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SCIENCE DIRECT
International Journal for Parasitology 36 (2006) 849–858
www.elsevier.com/locate/ijpara

Genotyping of *Giardia* in Dutch patients and animals: A phylogenetic analysis of human and animal isolates☆

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Received 30 November 2005; received in revised form 26 February 2006; accepted 2 March 2006

Abstract

Giardia duodenalis (syn. *Giardia lamblia*, *Giardia intestinalis*) is a protozoan organism that can infect the intestinal tract of many animal species including mammals. Genetic heterogeneity of *G. duodenalis* is well described but the zoonotic potential is still not clear. In this study, we analysed 100 *Giardia* DNA samples directly isolated from human stool specimens, to get more insight in the different *G. duodenalis* assemblages present in the Dutch human population. Results showed that these human isolates could be divided into two main Assemblages A and B within the *G. duodenalis* group on the basis of PCR assays specific for the Assemblages A and B and the DNA sequences of 18S ribosomal RNA and the glutamate dehydrogenase (*gdh*) genes. Genotyping results showed that *G. duodenalis* isolates originating from Dutch human patients belonged in 35% of the cases to Assemblage A (34/98) and in 65% of the cases to Assemblage B (64/98) whereas two human cases remained negative in all assays tested. In addition, we compared these human samples with animal samples from the Netherlands and human and animal samples from other countries. A phylogenetic analysis was carried out on the DNA sequences obtained from these *Giardia* and those available in GenBank. Using *gdh* DNA sequence analysis, human and animal Assemblage A and B *Giardia* isolates could be identified. However, phylogenetic analysis revealed different sub-clustering for human and animal isolates where host–species-specific assemblages (C, D, E, F and G) could be identified. The geographic origin of the human and animal samples was not a discriminating factor.

Keywords: *Giardia*; Molecular genetics; Phylogenetic analysis; 18S rRNA; Glutamate dehydrogenase; Zoonotic disease

1. Introduction

The flagellated protozoan *Giardia* is an intestinal parasite that can infect many species in the animal kingdom including mammalian, avian and reptilian wildlife, domesticated animals and humans (Thompson, 2004; Appelbee et al., 2005). Of the morphologically defined *Giardia* species, *Giardia muris*, *Giardia microti*, *Giardia agilis, Giardia psicatti* and *Giardia duodenalis* (syn. *Giardia lamblia, Giardia intestinalis*), only the latter is recovered from humans and a wide variety of other mammals. In humans, *G. duodenalis* can cause gastrointestinal infections ranging from mild to severe as well as chronic disease. In domestic animals, *G. duodenalis* is of considerable clinical importance and could have economic significance in cattle-based industries (Olson et al., 2004). Infection occurs by faecal oral route transmission, either by direct contact or by ingestion of contaminated food or water (Monis and Thompson, 2003). Despite morphological uniformity, considerable biotypic and genetic diversity exists within the *G. duodenalis* species (Monis et al., 1996; Thompson et al., 2000). The species includes several 'assemblages' or genotypes, A–G, that can be discriminated on the basis of host selection and genomic mutations (Monis et al., 1999). Although several genes encoding proteins involved in meiosis

☆ Note: Nucleotide sequence data reported in this paper are available in the Genbank under the accession numbers: AY826191–AY826210; AY827496–AY827499; DQ100287, DQ100288.

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0020-7519/$30.00
doi:10.1016/j.ijpara.2006.03.001

are present in *Giardia* (Ramesh et al., 2005), direct evidence for sexual recombination has not yet been shown. Phylogenetic multilocus analysis using 18S rRNA, glutamate dehydrogenase (*Gdh*), elongation factor 1 alpha (EF-1α) and triose phosphate isomerase (*Tpi*) gene based molecular methods have been used on representatives of each major genetic group to study the relations among assemblages from different hosts (Monis et al., 1999; van Keulen et al., 2002; Caccio et al., 2005). Zoonotic transmission of *G. duodenalis* is still under debate and despite increasing knowledge of the molecular identification of *Giardia* from different host species; the zoonotic potential of *G. duodenalis* is not clear (Monis and Thompson, 2003; Thompson, 2004; Hunter and Thompson, 2005). One report in the literature has identified a clear animal to human transmission: waterborne transmission of *Giardia* from a beaver to humans was identified (Isaac-Renton et al., 1993). A survey on the transmission of *Giardia* from dogs to humans indicated that there was no transmission from dogs to humans but that the reverse, transmission from humans to dogs, could be possible (Hopkins et al., 1997). Dogs, however, might not only transmit human adapted *Giardia* genotypes as was described by a study in an endemic area where humans and dogs were living closely together (Traub et al., 2004) but may also be infected with host-adapted *Giardia* genotypes (Caccio et al., 2005). Studies designed to investigate zoonotic potential are still based either on limited numbers of isolates from a diverse source or limited molecular identification tools.

