Parasitol Res (2014) 113:387–390
DOI 10.1007/s00436-013-3666-y

ORIGINAL PAPER

Characterization of *Giardia lamblia* genotypes in dogs from Tucson, Arizona using SSU-rRNA and β-giardin sequences

K. M. Johansen · N. S. Castro · K. E. Lancaster · E. Madrid · A. Havas · J. Simms · C. R. Sterling

Received: 26 August 2013 / Accepted: 18 October 2013 / Published online: 15 November 2013

Abstract The objective of this study was to determine if human genotypes of *Giardia lamblia* could be found in canine companion animals from urban and peri-urban environments in Tucson, Arizona. Canine fecal samples collected from the Humane Society of Southern Arizona between July 2006 and April 2009 were screened for *G. lamblia* infection using immunofluorescent microscopy and confirmed by polymerase chain reaction (PCR). Of the 672 samples screened, 196 were found positive by IFA and 185 of those positive were successfully amplified through PCR. Sequencing analysis showed samples were primarily of the C or D genotypes ($n=154$), or showing a mix of the C and D genotypes ($n=10$). One sample showed a mixed infection of a human genotype (A) and a dog-specific genotype (C). These data are consistent with previous studies showing dog specific genotypes to be dominant in environments where dog-to-dog transmission is likely to occur, and provides further evidence that multiple genes should be targeted for more accurate genotype characterization.

Introduction

Giardia lamblia (syn. *Giardia intestinalis*; *Giardia duodenalis*) is a flagellated protozoan parasite that is a major cause of diarrheal disease worldwide in a number of mammalian hosts, including humans and dogs (Sulaiman et al. 2003). The organism is transmitted via the fecal–oral route, with the ingestion of contaminated water being a typical source of infection (Feng and Xiao 2011). As few as ten cysts can elicit an infection that is self-limiting in otherwise healthy individuals (Ballweber et al. 2010; Feng and Xiao 2011). Giardiasis can be detrimental to the very young, the elderly, or the immunocompromised and can lead to refractory giardiasis in previously infected people. Symptoms of infection include dehydration, diarrhea, malabsorption, abdominal cramping, and weight loss; however, most cases of infection are asymptomatic (Ballweber et al. 2010).

G. lamblia is characterized into seven different genotypes, with only two major genotypes known to infect humans (Monis and Thompson 2003; Feng and Xiao 2011). The AI subgenotype and the B genotype can infect beaver, livestock, cats, dogs, and humans. The AII subgenotype has only been found in humans, except for one case of detection in a beaver (Baruch et al. 1996). Genotypes C and D are found in dogs and genotypes E–G are host specific in other mammals (Feng and Xiao 2011).

Although human genotypes have been characterized from samples of various mammals, the role of animals in transmission to humans and vice versa has yet to be confirmed (Cacciò and Ryan 2008; Cooper, et al. 2010; Feng and Xiao 2011) and data implicating pets as a risk factor for transmission of giardiasis is limited (Yoder et al. 2012). Previous studies have shown A and B genotypes to be found in both dogs and humans living in close proximity (Yong et al. 2000; Itagaki et al. 2005; Lalle et al. 2005a, 2005b; Volotão et al. 2007; Covacin et al. 2011). A study conducted in southern Germany confirmed a high prevalence of *G. lamblia* of the A genotype in asymptomatic dogs living in an urban environment (Leonhard et al. 2007). In contrast, the research completed by Sulaiman and colleagues (Sulaiman et al. 2003) in the USA showed that the dogs surveyed only displayed dog-specific genotypes and human samples showed only human genotype *G. lamblia* infections. Another study conducted in Aboriginal communities in Australia described dogs living in the same locality as humans

K. M. Johansen · N. S. Castro · K. E. Lancaster · E. Madrid · A. Havas · J. Simms · C. R. Sterling (✉)
School of Animal and Comparative Biomedical Sciences, University of Arizona, Bldg. 90, 1117 E. Lowell St., Tucson, AZ 85721, USA
e-mail: csterlin@u.arizona.edu

as being predominantly infected with the C and D genotypes (Hopkins et al. 1997). The aim of this study was to determine if human genotypes of *G. lamblia* could be found in canine companion animals in Tucson, Arizona by using direct immunofluorescence microscopy and polymerase chain reaction (PCR), thus providing additional molecular data to better understand transmission of the parasite.

