Title: Prevalence and Molecular characterization of *Giardia duodenalis* from dogs in La Habana, Cuba.

Authors: Luis Enrique Jerez Puebla¹, Fidel A. Núñez¹, Lázara Rojas Rivero¹, Yordan Robau Hernández², Iraís Atencio Millán¹, Norbert Müller³.

¹ Department of Parasitology, Tropical Medicine Institute "Pedro Kourí", Autopista Novia del Mediodía Km 6½ e/Autopista Nacional y Carretera Central, La Habana, Cuba.

² Veterinary Unit of Canine Shelter. La Lisa, La Habana, Cuba.

³: Institute of Parasitology. University of Berne, Switzerland.

Corresponding author: Lic. Luis Enrique Jerez Puebla. MsC.

E-mail address: luisjerezpuebla@infomed.sld.cu

Tel.: 537-255-3645.

Abstract

Giardia duodenalis is a worldwide protozoan parasite that infects humans and other mammals including dogs. Due to the risk of zoonotic transmission between dogs and humans we aimed in this study to determine the prevalence of *G. duodenalis* and distribution of assemblages of *Giardia* among dogs analysed. A descriptive cross-sectional study was carried out in La Habana from June 2014 to March 2015 in the Zoonosis Unit of La Lisa municipality. A total of 98 dogs were analysed by three different techniques, microscopy, ELISA, and PCR in order to detect *Giardia* in stool samples. Seven dogs were positive by microscopy after concentration technique, nine by NOVITEC® *Giardia* Microplate Assay, and ten and eleven samples were amplified by the β -giardin and 18ss RNA PCRs, respectively. After PCR sequence analysis of both genes only zoonotic assemblages (A and B) were detected. The 18ss RNA sequence results revealed a distribution of 8 assemblages A and 4 assemblages B, whereas only assemblages A were identified by the β -giardin analysis. Among sub assemblages classification by β -giardin phylogenetic tree, four isolates shown an AI pattern, and one isolate displayed an AII distribution. Mixed infections were detected in three isolates. These findings highlight the risk of zoonotic transmission of *Giardia duodenalis* between dogs and humans.

1 **Key words:** *Giardia duodenalis*, dog, prevalence, microscopy, ELISA, PCR, sequencing,

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1. INTRODUCTION

Intestinal parasitic infections in dogs are commonly recognized as a cause of gastrointestinal disorders with a high prevalence in developing countries (David et al., 2015). Among intestinal helminths occurring in dogs, *Toxocara canis*, represents the major concern as it can cause severe infection in humans leading to symptoms such as diarrhea, vomiting, loss of weight and fatigue (Nijse et al., 2014). Other zoonotic helminths like *Ancylostoma caninum*, *A. braziliensis* and *Uncinaria stenocephala* are primary causes of cutaneous, visceral, and ocular larva migrans and eosinophilic enteritis (McKenzie et al., 2010).

Among protozoan parasites, *Giardia duodenalis* has become an important pathogen with potential public health hazard due to possible zoonotic transmission to humans (Feng and Xiao, 2011). Giardiasis in dogs has emerged as one of the most prevalent infections in both household and kennelled dogs worldwide, with varying prevalence rates from 5% in privately owned dogs to up to 100% in kennelled animals (Dubná et al., 2007; Upjohn et al., 2010).

Dogs develop a clinical picture of this disease varying from subclinical to slight abdominal discomfort, or even resulting in severe abdominal pain and cramping. Diarrhea is characterized by soft to watery feces, being frequently mucoid with a strong odor, and also steatorrhea may occur (Tangtrongsup and Scorza, 2010).

1 Diagnosis of canine giardiasis mainly includes microscopical examination of feces,
2 Unfortunately, conventional coprology can lead to an underestimation of the prevalence of *G.*
3 *duodenalis* particularly in cases where the number of excreted cysts is very low or when
4 concurrent infections with multiple parasite species are present. However, the diagnostic
5 performance can be substantially improved by using PCR allowing unambiguous detection of
6 the parasite even in low-infection intensity cases and at the same time providing a basis for
7 genetic characterization of the corresponding *Giardia* isolates (David et al., 2015).

