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A RAPID ANTIGEN TEST SCREENING FOR *Giardia duodenalis* INFECTION IN DOGS AND CATS WITH DIGESTIVE DISORDERS

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Abstract

Giardia duodenalis, a flagellate protozoan with potential zoonotic risk, is one of the frequent causes of diarrhea in animals and humans. The aim of the present study was to investigate the prevalence of G. duodenalis infections and associated risk factors in carnivores. For this, a total of 107 client-owned animals living in Bucharest area (Southeast Romania), including 89 dogs and 18 cats with a history of digestive disorders, were included in the study. Animals were of different breeds and different ages; dogs aged between 2 months and 13 years (average 3.08 years; standard deviation - SD = 3.06) whereas cats aged between 5 months and 16 years (average 3.12 years; SD = 3.07). Fresh fecal samples were collected and tested for the presence of coproantigen (Ag) of G. duodenalis using a commercially available rapid immuno-chromatographic test. Additionally, a subset of 55 fecal samples (44 from dogs and 11 from cats) were subjected for a copro-parasitological examination for detection of Giardia cysts and other parasitic elements (protozoan oocysts, helminth eggs), using zinc sulphate flotation method. Overall, 21.4% and 5.6% of dogs and cats, respectively, were positive for G. duodenalis copro-Ag. Furthermore, 31.8% of the 44 copro-parasitologically tested dogs were positive for parasitic infections, of which 20.5% (9/44) were positive for G. duodenalis cysts, as single 11.4% (5/44) or mixed 9.1% (4/44) infections with other intestinal parasites, such as Isospora spp., Toxocara canis, Ancylostoma caninum and Trichuris vulpis. All 11 cats tested negative by coproscopy. A good correlation between the Ag rapid test and microscopic identification of cysts for Giardia infection was registered. These findings confirm G. duodenalis and other intestinal parasites as causative agents of enteric disorders in client-owned dogs and cats and emphasize on potential zoonotic risks.

Key words: Giardia duodenalis, copro-antigen, cysts, digestive disorders, carnivores.

INTRODUCTION

Giardia duodenalis is a protozoan parasite that represents one of the common causative agents of digestive symptoms (such as diarrhea) in animals and humans (Feng and Xiao, 2011). Its zoonotic potential is widely known in relation to companion animals, as pets live in the proximity of the owner (Thompson, 2004). *Giardia* infections in dogs, especially in puppies, are clinically important due to their high prevalence, serious symptoms in some occasions, and its potential zoonotic risk (Liu et al., 2014).

In cats, however, *Giardia* infections can occur in healthy specimens, as well as some with acute or chronic small bowel diarrhea, with or without weight loss (Scorza and Lappin, 2004). Symptomatic giardiosis is frequently addressed to decrease symptom length, eliminate complications and reduce parasite spread to other hosts (Dixon, 2020).

The management of giardiosis implies an accurate diagnosis, as in the case of other parasites. Laboratory tests focus on detection of microscopic cysts in stool samples, as well on immunological-based testing and molecular methods. In all diagnostic procedures, different sensitivities and specifics may be encountered. This condition depends on the testing methodology, competences and performances of the assays used (Hooshyar et al., 2019).

The aim of the present study is to investigate the prevalence of *G. duodenalis* infections and associated risk factors in carnivores with digestive disorders.

MATERIALS AND METHODS

Between August 2014 and October 2018, a number of 107 client-owned animals, 89 dogs and 18 cats, which exhibited digestive symptoms, were included in the study. The dogs and the cats were of different or mixed

breeds, with ages ranging from 2 months to 13 years, and from 5 months to 15 years, respectively, all living within the metropolitan area of Bucharest, Romania. Animals were grouped in three different age categories, as presented in Table 1.