Materials and methods

Sample collection and IFA

Canine fecal samples were collected from the Humane Society of Southern Arizona between July 2006 and April 2009. Both diarrheic and non-diarrheic samples were collected since *G. lamblia* infection may be present in either stool type (Ballweber et al. 2010). Samples were transported to the laboratory in a Styrofoam container immediately after collection and stored at 4 °C. Within 24 hours of collection, samples were concentrated for *Giardia* cysts using a standard ethyl acetate sedimentation technique (Ritchie 1948) and examined using Merifluor *Cryptosporidium/Giardia* direct immunofluorescence detection kit (Meridian Bioscience, Inc.).

DNA extraction

Sample DNA was isolated using the QIAamp DNA Stool Mini Kit (Qiagen, Inc.), per manufacturer's instructions, from samples found to be positive by the Merifluor kit and immunofluorescence microscopy. Positive control DNA (*G. intestinalis* ATCC 30957^{TM}) was extracted from cysts using the QIAamp DNA Mini Kit (Qiagen, Inc.). All DNA was suspended in AE buffer and stored at −20 °C until PCR was performed.

Polymerase chain reaction amplification

Nested PCR was performed using the following previously described primers to target small-subunit ribosomal DNA (SSU rDNA) and β-giardin sequences: RH11 and RH4, and GiarF and GiarR (Hopkins et al. 1997; Read et al. 2002), and G7 and G759 (Cacciò et al. 2002; Lalle et al. 2005a, b). The primers were chosen for their ability to amplify both human (A, B) and dog (C, D) genotypes in a single sample. PCR reactions for amplification of SSU rDNA consisted of 5.0 μL of DNA or 2.0 μL of external PCR product, 200 μM each of deoxynucleoside triphosphate (dNTP) (Fermentas, Inc.), 2.5 mM $MgCl_2$ included in 1× PCR buffer (Eppendorf-5 PRIME, Inc.), 1.25 μM of each corresponding primer, 5 % dimethyl sulfoxide (Sigma-Aldrich Corp.), 0.5 % nonacetylated bovine serum albumin (Sigma-Aldrich Corp.), and 1 unit of HotMaster *Taq* polymerase (Eppendorf-5 PRIME, Inc.) in a total 50 μL reaction. Reactions were performed as previously described (Read et al. 2002) using an Eppendorf Mastercycler Gradient (Eppendorf). PCR reactions for amplification of the β-giardin sequence consisted of 2.0 μL of template DNA or external PCR product, 200 μM each of dNTP, 2.5 mM $MgCl_2$ included in 1× PCR buffer, 0.4 μM of each corresponding primer, and 1 unit of HotMaster *Taq* polymerase in a total 25 μL reaction. The external PCR conditions were the following: first round of 95 °C for 5 minutes followed by 40 cycles of 95 °C for 30 seconds, 65 °C for 30 seconds, and 72 °C for 1 minute, and a final extension of 72 °C for 7 minutes. The nested PCR conditions were the following: first round of 96 °C for 5 minutes followed by 35 cycles of 96 °C for 45 seconds, 55 °C for 30 seconds, 72 °C for 45 seconds, and a final extension of 72 °C for 7 minutes.

Electrophoresis and purification of the polymerase chain reaction products

All nested PCR products were visualized on a 1.5 % ethidium bromide stained agarose gel for confirmation of amplification. A 100-bp ladder and both positive and negative controls were included. All PCR positive samples were subsequently subjected to DNA extraction via QIAquick Gel Extraction Kit (Qiagen, Inc.). DNA was suspended in EB buffer and stored at −20 °C until delivered for sequencing.

