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RIBOSOMAL RNA SEQUENCING REVEALS DIFFERENCES BETWEEN THE GENOTYPES OF *GIARDIA* ISOLATES RECOVERED FROM HUMANS AND DOGS LIVING IN THE SAME LOCALITY

Richard M. Hopkins, Bruno P. Meloni, David M. Groth*, John D. Wetherall*, James A. Reynoldson, and R. C. Andrew Thompson

World Health Organization Collaborating Centre for the Molecular Epidemiology of Parasitic Infections and State Agricultural Biotechnology Centre, School of Veterinary Studies, Murdoch University, South St., Murdoch, Perth, Western Australia 6150

ABSTRACT: A polymerase chain reaction-based method for genotyping *Giardia duodenalis* isolates using a polymorphic region near the 5′ end of the small subunit ribosomal (SSU) RNA gene is described. Analysis was performed using *Giardia* cysts purified directly from feces. Isolates were collected from humans and dogs living in isolated Aboriginal communities where *Giardia* infections are highly endemic. This is the first report of the genetic characterization of *Giardia* from dogs and humans living in the same locality. Comparison of the SSU-rRNA sequences from 13 human and 9 dog isolates revealed 4 different genetic groups. Groups 1 and 2 contained all of the human isolates, whereas groups 3 and 4 consisted entirely of *Giardia* samples recovered from dogs. One dog sample contained templates from both groups 2 and 3. These results suggest that zoonotic transmission of *Giardia* infections between humans and dogs does not occur frequently in these communities. The dog-associated SSU-rRNA sequences have not been reported before, suggesting a new *G. duodenalis* subgroup. A genetic basis for the differences observed between the groups was supported by sequence analysis of 9 in vitro cultured isolates that were placed into the same genetic groups established by enzyme electrophoresis.

Giardia duodenalis (syn. *Giardia intestinalis, Giardia lamblia*) is the most commonly reported intestinal parasite of humans and is a frequent cause of disease, particularly in the young (Thompson et al., 1993), *Giardia* is also capable of infecting a wide range of other vertebrate hosts including species of mammals, birds, and amphibians (Thompson et al., 1993). Whereas most authorities agree that *Giardia* has the potential to behave as a zoonosis, direct evidence for zoonotic transmission is currently lacking (Acha and Szyfres, 1987; Schantz, 1991; Thompson, 1996). The results of many cross-transmission studies to determine if humans are susceptible to infection with animal isolates of *Giardia* and vice versa have been largely inconclusive (Thompson et al., 1990), although Majewska (1994) recently described a symptomatic infection in a human volunteer inoculated with *Giardia* from a Gambian giant pouched rat. Circumstantial evidence for zoonotic transmission has also been provided by studies showing genetic and antigenic similarities between *Giardia* isolates taken from both human and animal hosts (Nash et al., 1985; Wenman et al., 1986; Forrest et al., 1989; De Jonckheere et al., 1990; Strandén et al., 1990; Meloni et al., 1992, 1995). Unfortunately, most of the *Giardia* isolates used in these studies were recovered from different geographical localities, which has limited any useful interpretation of their zoonotic potential. A better assessment of zoonotic transmission is more likely to be determined by studies examining the genotype of *Giardia* isolates collected from different hosts in the same localized areas. Such an approach was used by Isaac-Renton et al. (1993) to show that human isolates of *Giardia,* associated with an outbreak of giardiasis, were genetically identical to *Giardia* collected from a beaver living in the same locality. This result clearly demonstrated the potential for beavers to act as reservoirs for the waterborne spread of giardiasis.

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* School of Biomedical Sciences, Curtin University, Bentley, Perth, Western Australia 6102.