8 *G. duodenalis* is composed of a complex of species and is classified in eight assemblages (A-
9 H). Among these assemblages, only A and B have a zoonotic potential and they are present
10 in humans as well as in many mammals, including dogs (Ryan and Cacciò, 2013). Dogs were
11 shown to preferentially harbour canid host-specific assemblages C and D (Feng and Xiao,
12 2011). Compared to this finding, the identification of zoonotic assemblages in dogs are of
13 greater epidemiological importance, because in cases of a close contact between humans and
14 dogs, transmission of the parasite has to be considered a realistic scenario (Ryan and Cacciò,
15 2013).

16 In Cuba there are approximately 2 million of dogs controlled by clinical veterinary centers,
17 almost one per six habitants, and half of them are stray dogs according to data of the Ministry

of Cuban Public Health. (Hernández et al., 2007). The municipality of La Lisa with an extension of 37.5 square kilometers, in La Habana, has a Canine shelter center which receives stray and domestic dogs for analysis and veterinary control from different municipalities of La Habana. Given the lack of current knowledge about the prevalence of *Giardia* in canine population, we aimed in this study to identify by different methods *Giardia* cysts from stool samples, as well as to elucidate the pattern of distribution of *G. duodenalis* assemblages.

METHODS

2.1. Study area and dogs

A descriptive cross-sectional study was carried out from June 2014 to March 2015 in a population of dogs in the Canine Shelter located in the municipality of La Lisa, province of La Habana, Cuba. For a total population of 8371 dogs reported in La Lisa municipality (Hernández et al., 1997), using a prevalence of infections 10% (Meneses et al., 1994), a total sample size of 98 dogs of this area was calculated for 90% confidence level.

Dogs were randomly selected depending on probable parasitic infection and provenance. In total were checked 98 dogs divided in 56 shelter dogs and 42 household dogs. A standard questionnaire was used to collect information regarding each animal's origin, sex, age, gregarious behaviour, and symptomatology. The dogs assayed for the present study were

evaluated by veterinary professionals and stool samples were collected in cleaned containers and sending to the National Reference Laboratory of Intestinal Parasitic Infection from "Pedro Kourí" Institute for parasitological studies due to clinical signs of gastrointestinal disturbances, which varied from lose weight, diarrhea, vomiting and presence of ectoparasites. In case of shelter dogs samples were collected in shelter houses, and only from dogs kept in individual boxes to avoid sample mixture.

The research protocol was approved by the Ethics Committee of the "Pedro Kourí" Institute.

All *Giardia* positive cyst/trofozoites were preserved in potassium dichromate 2.5% and stored at -20°C for further molecular analysis.

2.2. Coproparasitological study

Faecal samples were examined for intestinal parasites by a wet smear stained with Lugol's iodine and followed by formalin ethyl acetate concentration technique. All samples were processed also by the Kato-Katz smear method and the flotation technique of Willis-Malloy for the identification of parasite eggs. All diarrheal faecal samples were stained by a modified acid-fast method for *Cryptosporidium* spp., *Cyclospora*, and *Cystoisospora* (Garcia, 2001).

2.3. Copro-antigen ELISA

The NOVITEC® *Giardia* Microplate assay was used for copro-antigen detection of *Giardia* - Specific Antigen 65 (GSA 65). All ELISAs were conducted on diluted faecal samples following the manufacturer's instructions. Both, visual observation and plate reader results were used to identify *Giardia*-positive samples. Spectrophotometric results were quantified by measuring the absorbance in a 96-wells spectrophotometer at a wave length of 450 nm.

2.4. Purification of *Giardia duodenalis* cyst and DNA extraction

Giardia cysts were purified and concentrated from faecal samples in a sucrose gradient with a specific gravity of 0.85 M and then washed with distilled water, following the protocol described elsewhere (Babaei et al., 2011). The cyst wall was disrupted by 8–10 freeze–thaw cycles in liquid nitrogen alternated with a 95 °C water bath. After that, total DNA from purified cysts was extracted from feces using a commercial kit (Gerbior (MO BIO Laboratories, USA).

