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**SUMMARY**

This study was conducted to determine the genotypes of *Giardia duodenalis* isolated from human faecal samples at Pos Betau, Pahang, Malaysia. Faecal specimens were collected and examined for *G. duodenalis* cysts using Trichrome staining techniques. Molecular identification was carried out by the amplification of a region of the small subunit of the nuclear ribosomal RNA (SSU rRNA) gene using nested PCR and subsequent sequencing. The sequences from 15 isolates from *G. duodenalis* were subjected to phylogenetic analysis (including appropriate outgroups) using the neighbor-joining and maximum parsimony methods. The trees identified *G. duodenalis* assemblages A and B, with a predominance of assemblage B. The predominance of anthroponotic genotypes indicates the possibility of anthroponotic transmission of these protozoa in this Semai Pahang Orang Asli community.

Key words: *Giardia duodenalis*, assemblage B, genotypes, Semai Pahang Orang Asli.

**INTRODUCTION**

Human giardiasis has been recognized as the most common cause of protozoal diarrhoea worldwide, leading to significant morbidity and mortality both in developing and industrialized nations (Marshall et al. 1997). Although person-to-person transmission of *Giardia duodenalis* is the major route of transmission, transmission *via* the ingestion of contaminated water and foods as well as zoonotic transmission of these protozoa have also been noted (Marshall et al. 1997). *Giardia* has caused multiple waterborne outbreaks in developed and developing countries.

*G. duodenalis* comprises at least 7 lineages; designated as assemblages A–G. Assemblage A has a wide range of hosts and has been considered as zoonotic assemblage. Although assemblage B has been reported to be an anthropoasitic assemblage, it has also been isolated from animals. The other assemblages appear to be host-adapted; assemblage C and D (dog), assemblage E (cattle and other ungulates), assemblage F (cats) and assemblage G (rats), and have not represented a potential public health concern (Monis et al. 1999).

In Malaysia, giardiasis is an endemic disease, predominantly in children, with prevalences ranging from 2·6% to 25% (Lim et al. 2008). *Giardia* has also been isolated from animals and environmental samples (Lim et al. 2008). However, the prevalence of individual *G. duodenalis* assemblages has yet to be estimated in Malaysia. This study aimed at determining the genotypes of *Giardia*, which will assist Malaysian public health authorities to build a more effective control strategy based on a better understanding of the epidemiology of this parasite.

**MATERIALS AND METHODS**

**Samples**

Faecal samples were collected from the Orang Asli (Peninsular Malaysia aborigines) community at Pos Betau, Pahang, Malaysia ~200 km north of Kuala Lumpur. The Orang Asli in this community belong to the Semai Pahang ethnic subgroup. The Orang Asli houses are made of wood or bamboo and they are provided with basic amenities, such as electricity and piped water by the Malaysian government. Although they have piped water supply, living beside a river is crucial to them, as most of their daily activities, including bathing, washing clothes and household items, swimming, playing, are linked to the river. Some inhabitants may also defecate along in the
river and use river water to wash themselves. A total of 400 stool containers were randomly distributed among 8 villages; faecal samples were collected randomly during several visits in 2 months from February to March 2006.

**Light microscopy, and purification of Giardia cysts using immuno-magnetic separation (IMS)**

For the identification of *Giardia* cysts, faecal smears were made from the preserved stool and stained with the Trichrome stain (Wheatley, 1951). The smears were then examined by light microscopy for *Giardia* cysts under 100× and 400× magnification respectively.

For purification of cysts from faecal specimens, a small portion of the faeces was mixed with 10 ml of distilled water and sieved through gauze. The suspension was then centrifuged at 1500 × g for 10 min, and the supernatant was discarded. The pellet was re-suspended in 2 ml of water and stored at 4 °C until use. IMS was carried out using Dynabeads® GC-Combo kit (Dynal, Oslo, Norway) according to method of Xiao *et al.* (2004).

**DNA methods and analyses**

Genomic DNA was extracted from the IMS-isolated cysts using QIAgen DNA Mini Kit (QIAGEN, Germany), according to Xiao *et al.* (2004). Briefly, IMS-isolated cysts were re-suspended in 180 μl of Buffer ATL provided in the kit, and then subjected to 5 consecutive cycles of freezing in liquid nitrogen for 1 min and thawing at 56 °C for 2 min, vortexing for 30 s following each cycle. DNA was then extracted, purified and stored at −20 °C until use.