In Australia, studies have shown that *Giardia* infections are highly prevalent in both humans and dogs living in Aboriginal communities (Meloni et al., 1993), and isolates recovered from these hosts fall into the *G. duodenalis* morphological group (Swan and Thompson, 1986). Whereas it is clear that poor levels of hygiene have contributed significantly to high levels of *Giardia* infections, the close physical association that exists between Aborigines and their dogs is also suspected of compounding the problem (Meloni et al., 1992; Thompson, 1992). However, it is not known whether there is a single cycle of transmission in which both humans and dogs are potential hosts, or if 2 separate transmission cycles exist. Until recently, a comparison of the genotypes of *Giardia* isolates harbored by humans and dogs has not been possible due to our inability to establish in vitro cultures of isolates from dogs (Meloni and Thompson, 1987) and our reliance on molecular techniques that require in vitro amplification of the parasite (Meloni et al., 1995). To circumvent this, we have applied the PCR to characterize *Giardia* isolates recovered directly from human and canine fecal samples, without the need for in vitro culture. The genotype of isolates was determined by PCR amplification and sequencing of a 292-bp region near the 5′ end of the small subunit-rRNA gene (SSU-rRNA). This report describes the application of this technique to characterize *Giardia* isolates taken from both humans and dogs living in the same local environment in order to determine the potential for zoonotic transmission. An understanding of this problem is important for examining the epidemiology of *Giardia* in these communities and also for the design of effective control strategies.

MATERIAL AND METHODS

Collection of *Giardia* cyst samples

Giardia cyst samples, recovered from both humans and dogs were obtained from Aboriginal communities in the Fitzroy Crossing area during a survey conducted in 1991 (Meloni et al., 1993). Fitzroy Crossing is located in the tropical Kimberley region of Western Australia, approximately 250 km inland from the coast. Samples were obtained from 4 Aboriginal communities located within a 10-km radius of the main town. The number of inhabitants in these communities ranged from 50

TABLE I. Geographic origin, excystation, and in vitro culture of *Giardia duodenalis* isolates genotype by rRNA sequencing.

Isolate	Host	Nature of sample	Geographical origin	Excystation/in vitro culture*
Human cyst samples				
H4	Human	Cysts	Fitzroy Crossing	+/−
H6	Human	Cysts	Fitzroy Crossing	+/+ (BAH 92)†
H7	Human	Cysts	Fitzroy Crossing	−/−
H12	Human	Cysts	Fitzroy Crossing	+/+
H15	Human	Cysts	Fitzroy Crossing	+/−
H17	Human	Cysts	Fitzroy Crossing	+/−
H18	Human	Cysts	Fitzroy Crossing	−/−
H19	Human	Cysts	Fitzroy Crossing	+/−
H21	Human	Cysts	Fitzroy Crossing	−/−
H25	Human	Cysts	Fitzroy Crossing	+/−
H26	Human	Cysts	Fitzroy Crossing	+/−
H39	Human	Cysts	Fitzroy Crossing	+/+
H43	Human	Cysts	Fitzroy Crossing	+/−
Dog cyst samples				
D3	Dog	Cysts	Fitzroy Crossing	+/−
D6	Dog	Cysts	Fitzroy Crossing	+/−
D9	Dog	Cysts	Fitzroy Crossing	−/−
D10	Dog	Cysts	Fitzroy Crossing	−/−
D17	Dog	Cysts	Fitzroy Crossing	+/−
D18	Dog	Cysts	Fitzroy Crossing	−/−
D19	Dog	Cysts	Fitzroy Crossing	−/−
D20	Dog	Cysts	Fitzroy Crossing	+/−
D27	Dog	Cysts	Fitzroy Crossing	+/−
D46	Dog	Cysts	Perth, WA	nd
D48	Dog	Cysts	Perth, WA	nd
D50	Dog	Cysts	Perth, WA	nd
D145	Dog	Cysts	Perth, WA	nd
Reference isolates				
Plc10	Cat	In vitro culture	Portland, Oregan	(ATCC/30888)
BAC 2	Cat	In vitro culture	Murdoch, WA	
BAH 3c3	Human	In vitro culture	Lockridge, WA	
BAH 7c5	Human	In vitro culture	Katanning, WA	
BAH 12c14	Human	In vitro culture	Wyndham, WA	
BAH 33c3	Human	In vitro culture	Broome, WA	
BAH 34c8	Human	In vitro culture	Perth, WA	
BAH 45c5	Human	In vitro culture	Fitzroy Crossing, WA	
BAH 92	Human	In vitro culture	Fitzroy Crossing, WA	

* + = Excystation observed or in vitro culture established; − = no excystation observed or in vitro culture established; nd = not done.
† BAH 92 from Meloni et al. (1995).

to 350. Dogs were kept in all communities and it was estimated that the 2 largest populations had approximately 70–100 dogs. These communities are provided with services in the town of Fitzroy Crossing and share a number of common facilities, including shopping complex, hospital, school, and day care center. Individuals and families are very mobile and commonly move from community to community.

