Veterinary Parasitology 179 (2011) 242–245

Contents lists available at ScienceDirect

Veterinary Parasitology

journal homepage: www.elsevier.com/locate/vetpar

Short communication

Intestinal parasites in dogs and cats from the district of Évora, Portugal

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ARTICLE INFO

Article history:
Received 30 July 2010
Received in revised form 1 February 2011
Accepted 7 February 2011

Keywords:
Intestinal parasites
Giardia duodenalis
Domestic animals
Évora

ABSTRACT

Intestinal parasites, both helminths and protozoa, are commonly found in domestic animals, and the possible transmission of enteric parasites from dogs and cats to humans may constitute a global potential health risk worldwide. In the present study, we analysed 148 stool samples from dogs ($n = 126$) and cats ($n = 22$) collected from animal shelters and veterinary clinics, in the district of Évora, Portugal. Microscopic examination confirmed that *Giardia* was the most frequent parasite in the studied population (34/148; 23%). Other parasites such as *Ancylostoma* sp., *Isospora* spp., *Toxocara*, *Trichuris* spp., *Toxascaris* and *Toxoplasma* were also found. Furthermore, molecular characterization of *Giardia duodenalis* analysis targeting the small subunit ribosomal RNA (*ssu-rRNA*) was performed revealing the presence of host-specific (C and D) and zoonotic assemblages (A and B). This work points out to the importance of protozoan parasites in companion animals, and reanalyses the need for parasite prophylaxis.

1. Introduction

Companion animals and their close relationship with humans offer several benefits although many potentially zoonotic parasites are associated with these animals (Martínez-Moreno et al., 2007). Companion animals placed in shelters are usually under the supervision of veterinarians, being properly vaccinated and given anthelmintic treatments to prevent the spread of infectious and parasitic diseases. However, protozoan parasites are missed with such treatments (Scaramozzino et al., 2009). *Giardia duodenalis* (syn. *Giardia intestinalis* or *Giardia lamblia*) is one of these pathogens that may represent a potential public health risk, since transmission of parasites from animals to humans may frequently occur (Claerebout et al., 2009). Molecular studies have shown that *G. duodenalis* comprises at least seven distinct genotypes or assemblages (A–G). Assemblages A and B have been the only, so far, associated with infection in humans, but have also been detected in a wide range of domestic and wild mammals. The remaining assemblages seem to be host-restricted and include assemblages C and D (dogs), E (hoofed animals), F (cats), and G (rats) (Xiao and Fayer, 2008).

The occurrence of zoonotic assemblages in domestic animals, such as dogs and cats, has recently been reported in a number of studies in urban areas of Mexico, Brazil, Japan, Italy, Poland and Thailand (Berrilli et al., 2004; Itagaki et al., 2005; Lalle et al., 2005; Eligio-Garcia et al., 2005; Zygner et al., 2006; Volotão et al., 2007; Inpankaew et al., 2007).

In recent years giardiasis has been recognized as a re-emerging infectious disease in developed countries. Updated information on the prevalence of intestinal par-

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doi:10.1016/j.vetpar.2011.02.003

Table 1
Intestinal parasites detected by light microscopy in animal population ($n = 148$) of Évora district.

