



Prevalence of *Giardia* spp. in dogs with gastrointestinal symptoms in Beijing, China

KEYWORDS

Giardia spp., Dogs, prevalence, Beijing

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ABSTRACT

The prevalence of *Giardia* spp. was studied in dogs in Beijing. Fresh faecal samples were taken from dogs (n=800) with gastrointestinal (GI) clinical signs presented to seven veterinary clinics between January 2012 and December 2012. All samples were tested for *Giardia* infection using the IDEXX SNAP *Giardia* test kit and the conventional microscopic method. The prevalence of *Giardia* spp. was 20.25% (162/800) by the SNAP test, and only 21.6% (35/162) of the SNAP-positive samples were positive by microscopy. A total of 13 risk factors associated with prevalence of *Giardia* infection were investigated in this study, Age ($P<0.001$), breed ($P=0.009$), defecation site ($P<0.001$), and boarding history ($P=0.014$) had a significant impact on *Giardia* infection according to the Chi square test. Puppies (< 6 months old) had the highest incidence (31.78%) of *Giardiasis*. (OR=14.556). While the prevalence of the other two categories was 18.45% (6 months to 2 years old) and 3.27% (≥ 2 years old), respectively. Purebred dogs had a higher prevalence (21.46%, OR 1.6) than that of crossbred dogs (10.34 %). Of *Giardia*-positive dogs, 54.3 % were anorexic. Compared with the results of previous study in Guangzhou China (Li et al., 2012), this study showed relatively high rates of *Giardia* spp. infection in dogs in Beijing. *Giardia* infection should be considered in the differential diagnosis of dogs presented to veterinary practices with GI symptoms.

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1. Introduction

Giardia spp is a common intestinal protozoan transmitted by faecal-oral contact and infects humans and most animal species including dogs (Solarczyk and Majewska, 2010; Wang et al., 2012). *Giardia* infection in dogs occurs worldwide, and the prevalence varies greatly among different studies, which might be related to the age and living condition of the dog population (e.g. shelter, kennel, household, and stray dogs), season, region, animal density, nutritional and immune status and assay method (Epe et al., 2010; Scaramozzino et al., 2009; Tangtrongsup and Scorza, 2010). Besides intermittent shedding, the protozoan cysts of *Giardia* are rather small and transparent, which makes it quite challenging to identify them. In addition, the motile trophozoite is more often identified in fresh unformed or watery faeces rather than formed faeces, and decomposes rapidly once released into the environment (Carlin et al., 2006). Thus, conventional diagnostic methods, e.g. microscopic examination of cysts and trophozoites with or without flotation, often result in the underestimation of prevalence, especially for untrained assay analysts (Dryden et al., 2006; Upjohn et al., 2010).

With the rapid improvement of user friendly ELISA-based methods over the last two years, the SNAP *Giardia* Test had been proved to be an easy to use, highly sensitive and specific method for epidemiological research (Geurden et al., 2008). As mentioned, diagnostic methodology plays an important role in the variance of the results obtained. Thus, with high accuracy and availability, commercial assays such as SNAP *Giardia* test, provide consistent and comparable investigations (Carlin et al., 2006; Epe et al., 2010).

Despite occasional clinical signs of diarrhea and/or vomiting, dogs with *Giardiasis* may also be asymptomatic and serve as a potential reservoir for human infection. *G. duodenalis* (syn.

G. intestinalis, *G. lamblia*) is the only species of *Giardia* spp. infecting both humans and animals, and increasing molecular evidence indicates that dogs may be infected with zoonotic genotypes A and B as well as host-specific genotypes C and D (Solarczyk and Majewska, 2010; Wang et al., 2012). Therefore, as ever increasing number of homes in Beijing have dogs as companion animals, *Giardia* infection in dogs is of great concern for public health, and thus a prevalence survey of canine *Giardia* infection is long overdue.

This is the first study to investigate the prevalence of *Giardia* spp. in dogs in Beijing by testing the faeces of symptomatic dogs using the SNAP *Giardia* Test.

2. Materials and methods**2.1. Faecal samples**

Fresh faecal samples (n=800) were collected from dogs with gastrointestinal (GI) symptoms in 7 large veterinary clinics in Beijing from January 2012 to December 2012. Animal age, gender, breed, vaccination status, anthelmintic treatment history, owner location, defecation site, diet, living environment (indoor moving freely, encaged or outdoor in the yard), presence of other dogs in the household, and pet-boarding history were recorded.

