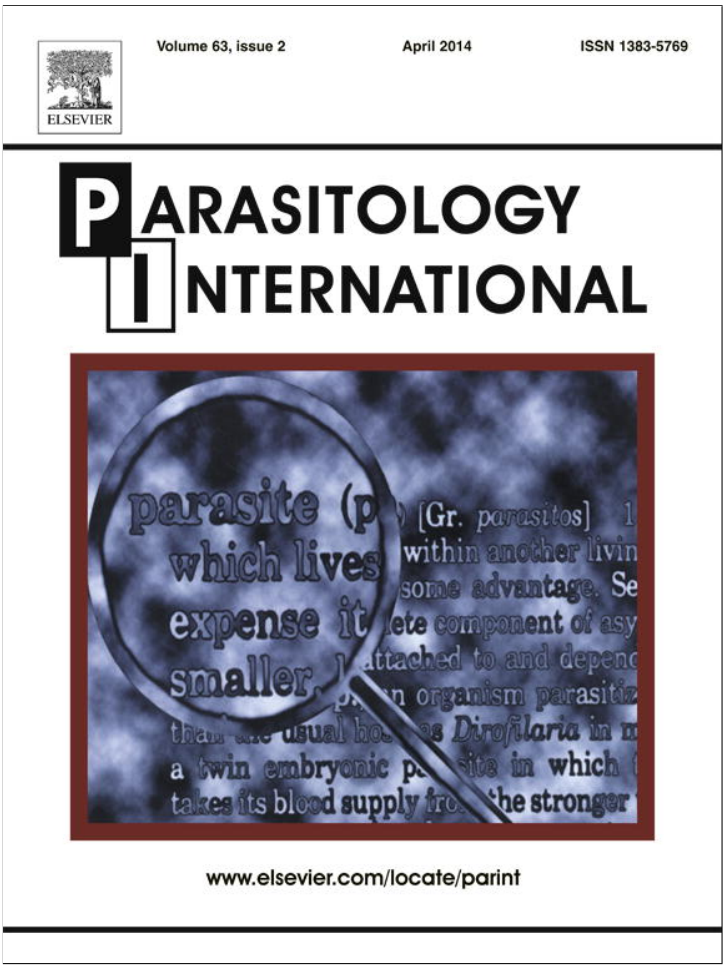

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Short communication

Genetic characterization of *Giardia duodenalis* by sequence analysis in humans and animals in Pemba Island, Tanzania

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ABSTRACT

Giardia duodenalis represents one of the most widespread human enteric parasites: about 200 million people in Asia, Africa and Latin America are infected. *Giardia* exerts a deep impact on public health because of high prevalence and possible effects on growth and cognitive functions in infected children. The major aim of this study was to detect and genetically characterize *G. duodenalis* in both human and animal fecal samples collected in Pemba Island, in the archipelago of Zanzibar (Tanzania), in order to deepen the knowledge of genotypes of *Giardia* in this area.

Between October 2009 and October 2010, we collected 45 human fecal samples from children from 2 primary schools and 60 animal fecal samples: 19 from zebus (*Bos primigenius indicus*) and 41 from goats (*Capra hircus*). Detection and genetic identification were performed by *multilocus* analysis of *ssu-rDNA* and *gdh* genes. In humans we found a higher prevalence of assemblage B (sub-assemblage BIV), in goats of assemblage E and in zebus of assemblage A. Our study represents an important contribution to the epidemiological knowledge of *G. duodenalis* in this area of Tanzania.

Giardia duodenalis (syn. *G. intestinalis*; *G. lamblia*) is a flagellated protozoan that infects the intestine of a wide range of vertebrate hosts and the only species of *Giardia* found in humans. Genetic studies have demonstrated that *G. duodenalis* is a multi-species complex comprising at least seven assemblages, identified from A to G [1]. In addition to these described assemblages, several novel genotypes have been reported including the assemblage H proposed by Lasek-Nesselquist et al. in 2010 [2]. Only assemblages A and B are capable of infecting humans but they are also found in a wide range of other mammalian hosts, so that they are considered potentially zoonotic. The distribution of the assemblages A and B is different in several studies and countries. In addition, a significant intra-assemblage genetic variability is recognized in both assemblages [1].

