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Molecular characterisation of *Giardia duodenalis* in captive non-human primates reveals mixed assemblage A and B infections and novel polymorphisms ☆

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ABSTRACT

Giardia is frequently detected in stools of non-human primates (NHP). However, a molecular identification has been rarely applied to *Giardia* isolates from NHP, and the distribution of the zoonotic assemblages A and B remains unclear. Moreover, little is known about the genetic variability among the isolates, although this may contribute to the elucidation of the different transmission pathways, including the role of NHP as a reservoir for human giardiasis. Therefore, 258 *Giardia* samples from 31 NHP species housed in nine zoological gardens and one sanctuary in Belgium and The Netherlands were characterised based on an assemblage-specific PCR targeting the *triose phosphate isomerase* (*tpi*) gene to identify both assemblage A and B infections. In addition, a multi-locus sequencing approach based on the *glutamate dehydrogenase*, the *tpi* and the *β-giardin* genes was used to examine both the genetic variability and the ability to allocate these isolates to different NHP groups. Overall, assemblage B was the most prevalent (78.6%), but mixed assemblage A and B infections occurred in 32.7% of the samples. Sequencing of the isolates revealed the presence of new polymorphisms for both assemblages and at the three loci examined. The majority of the assemblage B isolates could not be grouped into recently described sub-assemblages, particularly at the *tpi* gene. Isolates could only be allocated to a specific group when polymorphisms of the three loci were combined. The results confirm that NHP are a potential reservoir for zoonotic transmission and advocate the use of assemblage-specific primers in molecular epidemiological surveys, as mixed infections are likely to be underestimated. The high level of heterogeneity within assemblages indicates that a revised nomenclature of these sub-assemblages is needed, but points out the potency of a multi-locus sequencing approach to unravel the complex epidemiology of *Giardia duodenalis*.

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1. Introduction

Giardia in non-human primates (NHP) is of noteworthy importance both from a veterinary and a public health point of view. The parasite is commonly found in stools of wild and captive NHP (Ghandour et al., 1995; Hope et al., 2004; Salzer et al., 2007; Levecke et al., 2007) and is a significant cause of diarrhoea and failure to thrive, particularly in young animals (Hamlen and Lawrence, 1994; Kalishman et al., 1996). In addition, NHP might be a potential reservoir for zoonotic transmission, as these animals harbour the zoonotic *Giardia duodenalis* assemblages A (Monis et al., 1996; Graczyk et al., 2002; Vitazkova and Wade, 2006) and B (Monis et al., 1996; Karanis and Ey, 1998; Vitazkova and Wade, 2006; Cacciò et al., 2008). However, the distribution of these assemblages within NHP remains unclear. The majority of these studies examined only a limited number of isolates and/or animal species. Moreover, mixed assemblage A and B infections could not be ruled out, as the standard PCR approach will preferentially amplify the most abundant assemblage (Weiss et al., 1992; Geurden et al., 2007).

Furthermore, little is known about the genetic variation among isolates originating from different NHP populations, as a molecular characterisation was often based on a single genetic locus which frequently differed between studies. Yet, recent molecular analysis of *Giardia* isolates at the *glutamate dehydrogenase* (*gdh*),

☆ *Note:* Nucleotide sequence data reported in this paper are available in the GenBank™ under the accession numbers: FJ890942–78.

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triose phosphate isomerase (*tpi*) and the *β-giardin* (*bg*) genes indicates a high degree of genetic variability within both assemblages A and B (Wielinga and Thompson, 2007), which may contribute to the elucidation of different transmission pathways, including the role of animals as a reservoir for human giardiasis. In this study, we have conducted a molecular survey to determine the distribution of *G. duodenalis* assemblage A and B in NHP. *Giardia* samples previously found in NHP housed at nine zoological gardens and one sanctuary (Levecke et al., 2007; unpublished observations) were characterised based on an assemblage-specific PCR targeting the *tpi* gene. In addition, a multi-locus sequencing approach based on the *gdh*, *tpi* and *bg* genes was performed to examine the genetic variability and the ability to allocate these isolates to different NHP groups.

2. Materials and methods

2.1. Selection of the Giardia samples

A total of 258 *Giardia* samples from 47 NHP groups belonging to 31 animal species were withheld for further molecular analysis (Table 1). The samples were obtained from previously conducted epidemiological surveys at nine zoological gardens and one sanctuary in Belgium and The Netherlands (Levecke et al., 2007; unpublished observations) and were positive for at least one of the two techniques used (microscopic examination and a commercial immunofluorescence assay (MERIFLUOR® *Cryptosporidium*/*Giardia* immunofluorescence assay, Meridian Diagnostics Inc., Cincinnati, OH, USA)).