Sterile fecal containers were provided to all families with infants and children in the 4 major communities. Samples were collected from individuals ranging in age from 1 mo to 80 yr, although most were obtained from individuals under 6 yr of age. Dog feces and intestinal contents were obtained from animals that had been culled by the Regional Health Inspector and Shire Ranger. Fecal samples were examined for the presence of *Giardia* cysts by microscopy within 24 hr of collection. Positive specimens were stored at 4 C for up to 7 days before being transported by air to Murdoch University. Four *Giardia*-positive fecal samples from Perth-based dogs were obtained from the Murdoch University Veterinary School clinic. *Giardia* cysts were purified and concentrated from the feces using a modified sucrose concentration method (Meloni and Thompson, 1987). A portion of each cyst sample was removed for in vitro culture and the remainder stored in phosphate-buffered saline (PBS) at −80 C.

Excystation and in vitro culture

Giardia cyst samples collected from Fitzroy Crossing were excysted and placed in in vitro culture according to methods previously described (Meloni and Thompson, 1987) (Table I). Nine reference isolates that had been genetically characterized by enzyme electrophoresis (Meloni et al., 1995) were grown using the same conditions (Table I). Trophozoites obtained directly from the gut scrapings of the small intestine of culled dogs were placed into *Giardia* culture medium and maintained at temperatures between 25 and 30 C while in the field for no more than 2 days. When taken back to the laboratory, the media were changed and the cultures incubated at 37 C.

DNA isolation

Polymerase chain reaction (PCR)-amplifiable DNA was isolated from 13 human and 9 dog *Giardia* cyst samples collected from Fitzroy Cross-

