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ORIGINAL PAPER

Molecular epidemiology and multilocus sequence analysis of potentially zoonotic *Giardia* spp. from humans and dogs in Jamaica

Mellesia F. Lee[1,4] · Paul Cadogan[2] · Sarah Eytle[2] · Sonia Copeland[3] · Julia Walochnik[4] · John F. Lindo[1]

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Abstract *Giardia* spp. are the causative agents of intestinal infections in a wide variety of mammals including humans and companion animals. Dogs may be reservoirs of zoonotic *Giardia* spp.; however, the potential for transmission between dogs and humans in Jamaica has not been studied. Conventional PCR was used to screen 285 human and 225 dog stool samples for *Giardia* targeting the SSU rDNA gene followed by multilocus sequencing of the triosephosphate isomerase (*tpi*), glutamate dehydrogenase (*gdh*), and β-giardin (*bg*) genes. Prevalence of human infections based on PCR was 6.7 % (19/285) and canine infections 19.6 % (44/225). Nested PCR conducted on all 63 positive samples revealed the exclusive presence of assemblage A in both humans and dogs. Sub-assemblage A-II was responsible for 79.0 % (15/19) and 70.5 % (31/44) of the infections in humans and dogs, respectively, while sub-assemblage A-I was identified at a rate of 15.8 % (3/19) and 29.5 % (13/44) in humans and dogs, respectively. The predominance of a single circulating assemblage among both humans and dogs in Jamaica suggests possible zoonotic transmission of *Giardia* infections.

Keywords *Giardia* · Dogs · Humans · Jamaica · Zoonotic transmission · Assemblages

✉ John F. Lindo
john.lindo@uwimona.edu.jm

[1] Department of Microbiology, University of the West Indies, Mona, Kingston 7, Jamaica

[2] Jamaica Veterinary Medical Association, Kingston, Jamaica

[3] Ministry of Health, Kingston, Jamaica

[4] Institute of Specific Prophylaxis and Tropical Medicine, Center of Pathophysiology, Infectiology and Immunology, Medical University of Vienna, Vienna, Austria

Introduction

Giardia duodenalis (synonyms G. *lamblia* and *G. intestinalis*) is one of the most common intestinal parasites worldwide and an important cause of waterborne and foodborne infections in humans (Adam 2001; Adams et al. 2004). *Giardia* infections are re-emerging with just under 200 million symptomatic cases and up to 500,000 new cases per year (Pires et al. 2015). They are a common cause of diarrhea in developing countries and poor communities in the developed world (Pires et al. 2015). The global distribution of the parasite is attributable to its resilient infectious cysts, which can persist for long periods in harsh environmental conditions (Feng and Xiao 2011). Infection by *Giardia* is initiated when cysts are ingested from contaminated water, food, or direct fecal–oral contact (Thompson and Monis 2012). After ingestion, excystment triggers release of motile trophozoites in the small intestine and encystation in the large intestine (Thompson and Monis 2012). However, in symptomatic hosts with diarrhea, there is no encystment and trophozoites are excreted which die soon after passage (Thompson and Monis 2012).

Giardia isolated from mammals has been divided into host-specific assemblages (A-H) which have been proposed as separate species (Thompson and Monis 2004). Assemblages A, referred to as *G. duodenalis*, and assemblage B, referred to as *G. enterica*, are zoonotic having a broad host range and infect predominantly humans, dogs, and cats (McGlade et al. 2003; Paz e Silva et al. 2012). Unlike A and B, assemblages C and D have strong host specificities and are most commonly found in dogs, foxes, and other canines; assemblage E in livestock (cattle, sheep, pigs) and assemblages F and G are commonly found in cats and rodents, while H was identified in seals and gulls (Adam 2001; Feng and Xiao 2011). Initially, allozyme along with phylogenetic analysis resulted in subclustering within assemblages A and B, namely, A-I, A-II,

A-III, A-IV, and recently, B-I, B-II, B-III, and B-IV. This was derived from nucleotide sequence data of the glutamate dehydrogenase (*gdh*) gene. However, when compared to other genetic loci such as small subunit ribosomal RNA (SSU rRNA) and other housekeeping genes coding for β-giardin (*bg*), elongation factor 1 alpha (*ef1α*), and triosephosphate isomerase (*tpi*), this separation was not fully supported (Feng and Xiao 2011). These inconsistencies at the sub-assemblage level have resulted in the need of a multilocus genotyping approach to more accurately identify subtypes. Even though assemblages A and B have strong zoonotic potential, sub-assemblage A-I preferentially infects domestic animals and A-II is more common in humans (Sprong et al. 2009).

