Veterinary Parasitology 168 (2010) 231–239

Contents lists available at ScienceDirect

Veterinary Parasitology

journal homepage: www.elsevier.com/locate/vetpar

From mouse to moose: Multilocus genotyping of *Giardia* isolates from various animal species

Marianne Lebbad [a,b,*], Jens G. Mattsson [c], Bodil Christensson [c], Bitte Ljungström [d], Annette Backhans [e], Jan O. Andersson [f], Staffan G. Svärd [b,g]

[a] Department of Parasitology, Mycology and Environmental Microbiology, Swedish Institute for Infectious Disease Control, SE-171 82 Solna, Sweden
[b] Department of Microbiology, Tumor and Cell Biology, Karolinska Institute, SE-171 77 Stockholm, Sweden
[c] Department of Virology, Immunobiology and Parasitology, National Veterinary Institute, SE-751 89 Uppsala, Sweden
[d] Parasitology Unit Vidilab, SE-745 21 Enköping, Sweden
[e] Department of Clinical Sciences, Swedish University of Agricultural Science, SE-750 07 Uppsala, Sweden
[f] Department of Molecular Evolution, Evolutionary Biology Centre, Uppsala University, SE-752 36 Uppsala, Sweden
[g] Department of Cell and Molecular Biology, Uppsala University, BMC, SE-751 24 Uppsala, Sweden

ARTICLE INFO

Article history:
Received 3 April 2009
Received in revised form 23 October 2009
Accepted 3 November 2009

Keywords:
Giardia
Sweden
Multilocus genotyping
Zoonotic transmission
Assemblage

ABSTRACT

Giardia intestinalis is a protozoan parasite that consists of seven genetically distinct assemblages (A to G). Assemblage A and B parasites have been detected in a wide range of animals including humans, while the other assemblages (C to G) appear to have a narrower host range. However, the knowledge about zoonotic transmission of *G. intestinalis* is limited. To address this question, 114 *Giardia* isolates from various animals in Sweden including pets, livestock, wildlife and captive non-human primates were investigated by a sequence-based analysis of three genes (β-giardin, glutamate dehydrogenase and triose phosphate isomerase). Assemblage A infections were detected in nine ruminants, five cats and one dog, while three sheep were infected with both assemblages A and E. Multilocus genotypes (MLGs) were defined for assemblage A, and three of these MLGs have previously been detected in *Giardia* isolates from humans. The newly described sub-assemblage AIII, until now reported mainly in wild hoofed animals, was found in one cat isolate. Assemblage B occurred in three monkeys, one guinea pig and one rabbit. The rabbit isolate exhibited sequences at all three loci previously detected in human isolates. The non-zoonotic assemblages C, D, E, F or G were found in the remaining 83 *G. intestinalis* isolates, which were successfully amplified and genotyped, generating a wide variety of both novel and known sub-genotypes. Double peaks in chromatograms were seen in assemblage B, C, D and E isolates but were never observed in assemblage A, F and G isolates, which can reflect differences in allelic sequence divergence. No evidence of genetic exchange between assemblages was detected. The study shows that multilocus genotyping of *G. intestinalis* is a highly discriminatory and useful tool in the determination of zoonotic sub-groups within assemblage A, but less valuable for subtyping assemblages B, C, D and E due to the high frequency of double peaks in the chromatograms. The obtained data also suggest that zoonotic transmission of assemblages A and B might occur to a limited extent in Sweden.

1. Introduction

The parasitic protozoan *Giardia intestinalis* (syn. *G. lamblia*, *G. duodenalis*) has a global distribution and is one of the most common intestinal parasites in humans, in whom infection ranges from asymptomatic to acute or

* Corresponding author at: Department of Parasitology, Mycology and Environmental Microbiology, Swedish Institute for Infectious Disease Control, SE-171 82 Solna, Sweden. Tel.: +46 8 4572526; fax: +46 8 318450.
E-mail address: marianne.lebbad@smi.se (M. Lebbad).

0304-4017/$ – see front matter © 2009 Elsevier B.V. All rights reserved.
doi:10.1016/j.vetpar.2009.11.003