2.5. Amplification of SSU rRNA

Amplification of the small subunit ribosomal DNA (SSU rRNA) gene was performed by a nested PCR using a forward primer RH11, 5'-CATCCGGTCGATCCTGCC-3' and reverse primer RH4, 5'-AGTCGAACCCTGATTCTCCGCCAGG-3' as described by Hopkins et al.

(1997). The PCR reaction mix was prepared using 1 unit GoTaq® DNA Polymerase (Applied Biosystems, USA) with GeneAmp® 10X PCR buffer kit (Applied Biosystems, USA) in a total volume of 25µL.

The first-round PCR conditions were: 96 °C for 5 min for 1 cycle, 96 °C for 45 s, 50 °C for 30 s and 72 °C for 45 s for 35 cycles followed by 72 °C for 7 min.

Two microlitres from the first PCR were used in the second PCR using amplification conditions as follows: 96 °C for 5 min for 1 cycle, 96 °C for 45 s, 55 °C for 30 s and 72 °C for 45 s for 35 cycles followed by 72 °C for 7 min with PCR primers forward GiarF, 5'-GACGCTCTCCCCAAGGAC-3' and reverse primer GiarR, 5'-CTGCGTCACGCTGCTCG-3' (Read et al., 2004).

2.6. Amplification of β-giardin gene

In order to amplify the β-giardin gene, we used the forward primer G7 5'-AAGCCCGACGACCTCACCCGCAGTGC-3' and reverse primer G759 5'-GAGGCCGCCCTGGATCTTCGAGACGAC-3' described by Cacciò et al. (2002). The PCR reaction mixture was done using a GoTaq® DNA Polymerase with GeneAmp® 10X PCR buffer kit (Applied Biosystems, USA) in a total volume of 25µL.

1 The DNA was amplified using Gene Amp[®]PCR System 9700 (Applied Biosystem, USA).

2 The PCR products were visualized on 2% agarose gels.

3 DNA from axenic cultures of *G. duodenalis* strains WB-C6 (assemblage A) and GS
4 (Assemblage B), were used as positive controls, while ultrapure water was included in
5 negative controls.

6 2.7. PCR product purification and sequencing

7 In order to confirm the genetic characterization results, bands were excised from agarose gels,
8 purified using High Pure PCR product purification kit (Roche) and sequenced by an in-house
9 sequencing service kindly provided by the Institute of Veterinary Bacteriology in Berne
10 Switzerland. Phylogenetic analysis included alignment of the resulting SSU rRNA and β -
11 giardin sequences with those available in GenBank using Clustal X program. Sequence
12 similarity was also determined using the Basic Local Alignment Search Tool (BLAST) and
13 the phylogenetic and molecular evolutionary analyses were conducted using MEGA version
14 5. The evolutionary history was inferred using the Neighbor-Joining method (Saitou and Nei,
15 1987) and the respective evolutionary analyses were conducted using MEGA 5 (Tamura et al.,
16 2011).

2.8. Statistical analysis

All data were analysed using EPINFO 6.04 and EPIDAT 3.1 statistical programmes. The odds ratio (OR) with 95% confidence interval (CI) were performed as measures of association. McNemar test was used to compare paired proportions. The kappa index was used as measure of agreements between diagnostic tests. The P values less than 0.05 were considered as statistically significant.

3. Results

3.1. Coproparasitological study

Out of 98 dogs studied, 43 (43.9%) were infected with both zoonotic and non-zoonotic parasites. The zoonotic parasites *Ancylostoma caninum* (21.4%), *Trichuris vulpis* (16.3%), and the protozoan, *Giardia duodenalis* (7.2%) were the most prevalent parasites. Mixed infections were detected in 11 faecal samples (11.2%, 95% CI = 4.5-18.0). Dogs were represented from 9 out of 15 municipalities of La Habana. La Lisa (32/98; 32.7%), Boyeros (21/98; 21.4%), Marianao (17/98; 17.3%), Playa (12/98; 12.2%) were the municipalities more represented. No statistically difference was observed between dog's provenance and intestinal parasitic infection ($P > 0.05$).

