Title: Molecular epidemiology of giardiasis among Orang Asli in Malaysia: application of the triosephosphate isomerase gene

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Author's response to reviews: see over
Response to Reviewers

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All the authors would like to express their highest gratitude to the Editor, Miss Sheryl Ramos and the reviewers, Yaoyu Feng and Rachel Chalmers for the thoughtful responses to the manuscript. The authors appreciate the comments given and reviewer’s insight in reviewing this manuscript from a different perspective.

**Reviewer 1: Yaoyu Feng**

**Major Compulsory Revisions**

1. This is an interesting study and the results provided the practical intervention ways in preventing giardiasis in Orang Asli community. However, all the results were based on triosephosphate isomerase (tpi) gene only. Although the authors stated that the tpi gene was chosen because of the high genetic heterogeneity displayed by *Giardia* species at this locus at the end of the introduction, it is a common practice for detecting *G. duodenalis* on multiple loci including tpi, beta-giardin (β-giardin) and glutamate dehydrogenase (gdh) in all the recent publications because of the discrepant results based on different loci. Therefore, it is strongly suggested that the authors abided by the standard practice by adding data from the other two loci.

**Response**: Thank you for your honest comment and suggestion. The authors acknowledge that there is a limitation in this study; only one gene locus was used to identify *G. duodenalis* in the samples. The main reason why the authors only used one gene locus was to specifically determine the risk factors for each assemblage. The authors wanted to make this as a constant parameter so that the results will be more consistent and reliable. If the authors used more than one gene locus, it will definitely affect the outcomes as each gene will gave different results. The tpi gene was chosen based on the concrete results from the other researchers that showed this locus was more sensitive in detecting *G. duodenalis* infection and mixed assemblage than gdh or β-giardin. Moreover, comparison of the assemblage A sequences at the tpi locus in the pair wise identify matrix was more discriminatory in identifying
sub-assemblages than the other loci. Thus, detection methods targeting loci with a high degree of polymorphism such as *tpi* can be extremely useful when a common source of transmission is certainly involved. Table below shows the findings from others which also used *tpi* locus and proved to be far away better than other loci.

<table>
<thead>
<tr>
<th>Authors</th>
<th>Loci</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Huey et al. (2013)</td>
<td><em>tpi, gdh</em> and <em>β-giardin</em></td>
<td>The <em>tpi</em> locus achieved the highest percentage of amplicons produced (70%), followed by <em>gdh</em> (45%) and <em>β-giardin</em> (33%).</td>
</tr>
<tr>
<td>Laishram et al. (2012)</td>
<td><em>tpi, gdh</em> and <em>β-giardin</em></td>
<td>The <em>tpi</em> PCR was the most sensitive and detected <em>G. duodenalis</em> in all 74 microscopy-positive samples, while <em>gdh</em> and <em>β-giardin</em> PCR were positive in 62.2% and 56.8% of the samples.</td>
</tr>
<tr>
<td>David et al. (2011)</td>
<td><em>tpi</em> and <em>gdh</em></td>
<td>From the 71-microscopy positive samples, specific amplification of <em>gdh</em> and <em>tpi</em> fragments was noted in 95.7% and 90% samples, respectively. For 144 microscopy-negative samples, <em>gdh</em> and <em>tpi</em> gene amplification products were obtained from 8.3% and 35.9% samples, respectively.</td>
</tr>
<tr>
<td>Bertrand et al. (2005)</td>
<td><em>tpi</em> and <em>gdh</em></td>
<td>Among 26 faecal samples, the <em>tpi</em> gene was amplified</td>
</tr>
</tbody>
</table>
from 96% with the PCR assay, whereas only 81% samples were positive when the *gdh* gene was targeted.

<table>
<thead>
<tr>
<th>Authors (Year)</th>
<th>Gene(s) Targeted</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Traub et al. (2004)</td>
<td>tpi, SSU rRNA and ef1-α</td>
<td>Analysis of the <em>tpi</em> gene provided better genetic resolution.</td>
</tr>
<tr>
<td>Said E. R. Amer (2013)</td>
<td><em>tpi</em></td>
<td><em>Tpi</em> gene is a polymorphic gene providing a good target for analyzing and genotyping <em>Giardia</em>. In the study, assemblage A accounted for 33.3% and assemblage B accounted for 66.7%.</td>
</tr>
<tr>
<td>Ignatius et al. (2012)</td>
<td><em>tpi</em></td>
<td>Assemblage B (85.9%) and assemblage A (12.7%)</td>
</tr>
<tr>
<td>Atherton et al. (2013)</td>
<td><em>gdh</em></td>
<td>Of the 154 ELISA-positive samples analysed for the presence of the <em>gdh</em> gene, only 48% were positive.</td>
</tr>
</tbody>
</table>

However, the authors will definitely consider the brilliant ideas from the first reviewer in their future study.

**Minor Essential Revisions**

1. Figure 1 is unnecessary.

**Response:** Thank you for your suggestion. The authors agreed with the reviewer. Therefore, Figure 1 has been removed from the revised manuscript.
Reviewer 2: Rachel Chalmers

Minor Essential Revisions

1. Although the main paper is well written and clear, the abstract needs some clarification and editing as indicated in the PDF attached.

Response: Thank you for your valuable comments. The authors have made the corrections in the revised manuscript as suggested by the respective reviewer (page 2).

2. Other minor revisions are also indicated in the PDF.

Response: Thank you for your valuable comments. The authors have made the corrections in the revised manuscript as suggested by the respective reviewer (page 8).

3. Did the authors ask where the subjects had symptoms at the time of sampling or in the period preceding sampling? Such information would be useful in interpreting whether the findings relate to asymptomatic carriage or symptomatic infection.

Response: Thank you for raise up this point. The authors regret to inform that such question was not asked during the field work or sampling procedure. The following groups of variables (demographic data, socioeconomic background, behavioural risks, environmental sanitation and characteristics of living condition and close contact with household pets) were asked to identify G. duodenalis assemblage and the risk factors. This was to attain better understanding of the genetic diversity and transmission of giardiasis. Furthermore, G. duodenalis is known to vary widely in clinical manifestations including acute, chronic and asymptomatic courses. Therefore, the authors did not attempt to a priori define clinical giardiasis in the present study. Nevertheless, no evidence for causation of gastrointestinal symptoms was seen, with the potential exception of abdominal distension. However, the authors would definitely consider the reviewer suggestion in the next study.