Prevalence of *Giardia* antigen in stool samples from dogs and cats

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SUMMARY

A total number of 153 faecal samples (105 from dogs and 48 from cats) were examined for the presence of *Giardia* infection by a commercially available enzyme immunoassay (ProspecT® *Giardia* Rapid Assay). Infection rates were significantly (p<0.05) higher in dogs (19.04%) than in cats (4.16%), in female (28%) than in male (10.90%) dogs, and in winter (29.54%) than in the spring/summer period (11.47%). Though not significant, the prevalence of giardiasis was also found to be higher in owned (19.67%) than in refuge dogs (18.18%), in ≤ 3 year-old dogs (33.07%) than in older ones (7.40%), and in asymptomatic (37.50%) than in asymptomatic dogs (17.52%). These data demonstrate that *Giardia* is more widespread than had been previously thought in Italy. It is also concluded that dogs and, to a lesser extent, cats may serve as potential animal reservoirs in urban areas.

KEY-WORDS : *Giardia*, coproantigens, epidemiology, dog, cat, Italy.

RéSUMÉ

153 échantillons fécaux (105 issus de chiens et 48 de chats) ont été examinés afin de rechercher la présence de coproantigènes spécifiques de *Giardia* sp par un système immuno-enzymatique du commerce (ProspecT® *Giardia* Rapid Assay). Les taux d’infection se sont révélés significativement (p<0.05) plus élevés chez les chiens (19,04%) par rapport aux chats (4,16%), chez les femelles (28%) par rapport aux mâles (10,90%), et pendant l’hiver (29,54%) par rapport à la période printempst/été (11,47%). Bien que non significative, la présence des antigènes s’est également avérée plus fréquente chez les chiens de propriétaires (19,67%) que chez ceux issus de refuges (18,18%), chez les jeunes chiens (≤ 3 ans) (33,07%) que chez les animaux ayant dépassé cet âge (7,40%), et chez les animaux malades (21,42%) que chez les sujets asymptomatiques (13,63%). Ces données démontrent que l’infection par *Giardia* sp est plus répandue en Italie qu’on ne le pensait précédemment. Le chien et, dans une moindre mesure, le chat peuvent jouer le rôle de réservoirs animaux potentiels en milieu urbain.

MOTS-CLÉS : *Giardia*, coproantigènes, épidémiologie, chien, chat, Italie.

Introduction

The intestinal flagellate *Giardia* is a common protozoan parasite which has been reported throughout the world and can infect human beings as well as a great variety of domestic animals, especially livestock, dogs and cats, and numerous species of wild mammals [5, 29]. Even though *Giardia* is normally considered as non-pathogenic and many infections are asymptomatic, it has been shown that the organism may be a primary pathogen. Common symptoms of *Giardia* in animals and man include enteritis with diarrhoea [5, 10]. Malabsorption, dyspepsia, abdominal pain, asthenic neurosis, allergic dermatosis, elevated eosinophil levels and moderate dysproteinemia were also reported in children [10, 31]. Depression, anorexia, weight loss, poor condition and vomiting were occasionally observed in animals [5]. Diarrhoea may be intermittent or continuous and faeces are usually soft, pale, very foul smelling and steatorrhoeic [5]. The disease is usually acute and self-limiting. However, chronic infections can occur with or without an acute phase, may result in recurrent symptoms and are often associated with treatment failure. The chronic form may persist for weeks or months, or may last even for years, and is more common in immunocompromised individuals [5].

*Giardia* sp is transmitted in the cyst stage either directly, by contact with faeces, or indirectly, through water or food. Until recently, *Giardia* was considered to maintain rigid host specificity but this is now disputed since it was demonstrated experimentally that inter-species transmission can occur. Different animal species were successfully infected with cysts of *Giardia* isolates from other species, including man [9, 30]. It is therefore possible that animals and man may infect each other. Because of their close association with humans and because *Giardia* isolates which parasitize different mammalian hosts are morphologically indistinguishable, dogs and cats have been suggested as likely reservoirs of *Giardia* isolates capable of infecting humans [5, 18, 29].