Among the kind of dog investigated, shelter dogs had more frequency of infection due to *Ancylostoma caninum* (15/56, 26.8%) and *Trichuris vulpis* (11/56, 19.6%), whereas *G. duodenalis* was more prevalent in domestic dogs (4/42, 9.5%).

Among the two types of dogs (shelter versus household dogs) investigated, no statistical differences were found between frequencies of helminthic infections (data not shown).

3.2. Copro-antigen ELISA for detection of *Giardia*

From 98 stool samples 9 were positive by copro-antigen tests (9.2%). Seven of them were all positive by microscopical examination and concentration techniques, and two of them were previously negative by microscopy.

3.3. Molecular characterization of the 18ss RNA and β -giardin genes

In total, 11 out of 98 samples were positive for *Giardia* by PCR amplification of the SSU rRNA gene, for an overall prevalence of 11.2%. As expected, PCR-positive samples included also those 9 samples that turned out to be positive by microscopical and/or copro-antigen-based analyses. Among the SSU rRNA gene PCR-positive samples, 10 scored positive in the β -giardin PCR (10.2%)

3.4. Concordance between microscopy, ELISA and PCR

From 98 examined stool samples, 7 were positive by microscopically examination after concentration technique, 9 showed positive results (included previous 7 by microscopy) by ELISA test, and the most sensible test, the PCR amplified specific products in 11 out of 98. The kappa homogeneity tests showed excellent agreements among three methods with kappa values above 0.85 (data not shown).

3.5. Sequencing of PCR products of 18ss RNA and β -giardin

Phylogenetic analyses of sequences from SSU rRNA gene (Fig. 1) and β -giardin (Fig. 2) amplification products summarized in Table 1 revealed exclusively zoonotic assemblages (A or B) in faecal samples. Among these samples, 3 contained mixed infections (assemblages A + B).

The genetical assemblages of *Giardia* obtained at each locus by sequencing analysis are summarised in Table 1.

3.6. Clinical characteristic of *Giardia*-infected dogs identified in this study related to corresponding *Giardia* assemblages.

Clinical data of dogs diagnosed by SSU RNA gene-PCR as *G. duodenalis*-positive were as follows. Among domestic animals, 4 out of 8 (50%) were asymptomatic, whereas 2 out of 3

(66.6%) of stray dogs exhibited symptoms compatible with canine giardiasis (Table 2). In two symptomatic stray dogs co-infection with helminths of veterinary importance were identified.

4. Discussion

The development of epidemiological studies in dogs may be an important issue for integrated plans in National Health Control programmes for zoonotic infectious diseases. These studies can be useful tools to elaborate or modified such programmes, as they take into account the important roles of dogs in the zoonotic transmission from animal to humans (Traub et al., 2005).

Several studies have been addressed around the world concerning prevalence of intestinal parasites in canine population. These have shown a high prevalence rates of different intestinal parasitic infections, being *G. duodenalis* one of the most common intestinal parasites identified in dogs (Traub et al., 2005; Katagiri and Oliveira-Sequeira, 2008; Bajer et al., 2011).

Soil transmitted helminthes *Ancylostoma caninum*, and *Trichuris vulpis* accounted the most prevalent parasites in the studied population of dogs, whereas *G. duodenalis* was the third in frequency. According to this data and previous studies carried out in our country (Hernández et al., 2007, Sariego et al, 2012), one could estimate that nearly one fourth of dogs are

1 infected with intestinal nematodes which release eggs to the environment, resulting in places
2 where environmental contamination is high and an increase likelihood of infection by
3 humans. This problem has been supported by various studies in Cuba whom have showed a
4 strong environmental contamination with *Toxocara* eggs in parks from the city.

5 The main aim of our study was to identify and estimate the prevalence of *G duodenalis* in
6 dogs by using three different techniques, namely microscopical coprology, copro-antigen test,
7 and PCR, the latter allowing a phylogenetic analyses of the *Giardia* isolates.