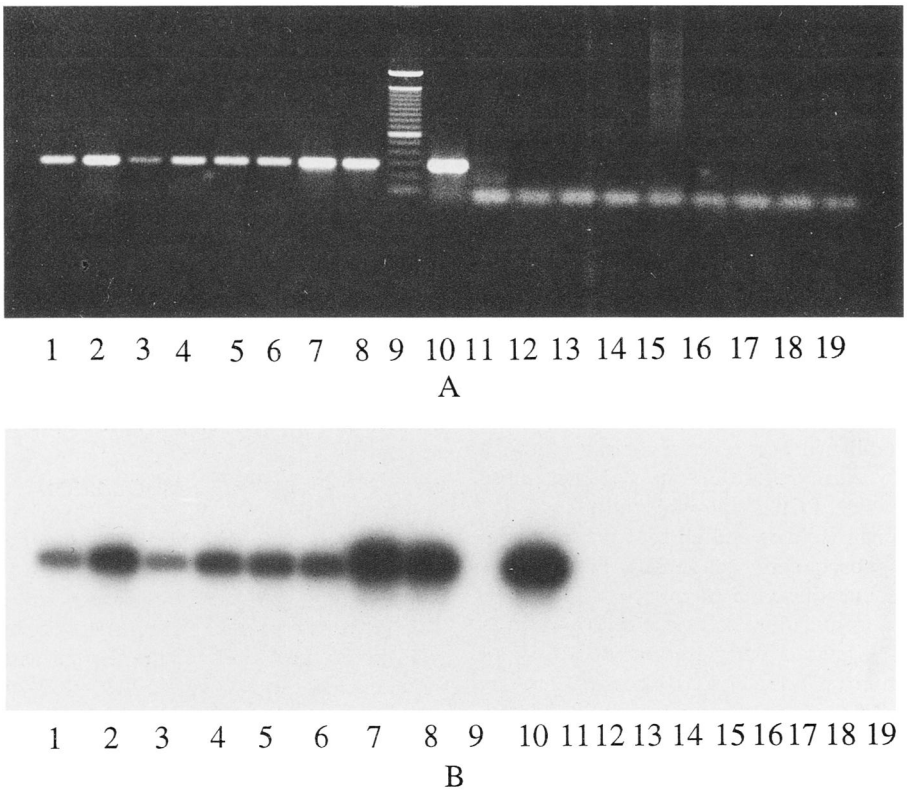

FIGURE 1. Specificity of RH11/4 PCR reaction and hybridization with probe RH 12. (A) Ethidium bromide-stained agarose gel. (B) Autoradiograph of transferred DNA probed with RH12 oligonucleotide. Lanes 1–8 contain *Giardia* samples D20, D46, D9 D18, H19, H18, BAH 12c14, and BAC2, respectively. Lane 9 = 100-bp marker, 10 = *Giardia ardeae,* 11–12 = DNA from *Giardia*-negative fecal samples, 13 = *Trichomonas foetus,* 14 = *Cryptosporidium parvum,* 15 = human DNA, 16 = *Serpulina hyodysenteriae,* 17 = *Staphylococcus aureus,* 18 = *Escherichia coli* and, 19 = no DNA control.

ing (Table I). DNA was extracted by resuspending sucrose-purified cysts in 500 μl of lysis buffer containing 1× PCR buffer (67 mM Tris-HCl pH 8.8, 16.5 mM $[NH_4]_2SO_4$, 1 mM mercaptoethanol, 6 μM ethylenediaminetetraacetic acid [EDTA], 0.2 mg/ml gelatin, 0.05 mg/ml proteinase K, 20 mM dithiothreitol, and 1.8 μM sodium dodecyl sulfate [SDS]) and incubated at 37 C for 1 hr and then at 95 C for 5 min to inactivate the proteinase K. DNA from cyst lysates was further purified using the Prep-A-Gene kit (BioRad, Hercules, California) according to the manufacturer's instructions and resuspended in H_2O.

As DNA yields using this method were low, an alternate SDS-lysis procedure was used to extract *Giardia* DNA from Perth dog cysts and in vitro culture-derived material as well as from all other bacterial, protozoan, and human specimens. Briefly, samples were freeze-thawed 5 times in liquid nitrogen, suspended in 600 μl of SDS lysis buffer (1% SDS, 20 mM Tris-HCl, 20 mM EDTA, 50 mM NaCl, pH 7.5), and incubated at 56 C for 2–3 hr. DNA was extracted with phenol/chloroform/isoamyl alcohol (25:24:1) (Sigma, St. Louis, Missouri) 3 times and with chloroform/isoamyl alcohol (24:1) once before the RNA was digested by adding 50 ng of RNAase (Boehringer Mannheim, Mannheim, Germany) and incubating at 37 C for 1–2 hr. The DNA was chloroform extracted once more, ethanol precipitated, and resuspended in 50 μl of TE buffer (10 mM Tris-HCl, pH 7.5, 0.1 mM EDTA, pH 8.0). DNA from the 9 cultured reference isolates was included in this study as they were representative of a broad range of genotypes, previously identified by enzyme electrophoresis (Meloni et al., 1995)