In many countries, *Giardia* is considered one of the most important parasitic diseases in terms of morbidity [5, 10, 31] and is one of the most common gastrointestinal pathogens in children with severe retardation of growth and development [10]. Children are frequently infected and there is a high prevalence in institutions such as orphanages or child-care...
centres [5]. The prevalence is often higher in individuals from developing countries or in the lower socio-economic groups where hygienic standards are compromised [8]. There are epidemiological data providing strong circumstantial evidence of zoonotic transmission [28, 32]. Therefore, there is an obvious need to determine the zoonotic potential of Giardia sp in different countries. Surveys in many countries have shown that infection with Giardia is common and widespread in both dogs and cats [1, 4, 12, 14, 17, 19, 26, 27]. However, little is known on the prevalence of Giardia sp in dogs and cats in Italy, despite its public health significance and possible zoonotic potential. Piergili Fioretti and Moretti [21] reported an overall prevalence of 4.3% in dogs by merthiolate-iodine-formaldehyde sedimentation. Rossi et al. [23] observed that 9.5% of dogs from Italian armed forces were infected by an indirect immunofluorescence test. Canestri-Trotti et al. [6] detected Giardia in 14% of refuge cats by flotation/sedimentation technique. Recently, Caelli et al. [7] have found a prevalence of 21.3% in dogs from regions of Northern and Central Italy by a centrifugation/flotation technique, combined with a sedimentation technique or with merthiolate-iodine-formaldehyde procedure. To the best of our knowledge, no other report on this subject has been published as yet. Therefore, in order to give further insight, the aim of the present study was to determine the prevalence of Giardia infections in dogs and cats by enzyme immuno-assay (EIA) technique.

Materials & Methods

The prevalence of Giardia in dogs and cats was examined among different groups according to species, sex, season, origin, and age (≤3 year and >3 year old). The presence of diarrhoea in each of the animals was also determined as an indication of the possible association of Giardia with clinical symptoms.

SAMPLES

A total of 153 faecal samples were collected and examined between January and July 2003: 105 were from dogs and 48 from cats of different sex and breed. Fresh stool specimens were collected in clean plastic containers, stored at +5°C and examined within 48 hours. According to the package insert, if fresh specimens could not be tested within 48 hours, they were frozen at -70°C. Frozen stool specimens were thawed at room temperature (20-25°C) before use.

ANIMALS

There were 55 male and 50 female (5 spayed) dogs, aged from 3 months to 11 years. There were 23 male (3 neutered) and 25 female (12 spayed) cats, aged from 3 months to 9 years. Sixty-one faecal samples of dogs and 6 of cats were from owner animals presented at the Veterinary Teaching Clinics of Pisa, and living in urban or suburban areas of the town. Forty-four faecal samples of dogs and 42 of cats were from animals living in a refuge shelter located in the metropolitan area of Florence.

EIA KIT

Each stool sample was analyzed by using a commercially available EIA for Giardia antigens (Prospect® Diarrhoea Rapid Assay, Alexon-Trend, Inc., 14000, Unity St., Ramsey, MN 55303, USA). This EIA kit detects the presence of a Giardia-specific antigen with a molecular weight of 65,000 (GSA 65), that is produced in abundant quantities by Giardia sp. trophozoites as they multiply within the host’s intestinal tract. The assays were conducted following the instructions of the manufacturer. Briefly, a negative result is represented by the appearance of a single dot in the positive control area of the membrane. A positive result is indicated by the presence of dots in both the positive control area and the test area of the membrane.

STATISTICAL ANALYSIS

All epidemiological data obtained from the survey were examined using χ² test. Results were considered significantly different with p<0.05.

Results

Overall, a total number of 22 out of 153 (14.37%) faecal samples was found to be positive for Giardia antigen. The infection was significantly (p<0.05) more prevalent in dogs (20/105, 19.04%) than in cats (2/48, 4.16%).

Prevalence rates from dogs are shown in Table I. Canine infections resulted to be significantly (p<0.05) more prevalent in females (14/50, 28%) than in males (6/55, 10.90%), and in winter (13/44, 29.54%) than in the spring/summer period (7/61, 11.47%). Giardia prevalence was also found to be higher in owner dogs (12/61, 19.67%) than in dogs from the refuge (8/44, 18.18%), and in ≤3 year-old dogs (18/78, 33.07%) than in older ones (2/27, 7.40%). Despite these trends, no statistically significant difference of Giardia infection was found among these groups.