8 Several studies were made around the world targeting the prevalence of *G. duodenalis* in dog
9 populations. These studies revealed that this epidemiological variable depends on several
10 factors including animal's age, living conditions, parasitological technique applied. In this
11 respect it is also relevant whether only one, or several, faecal sample(s) is/are examined given
12 the intermittent nature of cyst excretion, areas analyzed and health status of the animal
13 (Tangtrongsup and Scorza, 2010). Some reports based on surveys in canine populations
14 showed that the prevalence of *Giardia* is about 10% in household dogs, 36–50% in pups and
15 up to 100% in breeding establishments and kennels (Simonato et al., 2015).

16 In the present study, we analyzed only one sample per dog, but as cyst excretion occurs
17 intermittently, and in low number in symptomatic cases, false-negative results may arise in

1 these cases. For that reason, it is convenient to use diagnostic tools that increase the sensitivity
2 of examinations. Here the copro-antigen ELISA represents an important diagnostic tool in
3 human and veterinary parasitic infections. Accordingly, we applied the NOVITEC® *Giardia*
4 Microplate Assay, which detects the GSA65 antigen that is associated with the *Giardia* cyst
5 wall and trophozoite. This sensitive assay has been frequently used for diagnosis of giardiasis
6 in cats, dogs, and cattle (Decock et al., 2003; Cirak and Bauer, 2004). The test is simple to
7 perform and exhibits an excellent diagnostic sensitivity (92%) and specificity (99%) (Groat et
8 al., 2003). Using this test we identified 9 positive cases of *G. duodenalis* indicating an overall
9 prevalence of 9.2%. Despite of number of cases detected by copro-antigen test was higher than
10 microscopy, no statically differences were found, and this fact can explained because of low
11 number of *Giardia* positive cases detected in this study.

12 In the United States a survey carried out in dogs using a commercial ELISA-based copro-
13 antigen test scored positive for *Giardia* in fecal samples in 15.6% of 16,114 dogs that were
14 considered symptomatic (Carlin et al., 2006). Furthermore, a Canadian report on prevalence
15 and diagnosis of *Giardia* infections in dogs with another copro-antigen test exhibited a much
16 higher sensitivity as compared to faecal smear examination (13% versus 3.3%) (Olson et al.,
17 2010), but the number of total and positive samples analyzed in this last study was higher.

The introduction of molecular methods to the field of diagnosis of parasitic infections has provided powerful new tools for characterizing parasites, especially *Giardia*, and has allowed a better understanding of genetic differences within this genus, the taxonomy, and epidemiology of giardiasis in humans and domesticated animals (Feng and Xiao, 2011). Although molecular methods are more expensive and require specific equipment, they have a higher specificity and sensitivity compared to microscopy, and therefore their use has been increased to detect and characterize intestinal protozoa (Ryan and Cacciò, 2013).

In our study, we amplified DNA of *Giardia* targeting the SSU rRNA and β giardin genes. The first gene is a commonly used molecular marker for the species and assemblage differentiation and useful to determine the presence or absence of infections (Covacin et al., 2011; Feng and Xiao, 2011), although the short sequence does not provide subassemblage information (Bouzid et al., 2008): For this reason, previous subtyping studies primarily relied on the sequence analysis of fragments of single-copy gene targets, such as triosephosphate isomerase (*tpi*), glutamate dehydrogenase (*gdh*), or β -giardin (*bg*) (Ryan and Cacciò, 2013).

In the present study, one of these genes, β -giardin, has been used as an additional marker for subtyping of the *Giardia* isolates. The advantage of using giardin genes, such as β -giardin, as target for the molecular detection of *Giardia* cysts is that they are considered to be unique to

1 this parasite and appeared to be more sensitive to amplify single and multiple infections
2 (Covacin et al., 2011).

3 Dogs can be infected with either the zoonotic *G. duodenalis* assemblages A and B or the non-
4 zoonotic host-specific assemblages C and D (Ryand and Cacciò, 2013). The present analysis
5 among dog isolates revealed that zoonotic assemblages A and B were predominantly
6 identified in the sequence analysis with a 100% of cases (11 out of 11), as well, mixed
7 infection were detected in three isolates. It has been noticed according to some authors that
8 use of the β -giardin marker may preferentially amplify twice as many assemblage A as
9 assemblage B isolates (Sprong et al., 2009). The reason for these discrepancies is unknown,
10 although it has been speculated that primer sequence mismatches might reduce the ability to
11 amplify certain assemblage B isolates at the β -giardin locus (Robertson et al., 2007).