G. duodenalis is a common cause of diarrheal disease in humans, particularly among disadvantaged groups where recurrent infections contribute to growth deficits and malnutrition, especially in children in developing countries [3]. The human prevalence rates range from 2–7% in developed countries to 20–30% in most developing countries [4], due to poor hygiene and limited access to safe water supply. In September 2004, *Giardia* was included in the Neglected Diseases Initiative of the WHO [5]. Little data on the prevalence of *G. duodenalis* are available from sub-Saharan Africa and a genetic characterization of the parasite in these regions has been rarely performed. On the other hand collection of molecular data from endemic areas is necessary to better understand host and environmental interactions within the disease [6].

Considering the absence of molecular data related to *G. duodenalis* in Tanzania, in the present study we have genetically characterized at two loci isolates of *G. duodenalis* collected from humans and animals on Pemba Island, in order to identify the circulating assemblages and sub-assemblages and the transmission dynamics in this area.

Pemba Island is the second largest island of the archipelago of Zanzibar, located in the Indian Ocean south of the equator and 48 km from the eastern coast of the African continent. Between October 2009 and October 2010 45 fecal samples were obtained from humans in pediatric age (7–12 years) attending two primary schools in the district of Chake Chake, the main city of Pemba Island. In the same period, 60 samples from animals (41 from goat — *Capra hircus* — and 19 from zebu cattle — *Bos primigenius indicus*) were collected in a day, directly from the soil, immediately after observed defecation from each host in Kojani, a small village on the coast and separated from the island of Pemba by a narrow stretch of sea tidal. Children were selected at random from schools, located nearby the Public Health Laboratory Ivo de Carneri (PHL-IdC) where the first part of the study was carried out. Animals were selected from Kojani Island by local veterinary personnel. A continuous movement of people and livestock inter- and intra- the two

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islands was present. All samples were smeared on filter paper (8 cm^2), dried at room temperature for about 30 min and stored in plastic containers at room temperature before being transported to Italy as previously described in Berrilli et al. [7].

Informed verbal assent was obtained from children's parents or legal guardians. All investigations were performed in concordance with the WMA Helsinki Declaration (Edinburgh 2000). Ethical clearance was obtained by the Zanzibar Health Research Council.

The samples were carried to the University of Rome Tor Vergata where the molecular analysis was performed. DNA extraction was performed by cutting about 2 cm^2 of the smeared filter paper and using it for the extraction by QIAamp DNA Micro Kit (QIAgen, Gmbh, Hilden, Germany) in according to the manufacturer's recommendations. The final eluate was stored at −20 °C. In order to unequivocally assigned *Giardia* isolates at the assemblage level and identify sub-assemblages, PCR was carried out to amplify a 130 bp region from the small subunit ribosomal RNA (*ssu-rDNA*) [8], and a 432 bp region of the glutamate dehydrogenase (*gdh*) gene [9]. Positive (*Giardia* DNA) and negative (no template added) control samples were used in all PCR runs. PCR products were separated by electrophoresis in 1% agarose gel and amplicons were purified using the NucleoSpin® Extract kit (Macherey-Nagel GmbH & Co. KG, Germany). Both strands were sequenced by the Bio-Fab Research s.r.l. (Rome, Italy). Sequences were edited using the FinchTV 1.4 software (Geospiza, Inc., Seattle, WA). *Consensus* sequence was determined by alignment of respective forward and reverse sequences. Assignment to assemblage and sub-assemblage of *G. duodenalis* isolates was carried out by sequence comparison (*ssu-rDNA*) and phenetic analysis (*gdh*). Multiple alignments were performed using ClustalW2 software for DNA against known sequences available in GenBank for *Giardia* assemblages. Phenetic analysis was performed using the software MEGA5, conducted using the Tamura 3-parameter method and the phenetic tree was constructed by the Neighbor-Joining algorithm. Bootstrap values were calculated by analyzing 1000 replicates. All *gdh* sequences obtained in this study were deposited in the GenBank database and are available under the accession numbers: KF468653–KF468671.

As for humans, *ssu-rDNA* amplicons were obtained from 25 of the 45 samples, whereas 17 resulted positive in the *gdh* PCR. Eleven out of 25 *ssu-rDNA* positive samples were negative for *gdh* gene analysis, while 3 out of 20 *ssu-rDNA* negative samples were positive to *gdh* locus (Table 1). Sequences and phenetic analyses allowed the assignment of 6 isolates to assemblage A (3 of them to sub-assemblage AII) and 22 isolates to assemblage B, particularly 4 isolates to sub-assemblage BIII and 7 to sub-assemblage BIV. Three isolates K043 (KF468658), K051 (KF468659) and K060 (KF468663) were identified as belonging to the assemblage B but could not unambiguously be assigned to sub-assemblage level. In particular, their sequences showed several diagnostic nucleotides typical of BIII and some typical of BIV; moreover, the sequence K043 included one double peak (C/T). The high substitution rate in *gdh* sequences has been previously reported to limit the clear-cut identification of *Giardia* isolates at sub-assemblage level within assemblage B [10].