<table>
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<th></th>
<th>No. examined</th>
<th>No. positive</th>
<th>%</th>
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<tbody>
<tr>
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<tr>
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<td>55</td>
<td>6</td>
<td>10.90</td>
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<tr>
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<td>14</td>
<td>28.00</td>
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<td>61</td>
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<tr>
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</tr>
<tr>
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<td>97</td>
<td>17</td>
<td>17.52</td>
</tr>
<tr>
<td>Diarrhoeic</td>
<td>8</td>
<td>3</td>
<td>37.50</td>
</tr>
</tbody>
</table>

Table I. — Distribution of the prevalence of Giardia in dogs by EIA technique.

Legend: ‘statistically significant value by χ² test (p<0.05).

Eight of the dogs (1 with vomiting), which were included in the present survey, showed diarrhoea. Three dogs, among the animals that were noted as diarrhoeic, were positive for
Giardia coproantigen in stool. Two of these dogs were from owner dogs and had received a treatment combining antidiarrhoeal and antibacterial compounds, which was accompanied by a transient amelioration of clinical signs. Therefore, the rate of infection resulted to be higher in symptomatic (3/8, 37.50%) than in asymptomatic dogs (17/97, 17.52%). However, the association of Giardia with diarrhoea did not reach a statistically significant value.

As mentioned above, fewer cats (2/48, 4.16%) were infected than dogs (p<0.05). Consequently, an insufficient number of infected cats was available to perform further analyses. The only 2 cats that were infected with Giardia were 1 owner cat and 1 refuge cat. They were both females (1 intact and 1 spayed) and ≤3 years old. Six of the examined cats (1 with dehydration and anaemia) showed diarrhoea, but none of them was found to be Giardia positive (Table II).

<table>
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<tbody>
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<td>Clinical signs</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
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<td>3</td>
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<tr>
<td>Diarrhoeic</td>
<td>6</td>
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</table>

Table II. — Distribution of the prevalence of Giardia in cats by EIA technique.
Legend: 1 statistically lower than in dogs by \( \chi^2 \) test (p<0.05); 2 insufficient number to perform further analysis.

### Discussion

The present study examined the epidemiology of Giardia sp infections in different dog and cat populations, using sensitive EIA kit for the detection of specific coproantigen. The overall prevalence of the infection was found to be rather high with 14.37% of animals infected. Giardia sp antigen was significantly (p<0.05) more often detected in dogs than in cats. Many authors stated that the rate of the infection in cats is usually lower [17, 26, 27]. Although the infection rate in cats was statistically higher than in dogs in a comparable investigation [19], the results of the present study appear to support that statement.

Giardia is only occasionally identified as a cause of infection in dogs [5], but the present results show that this parasite can be rather common in these animals. Compared to former surveys conducted in Italy, the infection rate in our study (19.04%) is slightly lower than that reported by Capielli et al. (21.3%) [7] using a centrifugation/flotation technique, combined with a sedimentation technique or with a merthiolate-iodine-formaldehyde procedure. However, it is remarkably higher than those reported by Rossal et al. (9.5%) [23] using an indirect immunofluorescence test, and by Piergili Fioretti and Moretti (4.3%) [21] using merthiolate-iodine-formaldehyde sedimentation. Prevalence of excretion was determined in dogs from other countries by concentration of oocysts from faecal specimens with sedimentation techniques, using either formalin-ethyl acetate sedimentation combined with direct faecal smears [14], ether and acetoacetate buffer sedimentation supplemented with sodium chloride flotation and Baermann technique in some cases [1]. Otherwise, oocysts were concentrated by flotation methods, using saturated solutions such as sodium chloride/glucose, magnesium sulphate or zinc sulphate [4, 12, 17, 19, 26, 27]. In some studies, this relied on centrifugation and was supplemented with the formalin-ethyl acetate sedimentation technique [17, 27], or combined with direct smear of fresh faeces using isotonic saline solution and Lugol’s iodine stain [19]. Compared to those studies, the rate of infection in the present survey is similar to that reported from Denmark (17.1-20%) [12], higher than those from Japan (14.6%) [14], Yugoslavia (14.4%) [19], United States (4.7-7.2%) [17, 27] and Spain (1.8%) [1], but lower than those from France (30.4%) [4] and Australia (21%) [26]. There is no obvious explanation for these differences. These may be due to geographical variation or to differences in the number of animals and type of population surveyed, or may be attributed to different sensitivity of the diagnostic procedure used.