Sequencing analysis

DNA was sequenced with 3 μM nested primers using a 3730xl DNA Analyzer (Applied Biosystems) at the Genomic Analysis and Technology Core located at the University of Arizona. Sequencing data were analyzed, and contigs were made using Sequencer v4.5 (Gene Codes Corp.) or Vector NTI 11.1 (Life Technologies). The contig sequences were compared with sequences in GenBank (Hopkins et al. 1997; Covacin et al. 2011) using the Mult Alin website (Corpet 1988).

Results

A total of 672 canine stool samples were screened for *G. lamblia* cysts using a direct immunofluorescence detection kit, with 196 samples found positive. PCR amplification was successful for 185 of those positive samples, and sequencing data were obtained for 164 of those PCR products. These results mimicked similar studies surveying shelter dogs: 43.9 % of 357 dog samples were found to be *Giardia* positive in Belgium (Claerebout et al. 2009); 16.4 % of 604 dog samples were positive in Spain (Paz e Silva, et al., 2012); 35 % of 300 samples were found positive in Brazil (Dado et al. 2012).

A single sample showed the A genotype using β-giardin primers, while that same sample showed sequencing results of the C genotype for SSU-rDNA primers. Only one sample

provided the same genotype identification from both primer sets, identifying the sample as the C genotype. The majority of samples gave conflicting results between the C and D genotypes (Table 1). PCR amplification did not occur with the β-giardin primers for 66 % of the samples identified as the C genotype with SSU-rDNA primers. Similarly, no PCR amplification with the β-giardin primers could be obtained for 84 % of the samples identified as the D genotype with SSU-rDNA primers. These results conflict with data from a previous large-scale urban survey of *Giardia* in dogs in the Western USA (Covacin et al. 2011). The results from that study showed higher amplification rates with the same β-giardin primers (49 samples of 128) when compared to the same SSU-rDNA primers ($n=14$). Their study also showed a greater number of matching results from both primers ($n=47$). Although the samples were obtained from household dogs through local veterinarian offices, one would assume rates of amplification would be similar relative to the primer choice.

Ten samples showed mixed C/D genotype results with SSU-rDNA primers. Results using β-giardin primers on those ten samples showed that two were also positive for the D genotype, seven were PCR negative, and one gave inconclusive sequencing results. Mixed genotypes were seen as an identical sequence with the exception of the two single nucleotide polymorphism (SNP) differences between the C and D genotypes at positions 73 (A/G) and 173 (A/G) of the SSU-rDNA sequence. At those SNP differences, overlapping peaks on the chromatograms could be seen representing the presence of both genotypes in the sample.

Discussion

The present study is a large-scale survey for *Giardia* infections in the local canine population from urban and peri-urban environments in Tucson, Arizona to gain further insight into the possibility of zoonotic or anthroponotic transmission of infections. Previous studies have shown infections with human genotypes in dogs with frequent human contact (Yong et al. 2000; Itagaki et al. 2005; Lalle et al. 2005a, 2005b; Volotão et al. 2007; Covacin et al. 2011). The Humane Society of Southern Arizona was chosen as the sample site for this study in an effort to also sample canines with frequent human contact.

The data obtained in this study is consistent with previous studies that show predominantly C and D genotype infections in canine populations where dog-to-dog transmission is likely occurring (Hopkins et al. 1997; Thompson 2004; Leonhard et al. 2007). Dogs brought to the Humane Society are housed in groups of two or three, which increases the possibility for transmission amongst the animals. The dominance of the dog-specific genotypes in the dogs sampled in this study may be due to the C and D genotypes being better adapted to the host and out-competing any other *Giardia* infection that might be present when the animal is brought in (Hopkins et al. 1997; Thompson 2004; Ballweber et al. 2010). Although dog genotypes were the most abundant, a higher rate of human genotype infection ($n=1$) was anticipated based on frequent human contact prior to being housed in groups and based on data in previous studies (Covacin et al. 2011).