Cases of human giardiasis have been reported from Jamaica and other Caribbean countries, but data from the region are mostly limited to surveys and screening studies. For example, asymptomatic and symptomatic giardiasis was reported from 95 primary schoolchildren in Cuba at 57 and 43 %, respectively (Pelayo et al. 2008). The prevalence among a hospital cross-sectional population in Jamaica was 5.1 % (17 of 328) using microscopic and antigen detection methods (Lindo et al. 1998). Epidemiological studies on *Giardia* infections in dogs are largely lacking from the English-speaking Caribbeans. Dogs which may be infected with zoonotic assemblages of *Giardia* are important companion animals in the Caribbean and also occur in large numbers as strays due to the absence of leash laws (Brown et al. 2011). The prevalence of *Giardia* infection in dogs in Trinidad and Tobago was 25 % using PCR; however, these dogs were not infected with either assemblage A or B and zoonotic transmission was not considered likely (Mark-Carew et al. 2013).

The focus of this study was the identification and characterization of *Giardia* from humans and dogs in Jamaica using PCR and multilocus sequence analysis to gain insight into their zoonotic potential.

Materials and methods

Sample collection and microscopy

Two hundred and eighty five human fecal samples which were submitted for microscopic diagnosis to the Department of Microbiology at the University Hospital of the West Indies (UHWI) were randomly selected for the study. Two hundred and twenty five stool samples were randomly collected from dogs from 20 veterinary practices across Jamaica whether or not they presented with symptoms of *Giardia* infection. If transportation was delayed, samples were stored at 4 °C until transported to the University of the West Indies (UWI).

Parasitological diagnosis included the direct smear for the detection of *Giardia* trophozoites in fresh diarrheal stool samples and formalin–ether concentration on formed samples (Becker et al. 2011; Becker et al. 2013). Zinc sulfate flotation (specific gravity; 1.2–1.25), was used for the detection of *Giardia* cysts in stool samples collected from dogs (Dryden et al. 2005). Subsamples (~3 g) of all 510 samples were concentrated and stored in 85 % ethanol for DNA extraction.

Molecular investigations

DNA extraction was done by transferring 20 mg or 200 μl of stool into a 1.5-ml tube to 1000 ml of stool lysis buffer, heated at 70 °C for 10 min and then vortexed for 3 min. The remaining extraction procedure was done using the QIAamp Fast DNA Stool Kit® (Qiagen, Hilden, Germany) as described previously and the extracted DNA was stored at −20 °C until PCR was performed (Babaei et al. 2011).

Conventional and nested PCR amplification of the SSU rRNA, *tpi*, *bg*, and *gdh* genes

Conventional PCR was used to screen all stool samples by amplifying fragments of the SSU rDNA using primers RH11 and RH 4 (Table 1). Concentration of PCR master mix included 50 μl of reaction volume, molecular grade H_2O, 25 mM $MgCl_2$, 20 mM dNTP mix, HOT FIREPol® (5U/μl),10× Buffer B, and 10 pmol/μl of forward and reverse primer dilutions (Solis BioDyne®, Estonia). Amplification was done under the following cycle conditions: 35 cycles (95 °C for 15 min; 95 °C for 1 min; 52 °C for 2 min, and 72 °C for 2 min with a final extension of 7 min). Samples positive by conventional PCR were subjected to nested PCR targeting the *tpi*, *gdh*, and *bg* loci using primers specified (Table 1). Conditions for primary and nested amplifications were identical: 35 cycles (94 °C for 1 min, 45 °C for 30 s, and 72 °C for 45 s) using an Eppendorf® thermocycler which included preheating at 95 °C for 10 min and final extension at 72 °C for 7 min. The primary primer sets were used to amplify the external products, which were used as templates for the nested PCR reaction. Products were analyzed using 2 % agarose gel electrophoresis and a GelRed™ stain (BioTrend, Cologne, Germany) and visualized using a UV transilluminator box.

