

Original papers

Prevalence and multilocus genotyping of *Giardia* from animals at the zoo of Poznan, Poland[1]

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ABSTRACT. In this study total of 266 fecal samples from 242 animals belonging to 113 species kept in the Poznan Zoological Garden were examined for *Giardia*. The cysts of *Giardia* were found only in five samples of feces collected from a giant toad (*Bufo marinus*), tamandua (*Tamandua tetradactyla*) and three individuals of cactus mouse (*Peromyscus eremicus*). Fragments of *β-giardin* (*bg*), triose phosphate isomerase (*tpi*) and glutamate dehydrogenase (*gdh*) genes were successfully amplified only from the *Giardia* isolate obtained from the tamandua. Sequence and phylogenetic analyses showed that the *Giardia* isolate from the tamandua belonged to the B assemblage and showed homologies of 99% to 100% at *bg, gdh* and *tpi* loci of the same markers of parasites isolated from humans and animals in various parts of the world. This is the first molecular characterization of *G. duodenalis* from tamandua.

Key words: *Giardia*, assemblage B, captive animals, multilocus genotyping, zoonotic transmission

Introduction

Giardia duodenalis (*G. intestinalis, G. lamblia*) is one of the most frequently diagnosed parasitic intestinal protozoan in humans and many species of animals. The occurrence of morphologically identical populations of *G. duodenalis* in many species of hosts constitutes a significant limitation in the understanding of the epidemiology and transmission of this parasite.

The frequent use of molecular techniques for typing the isolates of *G. duodenalis* has demonstrated the significant genetic diversity within this species. Most of the isolates obtained from humans and animals from various regions of the world belong to two major assemblages A and B [1]. It has also been shown that there are additional genotype assemblages (C-H) characterised by a narrow host specificity [2,3]. Furthermore, sub-assemblages have been distinguished within some assemblages [1,4]. Moreover, new genotypes of *Giardia* are still being described, and these occur mainly in wild animals [5–7].

There are relatively few studies concerning the occurrence and/or identification of *Giardia* genotypes in animals raised in zoological gardens. The *Giardia* infection have been found in various species of mammals and birds kept in zoological gardens [8–15]. Since giardiasis has been found in zoo animals, further studies are necessary in order to explain which species of animals may constitute the reservoir of *G. duodenalis* genotypes infectious to zookeepers, veterinary surgeons, and visitors.

The aim of this study was to examine animals from the Zoological Garden in Poznan for *Giardia* infection and identification of *Giardia* genotypes.

Materials and methods

In the present study a total of 266 fresh fecal samples were collected from 242 animals belonging to 113 species kept in the Poznan Zoological Garden. Fecal samples were collected from 12 species of amphibians, 9 species of reptiles and 92 species of mammals. Stool specimens from some animals were taken twice. Fresh fecal material from an individual animal was placed separately into a plastic tube, labeled, and transported to the laboratory in a cooler.

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A direct wet smear was prepared from each specimen in duplicate by mixing a small amount of stools with 0.1 ml of phosphate-buffered saline (PBS), pH 7.4. A drop of Lugol's iodine was added while the smears were still wet to the first set of slides, the slides were coverslipped, and the entire coverslipped area was examined under ×60 microscope objective magnification. The second set of smears was fixed in Schaudinn's fixative and then stained with trichrome. Permanent stained smears were microscopically screened using an oil-immersion objective (×100).

Total genomic DNA was directly extracted from *Giardia* positive fecal samples. The FastDNA kit (BIO101, Vista, USA) was used for extraction of the *Giardia* DNA based on a protocol described earlier by da Silva et al. [16]. The eluted DNA was purified with the QIAquick PCR purification kit (Qiagen, Hilden, Germany) according to the kit instructions.

Three molecular markers were amplified and used for *Giardia* genotyping: a 753 bp fragment of the *bg* gene was amplified using the G7 forward PCR primer and the G759 reverse primer [17], a 530 bp fragment of the *tpi* gene was amplified using the AL3543 and AL3546 PCR primer pair as well as AL3544 and AL3545G7 primers [18], and a 430 bp fragment of the *gdh* gene was amplified using two forward (GDHeF, GDHiF) with one reverse (GDHiR) primers [19]. PCR was done using a GeneAmp 2400 thermocycler. In nested and semi-nested PCR 1 µl of PCR mixture from the first reaction was used. Both positive (*Giardia* DNA was extracted from cultured trophozoites of the Portland-1 reference strain) and negative (a reaction mixture without the DNA template) controls were included in the experiment. Spike control was provided to rule out inhibitors. The PCR products were sequenced using the ABI Prism 3130 XL BigDye v3.1, Terminator Cycle Sequencing in both directions with the same set of primers.

