Transactions of the Royal Society of Tropical Medicine and Hygiene (2009) **103**, 834—838

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High genetic polymorphism among *Giardia duodenalis* isolates from Sahrawi children

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Received 10 September 2007; received in revised form 16 April 2008; accepted 16 April 2008
Available online 27 May 2009

KEYWORDS
Giardiasis;
Giardia duodenalis;
Children;
Molecular typing;
Epidemiology;
Africa

Summary Human giardiasis, the gastrointestinal infection caused by two genetically different groups (or assemblages) of *Giardia duodenalis*, is very common worldwide, and its prevalence is higher in developing countries. However, few surveys in these regions have been performed to include a genetic characterization of the parasite, which is necessary to unravel the complex epidemiology of the infection. In this work, we screened 120 faecal samples collected from Sahrawi children in 2003—2005, and found 41 (34.2%) of them to be positive, using immunofluorescent microscopy, for the presence of *G. duodenalis* cysts. Molecular characterization of the isolates was performed by RFLP and/or sequence analysis of the triose phosphate isomerase (*tpi*) and the glutamate dehydrogenase (*gdh*) genes. The results disclosed an unexpectedly high genetic polymorphism among isolates of both assemblages A and B, and a large percentage of the sequences (50% for the *tpi* gene, and 90% for the *gdh* gene) from assemblage B isolates characterized by the presence of overlapping nucleotide peaks at specific positions in the chromatograms, which can be attributed to mixed infections or to allelic sequence heterozygosity of single cysts. Notably, this phenomenon was not observed in sequences from assemblage A isolates. These results suggest that the genetic structure is different in isolates of assemblages A and B.

1. Introduction

In 1987, the project 'Accoglienza Sahrawi' was created to provide aid to children of the Sahrawi population, an extremely poor population living in the Western Sahara Desert in Algeria (http://www.saharawi.it [accessed July 2008]). As part of the project, a number of children are

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0035-9203/$ — see front matter © 2009 Royal Society of Tropical Medicine and Hygiene. Published by Elsevier Ltd. All rights reserved.
doi:10.1016/j.trstmh.2009.04.017

brought to the Tuscany Region of Italy to spend summer holidays. During their stay, the children are provided with health care, part of which includes screening for a number of diseases, including infection with parasites. Consent for this testing is provided by the children's parents.

In 2006, we tested faecal samples that had been collected for screening with the aim of performing an in-depth analysis of *Giardia duodenalis* infection in these children, and included molecular characterization of the parasite to gain insights into the epidemiology of this pathogen in extreme environmental conditions, i.e. the Sahara desert.

Giardia duodenalis (syn. *G. intestinalis*, *G. lamblia*) is a protozoan enteric parasite that infects a wide variety of mammalian hosts, including humans.[1] Previous genetic analyses of human and animal isolates have shown that *G. duodenalis* is a species complex that consists of at least seven distinct groups, referred to as assemblages A to G, among which only assemblages A and B are human pathogens.[2] As a large proportion of the data are from surveys conducted in developed countries[3], investigations in endemic regions are useful for a more objective evaluation of the genetic diversity of these pathogens and will contribute to a better understanding of the epidemiology of human giardiasis.

2. Materials and methods

2.1. Study area

The Sahrawi population originally lived in the Western Sahara, in both settled and nomadic communities.[4,5] As a result of political changes in the 1970 s, about 200 000 Sahrawis moved away from their territory and found shelter near Tindouf, Algeria, in a desert plateau known as 'Hammada', where they currently live in refugee camps divided into four districts (known as 'wilayas') and 27 villages ('dairas'); each village currently hosts about 8000 people.

This region is a desert, with almost no rainfall, although the rain that does fall often causes floods, the most recent of which occurred in February 2006 and was calamitous. Drinking water usually has a high salt concentration, even that taken from low depths; since the occurrence of several cholera epidemics, the water has been chlorinated and is distributed using tankers. The temperature greatly varies, reaching 50—60 °C in the summer (with the Sirocco wind) and −5 °C on winter nights. For this reason, agriculture is problematic and the economy is based on the farming of animals such as dromedaries (about 30 000 heads in the areas outside of the villages and 300 within the camps), and goats and sheep (about 45 000 heads in the camps). The economy of the Sahrawi population also relies on international cooperation programmes and local trade with Mauritania and Algeria.[4]

2.2. Sample collection and parasite screening

The investigation was carried out on a group of Sahrawi children, all from the village of Auserd (27°38′ North, 7°55′ West), who were hosted for summer holidays in Tuscany. A total of 120 children (42% males, 58% females) between the ages of 8 and 13 years (mean age 9.5 years) were enrolled in the study. Faecal samples had been collected in 2003, 2004 and 2005, after having obtained informed consent from the children's parents and from the teachers. Samples were analysed for the presence of *G. duodenalis* cysts by immunofluorescence (IF) using FITC-conjugated cyst-wall-specific antibody (Merifluor, Meridian Biosciences, Cincinnati, OH, USA) and following the manufacturer's instructions.

2.3. DNA extraction and PCR assay

DNA was extracted from 400 μl concentrated faecal material using the FastDNA kit (Resnova, Rome, Italy), as previously described.[6] Nested PCR amplification of a triose phosphate isomerase (*tpi*) gene fragment (530 bp) was performed following the protocol of Sulaiman et al.[7] The amplification of a fragment (530 bp) of the glutamate dehydrogenase (*gdh*) gene was obtained using newly designed primers, Gdh1 (5′-TTCCGTRTYCAGTACAACTC-3′) and Gdh2 (5′-ACCTCGTTCTGRGTGGCGCA-3′) for the primary amplification, and Gdh3 (5′-ATGACYGAGCTYCAGAGGCACGT-3′) and Gdh4 (5′-GTGGCGCARGGCATGATGCA-3′) for the nested amplification. The conditions for the primary and secondary amplification of the *gdh* gene fragment were identical, and consisted of 35 cycles (94 °C for 30 s, 50 °C for 30 s and 72 °C for 1 min) in a T-personal thermocycler (Whatman-Biometra, Goettingen, Germany), with an initial hot start at 94 °C for 2 min and a final extension at 72 °C for 7 min. Reactions were visualized using 1% agarose gels stained with ethidium bromide (EtBr).

2.4. PCR-RFLP of the *tpi* gene

The sequences of the *tpi* gene fragment from *G. duodenalis* assemblages A to G (retrieved from the GenBank database) were analysed for restriction profiles. Restriction map analysis was carried out, and predicted restriction profiles were determined for each of the assemblages with the enzyme *Dde*I. Aliquots (10 μl) of *tpi* PCR products were digested using 5 units of *Dde*I (New England Biolabs Inc., Ipswich, MA, USA) in a final volume of 20 μl for 4 h at 37 °C. Restriction products were separated on EtBr-stained 3% MetaPhor agarose gel (BioSpa, Milan, Italy).

2.5. Sequence analysis

PCR products were purified and sequenced on both strands. A dataset was assembled containing the nine novel and unambiguous *tpi* nucleotide sequences obtained in the present study and 48 sequences retrieved from GenBank at the National Center for Biotechnology Information (http://www.ncbi.nlm.nih.gov/ [accessed July 2008]), which represented the diversity of *tpi* sequences in the *Giardia* genus and within *G. duodenalis*. A multiple alignment was generated using the ClustalX program.[8] No insertions, deletions or ambiguously aligned positions were identified within any of the 57 sequences.