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ORIGINAL PAPER

Prevalence and genotypes of *Giardia duodenalis* from dogs in Spain: possible zoonotic transmission and public health importance

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Abstract The prevalence of *Giardia duodenalis* was determinate in faecal samples from dogs and cats in Madrid, Spain and molecular characterisation of isolates. A total of 604 and 144 faecal samples from dogs and cats, respectively, were analysed by routine coprological methods. The prevalence of *G. duodenalis* was 16.4 % (99/604) in dogs and 4.2 % (6/144) in cats. Sixty-four *G. duodenalis* isolates (63 from dogs and 1 from a cat) were characterised using glutamate dehydrogenase and β-giardin genes by PCR-RFLP. The single cat sample showed a mixed infection by assemblages A + F. The assemblages found in the dog samples were A, B, C, D and E, both as single and as mixed infections. The zoonotic assemblages A and B were found in 56 (88.8 %) *G. duodenalis*-positive samples with 15.9 % of samples having assemblage A (10/63) and 73 % of samples with assemblage B (46/63), indicating high potential zoonotic risk and public health significance.

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Introduction

Giardia duodenalis (syn. *Giardia lamblia* and *Giardia intestinalis*) is an important cosmopolitan protozoan that affects a wide range of vertebrates, including humans and domestic animals such as cats and dogs. Genetic characterisation has revealed eight different assemblages (A to H). According to different surveys, these assemblages may be specific to certain hosts: assemblages A and B have been found mainly in humans (although they have been detected in other animals), C and D are specific to dogs, F is specific to cats, E is found in livestock (pigs, cattle, goat and sheep) and G is found mainly in rodents. Recently, assemblage H has been isolated in marine vertebrates (Feng and Xiao 2011). However, few characterisation studies have been undertaken to determine what the full range of assemblages infecting dog populations might be. In fact, in Spain, there have been no such characterisation studies with regard to dogs or cats despite the close relationship humans have with these animals as pets. The aims of the present study were to determinate the prevalence of *G. duodenalis* in stray dogs and cats from Madrid (Spain) and to molecularly characterise the isolates in order to assess the potential for zoonotic transmission and possible implications to public health.

Material and methods

Samples collection and faecal analysis

The present study was performed in dogs and cats individually housed at an animal shelter in the city of Madrid. A total of 604 and 144 faecal samples were collected from dogs and cats, respectively, and analysed using the modified Telemann sedimentation method and merthiolate–iodine–

formalin staining, followed by examination under a light microscope.

Molecular diagnosis and characterisation

DNA extraction

DNA was extracted from faecal samples identified as *G. duodenalis*-positive by microscopic examination, using the QIAmp DNA Stool Mini Kit (QIAGEN) and following the manufacturer's instructions. The extracted DNA was stored at 4 °C until PCR was performed.

Real-time PCR

Real-time PCR targeting the SSU RNA gene was performed in an ABI PRISM 7500 Sequence Detection System (Applied Biosystems) using the primers, probe and cycling conditions described by Verweij et al. (2003).

Genetic characterisation by nested PCR-RFLP

Molecular characterisation was carried out using two nested PCR protocols: one to amplify a fragment of the glutamate dehydrogenase (GDH) gene (Read et al. 2004), and the other to amplify a fragment of the β-giardin gene (Lalle et al. 2005). The *G. duodenalis* assemblages were determined by analysing the restriction patterns obtained following digestion of the purified amplification products.

Results and discussion

Results observed in the present study show that intestinal parasites were found in 28.1 % (180/604) of the faecal samples from dogs, including *G. duodenalis* (16.4 %), *Cystoisospora* spp. (0.8 %), *Toxocara canis* (6.5 %), *Toxascaris leonina* (4.8 %), *Trichuris vulpis* (1.5 %), family Ancylostomatidae (3.5 %), family Taenidae (2 %) and *Dipylidium caninum* (5.1 %). Intestinal parasites were found in 19.4 % (28/144) of the feline faecal samples, including *G. duodenalis* (4.2 %), *Cystoisospora* spp. (2.8 %), *T. canis* (11.8 %) and family Taenidae (2.8 %).

These results reveal a high prevalence of *G. duodenalis* infection in Madrid's dogs, in agreement with other studies performed in Spain (Ariza et al. 1998; Martínez-Carrasco et al. 2007), Europe and other regions of the world (Ballweber et al. 2010). Indeed, *G. duodenalis* is considered as one of the most prevalent parasitic intestinal protozoans found in dogs (Thompson et al. 2008). The prevalence of *G. duodenalis* infection in cats was lower (4.2 %), in accordance with an earlier study also performed in Madrid by Lopéz et al. (1995).