Both sequences were analyzed using Chromas and MEGA version 4.0 programs [20]. A phylogenetic tree was constructed by the neighbor-joining algorithm. Distance-based analyses were conducted using Kimura 2-parameter distance estimates using alignments obtained from ClustalW. Bootstrap proportions were calculated by the analysis of 1,000 replicates of the phylogenetic tree.

Results and discussion

In the present study low numbers of *Giardia* cysts were found in 5 of 266 fecal samples obtained from a giant toad (*Bufo marinus*), a tamandua (*Tamandua tetradactyla*) and three individuals of cactus mouse (*Peromyscus eremicus*). All *Giardia*-infected animals were asymptomatic. Likewise, asymptomatic giardiasis in zoo animals was reported by other authors [10,15]. The overall prevalence of *Giardia* infection was 2%. Similarly, a low *Giardia* infection rate (1%) in zoo animals was reported in Brazil [10]. However, higher prevalence of *Giardia* infection in animals kept in zoological gardens was found in Croatia (29%) [15], Belgium (41%) [12] and Australia (13.4%) [13]. The differences in *Giardia* prevalence might have resulted from using various diagnostic methods. In the present study diagnosis was based on microscopic examination of wet and permanent

Table 1. Sequence analysis of the *β-giardin* gene; single nucleotide substitutions (SNPs) in the T-001 isolate compared with sequences previously deposited in GenBank. Numbering was based on the start codon of the *β-giardin* complete coding sequence (X85958).

Isolate	Assemblage	Alignment position								
		222	285	339	366	369	450	660	744	756
T-001		T	A	C	T	T	T	G	C	A
H1-001	B	T	A	C	T	T	T	G	a	g
Nij5	B	T	A	t	T	T	T	a	C	g
LD18	B	c	g	C	c	c	c	G	C	g
BAH8	B	c	A	C	c	T	c	G	C	g
BG-Ber2	B	c	A	C	T	T	c	G	C	g

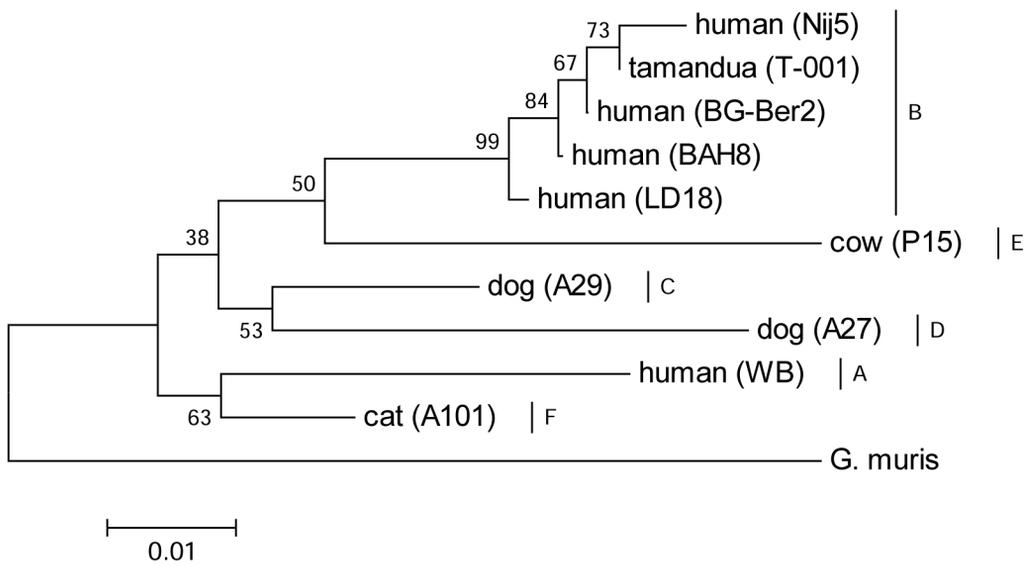

Fig. 1. Phylogenetic relationship of 11 *Giardia* isolates inferred by the neighbor-joining analysis of the *β-giardin* nucleotide sequences. Evolutionary distances were computed using the Kimura 2-parameter method and are given as units of the number of base substitutions per site.
T-001 – sequence of partial *β-giardin* gene of *Giardia* isolate obtained from tamandua;
WB, BAH8, LD18, Nij5 – reference human isolates (accession numbers: X85958, AY072727, AY072725, and AY072726); A101 – reference cat isolate (accession number AY647264); P15 – reference cow isolate (accession number AY072729); A27 and A29 – reference dog isolates (accession numbers: AY545644 and AY545646). *G. muris* (accession number AY258618) represents an outgroup.

stained fecal smears, whereas in other studies more sensitive diagnostic methods, e.g., concentration techniques and/or PCR and immunoassay, were performed [13,15]. On the other hand, according to the opinion of the employees of the Zoological Garden in Poznan, a continuous improvement in hygiene standards over the past 20 years had visibly influenced the decrease in the frequency of animal infections with intestinal parasites.

