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Prevalence of *Giardia* spp. in young dogs using a combination of two diagnostic methods

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Abstract

In this study, prevalence of the protozoan parasites from the genus *Giardia spp*, with zoonotic potential and worldwide dissemination, was accessed in young dogs, which are reported as having higher prevalence rates. With that purpose, 49 animals from the Grupo de Intervenção Cinotécnico of the Guarda Nacional Republicana (Portuguese Gendarmerie Canine Unit) were chosen. They were housed individually in areas with a high number of kennels (up to 100), with ages ragging from newborns to 10 years old. Dogs were divided in four groups, according their age: under 6 months (n = 16), 6–12 months (n = 6), 12–18 months (n = 13) and 18–24 months (n = 14), comprising 22 females and 27 males. Fecal samples were collected from every animal and all were submitted to two different diagnostic tests, a passive flotation technique with a $ZnSO_4$ solution and a detection of fecal antigen using a commercially available ELISA test (Witness® Giardia – Zoetis). From the 49 samples, 5 (10.2%) were considered positive with $ZnSO_4$ flotation technique and 6 (12.24%) with the Witness® Giardia test. When considering the combination of both tests, 5 animals (10.2%) were considered positive. Of these, 3 (60%) were from the group under 6 months old, 1 (20%) from the 6–12 months and 1 from the 18–24 (20%) months. Within each group, in the under 6 months group 18.75% (n = 16) were considered positive, 16.67% in the 6–12 month group (n = 6), 0% in the 12–18 month group (n=13) and 7.14% in 18–24 month group (n = 14). None of the animals had clinical signs and no significant differences were found when comparing prevalence according to age, breed or gender. A combination of fecal flotation and antigen ELISA tests have good sensitivity and are easy to perform in practice and, therefore, could be a good choice to perform a diagnostic and small animal veterinarians should have this possible diagnostic in mind when in the present of clinical signs, particularly in young dogs.

Keywords

Young dog, Giardia, fecal flotation, ELISA

Introduction

Flagellates from the genus *Giardia* infect human and animals worldwide with six recognized species (Hamnes *et al*. 2007). Of these, only *Giardia duodenalis* (syn. *G. intestinalis* or *G. lamblia*) is known to infect multiple hosts, namely people, dogs and cats (Hamnes *et al*. 2007; Scaramozzino *et al*. 2009; Tangtrogsup and Scorza 2010). Two million cases of symptomatic giardiasis are recorded in Asia, Africa and Latin America, representing the most common intestinal protozoan parasite in humans worldwide, as well as in animals (WHO 1995). In dogs, Giardia is one of the most prevalent parasite (Solarczyk and Majewska, 2010).

There are at least seven assemblages (A-G) in the species *Giardia duodenalis*. Some have host specificity and not all are associated with disease in humans (Ballweber *et al*. 2010; Tangtrogsup and Scorza 2010). Assemblages A and B occur in people and have also been reported in dogs and cats, with subgenotype A1 being the major responsible for dog-human transmission. Assemblages C and D are restricted to dogs, although mixed infections of assemblages A through D have already been reported, and assemblage F is restricted to cats (Thompson, 2004; Bowman and Lucio-Forster 2010; Solarczyk and Majewska 2010).

The main responsible for the transmission of the disease is an environmental resistant cyst, which can survive several months outside a host, and the infection starts through the ingestion of contaminated food or water (Tangtrogsup and Scorza 2010; Castro-Hermida 2011). The latter is being increasingly recognized as an important vehicle in the transmission of *Giardia*, including surface water, shallow wells and household water, but outbreaks have also been associated

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with drinking and recreational water, which is considered a serious public health problem as the cysts are capable of surviving the treatment process (Wolfe 1992; Thompson 2000; Carlin *et al*. 2006; Castro-Hermida 2011). For a veterinarian, the diagnosis of *Giardia* is, therefore, very important not only for the management of each particular animal's condition but also to control environmental contamination (Rishniw *et al*. 2010).

Prevalence rates vary according the study considered but are commonly between 5 and 15% in household pets and a 100% prevalence has been reported in kenneled dogs (Rishniw *et al*. 2010; Scaramozzino *et al*. 2009; Tangtrogsup and Scorza 2010), as can be seen in Table I. Young animals are more likely to be positive for *Giardia* with a reported prevalence of 30–50%, , with the greater number of infected animals being under 6 months and up to 12 months (Martínez-Moreno *et al*. 2007; Epe *et al*. 2010; Mircean *et al*. 2012). Liu, *et al*. (2007) found a prevalence rate of *Giardia duodenalis* of 14.9% in dogs under 2 years old, 8.7% in dogs 2–5 years old and 2.1% in dogs over 5 years.