DNA sequencing

Positive amplicons were purified using the illustra GFX PCR DNA and Gel Band Purification Kit® (illustra GFX, GE Healthcare, Austria) and stored at −20 °C until DNA sequencing was performed. Sequencing was done using the ABI BigDye® Terminator V3.1 sequencing kit and an ABI automated sequencer (Applied Biosystems, Vienna, Austria). Sequences were assembled to consensus sequences with the

Table 1 Primers used in conventional and nested PCR at the SSU rRNA, *tpi*, *bg*, and *gdh* loci

Primers	Primer sequence	PCR	bp	References
RH11 RH4	Forward (5′-ATC TTC GAG AGG ATG CTT GAG-3′) Reverse (5′-AGT ACG CGA CGC TGG GAT ACT-3′)	Conventional	292	(Feng and Xiao 2011)
TPI1 TPI2	Forward (5′-AAC GCA ATC ACT GTA TCT-3′) Reverse (5′-CAA TGA CAA CCT CCT TCC-3′)	Primary	650	(Zheng et al. 2014)
TPI3 TPI4	Forward (5′-CTT CAT CGG CGG TAA CTT-3′) Reverse (5′-GGC ACG CTT AGC CTT CTT-3′)	Nested	334	(Zheng et al. 2014)
GDH1 GDH2	Forward (5′-TTC CGT GTT CAG TAC AAC TC-3′) Reverse (5′-ACC TCG TCC TGA GTG GCG CA-3′)	Primary	754	(Lalle et al. 2009)
GDH3 GDH4	Forward (5′-ATG ACT GAG CTT CAG AGG CAC GT-3′) Reverse (5′-GTG GCG CAA GGC ATG ATG CA-3′)	Nested	530	(Lalle et al. 2009)
G7 G759	Forward (5′-AGG CCC GAC CTC ACC CGC AGT GC-3′) Reverse (5′-GAG GCC GCC CTG GAT CTT CGA GAC GAC-3′)	Primary	753	(Gelanew et al. 2007)
GiarF bg GiarR bg	Forward (5′-GAA CGA ACG AGA TCG AGG TCC G-3′) Reverse (5′-CTC GAC GAG CTT CGT GTT-3′)	Nested	511	(Gelanew et al. 2007)

GeneDoc® editor (version 2.7.000) and then compared to reference sequences from GenBank® by multiple alignments using the CLUSTAL W® alignment software (Gelanew et al. 2007; Zheng et al. 2014).

Results

Formalin–ether concentration revealed a prevalence of 0.7 % (2/285) of *Giardia* infections among humans while zinc sulfate flotation revealed 2.7 % (6/225) prevalence among dogs. Molecular analysis also demonstrated that *Giardia* infections were notably higher in dogs (19.5 %; 44/225) than in humans (6.7 %; 19/285) based on conventional PCR targeting the SSU rDNA.

Nested PCR showed that the *tpi* gene proved to be highly sensitive for the detection of the *Giardia* assemblages with a 100 % (63/63) detection rate. At the *bg* locus, a slightly lower detection rate of 70 % (44/63) was seen while only 55.6 % (35/63) revealed PCR amplicons at the *gdh* locus.

Sequence analysis of all 63 positive samples comparing the three genetic loci identified only assemblage A in both humans and dogs in Jamaica. Sub-assemblage A-II was dominant in humans at a rate of 84.2 % (16/19) and in dogs at 70.5 % (31/44). Assemblage A-I accounted for 15.8 % (3/19) of *Giardia* infections in humans and 29.5 % (13/44) in dogs, respectively.

At the sub-assemblage level, nucleotide sequence data provided conflicting results when comparing sequences at different genetic loci. Four samples gave inconsistent findings when compared at the SSU rRNA locus. Assemblage A-I was identified in three samples and assemblage B in a single sample based on sequences of this gene. When compared to all other genetic loci, the same samples were consistently identified as sub-assemblage A-II with one exception where sub-assemblage A-I was identified at the *bg* locus.

Sub-assemblage A-II was predominant among humans and dogs in Jamaica with clear evidence of genetic polymorphism within the *tpi* gene in this sub-assemblage.

Discussion

This study revealed prevalence of *Giardia* infections among humans and dogs in Jamaica of 0.7 and 2.7 %, respectively, based on microscopy. Conventional PCR yielded prevalence of 6.7 % in humans and 19.5 % in dogs. Further, multilocus sequencing at the *tpi*, *bg*, and *gdh* loci demonstrated that only assemblage A was found in both humans and dogs.