PCR amplification and sequencing

The primers, and their sequences, used to amplify a 292-bp region of the 5′ end of the 16S-rRNA gene were RH 11, forward primer (1–18), 5′CATCCGGTCGATCCTGCC3′ and RH 4, reverse primer (268–292), 5′AGTCGAACCCTGATTCTCCGCCAGG3′. PCR amplification was performed in 25-μl volumes with the final mix containing 5–50 ng DNA, 5% dimethyl sulfoxide (DMSO), 12.5 pmol of each primer, 1 unit Tth plus DNA polymerase (Biotech International, Perth, Australia), 200 μM of each dNTP, 2 mM $MgCl_2$, 67 mM Tris-HCl (pH 8.8), 16.6 mM $(NH_4)_2SO_4$, 0.45% Triton X-100, 0.2 mg/ml gelatin, and H_2O. Reactions were heated to 96 C for 2 min followed by 35 cycles of 96 C for 20 sec, 59 C for 20 sec, and 72 C for 30 sec and 1 cycle of 72 C for 7 min using a GeneAmp PCR System 2400 thermocycler (Perkin-Elmer, Foster City, California). Templates were purified from excess dNTPs and primers using Qiagen PCR spin columns (Qiagen, Hilden, Germany). Sequencing reactions were performed using an ABI Prism™ Dye Terminator Cycle Sequencing Core kit (Applied Biosystems, Foster City, California) according to the manufacturer's instructions, except that 6% DMSO was included in the reaction mix. PCR products were sequenced in both directions using either the RH11 or RH4 primers. Reactions were electrophoresed through an ABI 373 automatic sequencer and sequencing profiles analyzed using SeqEd v1.0.3 (Applied Biosystems). Phylogenetic analysis was performed using the DNA parsimony program available as part of the PHYLIP (v3.2) software package (Felsenstein, 1989).

Southern blotting and oligonucleotide probing

Aliquots (8 μl) of PCR product were electrophoresed in a 2% agarose gel, containing ethidium bromide, in TAE buffer and visualized using a UV transilluminator (Fig. 1). DNA was transferred to a Hybond N+ Nylon membrane (Amersham, Buckinghamshire, U.K.) under alkaline conditions using a vacuum blotting apparatus (BioRad). The membrane was prehybridized in 5× SSPE (0.9 M NaCl, 45 mM NaH_2PO_4, 5 mM EDTA, pH 7.4), 6% SDS, 1% bovine serum albumin (BSA), and 5 mM EDTA for 1 hr at 60 C. A *Giardia*-rDNA-specific oligonucleotide probe, RH 12 (123–144, 5′AGMCGGAYACCGCTGGCAACCC3′), was 5′ end labeled with 5 μl γ-^{32}P dATP (New England Nuclear, Boston, Massachusetts: specific activity 3,000 Ci mmol^{-1}) and T4 polynu-

cleotide kinase. Labeled probe was mixed with 10 ml of prehybridization solution and incubated with the membrane for 14 hr at 60 C. The filter was washed twice in 2× SSC/0.1% SDS (1× SSC contains 0.15 M NaCl, 15 mM Na-citrate, pH 7), once in 1× SSC/0.1% SDS, once in 0.2× SSC/0.1% SDS, and once in 2× SSC at 60 C. All washes were for 10 min. The membrane was sealed in a plastic bag and exposed to X-ray film with an intensifying screen for 1 hr at −70 C.

RESULTS

Excystation and in vitro culture of *Giardia* isolates

A total of 19 human and 12 dog *Giardia* cyst samples collected from the Aboriginal communities were excysted and placed in in vitro culture. Excystation was observed in 12 human and 5 dog samples; however, only 5 human isolates of *Giardia* successfully established and grew in culture. Table I shows the excystation/in vitro culture results only for those *Giardia* samples from which PCR-amplifiable DNA was obtained. Ten of the 12 human isolates and all 5 of the dog samples that excysted were subsequently genotyped. Trophozoites collected from the small intestine of 5 dogs also failed to establish in vitro. The consistent failure to establish dog isolates in culture confirmed our previous findings over a number of years using dog samples from both Fitzroy Crossing and the Perth metropolitan area (Meloni and Thompson, 1987; Meloni et al., 1992; B. P. Meloni and R. C. A. Thompson, unpubl. obs.).

Specificity of the SSU-rDNA PCR reaction

The specificity of the PCR reaction was determined by attempting to amplify a PCR product from human DNA, human fecal DNA, bacterial species including *Escherichia coli, Staphylococcus aureus,* and *Serpulina hyodysenteriae,* and protozoan DNA isolated from *Giardia, Cryptosporidium parvum,* and *Trichomonas foetus* (Fig. 1). A specific PCR product was only observed when *Giardia* DNA was used as the template. Occasionally, some *Giardia*-positive samples failed to amplify a PCR product; however, this was due either to poor quality, or insufficient amounts of template DNA. The specificity of the PCR reaction was further confirmed by probing the amplification products with an internal *Giardia*-specific SSU-rDNA oligonucleotide, RH 12. The probe hybridized only to the 292-bp PCR product present in the lanes containing *Giardia* DNA.