Although results show that all PCR positive samples contained dog genotypes, only one sample out of 41 showed the same genotype characterized by both primer sets. While this study shows a greater success of SSU-rDNA primers in detecting infection than the β-giardin primers, perhaps the SSU-rDNA sequence is too conserved to adequately differentiate between the C and D genotypes. There are only two SNP differences between the C and D genotypes in the SSU-rDNA sequence, while there are 29 SNP differences between the dog genotypes for the β-giardin sequence. These results provide further evidence that multiple primer sets should be used in *Giardia* genotyping studies, as suggested previously (Covacin et al. 2011; Beck et al. 2012). Our results also showed limited amplification of the β-giardin sequence compared to the SSU-rDNA sequence. Similar limited PCR amplification using β-giardin primers was found in a study in Brazil (Paz e Silva et al. 2012). The study showed β-giardin

Table 1 Genetic characterization and results from genotyping dog samples from the Humane Society of Southern Arizona, Tucson, AZ, USA

	β-giardin gene			
SSU-rDNA gene	C genotype	D genotype	PCR negative	Unconfirmed by sequencing
C genotype	1	29	57	1
D genotype	9	0	52	1
Mixed	0	2	7	1
PCR negative	2	1	18	0
Unconfirmed by sequencing	0	0	0	3

SSU-rDNA gene results read vertically while β-giardin gene results read horizontally. Number pertains to the number of dog samples obtained that showed one or more *Giardia* genotypes, with the exception of those that could not be confirmed through sequencing. Total PCR positive samples ($n=185$)

SSU-rDNA small-subunit ribosomal DNA; *PCR* polymerase chain reaction

amplification for 30 % of samples that tested PCR positive at another gene sequence, while ours showed 27 % amplification for almost triple the sample size in comparison. The greater sensitivity of the SSU-rDNA primers can be attributed to the multicopy nature of the sequence and strong sequence conservation (Cacciò and Ryan 2008).

A single human genotype was detected in this study using the β-giardin sequence specific primers. The SSU-rDNA primers did not detect the same genotype in the sample, which might be explained by the results found in Covacin et al. (2011). In that study, the same β-giardin primers used in this study appeared to preferentially amplify assemblages A and B. The A genotype has also previously been found in mixed infections of C and D genotypes in dogs (Leonhard et al. 2007). Detection of the human genotype in a canine fecal sample could be explained by a few possibilities. The city of Tucson hydrates parks and local lawns using reclaimed water, providing a previously suggested transmission cycle in urban environments for stray dogs brought in at the Tucson Humane Society (Amorós et al. 2010; Dado et al. 2012). If the animal was previously in a home and not a stray, then the animal could have possibly obtained the genotype from an infected family member (Covacin et al. 2011). However, presence of the human genotype in canine feces does not prove either zoonotic or anthroponotic infection because it does not show replication of the organism in its potential host.

Further study is required to determine the potential of *G. lamblia* genotypes as causes of zoonotic or anthroponotic infections. Infectivity studies measuring cyst numbers for amplification of the A and B genotypes in dogs would need to be done in order to definitively prove whether or not anthroponotic infections can occur between humans and their canine companions.

Acknowledgments We thank Andrew Clark and Debbie Schaefer for all their assistance and helpful discussions.

This project was funded by ARZT-136033-H-02-138, Enhanced detection of *G. lamblia* using PCR awarded to CRS.

References

Amorós I, Alonso JL, Cuesta G (2010) *Cryptosporidium* oocysts and *Giardia* cysts on salad products irrigated with contaminated water. J Food Prot 73:1138–1140

Ballweber LR, Xiao L, Bowman DD, Kahn G, Cama V (2010) Giardiasis in dogs and cats: update on epidemiology and public health significance. Trends Parasitol 26:180–189

Baruch AC, Isaac-Renton J, Adam RD (1996) The molecular epidemiology of *Giardia lamblia*: a sequence-based approach. J Infect Dis 174:233–236

Beck R, Sprong H, Pozio H, Cacciò S (2012) Genotyping *Giardia duodenalis* isolates from dogs: lessons from a multilocus sequence typing study. Vector Borne Zoonotic Dis 12:206–213