Generally, the prevalence was similar to a study previously conducted at the University Hospital of the West Indies (UHWI), which reported a prevalence of 1.8 % using microscopy and 5.2 % using antigen detection (Lindo et al. 1998). The UHWI is an urban tertiary referral hospital and has reported a general decline in intestinal parasitic infections, which was attributed to improved sanitation and living standards (Rawlins 1982). However, the true prevalence of *Giardia* infections in Jamaica, including also asymptomatic infections, is unknown, and it was last estimated at 6.3 % in a prospective community based cross-sectional study (Rawlins et al. 1991). In neighboring Cuba, the prevalence of infection was estimated at between 6.02 and 9.34 % but

can be as high as 54.8 % in children in day care (Cañete et al. 2012; Jerez Puebla et al. 2015; Puebla et al. 2014). Asymptomatic and symptomatic *Giardia* infections were reported in Cuba at 57 and 43 %, respectively, from primary school children (Pelayo et al. 2008). Prevalence of human *Giardia* infections in developing countries ranges between 8 and 30 % in children (Dib et al. 2008; Johnston et al. 2010; Lalle et al. 2009; Sánchez-Vega et al. 2006; Solarczyk et al. 2010; Tungtrongchitr et al. 2010; Usluca et al. 2010). Among adults, infection rates were 25.1 % in pregnant women in Mexico and up to 14 % in African refugees to the USA (Manzardo et al. 2008; Robertson et al. 2010).

Despite the low prevalence reported from many countries, *Giardia* infection has been suspected to be underreported due to the intermittent shedding of cysts in stool and low sensitivity of microscopy when a single sample is examined. In the USA, the total number of unreported cases was estimated at 20,000 annually with an incidence of 7.4–7.6 cases per 100,000 population (Yoder et al. 2010). Molecular screening, in the current study, estimated a prevalence of 6.7 % in humans which was similar in Australia (7.6 %), and slightly lower in South Korea (2.5 %), Germany (1.5 %), the USA (1.4 %), and the UK (1.3 %) (Church et al. 2010; Huh et al. 2009; Read et al. 2002; Sagebiel et al. 2009). While these estimates based on multilocus sequencing revealed increased prevalence over microscopy, it should be noted that variations in DNA extraction methods, PCR inhibitors, and whether multicopy or single copy genes are targeted will affect the accuracy of molecular methods in estimating the true prevalence of *Giardia* infections (Thompson and Ash 2016).

The infection rates in dogs were 2.7 % based on microscopy and 19.5 % using PCR. High prevalence rates in dogs were also reported from the USA and Europe (19 and 24.78 %, respectively) (Epe et al. 2010). In Trinidad and Tobago, 25 % of dogs in one study were positive for *G. duodenalis* using PCR (Mark-Carew et al. 2013).

Interestingly, multilocus sequence analysis revealed assemblage A as the only assemblage identified in both humans and dogs in Jamaica, where sub-assemblage A-II accounted for most infections (84.2 and 70.5 % in humans and dogs, respectively) and assemblage A-I for the rest (19.8 % in humans and 29.5 % in dogs). No other *Giardia* assemblages were identified. The findings mirror those of four studies from Mexico, which reported only assemblage A in both humans and animal samples including dogs (Cedillo-Rivera et al. 2003; Eligio-Garcia et al. 2008; Lalle et al. 2005; Ponce-Macotela et al. 2002). However, direct comparisons should be done with caution as many of these studies are based on amplification of a single gene, and there are differences in methods used such as the absence of sequencing (Eligio-Garcia et al. 2008; Lebbad and Svard 2008). In contrast, assemblage B was consistently more common than assemblage A in four studies from Cuba occurring in 70.4 % of cases in one study (Puebla et al. 2016) assemblage B was also the predominant species among humans in Leon, Nicaragua, with only the non-human-associated assemblages C and D found in dogs (Lebbad et al. 2008; Puebla et al. 2016; Puebla et al. 2014). A predominance of assemblage A has been reported from several countries, and in humans, particularly sub-assemblage A-II seems to be linked to symptomatic infections; however, predominance and also virulence of either assemblage A or B in humans seems to be geographically very different and also to depend on other factors such as age, diet, and immune and socioeconomic status of the study population (Feng and Xiao 2011).