Automatic sequencing analysis

Sequence analysis of the 292-bp SSU-rDNA PCR product showed that 4 different genetic groups of *Giardia* isolates could be established (Fig. 2). Group 1 consisted of 1 human isolate from Fitzroy Crossing (H6), 5 reference isolates (P1c10, BAC 2, BAH 3c3, 45c5, and 92), and 2 Perth dogs (D50 and D145). Group 2 contained 13 human isolates from Fitzroy Crossing, 4 reference isolates (BAH 7c5, BAH 12c14, BAH 15c1, and BAH 33c3), 1 dog from Fitzroy Crossing (D18), and 1 dog from Perth (D50). Group 3 contained only dog samples, 8 from Fitzroy Crossing and 1 from Perth; group 4 consisted of 1 dog specimen from each of Perth (D46) and Fitzroy Crossing (D6).

A total of 7 nucleotide differences were observed between groups 1 and 2, with the latter containing 6 substitutions at positions 22 (G→A), 23 (C→T), 24 (G→C), 44 (G→C), 62 (T→G), and 72 (C→G) and an insertion (—→T) at position 43. Group 3 and 4 sequences share many of the nucleotide changes found in group 2, including the same substitutions at positions 22, 23, 24, and 44 and an insertion at position 43. Samples in group 3 had additional substitutions at positions 62 (T→A) and 167 (G→T) and group 4 samples a further 2 substitutions at positions 72 (C→A) and 171 (G→A). The phylogenetic relationships between the groups are presented in Figure 3.

The presence of mixed automatic DNA sequencing profiles in samples H6, D18, and D50 suggested that these hosts were infected with 2 genetically different *Giardia* isolates. These mixed profiles were characterized by a clean sequence up to a point where a nucleotide difference occurred between the 2 templates. In the case of a substitution, 2 bases were present in the same position and where there was an insertion or deletion, 2 overlapping sequencing profiles were observed.

DISCUSSION

This study describes the PCR amplification and sequencing of a 292-bp region at the 5′ end of the SSU-rRNA gene from *Giardia* cysts purified from human and canine fecal samples. This is the first report of the genetic characterization of *Giardia* from dogs and humans living in the same local environment. Sequence comparison of the SSU-rRNA regions showed that 4 different groups of *Giardia* isolates could be distinguished. Groups 1 and 2 contained all of the human and 3 dog isolates, whereas groups 3 and 4 consisted entirely of *Giardia* samples recovered from dogs. The SSU-rRNA sequences found in groups 3 and 4 have not been reported before, suggesting a new genetic subgroup in the *G. duodenalis* species.

The results of this study confirm the recent findings of van Keulen et al. (1995), who classified *Giardia* isolates into 2 groups, "Polish" or "Belgian," based on the presence of either a GCG or ATC signature nucleotide sequence located at positions 22–24 of the SSU-rDNA gene. These groupings were the same as those previously established using enzyme electrophoresis and DNA profiling techniques (Homan et al., 1992). The ATC/GCG signature sequences described above can also be used to separate group 1 isolates used in the present study from isolates in groups 2–4 (Fig. 2). Of the 9 reference *Giardia* isolates examined, 5 contained the GCG signature and the remaining 4 the ATC sequence. Isoenzyme analysis has shown that reference isolates within either the GCG or ATC sequence groups are more closely related to each other than to isolates between each group (Meloni et al., 1995). Together, these rRNA results suggest that *Giardia* isolates can be separated into 2 and, with the inclusion of Western Australian dog *Giardia,* possibly 3 broad genetic groups.