Concerning animals, 9/41 samples from goats and 4/19 from zebus were found *Giardia* positive. The genetic characterization of goat isolates made it possible to assign 6 samples to the host-specific assemblage E, two samples to assemblage B (1 of them to sub-assemblage BIV) and 1 sample to assemblage A. Three isolates from zebus were found to belong to assemblage A and 1 to assemblage B (sub-assemblage BIV) (Table 1). Fig. 1 shows the phenetic tree forming two well-defined clusters, one corresponding to the assemblage A (sub-assemblage AII), the other including isolates matching which sequences including different subtypes of assemblage B (BIII and BIV).

No discrepancies were observed in assemblage types as determined by sequencing of the two genes in all 41 human and animal isolates, indicating single assemblage infections.

G. duodenalis has a cosmopolitan distribution. In developing countries, giardiasis is highly endemic in humans, especially in children [3]. On Pemba Island, prevalence of infection has been assessed during 3 epidemiological surveys among the general population, in particular school-aged children. In 1984 Pampiglione et al. [11] carried out a copro-parasitological study on 413 fecal samples and *G. duodenalis* was detected by microscopic examination in 23 patients (5.6%). Few years later Albonico and colleagues [12] reported a prevalence of 6.6% among children aged 9–17 years. Recently, the prevalence of *Giardia* in school-aged children was 16.4% [13].

In spite of its endemicity, to the best of our knowledge this is the first study on molecular characterization of *G. duodenalis* isolates of different origins conducted in Tanzania.

With regard to humans, by the sequencing of *ssu-rDNA* and *gdh* loci, we have assigned a higher number of isolates to the assemblage B, sub-assemblage BIV, known for its preference of human hosts [14]. As for assemblage A 3 isolates were assigned to the sub-assemblage AII, also apparently more specific for humans [6,14]. In no case were highlighted animal-specific assemblages (from C to G). The existing data on human genotypes characterized in African countries are still limited and fragmented, covering Egypt [15,16], Ethiopia [17], Western Sahara [18], Guinea Bissau [19] and Cote d'Ivoire [7]. The distribution of assemblages observed among our samples does not differ considerably from those observed in other African countries where a higher rate for assemblage B is already known, e.g. in Côte d'Ivoire [7] and Egypt [15]. Concerning *Giardia* infection in animals, the main presence of assemblage E found in goats and the identification of 3 out of 4 *Giardia* isolates from zebus as assemblage A is not surprising, since both host-adapted and zoonotic assemblages are reported worldwide in goats and livestock [20].

In the last decades, the studies of molecular epidemiology have revealed a wide variation in the distribution of assemblages and sub-

Table 1
Summary of genotyping results of *Giardia duodenalis* samples from humans and animals at the level of assemblage and sub-assemblage.

Humans						Animals		
Identification code	*ssu-rRNA*	*gdh*	Identification code	*ssu-rRNA*	*gdh*	Identification code	*ssu-rRNA*	*gdh*
A022	B	B (IV)	K053	B	–	G05	E	–
A031	B	B (IV)	K054	B	B (III)	G07	E	–
A035	A	–	K055	A	A (II)	G08	B	–
A039	A	A (II)	K057	B	B (III)	G11	E	–
A045	B	–	K060	B	B	G16	A	–
K009	A	A (II)	K062	B	–	G17	–	B (IV)
K012	A	–	K063	B	B (IV)	G22	E	–
K026	B	–	K064	–	B (IV)	G23	E	–
K031	B	–	K066	B	B (III)	G36	E	–
K035	B	B (IV)	K067	A	–	Z09	A	–
K043	B	B	K070	B	–	Z10	A	–
K047	B	–	K071	–	B (IV)	Z14	B	B (IV)
K049	B	–	K072	B	B (III)	Z19	A	–
K051	B	B	K073	–	B (IV)			