Parasite	Household ($n = 97$)		Kennel ($n = 51$)		Total ($n = 148$)
	Dogs ($n = 77$)	Cats ($n = 20$)	Dogs ($n = 49$)	Cats ($n = 2$)	
Giardia spp.	0	5.0% (1/20)	47.0% (23/49)	50.0% (1/2)	16.7% (25/148)
Ancylostoma sp.	7.8% (6/77)	5.0% (1/20)	4.1% (2/49)	0	6.1% (9/148)
Isospora spp.	5.2% (4/77)	5.0% (1/20)	6.1% (3/49)	0	5.4% (8/148)
Toxocara	1.3% (1/77)	10% (2/20)	0	0	2.0% (3/148)
Trichuris spp.	0	0	2.0% (1/49)	0	0.7% (1/148)
Toxascaris	1.3% (1/77)	0	0	0	0.7% (1/148)
Toxoplasma	0	5.0% (1/20)	0	0	0.7% (1/148)
Giardia spp. + *Isospora* sp.	0	0	4.1% (2/49)	50.0% (1/2)	2.0% (3/148)
Giardia spp. + *Toxocara* sp.	0	0	2.0% (1/49)	0	0.7% (1/148)
Giardia spp. + *Trichuris* spp.	0	0	4.1% (2/49)	0	1.4% (2/148)
Giardia spp. + *Toxascaris* sp	0	0	2.0% (1/49)	0	0.7% (1/148)
Isospora spp. + *Ancylostoma* sp.	2.6% (2/77)	0	2.0% (1/49)	0	2.0% (3/148)
Isospora spp. + *Toxocara* sp.	1.3% (1/77)	0	0	0	0.7% (1/148)
Isospora spp. + *Trichuris* sp.	1.3% (1/77)	0	0	0	0.7% (1/148)
Giardia spp. + *Isospora* sp. + *Toxocara* sp.	0	0	2.0% (1/49)	0	0.7% (1/148)
Giardia spp. + *Isospora* sp. + *Toxascaris* sp.	1.3% (1/77)	0	0	0	0.7% (1/148)

asites is available for different dog populations in several European countries as reviewed by Claerebout et al. (2009). The objective of the work was to determine the prevalence of intestinal parasites in the enrolled animals, with special attention on *G. duodenalis* isolates in dogs and cats from Évora district, Portugal.

2. Materials and methods

Faecal samples of 148 animals, including 126 canine and 22 feline samples, were collected during the period December 2007 to May 2008, in animal shelters and veterinary clinics within the Évora district. The majority of samples (97, dogs $n = 77$, cats $n = 20$) were gathered from household animals, and only 51 (dogs $n = 49$, cats $n = 2$) from shelter animals.

Freshly collected stool samples were screened for parasites detection by light microscopy, after a concentration step (modified *Faust* method, Faust et al., 1938).

Genomic DNA was extracted from *Giardia* positive samples identified through microscopic analysis, using a QIAamp DNA Mini Stool Kit (Qiagen, Germany) according to the manufacturer's instructions. For some samples DNA was re-extracted using elution volumes of 50, 25 or 15 μl.

DNA from every *Giardia* positive sample identified by microscopy was amplified using primers (Read et al., 2002) that target the small subunit ribosomal RNA locus (*ssu-rRNA*). For sequence analysis PCR products were purified using the Jetquick Gel Extraction Spin Kit/50 (Genomed, Germany) according to the manufacturer's instructions. DNA sequencing reactions were carried out in both directions using primers GiarF/GiarR for *ssu-rRNA* gene fragment (175 bp) (Read et al., 2002). The obtained sequences were aligned with previously published sequences of *G. duodenalis* isolates in GenBank database using ClustalW. Phylogenetic inference analyses were based on the construction of neighbour-joining (NJ) trees with MEGA vs. 4 (Tamura et al., 2007). The robustness of the obtained tree topology was assessed by bootstrapping using 1000 pseudo-replicates of the original sequence data.

The *ssu-rRNA* nucleotide sequences obtained in this study were deposited in GenBank under accession numbers FN689480–FN689509.

3. Results

3.1. microscopy

The overall calculated prevalence of infection with intestinal parasites was 39.2% (58/148). Infection with only one parasite species was most common (29.7%, 44/148), followed by infection with two (8.1%, 12/148), three (1.4%, 2/148) parasites species (Table 1). *G. duodenalis* was the most prevalent parasite in the studied population (23.0%, 34/148) with the majority of infected animals from shelters (28/34).

3.2. Molecular characterization of Giardia isolates

Thirty amplicons were successfully sequenced, resulting in 15 strains belonging to assemblage C, 12 to assemblage D two sequences (53AE and 98AE) to assem-

Table 2
Genotyping results of *Giardia* positive samples ($n = 30$).

Giardia assemblage	Household ($n = 2$)		Kennel ($n = 28$)	
	Dogs ($n = 2$)	Cats ($n = 0$)	Dogs ($n = 26$)	Cats ($n = 2$)
A	0	0	0	2
B	0	0	1	0
C	0	0	15	0
D	2	0	10	0
F	0	0	0	0

blage A while one (30AE) was found to be belong to assemblage B (Table 2).