Many infected animals are subclinical carriers and asymptomatic, with clinically ill animals presenting abdominal discomfort to severe abdominal pain, no fever, retarded growth, weight loss despite a normal appetite and diarrhea which is usually self-limiting (Díaz *et al*. 1996; Hamnes *et al*. 2007; Rimhanen-Finne, *et al*. 2007; Geurden *et al*. 2008; Tangtrogsup and Scorza 2010).

Primary testing for the diagnostic of *Giardia* includes direct fecal smear, passive or centrifugal fecal flotation using zinc sulfate ($ZnSO_4$) or sugar solutions, indirect immunofluorescence assay (IFA), detection of antigens by enzyme-linked immunosorbent assay (ELISA) and amplification of *Giardia* DNA by polimerase chain reaction assay (PCR) (Tangtrogsup and Scorza 2010). As sugar solutions tend to distort the *Giardia* cysts zinc sulfate solutions are preferred and have a higher recovery rate and maintain the integrity of cysts than sugar saturated ones (Carlin *et al*. 2006; Dryden *et al*. 2006; Geurden *et al*. 2008; Tangtrogsup and Scorza 2010). Dryden *et al*. (2006) found that a combination of fecal flotation with an antigen assay reached a combined sensitivity of 97,8%. Centrifugal flotation, with either $ZnSO_4$ or sugar solutions, is considered an optimal technique for the demonstration of *Giardia spp.* cysts and to be more sensitive than passive flotation techniques.

In a normal practice setting, passive flotation techniques and ELISA antigen test are easily available and can provide reliable results. Geurden *et al*. (2008) compared microscopical examination, a commercial available IFA and a commercial available ELISA test. This study showed that all tests had sensitivity above 90%, that the microscopical examination lacked sensitivity and that the ELISA test is a specific and fairly sensitive technique for the diagnosis of *Giardia spp*. Therefore, commercial diagnostic kits are being used more often in everyday practice, as they are easy to use and provide fast results.

Table I. Reported prevalence rates of *Giardia spp* worldwide

Country	Prevelance	Notes	Source
Argentina	9%	Privately owned dogs	Fontanarrosa *et al*., 2006
Australia	9,30%	Privately owned dogs	Palmer *et al*., 2008
Australia	22,10%	Privately owned dogs	Bugg *et al*., 1999
Belgium	9,30%	Privately owned, kenneled and with gastrointestinal disorders dogs	Claerebout *et al*. 2009
Brazil	16,90%	Privately owned dogs	Katagiri and Oliveira-Sequeira, 2008
Czech Republic	0,1–2,2%	Urban and rural area dogs	Dubná *et al*., 2007
Europe	24,78%	Privately owned dogs	Epe *et al*., 2010
Finland	5%	Asymptomatic dogs	Rimhanen-Finne, *et al*., 2007
Italy	20,50%	Kenneled dogs	Scaramozzino *et al*., 2009
Italy	55,20%	Shelter dogs	Papini *et al*., 2005
Japan	37,40%	Breeding kennels	Itoh *et al*., 2005
Norway	6,9–11,4%		Hamnes *et al*. 2007
Poland	2%	Privately owned and kenneled dogs	Solarczyk and Majewska, 2010
Poland	28%	Kenneled sled dogs	Bajer *et al*., 2011
Romenia	8,50%	Privately owned, from rural and urban areas, kenneled and shepherd dogs.	Mircean *et al*., 2012
Slovak Republic	1,60%		Szabová *et al*., 2007
South Korea	11,20%		Liu *et al*., 2008
Spain	22,07%	Inland and costal animals	Castro-Hermida *et al*., 2011
The Netherlands	15,20%	Privately owned dogs	Overgaauw *et al*., 2009
USA	15,60%	Privately owned dogs with gastrointestinal disorders dogs	Carlin *et al*., 2006

When comparing different tests for the diagnosis of *Giardia spp.*, a lack of concordance in results is registered, even in cases of high prevalence (Rishniw *et al*. 2010). This is due to peaks of cyst shedding that occur sporadically, making a single negative test not suitable to definitively rule out a possible infection and fecal flotation sensitivity is below 100% when a single fecal sample is tested. Sensitivity can be increased to >90% when at least 3 fecal samples are examined within 5 consecutive days and has the possibility of identifying other potential parasites, although this can be impractical in a private practice (Dryden *et al*. 2006; Tangtrogsup and Scorza 2010). Even when following all the correct principles, continued negative stool examinations are not sufficient to rule *Giardia* a causative agent (Wolfe 1992). Nevertheless, multiple samples are still advisable to increase sensitivity (Rimhanen-Finne *et al*. 2007; Geurden *et al*. 2008; Epe *et al*. 2010).