DNA was isolated from all above-mentioned *Giardia*-positive fecal samples. However, despite implementing three markers, PCR amplification of the gene fragments of *Giardia* isolates obtained from the giant toad and cactus mouse failed and therefore identification of *Giardia* species and genotypes was not possible. Probably, the lack of amplicons resulted from an insufficient amount of fecal materials and a low number of cysts because spike control ruled out polymerase inhibitors. Nevertheless, according to our knowledge, the giant toad and the cactus mouse are new hosts for *Giardia*.

Amplicons of all three genes were obtained only from the *Giardia* isolate (T-001) obtained from the tamandua, and were of the following sizes: 753, 530, and 430 bp of the *bg*, *tpi*, and *gdh* gene fragments, respectively. The amplification products were sequenced. No double peaks in the chromatograms occurred in the tested loci.

The gene sequence fragments of the *Giardia* isolate from the tamandua were aligned with the published reference sequences. It was found that the *Giardia* isolate from the tamandua belonged to the B assemblage. It was demonstrated that the DNA sequence of the *bg* gene fragment of the T-001 isolate obtained from the tamandua shared 99% similarity with the sequences from the reference isolates of *G. duodenalis* from humans: H1-001 (FJ009207), Nij5 (AY072725), BAH8 (AY072727), LD18 (AY072726), and BG-ber2 (DQ090523) [17,21,22]. The sequence of the molecular marker of the T-001 isolate differed only by two nucleotide substitutions (2 SNPs) from the H1-001 isolate, by 3 SNPs from the Nij5 and BG-ber2 isolates, and by 6 SNPs from the LD18 isolate (Table1). All mutations were synonymous.

The DNA sequence of the *tpi* gene fragment of the *Giardia* isolate obtained from the tamandua showed 100% homology with the sequences of the same marker of two *Giardia* isolates obtained from

humans (EU272169 and EU212761) and white-faced saki, *Pithecia pithecia* (AB569405), that were classified in the B assemblage [23,24].

The comparison of the sequence of the *gdh* gene fragment of the *Giardia* isolate from the tamandua with the sequences deposited in GenBank showed a 99% homology to the sequences of this gene of the parasite isolated from humans in various parts of the world, and belonging to the B assemblage (EF507682, AY826192, DQ923582). Also the *Giardia* isolate from the tamandua had a sequence identical to those that were isolated from dog (AY178750) and chinchilla (AY178751), and that were classified in the BIII-sub-assemblage.

Although asymptomatic *Giardia* infection had previously been documented in the tamandua raised in the zoological garden in Sǎo Paulo, Brazil, the isolate of this parasite was not molecularly characterized [10]. Thus, this is the first molecular characterization of *G. duodenalis* isolate from tamandua.

The sequences of the *bg*, *tpi* and *gdh* gene fragments from the *G. duodenalis* obtained from the tamandua were deposited in GenBank (NCBI) under accession numbers FJ009209, GU797247 and HM150749, respectively.

The phylogenetic analysis of *bg* nucleotide sequences showed that the *Giardia* isolate from tamandua clustered within sub-assemblage BIII together with the Nij5 isolate (Fig. 1). It should also be noted that *G. duodenalis* isolates classified in the BIII sub-assemblage have been predominantly found in humans [1]. This may indicate that the *Giardia* isolate from tamandua may be infectious for humans or originated from human. Moreover, a *Giardia* isolate obtained from the Gambian pouched rat (*Cricetomys gambianus*) from the Poznan Zoological Garden and characterized as a B assemblage was infectious to a human volunteer [25]. In addition, it was recently reported that the *bg* sequence obtained from zoo animals, e.g., a mantled guereza (*Colobus guereza*), a white-handed gibbon (*Hylobates lar*), and a ring-tailed lemur (*Lemur catta*), was identical to the tested T-001 isolate from tamandua [15]. Thus, it is possible that the tamandua may be a source of *Giardia* infections for other animals raised in zoological gardens as well as for visitors and animal handlers. Therefore, maintaining a suitable level of hygiene in the cages and rooms, as well as ensuring that the animal handlers follow strict sanitary procedures, may minimize the risk of *Giardia* infection spreading in zoological gardens that often keep numerous animal species of high economic and scientific value. The discovery of a new animal reservoir of *G. duodenalis* genotype infectious to humans is also important in order to eliminate the risk of infecting humans. This requires, however, the use of molecular methods and bioinformatics not routinely used by veterinarians in zoological gardens.

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