Assemblages C and D are considered host-specific in dogs and accounted for up to 76.5 % of cases in a study based on SSU rRNA gene sequencing in Italy (Berrilli et al. 2004). Mark-Carew et al. (2013) reported assemblages C and D at 15.4 and 80.8 %, respectively, among dogs in a study from the Caribbean islands of Trinidad and Tobago. In a recent study from Germany, high genetic diversity was seen in dogs presented to local veterinarians, including assemblages A, B, C, and D, with assemblages C, C/D, or D identified in 75.6 % of samples (93/123) (Pallant et al. 2015). However, in another study from Germany, similar to our study which included asymptomatic dogs, it also reported only very few host-specific strains (9 % assemblage C, 0 % assemblage D), while 60 % had assemblage A and another 27.3 % had mix of A and C (Leonhard et al. 2007). Generally, this seems to depend to a very high degree on the specific study setting (country, symptomatic/asymptomatic, pet/stray dogs Mexico).

The detection of a single circulating assemblage in dogs in the current study may be attributed to the cross-sectional nature of sample collection. Samples were not specifically collected from symptomatic animals presenting with gastrointestinal disturbances. As such, the predominance of assemblage A could indicate these dogs acted primarily as carriers. On the other hand, screening samples from dogs presenting with gastrointestinal disturbances might have shown a predominance of host-specific assemblages C and D, in addition to using species-specific primers. Moreover, *Giardia* infections are most commonly reported in young pups, while the vast majority of samples were collected from adult dogs (Alves and Santos 2016).

The predominance of sub-assemblage A-II in both humans and dogs was also unexpected. Sub-assemblages A-I and A-II differ in host preference. In accordance with our results, A-II has been most commonly reported from humans; however, in animals, A-I seems to be predominant (Sprong et al. 2009; Xiao and Fayer 2008).

While the high prevalence of *Giardia* infections in dogs coupled with the overlap in assemblages and sub-assemblages with the human infections strongly suggests a zoonotic transmission it does not provide definitive evidence. Nevertheless, zoonotic transmission is highly possible in Jamaica due to the

large number of free-roaming and uncared for dogs on the island which has no leash laws. Robust epidemiological evidence for zoonotic transmission from dogs to humans was seen in north eastern India where in addition to shared assemblages there was a significant correlation between the presence of a *Giardia* positive dog in the household and the prevalence of human infections (Traub et al. 2004). What also has to be taken into account is what has been described as "reverse zoonotic transmission" or anthropozoonotic transmission (Thompson 2013; Thompson and Ash 2016). This has been demonstrated in transmission between humans and wildlife such as non-human primates in Uganda and muskoxen in the Canadian Arctic (Johnston et al. 2010; Kutz et al. 2008).

Nested PCR at the *tpi* locus has proven to be highly sensitive and specific for detection and identification of *Giardia* spp. Inconsistencies when the *gdh* and *bg* genes were targeted—with a slightly lower amplification yield has been reported previously (Thompson and Ash 2016). It has been reported that the *Giardia*-specific SSU rRNA PCR has a detection limit of 10 pg DNA/microliter while the primers targeting the *tpi* locus have a detection limit of 1000 pg/microliter (Jaros et al. 2011). Moderate detection rates using the *gdh* locus were also seen in a study on *Giardia* infections in domestic cats (Jaros et al. 2011).

Multilocus sequence analysis is increasingly being used for characterization of *Giardia* isolates and is necessary to understand the distribution of different genotypes and the infection dynamics in and between different hosts. The findings from this study, demonstrating a predominance of *Giardia* assemblage A in both humans and dogs in Jamaica, suggest that zoonotic transmission of the parasite is likely occurring in this tropical endemic focus.

Compliance with ethical standards The study protocol was approved by the University Hospital of the West Indies/University of the West Indies/Faculty of Medical Sciences (UHWI/UWI/FMS) Ethics Committee (Ethics no. ECP 266, 13/14). It was conducted according to NIH, guidelines, ISO15189 standards for medical laboratories, and the Austrian Gene Technology Law. Moreover, the safety regulations by international standards and the European Community for working with biologically and chemically hazardous material were strictly adhered to.