Mixed DNA templates were observed in 1 human and 2 dog cyst samples. It was not possible to determine if the mixed sequencing profiles were due to each sample possessing an isolate with 2 different types of rDNA sequences or if a true mixed infection was present. However, mixed rDNA sequences were not observed in axenized or cloned *Giardia* isolates. Furthermore, sequence analysis of BAH 92, which was established in vitro from the mixed human sample H6 (Table I), revealed only 1 template containing the GCG signature. The loss of the ATC sequence suggests that the patient H6 had acquired 2 different *Giardia* infections and that in vitro culture had selected for the GCG genotype. Several other groups have reported finding

Group		Sequence	
Group 1	1	CATCCGGTCGATCCTGCCGGAGCGCGACGCTCTCCCCAAGGAC-GAAGCC	49
Group 2	1	.....ATC.....AC....	50
Group 3	1	.....ATC.....AC....	50
Group 4	1	.....ATC.....AC....	50
Group 1	50	ATGCATGCCCGCTCACCCGGGACGCGGCGGACGGCTCAGGACAACGGTTG	99
Group 2	51	.....G.....G.....	100
Group 3	51	.....A.....G.....	100
Group 4	51	.....A.....A.....	100
Group 1	100	CACCCCCCGCGGCGGTCCCTGCTAGCCGGACACCGCTGGCAACCCGGCGC	149
Group 2	101	.....	150
Group 3	101	.....	150
Group 4	101	.....	150
Group 1	150	CAAGACGTGCGCGCAAGGGCGGGCGCCCGCGGGCGAGCAGCGTGACGCAG	199
Group 2	151	.....	200
Group 3	151	.....T.....	200
Group 4	151	.....T...A.....	200
Group 1	200	CGACGGCCCGCCCGGGCTTCCGGGGCATCACCCGGTCGGCGCGGTCGCGG	249
Group 2	201	.....	250
Group 3	201	.....	250
Group 4	201	.....	250
Group 1	250	.CGCGCCGAGGGCCCGACGCCTGGCGGAGAATCAGGGTTCGACT	292
Group 2	251	.....	293
Group 3	251	.....	293
Group 4	251	.....	293

Group 1. Sequences for isolates P1c10, BAC 2, BAH 3c3 and BAH 45c8, BAH 92 H6*, D50*, D145.

Group 2. Sequences for isolates BAH 7c5, BAH 12c14, BAH 33c3, BAH 34c8, H4, H6*, H7, H9, H11, H12, H15, H18, H19, H21, H25, H26, and H43, D18*, D50*

Group 3. Sequences for isolates D3, D9, D10, D17, D18*, D19, D20, and D27, D48.

Group 4. Sequences for isolates D6 and D46.

* = automatic sequencing profile suggested that mixed template was present

FIGURE 2. Continued.

mixed *Giardia* infections and have also described the problem of selection of genotypes during in vitro culture (Mayrhofer et al., 1992; Weiss et al., 1992; Upcroft and Upcroft, 1994).

Examination of the genotype of *Giardia* isolates sampled from Aboriginal communities in the Fitzroy Crossing area showed that all human cyst samples contained either group 1- or group 2-type sequences and all dog samples, group 3- or 4-type sequences. Although the sample sizes used in this study are relatively small, these results suggest that 2 distinct cycles of *Giardia* transmission are present within these communities and that cross transmission does not appear to occur frequently. It was not possible to determine if the mixed infection observed in dog 18 was contracted from within the dog population, in which group 2-type *Giardia* might be cycling, or from a human source. However, our observations regarding the behavior of dogs in Aboriginal communities would not discount the possibility of zoonotic transfer. Coprophagy of exposed human feces is known to occur and we have found eggs of the human parasite, *Hymenolepis nana,* in the stools of dogs from these communities (Meloni et al., 1993).