Faeces were collected from defecation induced by glycerine anal injection, or directly from the rectum either by hands or by a catheter according to the dog's size and body condition. Faecal consistency was scored on a five-point scale ranging from watery stool (5 points), unformed stool (4 points), normal stool (3 points), firm stool (2 points) to hard stool with concomitant constipation (1 point) (Zentek et al., 2004).

2.2. Detection of *Giardia* spp. in faecal samples

All faecal samples were analysed for *Giardia* cysts or tropho-

zoites by microscopy and *Giardia* antigens by the SNAP *Giardia* test (IDEXX Laboratories, Westbrook, ME, USA). Fresh faecal samples were diluted with 5 ml saline solution and were prepared for microscopic examination by direct smear within 1 h of faeces collection. The SNAP *Giardia* Test for the detection of *Giardia* spp antigens was performed according to the manufacturer's instructions.

2.3. Statistical analysis

Data were analyzed statistically using SPSS for Windows (Release 20.0 standard version, SPSS Inc., Chicago, USA). The positive or negative *Giardia* spp. samples was categorized by different risk factors. To assess whether these factors are in association with *Giardiasis*, Chi-square test is initially applied to the variables (considered significant if $P < 0.05$). A multinomial logistic regression model was then utilized to determine the level of significance based on the odds ratio (OR) (Hosmer and Lemeshow, 1989).

Results of the two diagnostic methods were compared by the Kappa test for consistency. The two methods were regarded to have consistent results if $P < 0.05$, and the consistency was considered good if Kappa value ≥ 0.75 and poor if Kappa < 0.4 .

3. Results

Of 800 samples, 162 samples (20.25%) were positive to *Giardia* spp. by the SNAP test. According to the Chi-square test, which analyses the correlation between the risk factor and *Giardiasis* separately, age ($P < 0.001$), breed ($P = 0.009$), defecation site ($P < 0.001$), and boarding history ($P = 0.014$) had a significant impact on *Giardia* infection. Based on the multinomial logistic regression test, which take the integrated effect of all the risk factors into consideration, age ($P < 0.001$) and vaccination ($P = 0.016$) had a significant correlation with *Giardia* infection (Table 1).

The prevalence of *Giardia* infection in pups ≤ 6 months old and 0.5-2 years old was 31.78% (OR 14.6) and 18.45% (OR 6.7), respectively, compared with 3.27% for ≥ 2 years older dogs. Pure breed dogs had a prevalence of 21.46% (OR 1.6), higher than 10.34% in crossbreed dogs (Table 1).

As revealed by the multinomial logistic regression test, dogs defecating indoor without a fixed site were more predisposed to *Giardia* infection than that defecating indoor at a fixed site (OR 0.878) or outdoor (OR 0.972). Moreover, dogs who had a pet-boarding history in 2 weeks got a slightly higher prevalence of *Giardia* (OR 1.173). As expected, *Giardiasis* was more likely to be positive in dogs who had not been vaccinated.

For dogs with diarrhoea, 22.08% was positive, dogs with vomiting 18.26% and those having both clinical signs 19.36%. Only 3.75% of the dogs presented chronic diarrhea (lasting for more than 3 weeks).

Of *Giardia*-positive dogs, 26.5% had decreased appetite, 27.8% was anorexic and 45.7% had normal appetite. A total of 48.1% *Giardia*-positive dogs appeared lethargic while the others had normal activity.

Dogs surveyed in this study were mostly from Haidian ($n = 448$) and Chaoyang ($n = 123$) Districts. All districts were analysed by Chi-square test and no significant difference was found between them ($P = 0.622$). The detailed distribution of positive samples by districts are presented in Table 2.

Only 35 (4.38%) samples were positive by microscopic examination, and neither cysts nor trophozoites were found in the remaining samples. All positive samples by microscopy were also positive by the SNAP test. There was a poor consistency in the detection of *Giardia* infection between the IDEXX SNAP *Giardia* test and routine microscopic examination (Kappa test, $k = 0.305$, $P = 0.000$).