Cacciò SM, Ryan U (2008) Molecular epidemiology of giardiasis. Mol Biochem Parasitol 160:75–80

Cacciò SM, de Giacomo M, Pozio E (2002) Sequence analysis of the β-giardin gene and development of PCR-RFLP assay to genotype *Giardia duodenalis* cysts from human faecal samples. Int J Parasitol 32:1023–1030

Claerebout E, Casaert S, Dalemans AC, De Wilde N, Levecke B, Vercruysse B, Geurden T (2009) *Giardia* and other intestinal parasites in different dog populations in Northern Belgium. Vet Parasitol 161:41–46

Cooper MA, Sterling CR, Gilman RH, Cama V, Ortega Y, Adam RD (2010) Molecular analysis of household transmission of *Giardia lamblia* in a region of high endemicity in Peru. J Infect Dis 202: 1713–1721

Corpet F (1988) Multiple sequence alignment with hierarchical clustering. Nucleic Acids Res 16:10881–10890

Covacin C, Aucoin DP, Elliot A, Thompson RC (2011) Genotypic characterization of Giardia from domestic dogs in the USA. Vet Parasitol 177:28–32

Dado D, Montoya A, Blanco MA, Miró M, Saugar JM, Bailo B, Fuentes I (2012) Prevalence and genotypes of *Giardia duodenalis* from dogs in Spain: possible zoonotic transmission and public health importance. Parasitol Res 111:2419–2422

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

Hopkins RM, Meloni BP, Groth DM, Wetherall JD, Reynoldson JA, Thompson RCA (1997) Ribosomal RNA sequencing reveals differences between the genotypes of *Giardia* isolates recovered from humans and dogs living in the same locality. J Parasitol 83:44–51

Itagaki T, Kinoshita S, Aoki M, Itoh N, Saeki H, Sato N, Uetsuki J, Izumiyama S, Yagita K, Endo T (2005) Genotyping of *Giardia intestinalis* from domestic and wild animals in Japan using glutamate dehydrogenase gene sequencing. Vet Parasitol 133:283–287

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

Lalle M, Pozio E, Capelli G, Bruschi F, Crotti D, Cacciò SM (2005b) Genetic heterogeneity at the β-giardin locus among human and animal isolates of *Giardia duodenalis* and identification of potentially zoonotic subgenotypes. Int J Parasitol 35:207–213

Leonhard S, Pfister K, Beelitz P, Wielinga C, Thompson RCA (2007) The molecular characterization of *Giardia* from dogs in southern Germany. Vet Parasitol 150:33–38

Monis PT, Thompson RCA (2003) Cryptosporidium and Giardia-zoonoses: fact or fiction? Infect Genet Evol 4:233–244

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

Ritchie LS (1948) An ether sedimentation technique for routine stool examinations. Bull U S Army Med Dept 8:326

Silva FM P e, Monobe MM, Lopes FS, Araujo JP Jr (2012) Molecular characterization of *Giardia duodenalis* in dogs from Brazil. Parasitol Res 110:325–334

Sulaiman IM, Fayer R, Bern C, Gilman RH, Trout JM, Schantz PM, Das P, Lai AA, Xiao L (2003) Triosephosphate isomerase gene characterization and potential zoonotic transmission of *Giardia duodenalis*. Emerg Infect Dis 9:1444–1452

Thompson RCA (2004) The zoonotic significance and molecular epidemiology of *Giardia* and giardiasis. Vet Parasitol 126:15–35

Volotão AC, Costa-Macedo LM, Haddad FSM, Brandão A, Peralta JM, Fernandes O (2007) Acta Trop 102:10–19

Yoder JS, Gargano JW, Wallace RM (2012) Giardiasis Surveillance – United States, 2009–2010. MMWR 61:13–23

Yong TS, Park SJ, Hwang UW, Yang HW, Lee KW, Min DY, Rim HJ, Wang Y, Zheng F (2000) J Parasitol 86:887–891