Given that the close physical contact that occurs between humans and dogs in Fitzroy Crossing would seem to favor zoonotic transfer, why do levels of cross transmission appear to be so low? It is possible that some *Giardia* genotypes observed in different hosts may represent discrete genetic subgroups capable of exhibiting some level of host adaptation. There is some evidence for this. We have been unable to establish in vitro any *Giardia* isolates retrieved from Australian dogs (Meloni and Thompson, 1987; Meloni et al., 1992; B. P. Meloni and R. C. A. Thompson, unpubl. obs.) and isoenzyme analysis of *Giardia* cysts purified from 1 Australian dog showed that this isolate was genetically distinct from all human isolates (Meloni et al., 1992, 1995). Where other researchers have succeeded in culturing dog *Giardia* in vitro, subsequent analysis has shown them to be antigenically and genetically very similar to other human and animal isolates (Wenman et al., 1986; Forrest et al., 1989; Strandén et al., 1990; Meloni et al., 1992). This suggests that these isolates were not the same as the group 3/4 isolates described in this study. The possibility that human isolates that are refractory to in vitro culture may contain group 3- or 4-type sequences is also unlikely because all 7 of the human cyst samples from Fitzroy Crossing that excysted but failed to grow in vitro contained group 1- or 2-type sequences. Finally, the results from both Perth and Fitzroy Crossing show that dogs can harbor all 4 genetic groups of *Giardia* isolates. Despite this, group 3/4-type sequences are overrepresented in dog samples recovered from Aboriginal communities. This may be due to group 3/4 isolates being better suited for survival in the dog so that they are able to out-compete group 1/2 *Giardia* isolates (Thompson et al., 1996). The possibility that zoonotic transfer is occurring but is not being detected due to selection of particular genotypes in different hosts requires further investigation.

In contrast to Fitzroy Crossing, 2 of 4 dog samples examined from urban areas of Perth contained only group 1- or 2-type sequences. Although the sample size is too small to make any useful conclusions, this result may reflect a different epidemiological picture of *Giardia* infections for domesticated animals in larger urban areas. Dog to dog contact in Perth is likely to be less frequent than between animals living in Aboriginal communities. As such, it is more likely that the source of *Giardia* infections for Perth-based animals is from humans. It is also

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FIGURE 2. Sequence alignments of the rDNA sequences determined from the 292-bp PCR fragment amplified from a range of different *Giardia* isolates.

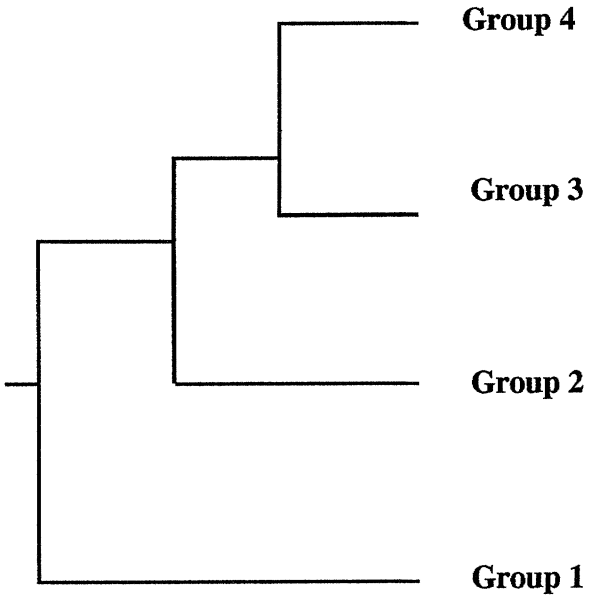

FIGURE 3. Phylogenetic relationship between the 4 *Giardia duodenalis* SSU-rRNA groups as determined by parsimony.

possible that dogs infected with type 1 or 2 sequences may represent more of a zoonotic risk to humans than those that contain group 3/4 sequences.

In conclusion, this study describes a PCR-based sequencing technique for genotyping *Giardia* isolates purified directly from fecal samples. This approach makes it possible to characterize *Giardia* isolates without the need for in vitro culture. The results describe a new SSU-rDNA genotype within the *G. duodenalis* group that is associated with *Giardia* infections in dogs. In Aboriginal communities where the prevalence of *Giardia* is high in both humans and dogs, transfer of infections was found to occur infrequently between the 2 groups. This contrasts with Isaac-Renton et al. (1993), who found evidence for zoonotic transfer of *Giardia* infections between a beaver and humans living in the same environment. These results highlight the importance of examining *Giardia* isolates from the same locale in order to determine the potential for zoonotic transmission. Studies from other geographical areas are required to determine if the dog-associated genotype is present in other canine populations and cross-transmission experiments are also needed to determine the extent, if any, to which the dog *Giardia* genotype is host adapted.

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