Female sex appeared to be a statistically significant (p<0.05) predisposing factor for canine giardiasis. In the present study, hormonal conditions of the examined dogs were not known; anyway, there was no evidence for detection of oestral and pre-oestral status, pregnancy or lactation in females. Hormonal factors are known to influence the outcome of parasitic infections in host species other than dogs [25]. The onset of Giardia cyst excretion in lactating bitches was previously reported in dogs [22]. Therefore, the direct and indirect effects on parasite burden that differences in sex hormonal levels may have in females, as opposed to males, cannot be dismissed.

Regarding seasonal variation in the prevalence of Giardia, seasonal effects on the infection rate may reflect climatic changes, which affect the parasite itself, as well as changes in the photoperiod which may, in turn, influence the host’s physiology [3]. In the present study, a significantly (p<0.05) lower prevalence of infections in dogs was detected in June and July, when the weather is hot and dry in Italy. Such conditions are not favourable for cyst survival. Probably, it would have been better to follow the same animals from January to December detecting possible differences in positivity to Giardia. Nevertheless, it can not be excluded that different seasons of sampling may affect the diagnosis of giardiasis in dogs and cats.

Giardia was equally prevalent in both owned and refuge dogs. The fact that Giardia sp is more common in owned dogs than is normally thought is supported by a previously published survey in Japan, which revealed that 14.6% of household dogs were infected [14]. Many of the owned dogs in our study were from breeding kennels, compared to dogs from individual households (32 vs 29). The prevalence of Giardia sp is considered to be highest in dogs housed in crowded environments, such as breeding kennels and shelters [5]. Therefore, our results may link up with the high density of dogs in limited areas, the high level of environmental faecal contamination with Giardia cysts and, consequently, the increased rate of transmission.

Though younger dogs were not statistically more susceptible to Giardia infection, the data presented here suggest...
that the prevalence of *Giardia* was higher in dogs below 3 years of age. A similar age predisposition to giardiasis has been previously reported in dogs and humans [5, 10, 31]. This may suggest that specific immunity to the parasite develops with age, probably as a consequence of one or more exposures. Older animals may be more susceptible if natural resistance is compromised [5].

Although molecular characterisation of morphologically identical isolates from humans and numerous other species of mammals has confirmed the genetic heterogeneity of the parasite, only one species has been recognised as causing disease in humans and most other animals [5, 28, 29]. Some of the genotypes appear to have a limited host range, whereas others appear to be infective to a wide range of host species [28, 29]. In particular, since humans and cats may have genetically identical forms of *Giardia*, some findings suggest that the cat could act as a reservoir of infection for humans and play a zoonotic role [18]. In the present study, only 4.16% of cats were positive by the EIA technique. Similarly, in the United States, Nolan and Smith [27] found a prevalence of 2.4% in their study, and a prevalence of 3.5% was recorded by Kirkpatrick [17], using the zinc sulphate centrifugal flotation method, combined in some cases with the formalin-ethyl acetate sedimentation technique. However, previous investigations conducted in Australia by concentration using zinc sulphate flotation, and in Italy using flotation/sedimentation technique reported prevalence of 14%, [6, 26]. Moreover, Kirkpatrick [16] found a prevalence rate of 50% in a cattery of Persian cats in the United States by formalin-ethyl acetate sedimentation technique, and results from a recent study have shown that *Giardia* prevalence reached 22.2% in Yugoslavia by direct smear of fresh faeces using isotonic saline solution and Lugol’s iodine stain, combined with the zinc sulphate concentration technique [19]. However, based on the present findings, cats are probably poor hosts for *Giardia* sp and serve as limited reservoirs of *Giardia*. Since these animals infrequently carry this zoonotic agent, it is unlikely that significant environmental contamination by cats with *Giardia* cysts infective for humans can occur. Further investigations are probably needed to better understand the role of cats, if any, in the epidemiology of *Giardia*.

The high prevalence of infection with *Giardia* in healthy animals raises further considerations. Giardiasis is commonly regarded as an occasional cause of enteric disease in dogs [5], which suggests that either the parasite is rarely pathogenic or that it is not often considered in the diagnosis of enteritis. In the present study, 21.42% of the symptomatic and 13.63% of the asymptomatic dogs were positive for *Giardia* coproantigen. However, there was no evidence of a statistically significant association between the presence of *Giardia* and clinical disease. Since there was no significant relationship between the presence of *Giardia* and diarrhoea, this may indicate that dogs and cats are less susceptible than man to the clinical effects of *Giardia*. It is possible that a number of factors affecting the host’s defence mechanisms must be present before *Giardia* can cause clinical signs [5].