References

Adam R (2001) Biology of *Giardia lamblia*. Clin Microbiol Rev 14:447–475

Adams PJ, Monis PT, Elliot AD, Thompson RCA (2004) Cyst morphology and sequence analysis of the small subunit rDNA and ef1α identifies a novel *Giardia* genotype in a quenda (Isoodon obesulus) from Western Australia. Infect Genet Evol 4:365–370

Alves J, Santos A (2016) Prevalence of *Giardia* spp. in young dogs using a combination of two diagnostic methods. Acta Parasitol 61:261–266

Babaei Z, Oormazdi H, Rezaie S, Rezaeian M, Razmjou E (2011) *Giardia intestinalis*: DNA extraction approaches to improve PCR results. Exp Parasitol 128:159–162

Becker S, Lohourignon L, Speich B, Rinaldi L, Knopp S, N'Goran E, Cringoli G, Utzinger J (2011) Comparison of the Flotac-400 dual technique and the formalin-ether concentration technique for diagnosis of human intestinal protozoon infection. J Clin Microbiol 49: 2183–2190

Becker SL, Vogt J, Knopp S, Panning M, Warhurst DC, Polman K, Marti H, von Müller L, Yansouni CP, Jacobs J, Bottieau E, Sacko M, Rijal S, Meyanti F, Miles MA, Boelaert M, Lutumba P, van Lieshout L, N'Goran EK, Chappuis F, Utzinger J (2013) Persistent digestive disorders in the tropics: causative infectious pathogens and reference diagnostic tests. BMC Infect Dis 13:37–37

Berrilli F, Cave DD, Liberato CD, Franco A, Scaramozzino P, Orecchia P (2004) Genotype characterisation of *Giardia duodenalis* isolates from domestic and farm animals by SSU-rRNA gene sequencing. Vet Parasitol 122:193–199

Brown PD, McKenzie M, Pinnock M, McGrowder D (2011) Environmental risk factors associated with *leptospirosis* among butchers and their associates in Jamaica. J Occup Environ Med 2:47–57

Cañete R, Díaz MM, Avalos García R, Laúd Martinez PM, Manuel Ponce F (2012) Intestinal parasites in children from a day care centre in Matanzas City, Cuba. PLoS One 7:e51394

Cedillo-Rivera R, Darby JM, Enciso-Moreno JA, Ortega-Pierres G, Ey PL (2003) Genetic homogeneity of axenic isolates of *Giardia intestinalis* derived from acute and chronically infected individuals in Mexico. Parasitol Res 90:119–123

Church C, Neill A, Schotthoefer A (2010) Intestinal infections in humans in the Rocky Mountain region, United States. J Parasitol 96:194–196

Dib H, Lu S, Wen S (2008) Prevalence of *Giardia lamblia* with or without diarrhea in South East, South East Asia and the Far East. Parasitol Res 103:239–251

Dryden M, Payne P, Ridley R, Smith V (2005) Comparison of common fecal flotation techniques for the recovery of parasite eggs and oocysts. Vet Ther 6:15–28

Eligio-Garcia L, Cortes-Campos A, Cota-Guajardo S, Gaxiola S, Jimenez-Cardoso E (2008) Frequency of *Giardia intestinalis* assemblages isolated from dogs and humans in a community from Culiacan, Sinaloa, Mexico using beta-giardin restriction gene. Vet Parasitol 156:205–209

Epe C, Rehkter G, Schnieder T, Lorentzen L, Kreienbrock L (2010) *Giardia* in symptomatic dogs and cats in Europe-results of a European study. Vet Parasitol 173:32–38

Feng Y, Xiao L (2011) Zoonotic potential and molecular epidemiology of *Giardia* species and giardiasis. Clin Microbiol Rev 24:110–140

Gelanew T, Lalle M, Hailu A, Pozio E, Caccio SM (2007) Molecular characterization of human isolates of *Giardia duodenalis* from Ethiopia. Acta Trop 102:92–99

Huh J-W, Moon S-G, Lim Y-H (2009) A survey of intestinal protozoan infections among gastroenteritis patients during a 3-year period (2004–2006) in gyeonggi-do (province), South Korea. Korean J Parasitol 47:303–305

Jaros D, Zygner W, Jaros S, Wedrychowicz H (2011) Detection of *Giardia intestinalis* assemblages A, B and D in domestic cats from Warsaw, Poland. Pol J Microbiol 60:259–263