In this study, a combination of a passive flotation technique with a $ZnSO_4$ solution and a commercially available ELISA test (Witness® Giardia – Zoetis) was used in order to access the prevalence of *Giardia spp* in young asymptomatic dogs up to two years.

Materials and Methods

For the present study, 49 animals from the Grupo de Intervenção Cinotécnico of the Guarda Nacional Republicana (Portuguese Gendarmerie Canine Unit) were chosen. They were housed in two different locations from the Lisbon area, in Ajuda (n = 24) and Queluz (n = 25). These animals were housed individually in locations with a high number of kennels (up to 100), with ages ragging from newborns to 10 years old. All dogs follow a biannual deworming protocol based on a commercial formulation of praziquantel, pyrantel pamoate and febantel. Puppies are dewormed 15 and 30 days after birth and then once a month until they reach 6 months of age, using pyrantel pamoate.

The 49 dogs involved the study were divided in four groups, according to their age: under 6 months (n = 16), 6–12 months (n = 6), 12–18 months (n = 13) and 18–24 months (n = 14), comprising 22 females and 27 males, with 14 being German Shepherd Dog, 18 Belgian Malinois Shepherd Dog, 10 Dutch Shepherd Dog, 4 Labrador Retriever and 3 animals of other breeds (Table II). Regarding their origin, 67.35% (n = 33) were bread internally and 32.65% (n = 16) were donated to the Grupo de Intervenção Cinotécnico.

Samples were collected in the morning from the animals' individual kennel and processed in the following 30 minutes, over a 6 days period, by the end of November, 2014. All samples were submitted to two different tests, a passive flotation technique with a $ZnSO_4$ solution using a HenrySchein® fecal diagnostic kit and a detection of fecal antigen using a commercially available ELISA test (Witness® Giardia – Zoetis). This latter having 83% sensitivity and 100% specificity, according to its manufacturer.

With the HenrySchein® fecal diagnostic kit, a sample was collected using the internal piece and then inserted in the external piece. The $ZnSO_4$ solution was added up to the marked limit and homogenized by rotating the internal piece. More $ZnSO_4$ solution was then added until a convex meniscus is formed and a microscopy slide placed over it. The sample thus obtained was observed after a 15–20 minute period.

With the Witness® Giardia test, samples were collected using the provided swab and placed in the tube with the buffer solution and then homogenized. The swab tip was then broken and 5 drops placed in the appropriate window in the test slide. Results were read 5 minutes after.

All data were statistically analyzed using IBM SPSS Statistics version 20. A $\chi 2$ was used to compare prevalence according to age, breed and gender, with a significance level of $p<0,05$.

Results

From the 49 samples, 5 (10.2%) were considered positive with the $ZnSO_4$ flotation technique and 6 (12.24%) with the Witness® Giardia test. When considering the combination of results from both tests, 5 animals (10.2%) were considered positive. Of these, 3 (60%) were from the group under 6 months old, 1 (20%) from the 6–12 months and 1 from the 18–24 (20%) months group.

Results within each group revealed that: in the under 6 month group 18.75% (n = 16) were considered positive,

Table II. Sample characterization

Group	Breed					
	GSD	BMSD	DSD	LR	Other	Total
≤6m	0	11	3	0	2	16
6<x≤12m	6	0	0	0	0	6
12<x≤18m	6	0	6	0	1	13
18<x≤24m	2	7	1	4	0	14
Total	14	18	10	4	3	49

GSD – German Shepherd Dog; BMSD – Belgian Malinois Shepherd Dog; DSD – Dutch Shepherd Dog; LR – Labrador Retriever

16.67% in the 6–12 month group (n = 6), 0% in the 12–18 month group (n = 13) and 7.14% in 18–24 month group (n = 14).

None of the animals had diarrhea, 6 (12.24%) had soft stool and the remaining animals had normal feaces. Of the 6 with soft stool, none was considered positive with either test.

Regarding the animals that were considered positive, 3 (60%) were Belgian Malinois Shepherd Dog, 1 (20%) was a German Shepherd Dog and 1 (20%) was a Labrador Retriever. Of these, 3 (60%) were females and 2 (40%) were males.

No significant differences were found when comparing prevalence according to age, breed or gender.

Discussion

For this study, a single sample was collected, based on the fact that all animals were asymptomatic at the time of the study and the described 97.8% combined sensitivity of the combination of fecal flotation and antigen assay (Dryden *et al*. 2006). Also, a good concordance between test results was found, with all positive results with the flotation technique being also positive with the antigen ELISA test. Only one case was not concordant in both techniques, being only positive with the antigen ELISA test.