It was our aim to analyse the genetic diversity of *Giardia* isolates from human clinical cases in the Netherlands by different molecular typing methods and to compare the results of the different methods. We used the following methods: *gdh* PCR-restriction fragment length polymorphism (RFLP) assay and the two discriminative PCR assays. One PCR assay specifically detected Assemblage A and one was specific for Assemblage B genotypes of *G. duodenalis* as described by Homan et al. (1998). In addition, we performed DNA sequence analysis of two different genes, 18S rDNA and the *gdh* gene. As well as the human isolates, we sequenced these loci for several animal *Giardia* isolates. The different sequences were used to construct a database so we could compare our findings for human patient *Giardia* isolates with those from animals in the Netherlands and those published previously and available in GenBank. Using phylogenetic analysis, it was our aim to further elucidate the relationship of the different genotypes to each other, their hosts and the geographic origin to study the possibilities for zoonotic transmission.

2. Materials and methods

2.1. Origin of the samples

One hundred microscopically confirmed *Giardia*-positive faecal samples from humans with symptoms of diarrhoea were analysed. In addition, *Giardia*-positive faecal samples of two dogs three sheep/goat and one Dutch roe deer from the Netherlands were analysed using the same methods (Table 1A). The details of the DNA sequences acquired from Genbank and used in this study are shown in Table 1B and C.

2.2. DNA isolation

Total DNA from *Giardia* cysts from fresh non-preserved stool samples was isolated as described earlier (Homan et al., 1998) with some modifications. Briefly, stools were broken up in distilled water and filtered through a 70 μm cell strainer (BD Falcon, Belgium), 2.5 ml stool suspensions were layered on 3 ml of 1.6 M sucrose and centrifuged at 750×*g* for 5 min. Cysts at the sucrose–water interphase were collected and washed with distilled water. The enriched cysts were resuspended in 1 ml distilled water and 100 μl of 10×buffer A, 100 μl of 10×buffer B and 10 μl anti-*Giardia* magnetic beads provided with the *Giardia*/*Cryptosporidium* purification kit (Dynal Biotech GmbH, Hamburg, Germany). After 1 h of gently mixing the suspension at room temperature the magnetic beads were washed with buffer A using a tube-holder with a magnetic strip (Dynal Biotech). For DNA isolation the beads

Table 1A
Giardia 18S rDNA and *gdh* DNA sequences submitted to Genbank for human and animal *Giardia* isolates determined in this study

Isolate	Host	Geographical origin	18S rRNA GenBank GI[a]	GDH GenBank GI	Assemblage[b]
NLH13	Human	The Netherlands	AY826201	AY826191	B
NHL20	Human	The Netherlands	AY826204	AY826194	A
NHL25	Human	The Netherlands	AY826203	AY826193	B
NHL28	Human	The Netherlands	AY826202	AY826192	B
NHL35	Human	The Netherlands	AY826207	AY826197	B
NHL37	Human	The Netherlands	AY826206	AY826196	A
NHL45	Human	The Netherlands	AY826205	AY826195	A
NLDE3	Dog	The Netherlands	AY827497	AY827498	D
NLD37	Dog	The Netherlands	AY827496	AY827499	D
NLG409	Goat	The Netherlands	AY826210	AY826198	E
NLR118	Roe deer	The Netherlands	DQ100287	DQ100288	A
NLS352	Sheep	The Netherlands	AY826208	AY826199	E
NLS387	Sheep	The Netherlands	AY826209	AY826200	E

[a] GenBank accession/GI number.
[b] Assemblage A corresponds to a positive A PCR, type 1 18S rRNA sequence and G1 glutamate dehydrogenase (GDH) restriction fragment length polymorphism (RFLP), Assemblage B to a positive B PCR, type 2 18S rRNA sequence and G2 GDH RFLP.