Nested PCR assay was used to amplify a region (~200 bp) of the small subunit of the nuclear ribosomal RNA (SSU rRNA) gene, according to Read *et al.* (2002). Primary PCR was carried out with the primers RH11 (5'-CAT CCG GAT CCT GCC-3') and RH4 (5'-AGT CGA ACC CTG ATT CTC CGC CAGG-3') (Hopkins *et al*. 1997), and the secondary PCR with primers Giar-F (5'-GAC GCT CTC CCC AAG GAC-3') and Giar-R (5'-CTG CGT CAC GCT GCTC-3') (Read *et al*. 2002). Both primary and secondary PCRs were performed in a 25 μl reaction volume containing 12.5 pmol of each primer (Research Biolab, Singapore), 2 U of *Taq* polymerase (New England Biolabs, Ipswich, USA),

<table>
<thead>
<tr>
<th>Assemble</th>
<th>Host species</th>
<th>GenBank Accession number</th>
</tr>
</thead>
<tbody>
<tr>
<td>Test samples representing samples</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3s</td>
<td>Human</td>
<td>FJ460002</td>
</tr>
<tr>
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<td>Human</td>
<td>FJ460014</td>
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<tr>
<td>12</td>
<td>Human</td>
<td>FJ460013</td>
</tr>
<tr>
<td>17a</td>
<td>Human</td>
<td>FJ460003</td>
</tr>
<tr>
<td>25a</td>
<td>Human</td>
<td>FJ460004</td>
</tr>
<tr>
<td>26a</td>
<td>Human</td>
<td>FJ460005</td>
</tr>
<tr>
<td>29</td>
<td>Human</td>
<td>FJ460006</td>
</tr>
<tr>
<td>32a</td>
<td>Human</td>
<td>FJ460007</td>
</tr>
<tr>
<td>44</td>
<td>Human</td>
<td>FJ460009</td>
</tr>
<tr>
<td>45a</td>
<td>Human</td>
<td>FJ460008</td>
</tr>
<tr>
<td>47a</td>
<td>Human</td>
<td>FJ460010</td>
</tr>
<tr>
<td>51</td>
<td>Human</td>
<td>FJ460011</td>
</tr>
<tr>
<td>S</td>
<td>Human</td>
<td>FJ460012</td>
</tr>
<tr>
<td>Group I=(53,58a, 64, 68, 73, 74, 87, 101, 115a, 119, 124, 138, 141, 147a, 149a, 162a, 197, 199, 200, 201, L12, L23, L24, L41, M1, M2, M6, M10)</td>
<td>Human</td>
<td>FJ460016</td>
</tr>
<tr>
<td>43</td>
<td>Human</td>
<td>FJ460015</td>
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</tbody>
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Reference sequences representing assemblages

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<th>Assemble</th>
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</thead>
<tbody>
<tr>
<td>A</td>
<td>—</td>
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<tr>
<td>B</td>
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<td>U09491</td>
</tr>
<tr>
<td>C</td>
<td>Dog</td>
<td>AF113899</td>
</tr>
<tr>
<td>D</td>
<td>Dog</td>
<td>AF113900</td>
</tr>
<tr>
<td>E</td>
<td>Cattle</td>
<td>AF199448</td>
</tr>
<tr>
<td>F</td>
<td>Cat</td>
<td>AF199444</td>
</tr>
</tbody>
</table>

Table 1. Sequences of *Giardia duodenalis* assemblages used in the phylogenetic analyses
Among genotypes (Hopkins nucleotide positions shown previously to differentiate and MP methods), utilizing 140 informative-nu-
Table 1) were used in the phylogenetic analyses (NJ (A-F) obtained from the GenBank database (see
strong support (100%) and G. duodenalis
21 G. duodenalis shown as a representative in Fig. 1. In this tree, all
of the 42 isolates and 6 reference sequences re-
in both directions. Fifteen sequences representing all
samples (23.7%) contained G. duodenalis
21 of assemblage B (GenBank Accession number
100% for isolate 43 was placed
isolate 43 was 98% similar to assemblage A
assemblage B was the predominant assemblage in
resulting from microscopically, 76 samples (23.7%) contained Giardia. Amplicons (~200 bp) were produced for 42 of the 76 samples. All of these amplicons were successfully sequenced in both directions. Fifteen sequences representing all of the 42 isolates and 6 reference sequences representing all known G. duodenalis assemblages (A-F) obtained from the GenBank database (see Table 1) were used in the phylogenetic analyses (NJ and MP methods), utilizing 140 informative nucleotide positions shown previously to differentiate among genotypes (Hopkins et al. 1997). The NJ and MP trees were concordant in topology; the NJ tree is shown as a representative in Fig. 1. In this tree, all 21 G. duodenalis sequences grouped together with strong support (100%) and G. duodenalis isolates (3s, 10, 17a, 25a, 26a, 29, 32a, 44, 45a, 47a, 51, S and Group I) grouped with a representative of assem-
blage B (82%). G. duodenalis isolate 43 was placed with assemblages A, E and F (96%).