12 This study showed that the SSU rRNA in conjunction with the β -giardin PCR sequencing
13 methods were able to type the highest number of samples, and also to identify mixed
14 infections. The SSU rRNA is a highly conserved multi-copy gene, which may explain why
15 SSU rRNA gene PCR sequencing is more successful than other sequencing methods (Cacciò
16 and Ryan 2008).

1 These data show that dogs in our settings harbor zoonotic *Giardia*, and should therefore be
2 considered a potential reservoir for infection in humans. As is known all *Giardia* assemblages
3 can be transmitted in cycles where only dog to dog transmission occurs, but humans may be
4 involved in a zoonotic cycle of transmission with assemblages A and B, either contracting
5 infection from dogs, or acting as a source of infection for dogs (Covacin et al., 2011).

6 When general primers are used, only the most abundant *G. duodenalis* assemblage is
7 amplified in the PCR reaction, and co-infections with less abundant assemblages may remain
8 undetected (Covacin et al., 2012; Ryan and Cacciò, 2013). That could be a reason for which
9 only zoonotic assemblages were found in the present study. It is recommended a detailed
10 analysis when interpreting genotyping data from dog isolates of *Giardia*, particularly when
11 only single locus is investigated (Beck et al., 2012). A close approach using a multilocus
12 analysis would be more specific in order to determine assemblages, sub assemblages and
13 genotypes present in faecal samples.

14 Several studies have explored the zoonotic potential of *Giardia* in the direct transmission that
15 might occur between dogs and humans (Traub et al., 2005; Inpankaew et al., 2007; Cacciò
16 and Ryan 2008). In household or domestic dogs, most of studies identified a high prevalence
17 of host-specific assemblages C and D (Berrilli et al. 2004; Souza et al.2007; Lebbad et al.

2010). In addition, worldwide studies found assemblages C and D being prevalent in stray/shelter dog populations (Claerebout et al. 2009; Upjohn et al. 2010).

Although assemblages C and D seem to be predominant in dogs, the exclusive identification of assemblages A and B in the 98 dogs examined in the present study is not extremely surprising. This is the case because some reports in community-based studies in developed countries, such as, Brazil and Thailand, only reported the zoonotic assemblage A in dogs (Volotão et al. 2007; Traub et al. 2004). Furthermore, in Latin America, specifically Mexico, Lalle et al. (2005) founded that five puppies from the Mexican Sinaloa state were exclusively infected with assemblage A (Lalle et al., 2005). In the same way, a high prevalence of assemblage A was found in domestic dogs from Germany (Leonhard et al. 2007).

In other studies made in overcrowded communities from India and Thailand, however, assemblage B was also identified in dogs (Traub et al., 2004; Inpankaew et al., 2007). In a Brazilian study on canine *Giardia* infections (Volotão et al, 2008) assemblage A was identified as the only genotype as assessed by PCR sequencing targeted to a β -giardin gene fragment. Here, sixty samples were typed as subassemblage AI and two as sub-assemblage AII (Volotão et al., 2008).

Phylogenetic analysis using different genes divided assemblage A into sub-assemblages, such as, AI which is considered potentially zoonotic, and sub-Assemblage AII considered anthroponotic (Thompson and Monis, 2004). A third classification, AIII, restricted to wild hoofed stock has been reported (Cacciò et al., 2008), and was identified in Mexico (Lalle et al., 2005). Previous genotyping analyses revealed that humans are mostly infected with AII and dogs with AI (Feng and Xiao, 2011). In the present study, β -giardin sequence-based analysis identified one dog isolate as AII. This observation was rather surprising because at least to our knowledge there is only one publication in current literature that reported on the presence of subassemblage AII in dogs (Traub et al., 2004).