Table 1. Animals (dogs and cats) included in the study, categorized by age and gender

The animal species	Number of animals included in the study							
	Animal age and gender categories							
	≤ 1 year		1 - ≤ 8 years		> 8 years		Animal's average age (years) [standard deviation:SD]	Total number of animals
	Male	Female	Male	Female	Male	Female		
Dogs	19	7	35	23	4	1	3.08 [3.06]	89
Total	26		58		5			
Cats	2	4	6	3	2	1	3.12 [3.07]	18
Total	6		9		3			

Fresh fecal samples collected from the animals (89 dogs and 18 cats) were examined within 24-48 hours after sampling, using a rapid immunochromatographic *Giardia* copro-antigen test (Bionote Anigen Rapid Giardia Ag Test Kit.), in accordance with the manufacturer' recommendations.

Additionally, a subset of 55 fecal samples (44 from dogs and 11 from cats) were subjected for copro-parasitological examination for detection of *Giardia* cysts and other parasitic elements (protozoan oocysts, helminth eggs), using a flotation method (33% zinc sulphate solution, and Lügol staining) (Ioniță and Mitrea, 2013).

Moreover, for 7 samples (4 of dogs and 3 cats), a bacteriological exam was also performed.

RESULTS

In order to assess the prevalence of *Giardia* infection among owned dogs and cats showing digestive disorders, we conducted an investigation using rapid immunochromatographic test and copro-parasitological exams. In some cases, a bacteriological investigation was also carried out.

Animals were of different breeds and different ages: dogs' age varied between 2 months and 13 years (average 3.08 years; standard deviation - SD = 3.06 years), while for cats, the age varied between 5 months and 16 years (average 3.12 years; SD = 3.07 years).

Results of the Giardia - copro Antigen test

Out of the 107 animals tested, 20 were positive for the *Giardia* copro-Ag test, 19 dogs (19/89; 21.4%) and 1 cat (1/18; 5.6%). By age groups, the test was positive for 30.8% of the dogs up to 1-year-old; 17.2% of adult dogs and 20% dog over 8 years old. The positive cat was 10 year-old, belonging to the age group of over 8 years (Table 2).

Table 2. Data on positive animals (dogs and cats) for the *Giardia* - coproantigen test (stratification by age and gender)

Animal species		Positive animals/total number examined animals [number and percentage]						Total positive animals
		≤ 1 year		<1 - ≤ 8 years		>8 years		
		Male	Female	Male	Female	Male	Female	
Dogs		5/19 (26.3%)	3/7 (42.9%)	5/35 (14.3%)	5/23 (21.7%)	1/4 (25%)	0/1 (0%)	19/89; (21.4%)
	total	8/26 (30.8%)		10/58 (17.2%)		1/5 (20%)		*p = 0.331
Cats		0/2	0/4	0/6	0/3	1/2	0/1	1/18 (5.6%)
	total	0/6 (0%)		0/9 (0%)		1/3 (33%)		*p = 0.167

*p>0.05 with no statistic significant regarding positive *Giardia* Ag tests among age groups.

Results of the flotation test

The coproparasitological examination of the 44 dog samples revealed that 31.8% (14/44) were infested with at least one parasitic species as follows: *Giardia* sp. (20.45%; 9/44), *Isospora* spp. (6.8%; 3/44), *Toxocara canis* (11.4%; 5/44), *Ancylostoma caninum* (4.5%; 2/44) and *Trichuris vulpis* (2.3%; 1/44) (n = 44) (Table 3).

Additionally, mixed infections were identified in the case of the 9 dogs positive by flotation for *Giardia*: *Isospora* spp. (n = 3), *T. canis* (n = 1), *A. caninum* (n = 1) and *T. vulpis* (n = 1). It is worth noting the case of a dog identified with 4 parasitic species: *T. canis, A. caninum, Isospora* spp., *G.duodenalis*, but also the case of a positive antigen result and microscopic examination illustrating just the presence of *T. canis* (Table 3).

None of the cats (11) which were the subject of the coproparasitological examination was positive (the cat that was positive for the *Giardia* Ag test was not tested by flotation).