One hundred faecal samples (96 from dogs and 4 from cats) shown positive for *G. duodenalis* by microscopy were selected for molecular characterisation, of which 90 were confirmed positive by real-time PCR. On the other hand, with nested PCR-RFLP, only 64 of these 100 faecal samples yielded amplification products for either one or both of the target genes: 28 (43.75 %) isolates with both PCR-RFLP protocols (β-giardin and GDH), 6 (9.4 %) for the β-giardin gene only and 30 (46.8 %) for the GDH gene only.

The difference we report between the real-time PCR and PCR-RFLP results is similar to that previously reported by Adamska et al. (2010) who observed that real-time PCR was more sensitive than nested PCR. It is likely that the samples that tested positive with real-time PCR, but in which nested PCR-RFLP failed to amplify the target sequences, had insufficient numbers of parasites for achieving such amplification (Adamska et al. 2011). They may have been detected if larger faecal samples had been available or if these samples had been concentrated (Castro-Hermida et al. 2007; Adamska et al. 2011).

Nested PCR-RFLP amplified the GDH target sequence in 58 samples and the β-giardin target in 34 samples (some of which returned no amplicons with the GDH protocol). Thus, both protocols should always be used to ensure the best detection results.

The characterisation study shows that in 33 of the dog faecal samples only one type of assemblage was identified (single infection): AI in 3 samples, BIII in 7 samples, BIV in 15 samples, C in 1 sample and D in 7 samples. Thirty mixed infections were also detected (Table 1). Table 2 shows the overall detection percentage for each assemblage identified in single and mixed infections. The global genotype results showed that assemblage AI was present in 10 samples (15.9 %), assemblage B in 46 samples (73 %) with 21 samples having assemblage BIII (33.3 %) and 25 samples having assemblage BIV (39.7 %). The assemblages specific to dogs were present in 34 samples: 12 samples with assemblage C (19 %) and 22 samples with assemblage D (35 %). Finally, assemblage E (9.5 %) was identified in six samples. *G. duodenalis* was successfully identified in only one cat sample as a mixed infection by assemblages AI and F.

In Spain, few characterisation studies have been performed on *G. duodenalis* and never in companion animals

Table 1 Mixed infections (n=30): combination of assemblages

Assemblages	BIII	BIV	C	D	E
AI	1	2	3	0	1
BIII	–	0	2	4	4
BIV	–	–	1	7	0
C	–	–	–	1	1

Besides, three mixed (triple) infection were assemblages BIII + C + D

Table 2 *G. duodenalis* genotypes in dog samples

Assemblages	Number (*n*)	Percent
AI	10	15.9
BIII	21	33.3
BIV	25	39.7
C	12	19
D	22	34.9
E	6	9.5

Number pertains to the number of dog samples in which the assemblages mentioned were detected. Percent pertains to percentage of dog samples in which the assemblage mentioned was detected (*n*/total of dog samples). Total dog samples studied=63

(dog and/or cats). Two studies in human beings detected a predominance of assemblages A and B (Goñi et al. 2010; Sahagún et al. 2008), one study in nonhuman primates found assemblage A as the most common (Martínez-Díaz et al. 2011) and three studies in farm animals recorded assemblage E to be the most prevalent (Castro-Hermida et al. 2007; Ruiz et al. 2008; Gómez-Muñoz et al. 2009). However, recently, a high proportion of assemblage A or mixed assemblage A/E had been detected in samples from lambs (Gómez-Muñoz et al. 2012). Our results showed that the host-specific assemblages for dogs (C and D) and cats (F) were detected; however, a high prevalence of assemblages A and B, which are preferentially found in humans, were also detected in the dog samples, and the assemblage A was also detected in the positive cat sample.

Recent studies in certain European countries and Brazil (Paz e Silva et al. 2011), Canada (Himsworth et al. 2010), Japan, Peru (Cooper et al. 2010) and Thailand have reported dogs infected with the canine host-specific C and D assemblages, and cats infected with the feline host-specific assemblage F; however, zoonotic assemblage A was also detected in both cats and dogs, as was assemblage B, although to a lesser extent (Sprong et al. 2009; Ballweber et al. 2010).

These results suggest that dogs and cats could play an important epidemiological role in the transmission of *G. duodenalis* to humans. The high prevalence of assemblages A (15.9 %) and B (39.7 %) detected may be the result of these dogs having become infected by drinking water contaminated with human faeces or those of other domestic animals (Thompson 2004; Feng and Xiao 2011). This transmission cycle may be possible in urban environments where parks are irrigated with 'recycled' water that has not been fully treated for human consumption; thus, *Giardia* cysts could still be infective (Amorós et al. 2010). Transmission is also favoured given the close relationship of human beings with their pet dogs and cats (Feng and Xiao 2011).

This is the first report of genotyping of *G. duodenalis* in dogs and cats of Spain. The most commonly circulating assemblages were B, D, followed by C, A, E and F. The implications for public health of detecting zoonotic assemblages A and B in these companion animals should be investigated.

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