In order to confirm the genetic characterization results, the evolutionary relationships between several *ssu-rRNA Giardia* sequences were investigated phylogenetic analysis. On the basis of the analysis of the obtained trees, the assignment of sequences obtained from *G. duodenalis* from two cat samples, 98AE and 53AE to assemblage A and the dog sample 30AE to assemblage B was unambiguous, and statistically supported. However, although the tree topologies suggest a phylogenetic association of 15 and 12 of the sequences presented here with assemblages C and D, respectively, this result was not supported statistically (data not shown).

4. Discussion

In the present work faecal samples from 148 companion animals were screened microscopically for the presence of intestinal parasites. The overall prevalence of intestinal parasites in dogs and cats from the Évora district analysed was 39.2% (58/148). This result is similar with other studies (Coggins, 1998; Ramírez-Barrios et al., 2004) or even higher (Anene et al., 1996; Fok et al., 2001; Eguia-Aguilar et al., 2005; Fontanarrossa et al., 2006).

In our study single infection was the most frequent situation (39.2%, 58/148), in contrast with multiple parasitism, which was less frequent being only detected in 9.5% (14/148) of sampled animals, as shown above. Intestinal parasites were mainly detected in shelter animals, as opposed to household animals. This finding is largely consistent with those described in other works where infection rates with intestinal parasites in shelter animals are very high (Dubná et al., 2007; Palmer et al., 2008; Scaramozzino et al., 2009; Claerebout et al., 2009). In shelters, the large number of animals in a limited space contributes to the propagation of these parasites, thereby increasing the risk of infection (Paoletti et al., 2008). As these animals are often re-homed, they pose a potential risk for the health of future owners (Little et al., 2009).

Globally, protozoan cysts were more commonly identified in faecal samples rather than helminth eggs (Table 1). Veterinarians recommend the administration of prophylactic anthelmintics. Ideally, these control products should be routinely applied (Palmer et al., 2008). However, the currently available products are not effective against protozoan infections (Little et al., 2009) which might explain the differences between protozoan and helminths infections rates.

G. duodenalis was the most frequent parasite found in stools (23.0%). The prevalence of *Giardia* in companion animals is not consistent between studies, and is often influenced by the sensitivity of the diagnostic test used and the number of samples examined, due to the intermittent nature of cyst excretion (Thompson et al., 2008). Some authors suggest that *G. duodenalis* infection prevalence may be underestimated as the presence of parasite cysts might not reflect any clinical signs of infection (Palmer et al., 2008; Scaramozzino et al., 2009). This fact may be relevant given the potential for these animals to transmit the infection to other animals and humans, thus contaminating a shared environment (Scaramozzino et al., 2009).

Companion animals are known to harbour both zoonotic and host-specific genotypes of *G. duodenalis* (Monis et al., 1998; Monis et al., 1999; Read et al., 2004; Thompson et al., 2008). *G. duodenalis* genotypes C, D (dog-specific) and F (cat-specific) are not known to be zoonotic and therefore are not thought to constitute public health concern (Papini et al., 2007).

In our study, molecular characterization of *G. duodenalis* revealed that host-specific assemblages C and D were predominantly found corresponding to 90.0% (27/30) of analysed DNA sequences. We are aware that the use of a genotyping approach with a single locus may constitute a limitation, namely in what concerns to the determination of the zoonotic potential. Even though assemblages A and B were also determined in three isolates (two cats and one dog, respectively).

The present work contributes to the body of knowledge regarding the epidemiologic status of intestinal parasites, especially *G. duodenalis* infection in companion animals, both from shelters and households from the Évora district. This kind of studies is very helpful in order to clarify veterinarians and owners concerning to intestinal parasites prevalence and aiming to the development of control and treatment strategies.

Conflict of interest statement

No competing financial interests exist.

Acknowledgments

Study supported by Unidade de Clínica das Doenças Tropicais and Centro de Malária e outras Doenças Tropicais – Laboratório Associado, Instituto de Higiene e Medicina Tropical, Lisboa, Portugal.

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