Table 3. Data on positive dogs for the flotation test, *Giardia* infection prevalence and other intestinal parasites, including co-infections (stratified by age category)

Parasite species and/or associations	Dog age category (number and percentage)						Total	
	Puppies (≤1 year)		Adult (>1-≤8 yrs.)		Old (>8 years)			
	No.	%	No.	%	No.	%	No.	%
Giardia duodenalis total	6	37.5(6/16)	2	8.3(2/24)	1	25.0(1/4)	9	20.5 (9/44)
G. duodenalis as single parasite	4	25.0(4/16)	1	4.2(1/24)	0	0 (0/4)	5	11.4 (5/44)
G. duodenalis and *Isospora* spp.	1	6.3(1/16)	1	4.2 (1/24)	0	0/4	2	4.5 (2/44)
G. duodenalis and *T. vulpis*	0	0/16	0	0/24	1	25.0(1/4)	1	2.3 (1/44)
G. duodenalis, Isospora spp., *A. caninum* and *T. canis*	1	6.3(1/16)	0	0/24	0	0/4	1	2.3 (1/44)
Ancylostoma caninum	0	0/16	1	4.2(1/24)	0	0/4	1	2.3 (1/44)
Toxocara canis (one sample was positive for *Giardia* Ag)	3	18.8(3/16)	1	4.2(1/24)	0	0/4	4	9.1 (4/44)

Results of the bacteriological test

Some of the bacteriological tested dogs that tested negative for both *Giardia* Ag test and coproscopical exam, were diagnosed with bacterial infections that consisted of: *Escherichia coli* (4/4), *Enterococcus* spp. serological group D (4/4), *Proteus mirabilis* (1/4), *Citrobacter youngae* (1/4), *Pseudomonas aeruginosa* (1/4), *Streptococcus canis* serologic group G (1/4). Bacterial infections were mixed, as 4 to 5 bacterial species were found in all of the dogs' sample: 1 with *Citrobacter youngae*, *Escherichia coli*, *Enterococcus* spp. serologic group D, *Pseudomonas aeruginosa*; another 1 with *Escherichia coli*, *Enterococcus* spp. serologic group D; an additional 1 with *Escherichia coli*, *Enterococcus* spp. serologic group D, *Streptococcus* serologic group G; 1, with *Escherichia coli*, *Proteus mirabilis*, *Enterococcus* spp. serologic group D (n = 4).

The cat which tested positive for *Giardia*-Ag was also positive at the bacterial exam, showing positive results for the following species: *Escherichia coli; Enterococcus* spp. *Serological group D; Proteus mirabilis.*

In the two case of cats that tested negative for both *Giardia* Ag test and coproscopical exam, they were positive for bacterial infections as following: *Escherichia coli* (2/2), *Enterococcus* spp. serological group D (1/2), *Enterobacter cloacae* (1/2). Similarly, bacterial infections were mixed in some cases, either with *Escherichia coli*, *Enterococcus* spp. serologic group D, *Proteus mirabilis* (1/3); or with *Escherichia coli*, *Enterobacter cloacae*, *Enterococcus* spp. serologic group D (1/3).

Thus, the clinical signs of the examined dogs (*Giardia* Ag test and coproscopical exam) were associated with the following parasite infections: *G. duodenalis,* single infection (22.7%; 10/44), co-infestations of *G. duodenalis* and other species, 11.4% (5/44), infestations with other parasitic species (other than *Giardia*) (9.1%; 4/44).

Bacterial infections represented a factor of symptomatology for 4 dogs and 2 cats (having negative results for the rapid test and coproparasitological examination). For cats it was found one co-infection of *G. duodenalis* and bacteria.

DISCUSSIONS

The aim of the present study was to investigate the prevalence of *G. duodenalis* infections and associated risk factors in carnivores, owned dogs and cats with digestive disorders. Our study has showed that the ascertained *Giardia* prevalence varies according to the animal species included in the study and the method used. The prevalence of *G. duodenalis* was higher in the case of dogs (21.4%) than cats (5.6%), even if the cats investigated were in lower number.

In Romania, screening studies were carried out, most of them in dogs, by means of coproscopic methods. *Giardia* prevalence in these studies ranged from 8.5% (Mircean et al., 2012), 21.3% (Soran et al., 2017) to 42.62% (Sorescu et al., 2014). Our study has shown a *Giardia* prevalence of 20.5% by coproparasitological exam.