chronic disease (Gardner and Hill, 2001). A wide range of animal species including pets, livestock and wildlife can also be infected (Thompson, 2000). *G. intestinalis* consists of seven genetically distinct, though morphologically identical, assemblages (Monis et al., 2003). Assemblage A and B parasites have been detected in a wide range of mammals including humans, while the other assemblages (C to G) appear to have a narrower host range. The role of animals in transmission of infection to humans is still uncertain (Monis and Thompson, 2003; Caccio and Ryan, 2008). Studies from India and Thailand suggest that zoonotic transmission of *Giardia* can occur between dogs and humans living in the same locality (Traub et al., 2004; Inpankaew et al., 2007). Nonetheless in-depth studies of genes with greater variation are required to clarify the issue of zoonotic transmission. Several genetic loci have been described for the investigation of *Giardia* assemblages and genotypes and most published studies rely on sequence analyses of the small subunit ribosomal RNA (ssrRNA), β-giardin, glutamate dehydrogenase (gdh) and triose phosphate isomerase (tpi) genes. PCR based on the ssrRNA gene is a sensitive method for the detection of different assemblages, but intra-assemblage variation of this gene is limited. The β-giardin, gdh and tpi genes show more intra-assemblage variation, and sub-genotypes within assemblages A (AI and AII) and B (BIII and BIV) are used as discriminatory markers. However, even though it is clear that more variability is present within these assemblages (Monis and Thompson, 2003; Wielinga and Thompson, 2007; Caccio and Ryan, 2008), investigations using only a single locus do not provide enough information to understand the possible zoonotic linkage. To address this problem a multilocus genotyping approach has been suggested for *Giardia*, in order to estimate the occurrence of zoonotic transmission by comparing multilocus genotypes (MLGs) from human and animal isolates (Caccio et al., 2008). However, the impact of infections with mixed genotypes or subtypes, a common occurrence in giardiasis, on multilocus genotyping tools is not clear.

Many studies concerning the prevalence and genotypes of *Giardia* in animals have been performed worldwide, yet data from Sweden are very scarce. The occurrence of *Giardia* in dogs, sheep and calves has been previously investigated (Castor and Lindqvist, 1990; Ljungström et al., 2001; Bjorkman et al., 2003), but no information on *Giardia* genotypes in Swedish animals or their zoonotic potential is available. Furthermore, MLG studies of *Giardia* isolates representing all seven assemblages are lacking and few sequences from the host-specific assemblages C, D, E, F and G at different loci are available in the public databases. To address these questions, we performed a multilocus characterization of *Giardia* isolates from different animal species in Sweden.

2. Materials and methods

2.1. Source of isolates

Fecal samples from 114 animals infected with *Giardia* were collected from October 2002 through March 2008. These samples were from 31 dogs, 19 cats, 26 sheep, 17 cattle, one moose (*Alces alces*), one yak (*Bos grunniens*), two fallow deer (*Dama dama*), one guinea pig, one rabbit, 10 rats, two mice and three captive non-human primates [a pygmy marmoset (*Callithrix pygmaea*), a cotton-top mandarin (*Saguinus oedipus*) and a vervet monkey (*Cercopithecus* sp.)]. One hundred and two samples were initially analyzed for diagnostic purposes and the remaining 12 samples (10 rats and 2 mice) were part of a study concerning the importance of wild rodents as vectors for transmission of infections to farm animals. Rodents were handled and cared for in accordance with the Swedish national guidelines (Swedish permit no. C247/5). Seventeen samples (two from sheep, 11 from dogs and four from cats) consisted of pooled fecal material from two to four animals, while the remaining samples were from individual animals.

2.2. Cyst isolation, microscopy and DNA extraction

A sucrose gradient purification was performed on all fecal samples (Lebbad et al., 2008) and the isolated *Giardia* cysts were studied using direct immunofluorescence (IF) with a monoclonal antibody (Agua-Glo, Waterborne Inc., New Orleans, LA). DNA was extracted using the QIAamp DNA mini kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. A disruption of the purified cysts using a Mini-BeadBeater (Biospec Products Inc., Bartlesville) was performed prior to DNA extraction (Lebbad et al., 2008).

2.3. Molecular methods

All 114 samples were analyzed using a nested β-giardin PCR (Lalle et al., 2005), a semi-nested gdh PCR (Read et al., 2004) and a nested tpi PCR (Sulaiman et al., 2003). Fourteen assemblage A isolates were also analyzed using a second nested gdh PCR with a different set of primers (Caccio et al., 2008). Modified tpi primers based on GenBank accession number EU781028 (TPIDF 5′-CCG TTC ATA GGT GGC AAC TT-3′ and TPIDR 5′-GTA GCC ACT ACA CCA GTT CC-3′) were used for all dog isolates ($n = 31$) following the same PCR conditions as for the original nested tpi PCR. Moreover, a 292 bp fragment of the ssrRNA gene was amplified from a subset of samples (Appelbee et al., 2003).

All amplicons were sequenced on both strands. Chromatograms and sequences were examined using BioEdit (http://www.mbio.ncsu.edu/BioEdit/page2.html). Nucleotide sequence searches were conducted using BLAST (http://www.ncbi.nlm.nih.gov/blast/).

Nucleotide sequences without ambiguous positions, either unique for this study or obtained from a new host, have been deposited in GenBank under the accession numbers EU769204–EU769234, EU781000–EU781028, EU921646 and EU921647. In addition, 270 sequences generated in this study were deposited in the Zoop-Net Sequence Database (www.zoopnet.eu).

2.4. Phylogenetic analysis

Of the 89 isolates successfully amplified at all three genes, 46 had no double peaks at any loci and a dataset containing unambiguous β-giardin, gdh and tpi nucleotide