, these values were lower (36.1%, South-Eastern Romania; 51.08% in Satu Mare county) than reported in studies by imunoenzymatic test ELISA (Jarca et al., 2008; Sommer et al., 2015) and 42.62% by Lügol staining in stray dogs from Timis county (Sorescu et al., 2014).

All these studies report occurrence of *Giardia* infection, based on different size/types of studies (epidemiological, clinical, etc.), therefore, variations are expecting.

Based on anamnesis or reports of clinicians, pets are often dewormed using common products primarily against worms, but that do not act on *G. duodenalis* and *Isospora* spp., increasing the risks of subclinical infections. The absence of clinical signs and the proximity to humans of this category of animals, living in the same space can increase the risk of zoonotic transmission of infection. Therefore, the owner needs to be instructed by the veterinarian to perform regular checks for coproparasitological of their animals.

Similar values are found in many places, almost independent of geographical area. Therefore in Northern Italy (Zanzani et al., 2014) in metropolitan and micropolitan area of Milan, G. *duodenalis* was the most common of the detected parasites. In dogs from micropolitan areas the prevalence varied between 20.37% - 25.58% and in the metropolitan area was of 16.05%. In cats, the prevalence in two micropolitan areas varied between 25.00% - 36.84% and in the metropolitan areas was 24.7%. In Germany, a study conducted between 2003 and 2010 (Barutzki et al., 2011) highlighted the overall value of G. *duodenalis* prevalence of 18.6% for dogs and 12.6% for cats, as well as the distribution according to age. The highest values of prevalence were up to 3 months (37.5% for dogs and 19.5% for cats) and between 3 and 6 months (38.2% for dogs and 24% for cats) (Barutzki et al., 2011).

In the Parisian area (Beugnet et al., 2000) carried out a parasitological study on owned dogs and cats. It was found that 25% of dogs and 20% of cats were infected with parasites; age was the main prevalence factor: 56.5% of dogs under 6 months being infected with at least one parasite. G. *duodenalis* was the most widespread, 30.4% of the animals younger than 6 months were infected with this parasite.

Other studies, for Canada, provide data on the prevalence of G. *duodenalis* in dogs. Animals under one year old were infected at a rate of 17.36%, and those over two years old just at a rate of 4.15% (Joffe et al., 2011).

CONCLUSIONS

This study provides data on the prevalence of *Giardia* infection in owned dogs (21.28%) and cats (22.78%) from urban area of Bucharest. Animals younger than one year old were more susceptible to infection. Mixed parasite infections were recorded in the both dogs (6.38%) and in cats (6.33%), including parasites with zoonotic potential. Altogether, the findings of the present study are of relevance for the both animal and public health, emphasizing potential high risks for infection.

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