Jerez Puebla LE, Nunez FA, Martinez Silva I, Rojas Rivero L, Martinez Gonzalez M, Mendez Sutil Y, Ayllon Valdes L, Atencio Millan I, Muller N (2015) Molecular characterization and risk factors of *Giardia duodenalis* among school children from La Habana, Cuba. J Parasitol Res 2015:378643

Johnston AR, Gillespie TR, Rwego IB, McLachlan TL, Kent AD, Goldberg TL (2010) Molecular epidemiology of cross-species *Giardia duodenalis* transmission in western Uganda. PLoS Negl Trop Dis 4:e683

Kutz SJ, Thompson RA, Polley L, Kandola K, Nagy J, Wielinga CM, Elkin BT (2008) *Giardia* assemblage A: human genotype in muskoxen in the Canadian Arctic. Parasit Vectors 1:32

Lalle M, Jimenez-Cardosa E, Caccio SM, Pozio E (2005) Genotyping of *Giardia duodenalis* from humans and dogs from Mexico using a beta-giardin nested polymerase chain reaction assay. J Parasitol 91: 203–205

Lalle M, Bruschi F, Castagna B, Campa M, Pozio E, Caccio SM (2009) High genetic polymorphism among *Giardia duodenalis* isolates from Sahrawi children. Trans R Soc Trop Med Hyg 103:834–838

Lebbad M, Svard S (2008) Comment on article by Eligio-Garcia et al. entitled "Frequency of *Giardia intestinalis* assemblages isolated from dogs and humans in a community from Culiacan, Sinaloa, Mexico using beta-giardin restriction gene". Vet Parasitol 158: 159–160

Lebbad M, Ankarklev J, Tellez A, Leiva B, Andersson JO, Svärd S (2008) Dominance of *Giardia* assemblage B in Leon, Nicaragua. Acta Trop 106:44–53

Leonhard S, Pfister K, Beelitz P, Wielinga C, Thompson RCA (2007) The molecular characterisation of Giardia from dogs in southern Germany. Veterinary Parasitology 150(1–2):33–38

Lindo JF, Levy V, Baum M, Palmer C (1998) Epidemiology of giardiasis and cryptosporidiosis in Jamaica. Am J Trop Med Hyg 59:717–721

Manzardo C, Trevino B, Gomez i Prat J, Cabezos J, Mongui E, Claveria I, Luis Del Val J, Zabaleta E, Zarzuela F, Navarro R (2008) Communicable diseases in the immigrant population attended to in a tropical medicine unit: epidemiological aspects and public health issues. Travel Med Infect Dis 6:4–11

Mark-Carew MP, Adesiyun AA, Basu A, Georges KA, Pierre T, Tilitz S, Wade SE, Mohammed HO (2013) Characterization of *Giardia duodenalis* infections in dogs in Trinidad and Tobago. Vet Parasitol 196:199–202

McGlade T, Robertson I, Elliot AD, Thompson R (2003) High prevalence of *Giardia* detected in cats by PCR. Vet Parasitol 110:197–205

Pallant L, Barutzki D, Schaper R, Thompson RC (2015) The epidemiology of infections with *Giardia* species and genotypes in well cared for dogs and cats in Germany. Parasit Vectors 8:2

Paz e Silva F, Monobe M, Lopes R, Araujo JJ (2012) Molecular characterization of *Giardia duodenalis* in dogs from Brazil. Parasitol Res 110:325–334

Pelayo L, Nunez F, Rojas L, Furuseth Hansen E, Gjerde B, Wilke H, Mulder B, Robertson L (2008) *Giardia* infections in Cuban children: the genotypes circulating in a rural population. Ann Trop Med Parasitol 102:585–595

Pires SM, Fischer-Walker CL, Lanata CF, Devleesschauwer B, Hall AJ, Kirk MD, Duarte AS, Black RE, Angulo FJ (2015) Aetiology-specific estimates of the global and regional incidence and mortality of diarrhoeal diseases commonly transmitted through food. PLoS One 10:e0142927

Ponce-Macotela M, Martinez-Gordillo MN, Bermudez-Cruz RM, Salazar-Schettino PM, Ortega-Pierres G, Ey PL (2002) Unusual prevalence of the *Giardia intestinalis* A-II subtype amongst isolates from humans and domestic animals in Mexico. Int J Parasitol 32: 1201–1202