This combination of techniques can not only improve diagnostic capabilities but also help to reduce false positives as *Giardia spp* is one of the most commonly misdiagnosed parasites, being confused with yeasts, plant remnants and debris. Under-diagnose is also possible, in face of improper diagnostic techniques (Thompson 2000; Dryden *et al*. 2006; Tangtrogsup and Scorza 2010).

Prevalence of *Giardia spp.* is influenced by host and environmental factors, including age, pure/mixed breed, treatment, immune status, living conditions, feeding, husbandry and others (Hamnes *et al*. 2007). In a study comparing different groups of kenneled dogs, the animals in the kennel with greater number of dogs and higher turnover had also a higher prevalence of *Giardia duodenalis* (46.9% in contrast with 10.5% and 15.8% of the remaining kennels (Scaramozzino *et al*. 2009). Dogs in a multi-dog household are also more commonly affected with *Giardia spp.* than dogs in single-dog households (Thompson, 2004). Confinement to a limited area is also associated with increased risk (Capelli *et al*. 2003). The animals in this study are individually kenneled but in settings with a great number of kennels. An overall prevalence of 10.2% was found, a number in line with the prevalence registered in household dogs.

Dogs seem to develop some immunological competence, since animals that are kenneled for more than 12 months have a lower prevalence of infection (Scaramozzino *et al*. 2009). Cases of dogs that seem to spontaneously clear its infection before becoming re-infected have been reported (Rishniw *et al*. 2010). In this study a decrease in prevalence with age was recorded which might support this fact.

Pure-breed dogs have been found to have a higher prevalence of *Giardia spp.* than mixed-breed dogs (Fontanarrosa *et al*. 2006; Hamnes *et al*. 2007; Claerebout *et al*. 2009; Scaramozzino *et al*. 2009). In this study, no difference was found between the breeds included.

In one study, recorded prevalence in puppies was 21.9% while adult dogs had a prevalence of 10.82% (Díaz *et al*. 1996), while breeding kennels in Japan have registered overall prevalence of 54.5% in puppies (Itoh *et al*. 2005). Overall prevalence found in this study was much lower than the ones found previously for puppies in breeding kennels or in other settings. This may be due to the fact that the animals included in this study are housed individually since their 7th week after birth, due to the nature of the work that they are destined to.

Stress has an effect on immunological capability which also leads to the higher prevalence recorded in environments such as dog shelters or kennels (Fiechter *et al*. 2012). Working dogs may have high levels of physiological stress (Rooney *et al*. 2009) and, therefore, the animals included in this study could be in greater risk of infection. Despite these potential factors, the prevalence found in the study was similar to that of household dogs which might indicate that stress, by itself, is not a determinating factor.

The primary goal of anti-*Giardia* treatment is to stop clinical signs, mainly diarrhea, and the Companion Animal Parasite Council recommends that animals with this clinical sign are tested with a combination of direct smear, fecal centrifugal flotation and *Giardia spp.* antigen assay (Tangtrogsup and Scorza 2010). A rapid detection of *Giardia spp.* is necessary so that dogs can be timely treated and develop properly and people which they come in contact with are protected (Solarczyk and Majewska 2010). With this goal in mind, a combination of the two diagnostic methods seems to be a good approach, being easy and fast to perform, without significant monetary investment necessary. Current data suggest that house pets, even when sharing the same household as their owners do not frequently share their infections with healthy people (Bowman and Lucio-Forster, 2010). Nonetheless, a high percentage of infected dogs increases the risk of infection for humans (Solarczyk and Majewska 2010).

Giardia spp. cysts can also be detected in clinically normal dogs, which can be relevant since they can transmit the infection to other dogs and contaminate a shared environment (Scaramozzino *et al*. 2009).

Treatment of positive but otherwise healthy animals is controversial, since animals with normal stool are not considered a human health risk and treatment is unlikely to eliminate the infection and a re-infection can occur soon after. Nevertheless, *Giardia* treatments have been recommended for all positive animals because of the possible zoonotic risk (Rishniw *et al*. 2010). The animals that were found positive in this study were not submitted to any treatment since they presented no clinical signs.

In areas of high animal concentration good hygiene measures are essential and must be carefully observed, and all pos-

sibly contaminated surfaces should be submitted to a thorough cleaning and disinfection with quaternary ammonium compounds (Tangtrogsup and Scorza 2010).

Conclussions

The parasites from the genus *Giardia spp* have a worldwide dissemination and a zoonotic potential. Dogs, and young ones in particular, can be a source of infection. The diagnosis of an *Giardia* infection could be challenging and should be made by a combination of history, physical signs and diagnostic tests.

A combination of fecal flotation and antigen ELISA tests have good sensitivity and are ease and fast to perform in practice, without a significant investment in equipment or reagents and, therefore, could be a good choice to perform a diagnostic.

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