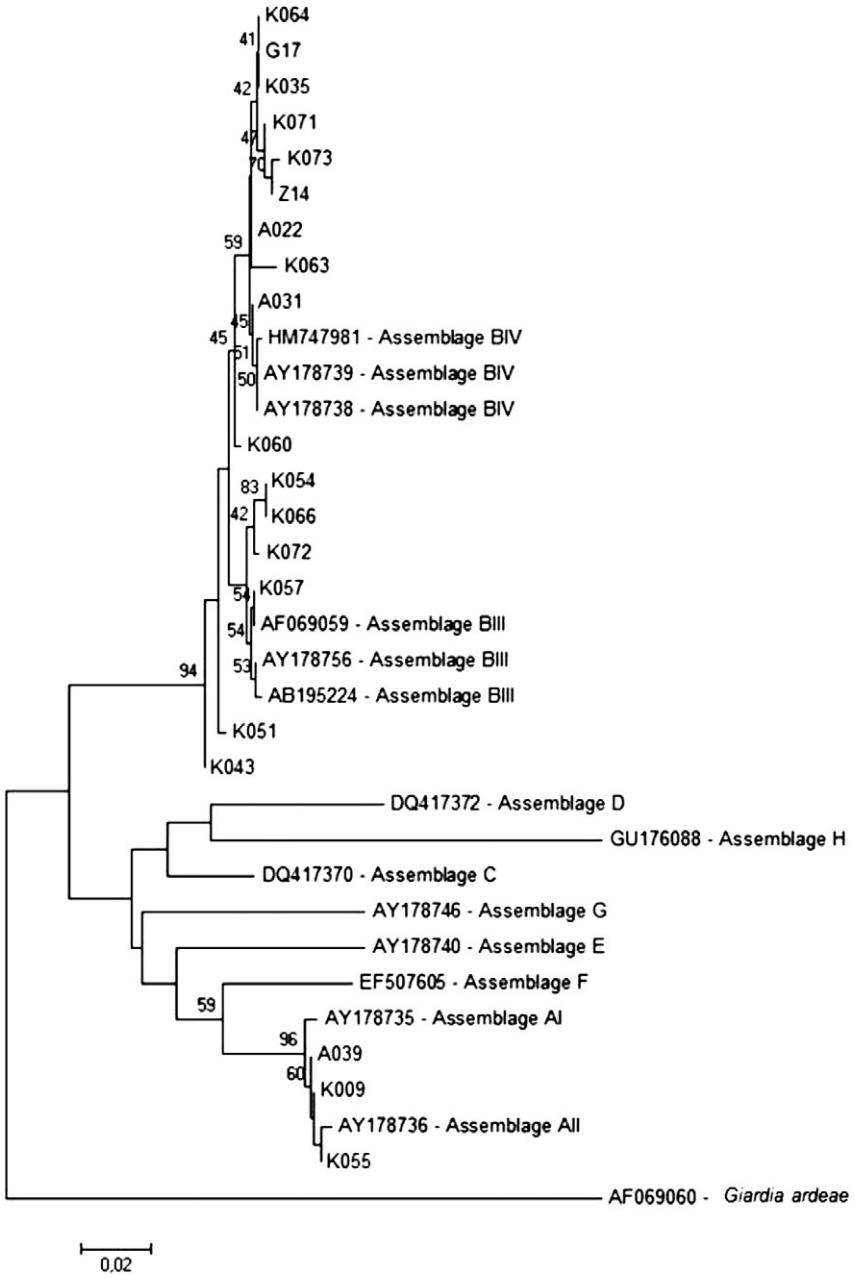

Fig. 1. Phenetic relationships inferred using the Neighbor-Joining method of the consensus *gdh* gene sequences obtained from PCR positive isolates.

assemblages among human and animal populations in different geographical areas worldwide, likely in correlation to the different transmission cycles identifiable at local scale [21]. Comparison of assemblages and sub-assemblages identified in humans, goats and zebus on Pemba Islands seems related to separated transmission cycles suggesting that zoonotic risk of *Giardia* transmission could have a minor impact on human giardiasis in the studied area. The paucity of data, however, does not yet provide enough information to better understand the epidemiological meaning of our finding that may simply reflect a sample bias.

This study has contributed to add new data about *Giardia* assemblages on Pemba Island and has improved the knowledge of the occurrence of these pathogens among humans and animals. Overall, the relatively high level of infection observed may pose a specific public health issue reflecting the poor availability of sanitation where people live, characterized particularly by limited access to safe water supplies and a high degree of environmental contamination due to the lack of fecal and waste disposal. More *Giardia* samples from much broader geographical localities of sub-Saharan Africa and from both humans and animals should be employed in further studies to better clarify the epidemiology and the zoonotic risk of giardiasis in endemic areas.

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