Genetic characterization was further confirmed by similarity search using BLAST. It was found that all the isolates that were clustered in assemblage B were similar to a small ribosomal subunit RNA sequence of assemblage B (GenBank Accession number DQ789112), which was isolated from beavers in Massachusetts, USA (Fayer et al. 2006), assemblage B (GenBank Accession number DQ385547) obtained from Canis latrans in northeastern Pennsylvania, USA (Trout et al. 2006) and assem-
blage B (GenBank Accession numbers AY826201, AY826202, AY826203, AY826207) from faecal samples from humans in the Netherlands (van der Giessen et al. 2006). The similarity was 100% for isolates 44 and S, 99% for isolate 10, 98% for isolates 25a, 29, 32a, 51 and group 1, 97% for isolates 3s, 45 and 17a, 95% for isolate 26a and 93% for isolate 47a. The isolate 43 was 98% similar to assemblage A (GenBank Accession number EU562195).

The present study showed that G. duodenalis assemblage B was the predominant assemblage in Semai Pahang Orang Asli communities in Malaysia.
The predominance of this assemblage has been documented in several studies in the UK (Caccio et al. 2005), Australia (Read et al. 2004), Albania (Berrilli et al. 2006), Turkey (Aydin et al. 2004), Peru (Sulaiman et al. 2004), the Philippines (Yason and Rivera, 2007) and India (Sulaiman et al. 2004). In contrast, assemblage B appeared to be less prevalent compared with assemblage A in the USA and Canada (van Keulen et al. 2002), Italy (Lalle et al. 2005), Mexico (Eligio-García et al. 2005) and Ethiopia (Gelanew et al. 2007).

The 2 main assemblages responsible for human infections are assemblages A and B. Although assemblage B appears to be predominantly human specific, it has been isolated from dogs, calves, cats and monkeys (van Keulen et al. 2002; Itagaki et al. 2005). The greatest zoonotic risk is considered to be posed by assemblage A, which infects humans and a wide range of animals (Itagaki et al. 2005). The predominance of assemblage B in this Semai Pahang Orang Asli community indicates the possibility of anthropogenic transmission from human to human. Furthermore, the suggestion of anthropogenic transmission was supported by epidemiological findings indicating the rearing of pets is not a risk factor for giardiasis in this Orang Asli community (Mohammed Mahdy et al. 2008). Anthropogenic transmission may be facilitated by low hygienic practices which include consuming food with unwashed hands, consuming raw unwashed vegetables and storing drinking water in dirty containers which are not covered. Drinking tap water and eating raw vegetables were identified as significant risk predictors of giardiasis in this Orang Asli community (Mohammed Mahdy et al. 2008). Another study showed that contamination of Giardia in drinking water occurred after treatment in the Temuan Orang Asli community (Lim and Ahmad, 2004). In addition, it has also been reported that some of the Orang Asli children feel more comfortable defecating in an open space, thereby contaminating the environment. Unfortunately, there is a void of information regarding Giardia genotypes in aboriginal populations within Malaysia and in other countries, which currently makes the comparison of Giardia genotypes between communities that have similar behavioural, cultural background and share the same public health problems impossible.

In conclusion, assemblage B is the predominant assemblage of G. duodenalis in this Semai Pahang Orang Asli community, suggesting that human giardiasis is mainly linked to anthropogenic transmission. However, the inference of such transmission is limited by the fact that domestic animals were not included in the present investigation and that genetic characterization of Giardia isolates was based on a single locus. Therefore, further molecular studies of a range of Giardia isolates from humans, animals and the environment using multiple loci are warranted in the Semai Pahang Orang Asli community. Better insights into the genetic make-up of Giardia populations as well as improved sanitary measures with health education will be the key factors in the control of giardiasis in this community.

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