4. Discussion

There has been no report of the prevalence of *Giardia* infection in dogs in Beijing, China. The prevalence of *Giardia* in pet dogs in Guangzhou, China was reported to be 8.61% and 11.00%, respectively, by microscopic and PCR examination of faecal samples (Li et al., 2012). It is expected that prevalence may vary in different dog populations because of different risk factors in each population (Carlin et al., 2006; Epe et al., 2010; Itoh et al., 2011). The prevalence could also be affected by sensitivity of the diagnostic method, and intermittent shedding of *Giardia* cysts (Geurden et al., 2008). Using the IDEXX SNAP *Giardia* test kit, we found that the prevalence of *Giardia* infection in dogs with GI symptoms was 20.25% in Beijing, China. This relatively high rate of *Giardia* positivity indicates that *Giardia* infection may be a concern in dogs with GI symptoms in Beijing.

There is no gold standard in the diagnosis of *Giardia* spp. infection (Traub et al., 2009), and four diagnostic methods are available to evaluate prevalence of *Giardia* spp. in dogs, including zinc sulphate flotation and microscopy (ZME), immune-fluorescence antibody testing (IFAT), nested PCR, and commercial CELISA, and the sensitivity and specificity varied among these methods (Traub et al., 2009). As a routine examination method of faecal samples for parasite infection, microscopy has very low sensitivity as *Giardia*-infected dogs do not excrete cysts all the time (Geurden et al., 2008), but it has high specificity. Floatation with saturated solutions such as zinc sulphate, sodium chloride, and sucrose is commonly used for *Giardia* cysts identification. However, sodium chloride flotation and a commercial ELISA kit used in a survey of the same population for *Giardia* spp. gave marked different prevalence rates (8.5% and 34.6%, respectively) (Mircean et al., 2011). In another study in Canada, only 0.1% dogs were found to be *Giardia*-positive by the flotation method (Shukla et al., 2006). Similarly, we found only a small number of *Giardia* positive samples (35/800, 4.38%), compared to 20.25% by the ELISA-based SNAP assay. If sucrose is used in the flotation method, the high specific gravity of sugar solutions distorts parasite cysts, reducing the detection rates. Even using the recommended zinc sulfate as the flotation medium, identification by inexperienced staff may still be an issue (Carlin et al., 2006). IFAT and PCR can be time-consuming and expensive. The presence of soluble cyst antigen in fecal samples indicates the existence of *Giardia* trophozoites or cysts in the intestine and may be shedding cysts in the faeces (Olson et al., 2010), although the cysts or trophozoites may escape from detection by microscopy. For this reason, the SNAP *Giardia* test is the method of choice for use in veterinary clinics.

There were about 50 breeds in our survey, and they were grouped to pure breeds and crossbreeds. Although there was a significant difference between the two groups, the small sample size of the crossbred dogs ($n = 87$ cf. 713 pure breeds) made the comparison unreliable. One study in Romania (Mircean et al., 2012) found no difference between the two categories ($P = 0.15$) and another study of dogs in a London rescue shelter, Rottweilers were found to be more predisposed to infection than Staffordshire Bull Terriers (Upjohn et al., 2010).

Consistent with findings of many other surveys (Claerebout et al., 2009; Epe et al., 2010; Upjohn et al., 2010), gender was not a risk factor of *Giardia* infection according to our study. Unlike gender, age played an important role in *Giardia* infection. Puppies of ≤ 6 months old, whose immune system are not well-established, were most susceptible to *Giardiasis*, which is in agreement with many previous studies (Claerebout et al., 2009; Epe et al., 2010; Itoh et al., 2011; Li et al., 2012; Mircean et al., 2012).

No obvious seasonality and regional difference were found to be associated with *Giardia* infection in our survey, different from a US and an Italian study. A large difference between

geographic locations was reported in a US study (Carlin et al., 2006), while a higher prevalence in winter than in spring or summer among dogs and cats was noted in an study in Pisa, Italy (Bianciardi et al., 2004).