The role of the dog as reservoir host for *G. duodenalis* genotypes potentially pathogenic for humans was widely studied and recognized as being a public health problem, especially in developing countries (Traub et al., 2005). The finding in the present work that only zoonotic assemblages A and B were detected in dogs from, either domestic or shelter animals has a serious epidemiological importance due to the greater risk of transmission by close contact between humans and dogs in the municipality of La Lisa, province of La Habana.

To date, no reports are available in current literature that describes the composition of *G. duodenalis* genotypes in Cuban dogs. Surprisingly, we now exclusively identified zoonotic

1 assemblages of *Giardia* in a cohort of dogs originating from a defined area in Havana city. In
2 future, a more comprehensive study with a larger number of samples from various regions of
3 the country and by using more gene markers for genotyping will provide a better
4 understanding of the zoonotic potential of canine *G. duodenalis* infections in Cuba.

5 **4. Conclusions**

6 In conclusion, the present results indicate that *G. duodenalis* is the most identified intestinal
7 protozoa in dogs from La Habana, Cuba. The combined use of three different coprological
8 techniques including microscopy, copro-antigen analysis, and PCR for canine faecal sample
9 testing showed that PCR has the highest sensitivity, detecting 11 out of 98 (11.2%) samples
10 being positive for *Giardia*. Comparisons of sequences from PCR-amplified SSU rRNA and β -
11 giardin genes revealed that faecal canine samples investigated exclusively contained zoonotic
12 assemblages A and B. Mixed assemblage A and B infections were detected in three samples.
13 This study contributes to the body of knowledge regarding the epidemiological status of
14 intestinal parasitic infections; especially *G. duodenalis* infections in dogs from Cuba. The
15 present data also provides initial information about the risk of zoonotic transmission for this
16 intestinal parasite infection.

1 **Competing interests**

2 The authors declare that they have no competing interests.

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Table 1. Results of genetic characterization of *Giardia* isolates by 18ss RNA and β -giardin sequencing from dogs in Havana, Cuba.

GenBank			GenBank	
Accession No.	Location	ss18RNA	Accession No.	β -giardin
KT595649	Domestic dog	A		
KT595650	Domestic dog	B	KT907056	AI
KT595651	Domestic dog	B	KT907057	AI
KT595652	Domestic dog	A	KT907058	AI
KT595653	Domestic dog	B	KT907059	AII
KT595654	Domestic dog	A	KT907060	AI
KT595655	Domestic dog	A		
KT595656	Domestic dog	A		
KT595657	Stray dog	A		
KT595658	Stray dog	A		
KT595659	Stray dog	B		

Table 2. Clinical characteristic of *Giardia*-infected dogs identified in this study related to corresponding *Giardia* assemblages.

Dogs	Symptoms	Co-infection with other parasites	<i>Giardia</i> assemblage
Domestic dog 1	Asymptomatic	-	A
Domestic dog 2	Asymptomatic	<i>Blastocystis</i> spp.	A+B
Domestic dog 3	Loss of weight	-	A+B
Domestic dog 4	Loss of appetite	-	A
Domestic dog 5	Asymptomatic	-	A+B
Domestic dog 6	Flatulence	-	A
Domestic dog 7	Constipation	-	A
Domestic dog 8	Asymptomatic	-	A
Stray dog 1	Diarrhea	<i>Trichuris vulpis</i>	A
Stray dog 2	Loss of weight	-	A
Stray dog 3	Diarrhea, flatulence	<i>Ancylostoma caninum</i>	B

Due to the low number of *Giardia* as sole infection in dogs analysed, correlation between symptomatology and genetic assemblage of this parasite was not done.

Fig. 1 Phylogenetic relationship of 11 *Giardia* isolates inferred by the neighbor-joining analysis of the ss18RNA nucleotide sequences. The evolutionary distances were computed using the Kimura 2-parameter method.

1

0.1

2

3 **Fig. 2** Phylogenetic relationship of 5 *Giardia* isolates obtained by the neighbor-joining
 4 analysis of the β -giardin nucleotide sequences. The evolutionary distances were computed
 5 using the Kimura 2-parameter method and are in the units of the number of base substitutions
 6 per site.

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