Higher percentages were obtained by using ELISA tests: 34.6% (Mircean et al., 2012), 36.1% (29.5% in client-owned dogs) (Sommer et al., 2015), 42.1% (Sorescu et al., 2014) and 51.1% (Jarca et al., 2008). This can be explained by the better sensitivity of the tests, although in our study a good correlation was found between the microscopy and the tests. Furthermore, similar percentages (42.6%, 42.1%) have been observed in dogs from Timis county (Sorescu et al., 2014).

In cats, the prevalence observed (5.56%) is lower than reported in previous studies, of 27.9% (51/183), increasing up to 32% (16/50) in symptomatic cats (Mircean et al, 2011). By using flotation and Lügol staining, a 22.8% (18/79) prevalence was reported in owned cats in the urban area of Bucharest (Soran et al., 2017).

In comparison with similar studies in Europe, *Giardia* prevalence in dogs obtained by using microscopy ranged from 7.8% (Italy; Scaramozzino et al. 2018) to 25% (France; Osman et al, 2015). In other studies, by using rapid tests, a prevalence of 11.4% in dogs and 6.8% in cats were registered, all animals without owner, from foster homes (Germany; Becker et al., 2012), while other studies showed a prevalence of 18.6% in dogs and 12.6% in cats, from privately-owned pets (Germany; Barutzki and Schaper, 2011).

Using similar study designs, animals with digestive disorders and rapid copro-Ag testing, the reported prevalence was of 16% for dogs (Italy; Symeonidou et al., 2020) and 15.3% for cats (France; Epe et al., 2010). The prevalence of *Giardia* infection in dogs in our study (21.4%) is closer to the one from Italy, with cats having a smaller percentage (5.6%).

It is clear that the observed prevalences differ greatly depending on the diagnostic method used. In our study, the results of the fecal Ag rapid test and microscopic identification of cysts for detecting *Giardia* infections were consistent, confirming the copro-Ag test as a useful diagnostic tool for *Giardia* infections. Similarly, Giardia cysts were demonstrated in the majority of the ELISA-positive samples in the IFA, also for samples from Romania (82.4 %) (Sommer et al., 2015).

Recently, some research (Symeonidou et al., 2020) has shown that fecal examination with the Speed™ *Giardia* test was more sensitive than the parasitological method and results differed consequently.

Similarly, a recent study on comparing diagnostic tests for *G. duodenalis* in dogs showed that the copro-Ag-test had the highest specificity, followed by coproscopy (centrifugation sedimentation flotation microscopy), while qPCR showed the highest sensitivity. Therefore, depending on the purposes, qPCR is recognized as valuable screening tool, due of its high sensitivity, whereas for studies in which high specificity is required microscopy-based methods or Ag-test are recommended (Uiterwijk, 2018).

However, fecal flotation has the ability to detect also mixed infections with other parasites, as reported also for other parasite infections (Mitrea et al., 2013).

Thus, a combination of both immunoassay and microscopic techniques would provide more sensitive approach for detection of *Giardia* infection and other internal parasites (Saleh et al., 2019). Therefore, in the case of negative results but with reasonable suspicions, complementary tests should be carried out in order to have a certain diagnosis, as a base for an appropriate therapy and management protocol to be applied.

To be noted, 34 animals from our study (25 dogs and 9 cats) that showed digestive

symptoms had negative *Giardia* Ag tests and coproparasitological examinations. In these cases, a bacteriological examination would have been recommended, however this is largely a matter of availability.

Various studies have shown that the prevalence of *G. duodenalis* could be underestimated (Adell-Aledón et al., 2018; Epe et al., 2010), therefore additional studies are always necessary to shed more light into the real depth of such parasitoses.

CONCLUSIONS

These findings of the present study confirm *G. duodenalis* and other intestinal parasites as causative agents of enteric disorders in client-owned dogs and cats and emphasize on potential zoonotic risks.

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