For most intestinal parasites, infection is manifested clinically only when parasite load is sufficiently high, while infections with low parasite load are usually asymptomatic (Claerebout et al., 2009). Dogs with *Giardiasis* may look healthy with normal appetite and rare vomiting (Villeneuve, 2009). Immature dogs infected with *Giardia* are more likely to present clinical signs, while adults are usually asymptomatic (Kirkpatrick, 1987). Acute and self-limiting diarrhea is often presented in younger dogs, while diarrhea in older dogs can be acute or chronic, or intermittent or continuous (Barr, 2006). Diarrhea increases the risk of excreting *Giardia* cysts (OR 1.376), and significantly higher prevalence was found in symptomatic dogs than in asymptomatic animals (Itoh et al., 2011; Li et al., 2012; Liu et al., 2008). The lack of association between faecal consistency and *Giardia* infection partly proved the existence of sub-clinical infection (Upjohn et al., 2010), in spite of this, the positive rate in our survey was a little higher for unformed/watery stools than normal/firm stools (20.55% cf. 14.63%).

Giardiasis is usually transmitted through the ingestion of cysts via faeces or faeces-contaminated food or water. Infection can be exacerbated by poor dietary hygiene and sanitation (Carlin et al., 2006; Traub et al., 2009). Dogs fed on commercial wet food was previously found to have higher prevalence of *Giardia* infection (Traub et al., 2009). In our study, most dog owners (85.13%) fed their dogs commercial dog food, occasionally supplemented with rice, meat, egg yolk, and/or milk, but no significant correlation was found between the food types and *Giardia* infection. *Giardia* can also be caused by outbreaks of waterborne infection (Olson et al., 2010). However, in our survey, only 3.4% of the dogs had free access to public water sources, swimming pool or pond, and 14.8% of them were *Giardia* positive. The majority of the dogs investigated (76.1%) were drinking purified or boiled water. So, our findings can not explain whether drinking water was the source of *Giardia* infection.

Cysts are environmentally resistant and can survive several months in cold, wet conditions. Cysts could be ingested by the same host known as re-infection, which is especially more likely to occur in dogs confined in a small cage at home and those defecating indoors without prompt disposal by the owner (Carlin et al., 2006; Rinaldi et al., 2008). Supporting the above, we found that *Giardia* infection was associated with defecation site, with defecation indoors having higher prevalence (26.8%) than that outdoors (15.06%).

As was demonstrated in some other surveys (Li et al., 2012; Claerebout et al., 2009), *Giardia* prevalence was relatively

high in shelters or kennels where dogs were intensively reared with poor sanitary conditions. In our study, two dogs housed in one household had a higher infection rate (23.97%) than one dog per household. This is different from a European survey where one dog per household had a surprisingly higher *Giardia* infection rate (31.52% no other animal in the household cf. 20.16% with one other animal) (Epe et al., 2010).

As was the case with other infectious disease, immune-compromised individuals were susceptible to *Giardiasis* (Upjohn et al., 2010). In accordance with our study, dogs without vaccination had higher prevalence. Dogs coming into a new environment might have lower immune function, which was possibly the reason why dogs having been pet-boarded had higher infection rate. Nevertheless, We also found that anthelmintic treatment history had no impact on *Giardia* infection, similar to the study of 27 breeding kennels in northern Belgium (Claerebout et al., 2009).

The genotype of *Giardia spp.* was not the focus of our study. Cautions have to be made with the underlying zoonotic infection of *Giardiasis*. According to a genetic analysis, *Giardia duodenalis*, which can infect several species, including human, dogs and cats, has at least 7 distinct assemblages (A-G), with assemblages A, and B considered zoonotic (Tangtrongsup and Scorza, 2010). A study conducted in Guangzhou, China detected 21.7% assemblage A from canine faecal samples using PCR (Li et al., 2012). In another study in Bangkok, Thailand, assemblage A comprised 79% of the genotypes isolated in dogs, 12% assemblage C and 31% D (Traub et al., 2009). *Giardia* infection is a public health issue. People who may have close contact with dogs must be aware of the potential risk and take appropriate protective measures to prevent infection (Upjohn et al., 2010).

In conclusion, this is the limitative survey of the prevalence of *Giardia. Spp* in household dogs with GI symptoms presented to veterinary practices in Beijing. Using the IDEXX SNAP *Giardia* test kit, we found a high prevalence of 20.25% in dogs. Thus, it is recommended that *Giardia* infection should be considered in the differential diagnosis of dogs presented to veterinary practices with GI signs, particularly in dogs with the high risk factors (Age, breed, defecation site, vaccination status and boarding history). Compared to the limitation of traditional diagnostic techniques, the IDEXX SNAP *Giardia* test is easy to operate with satisfactory sensitivity and specificity, and it can be widely used in veterinary practices.