2.2. Molecular identification

DNA was extracted using the QIAamp® Stool Mini Kit (Qiagen) according to the manufacturer's instructions, incorporating an initial step of three freeze-thaw cycles (freezing in liquid nitrogen for 5 min and heating at 95 °C for 5 min) in the protocol to maximise rupture of the cysts.

The molecular identification was based on two approaches: assemblage-specific amplification and multi-locus sequencing.

2.3. Assemblage-specific amplification

The assemblage-specific amplification was performed on all selected *Giardia* isolates and consisted of a nested PCR targeting the *tpi* gene. The first reaction was based on the internal set of primers described by Sulaiman et al. (2003), followed by two separate assemblage-specific nested PCRs. The amplification of *G. duodenalis* assemblage A was according to Geurden et al. (2007). For the detection of assemblage B, new primers (AssBF: 5′ GTT GTT GTT GCT CCC TCC TTT 3′ and AssBR: 5′ CCG GCT CAT AGG CAA TTA CA 3′) were designed based on Genbank accession no. L02120 (assemblage A), AY228628 (assemblage BIII), AY228632 (assemblage BIII-like), AF069560 (assemblage BIV), AY228634 (assemblage BIV-like), AF069563 (assemblage C), DQ246216 (assemblage D), AY655705 (assemblage E), AF069558 (assemblage F) and AF069562 (assemblage G). The GoTaq® Flexi DNA Polymerase kit (Promega) was used for each of the PCR mixtures and consisted of 2.5 μl DNA, 0.5 μl of each primer (10 μM), 1 μl $MgCl_2$ (25 mM), 5 μl GoTaq® Flexi Buffer, 14.875 μl PCR H_2O and 0.125 μl GoTaq® Flexi DNA polymerase. Identical conditions were

Table 1
The number of isolates and mono-and mixed infections of *Giardia duodenalis* assemblage A and B in 31 non-human primate species based on assemblage-specific PCR targeting the *tpi* gene.

Common name	Country	Isolates	Assemblage			NA
			A	B	A & B	
Alaotran lemur	The Netherlands	2	0	2	0	0
Barbary macaque	The Netherlands	20	1	1	5	13
Black-and-white ruffed lemur	Belgium	3	0	1	0	2
Black-capped squirrel monkey	The Netherlands	12	3	1	4	4
Black-headed spider monkey	Belgium/The Netherlands	12	2	3	0	7
Brown howler	The Netherlands	29	7	4	0	18
Brown woolly monkey	The Netherlands	8	2	0	0	6
Chimpanzee	Belgium	15	1	14	0	0
Common squirrel monkey	Belgium	9	1	4	0	4
Crab-eating macaque	The Netherlands	2	1	0	1	0
Eastern gorilla	Belgium	1	0	0	0	1
Golden lion tamarin	The Netherlands	1	0	0	0	1
Golden-bellied capuchin	Belgium	1	0	0	0	1
Grivet/Tantalus monkey	The Netherlands	1	0	0	0	1
Hamadryas baboon	Belgium/The Netherlands	7	2	3	2	0
Javan lutung	Belgium/The Netherlands	4	0	4	0	0
Mantled guereza	Belgium	12	0	10	0	2
Northern white-cheeked gibbon	The Netherlands	1	0	0	0	1
Pygmy marmoset	The Netherlands	1	0	0	0	1
Red ruffed lemur	Belgium/The Netherlands	7	0	0	0	7
Rhesus monkey	The Netherlands	1	1	0	0	0
Ring-tailed lemur	Belgium/The Netherlands	85	8	20	36	21
Ring-tailed lemur/White-fronted lemur	Belgium	2	0	0	0	2
Siamang	Belgium	1	0	1	0	0
Silvery woolly monkey	The Netherlands	1	0	0	0	1
Sunda pig-tailed macaque	The Netherlands	6	2	2	1	1
Vervet monkey/Tantalus monkey	The Netherlands	5	1	1	1	2
Western gorilla	The Netherlands	4	1	2	0	1
White-handed gibbon	Belgium	2	0	0	0	2
White-headed geoffroyi	The Netherlands	3	1	0	2	0
Total		258	34	73	52	99

NA: no amplification.

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