Puebla LJ, Nunez FA, Fernandez YA, Fraga J, Rivero LR, Millan IA, Valdes LA, Silva IM (2014) Correlation of *Giardia duodenalis* assemblages with clinical and epidemiological data in Cuban children. Infect Genet Evol 23:7–12

Puebla LEJ, Núñez FA, Rivero LR, Silva IM, Valdés LA, Millán IA, Müller N (2016) Distribution of *Giardia duodenalis* assemblages by PCR-RFLP of β-Giardin gene in Cuban children. J Pediatr Infect Dis 11:6–12

Rawlins S (1982) Changing patterns in the prevalence of intestinal parasites at the University Hospital of the West Indies (1964–1981). West Indian Med J 31:111–120

Rawlins S, Campbell M, Fox K, Bennett F, Gibbs W, Greene M (1991) Parasitic infections in young Jamaicans in different ecological zones of the island. Trop Geogr Med 43:136–141

Read C, Walters J, Robertson I, Thompson R (2002) Correlation between genotype of *Giardia duodenalis* and diarrhoea. Int J Parasitol 32: 229–231

Robertson LJ, Gjerde BK, Hansen EF (2010) The zoonotic potential of *Giardia* and *Cryptosporidium* in Norwegian sheep: a longitudinal investigation of 6 flocks of lambs. Vet Parasitol 171:140–145

Sagebiel D, Weitzel T, Stark K, Leitmeyer K (2009) Giardiasis in kindergartens: prevalence study in Berlin, Germany, 2006. Parasitol Res 105:681–687

Sánchez-Vega J, Tay-Zavala J, Aguilar-Chiu A, Ruiz-Sánchez D, Malagón F, Rodríguez-Covarrubias J, Ordóñez-Martínez J, Calderón-Romero L (2006) Cryptosporidiosis and other intestinal protozoan infections in children less than one year of age in Mexico City. Am J Trop Med Hyg 75:1095–1098

Solarczyk P, Werner A, Majewska A (2010) Genotype analysis of *Giardia duodenalis* isolates obtained from humans in west-central Poland. Wiad Parazytol 56:171–177

Sprong H, Caccio S, van der Giessen J, network Z, partners (2009) Identification of zoonotic genotypes of *Giardia duodenalis*. PLoS Negl Trop Dis 3:e558

Thompson RC (2013) Parasite zoonoses and wildlife: one health, spillover and human activity. Int J Parasitol 43:1079–1088

Thompson RC, Ash A (2016) Molecular epidemiology of *Giardia* and *Cryptosporidium* infections. Infect Genet Evol 40:315–323

Thompson R, Monis P (2004) Variation in *Giardia*: implications for taxonomy and epidemiology. Adv Parasitol 58:69–137

Thompson R, Monis P (2012) *Giardia*-from genome to proteome. Adv Parasitol 78:57–95

Traub RJ, Monis PT, Robertson I, Irwin P, Mencke N, Thompson RC (2004) Epidemiological and molecular evidence supports the zoonotic transmission of *Giardia* among humans and dogs living in the same community. Parasitology 128:253–262

Tungtrongchitr A, Sookrung N, Indrawattana N, Kwangsi S, Ongrotchanakun J, Chaicumpa W (2010) *Giardia intestinalis* in Thailand: identification of genotypes. J Health Popul Nutr 28:42–52

Usluca S, Inceboz T, Over L, Tuncay S, Yalçin G, Arcak S, Ozkoç S, Aksoy U, Akisü C (2010) The distribution of intestinal parasites detected in The Dokuz Eylul University Medical Faculty Hospital between 2005 and 2008. Turkiye Parazitol Derg 34:27–31

Xiao L, Fayer R (2008) Molecular characterisation of species and genotypes of *Cryptosporidium* and *Giardia* and assessment of zoonotic transmission. Int J Parasitol 38:1239–1255

Yoder JS, Harral C, Beach MJ, Centers for Disease C, Prevention (2010) Giardiasis surveillance—United States, 2006–2008. MMWR Surveill Summ 59:15–25

Zheng G, Alsarakibi M, Liu Y, Hu W, Luo Q, Tan L, Li G (2014) Genotyping of *Giardia duodenalis* isolates from dogs in Guangdong, China based on multi-locus sequence. Korean J Parasitol 52:299–304