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Table 1 Prevalence of *Giardia* infection associated with 13 risk factors analyzed by the Chi-square test and multinomial logistic regression test.

Risk factor	Sample size (n)	Positive to <i>Giardia</i> (n)	Prevalence(%)	P(chi-square Test)	P(regression test)	Odds Ratio (95% Confidence Interval)
Total	800	162	20.25			
Season						
Spring	223	49	21.97	0.588	0.967	0.984(0.462-2.098)
Summer	216	37	17.13		0.551	0.792(0.367-1.708)
Autumn	297	63	21.21		0.798	0.907(0.431-1.909)
Winter	64	13	20.31			1

Breed							
Pure breed	713	153	21.46	0.009	0.215	1.635(0.751-3.557)	
Cross breed	87	9	10.34			1	
Gender							
Male	487	101	20.74	0.667	0.801	1	
Female	313	61	19.49			0.951(0.645-1.403)	
Age							
≤6 months	387	123	31.78	0.000	0.000	14.902(6.793-32.693)	
0.5-2 years	168	31	18.45			6.843(3.026-15.475)	
≥2 years	245	8	3.27			1	
Number of dogs per household							
One dog	559	109	19.5	0.473	0.985	0.994(0.529-1.865)	
two dogs	146	35	23.97			0.512	1.266(0.626-2.561)
≥three dogs	95	18	18.95			1	
Vaccination ¹							
Yes	550	106	19.27	0.616	0.016	0.586(0.379-0.907)	
No	250	52	20.80			1	
Antiparasite treatment history							
Yes	325	60	18.46	0.296	0.091	1	
No	475	102	21.47			1.418(0.945-2.127)	
Clinical signs of GI disorder							
Diarrhea	308	68	22.08	0.577	0.123	1.376(0.918-2.062)	
Vomiting	115	21	18.26			0.772	1.104(0.564-2.164)
Both	377	73	19.36			1	
Fecal consistency score ²							
2&3	41	6	14.63	0.623	0.943	1.044(0.317-3.445)	
4	610	125	20.49			0.884	1.038(0.625-1.725)
5	149	31	20.81			1	
Food							
A*	681	145	21.29	0.069	0.800	1.084(0.581-2.022)	
B*	119	17	14.29			1	
Living environment							
Indoor – moving freely	654	128	19.57	0.446	0.640	1.197(0.564-2.538)	
Indoor – encaged	86	22	25.58			0.761	1.146(0.477-2.750)
Outdoor in the yard	60	12	20.00			1	
Defecation site							
Outdoor	445	67	15.06	0.000	0.910	0.972(0.598-1.582)	
Indoor at a fixed site	168	42	25.00			0.612	0.878(0.530-1.453)
Indoor not at a fixed site	187	53	28.34			1	
Pet-boarding							
Yes	156	43	27.56	0.014	0.495	1.173(0.742-1.853)	
No	644	119	18.48			1	

1 Vaccination meant dogs were vaccinated against canine distemper virus, adenovirus, parvovirus, parainfluenza virus, and rabies virus.

2 Faecal consistency was scored on a five-point scale ranging from watery stool (5 points), unformed stool (4 points), normal stool (3 points), firm stool (2 points) to hard stool with concomitant constipation (1 point) (Zentek et al., 2004).

* A: commercial dog food supplemented occasionally with rice, meat, egg yolk or milk; B: commercial dog food, occasionally adding home-made or raw/unprocessed food.

Table 2 Prevalence of Giardia infection in different districts in Beijing

Districts	Number of Positive cases	Number of negative	Prevalence
HaiDian	88	360	19.64
ChaoYang	25	98	20.33
CangPing	14	54	20.59
FengTai	13	28	31.71
XiCheng	8	21	27.59
ShiJingShan	2	15	11.76
DaXing	2	15	11.76
TongZhou	2	13	13.33
Other districts*	8	34	19.05

* Other districts included FangShan, DongCheng, ShunYi, MenTouGou, PingGu, HuaiRou, YanQing, MiYun districts in Beijing.

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