*Giardia* can often be overlooked as a cause of infection in dogs and cats due to the poor recovery of faecal cysts by the sodium chloride, sucrose, or sodium nitrate flotation methods and to the fact that shedding of the cysts may fluctuate over time. The trophozoites can be seen in fresh faecal smears but they rapidly disintegrate in faecal samples. Moreover, the size of cysts, approximately 8 x 11 µ, make it necessary the use of high-dry or oil immersion microscope objectives [5]. For these reasons, faeces of dogs and cats were examined by the EIA technique in the present survey. The commercial availability of EIAs for detecting *Giardia*-specific antigens in stool specimens has provided a potentially attractive alternative to conventional microscopy for diagnosis of giardiasis. Various studies looking at antigen detection in stool specimens for the diagnosis of human *Giardia* infection were published [2, 11, 24]. In a number of clinical evaluations, *Giardia* EIAs were found to be rapid and, perhaps more importantly, cost effective tools for diagnosing the infection [2]. In particular, the sensitivity of ProSpecT® *Giardia* EIA in certain studies was comparable, at worst, but in most cases somewhat superior to conventional microscopy, with reported sensitivity ranging from 94% to 95% and specificity of 100% [11, 24]. However, the sensitivity of this assay was questioned in veterinary medicine, especially in dogs [20]. In a therapeutic trial on naturally infected dogs, the assay did appear to be specific, as results were positive in only 4.3% of cyst negative samples. Unfortunately, the immunoassay gave 31.6% of false negative results, when compared to the results obtained with a zinc sulphate flotation technique [20]. According to the package insert, *Giardia* EIA is a commercial test which was developed to detect a specific antigen (GSA 65) that is produced in abundant quantities by *Giardia* sp. trophozoites as they multiply within the host’s intestinal tract. It was clearly shown that the estimated amount of *Giardia* antigens can widely range in positive stool samples, for instance from 50 to 5,000 ng/ml in human samples found positive to *Giardia* by both microscopy and EIA test [32]. In a negative reaction, either there is no GSA 65 present or the level of antigen present is below detectable levels, as reported in the insert. When a host ingest a cyst, the trophozoites leave the cyst and divide repeatedly until they have populated the small intestine of the new host. Thereafter, they probably divide less actively, as soon as host’s defence mechanisms can play their role. Nevertheless, some trophozoites periodically are carried in the faecal stream towards the anus, encysting on the way, and cysts are passed in the faeces. Therefore, it is probably possible to find cysts in the faeces even when *Giardia* sp. trophozoites are less intensely multiplying within the host’s intestinal tract and animals are thus passing amounts of GSA 65 which could be below levels detectable by EIA kit [20]. As a consequence, EIA technique appears to be sufficiently sensitive to detect cases of giardiasis when fairly high levels of antigen are shed [20], but more sensitive techniques are necessary in cases of light infection. Therefore, a number of *Giardia* infections in dogs and cats might have been overlooked in the present study. In particular, a recent study has pointed out that a polymerase chain reaction may be necessary, and may yield more reliable results for the detection of low grades of *Giardia* infection in domestic cats [18]. Otherwise, the examination of two specimens, collected independently within a seven day period, might be useful.
to achieve better diagnostic sensitivity with EIA technique in animals; analysis of the second sample should be performed if the first assay yielded a negative result, as it was recommended for human patients [13].

Despite this, the use of Giardia EIA in our evaluation was a considerable improvement to perform epidemiological screening over faecal flotation for conventional microscopy. The EIA was found to be a rapid and simple method for detecting Giardia, while requiring almost no handling of the stool [15]. Although more sensitive, zinc sulphate centrifugation is not often performed as a screening procedure in most general practices. Duodenal aspiration via endoscopy was reported to indentify Giardia cysts but is no longer recommended as a diagnostic tool. Fluorescent antibody testing requires specialized equipment and is thus impractical in most clinical situations. In our opinion, the ability to detect Giardia antigen in fresh or frozen faecal samples with an approximately 15-min qualitative EIA technique provides the veterinary practitioner with a very useful, convenient, and alternative diagnostic method for Giardia infections in dog and cat faecal specimens.

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References