Molecular detection of *Giardia duodenalis* from Long-tailed macaque (*Macaca fascicularis*) at San Phra Kan shrine, Lopburi, Thailand

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**ABSTRACT**

*Giardia* spp. is a flagellated protozoan parasite infecting humans and various animals and can cause gastrointestinal diseases worldwide. *Giardia duodenalis* was classified as assemblage A and B that were frequently found in feces of non-human primates recently reported in humans. The objectives of this study were to determine the prevalence of *Giardia* infection and to classify their species found in long-tailed macaque (*Macaca fascicularis*). In 2014, a total of 200 fecal samples were randomly collected from long-tailed macaques living in different areas of San Phra Kan shrine, Lopburi, Thailand. Extracted DNAs from fecal samples were amplified using nested-PCR of the ssu-rDNA gene. Determination of *Giardia* spp. among PCR positives was confirmed by using nucleotide sequence analysis. The results revealed that 7% (14/200) of fecal samples were positive for *Giardia duodenalis* and the prevalence was majorly found in male macaques (7.9%; 9/114), compared to female (5.8%; 5/86). No significant association were found between positive *Giardia*, sex and location of macaques. Using sequence analysis, assemblage B of *G. duodenalis* was majorly found in these positives (78.6%, 8/14) while the others were inconclusive assemblage. Assemblage B was one of the important assemblage that was previously reported in humans. The present study demonstrated that long-tailed macaques might be potential reservoir for *G. duodenalis* assemblage B with zoonotic concern for nearby humans.

Key words: *Giardia duodenalis*, *Macaca fascicularis*, PCR, San Phra Kan shrine, Thailand

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INTRODUCTION

*Giardia* spp. is a flagellated protozoan parasite infecting humans and animals with its implication of gastrointestinal diseases worldwide. This parasitic protozoa is transmitted via the fecal-oral route or ingestion of contaminated food or water (Xiao et al., 2008). *Giardia* infections were found in a variety of animals including dogs (Leonhard et al., 2007, Palmer et al., 2008, Traub et al., 2009), cats (Palmer et al., 2008, Souza et al., 2007), cattle (Becher et al., 2004, Coklin et al., 2007, Trout et al., 2007), buffaloes (Caccio et al., 2007), sheep (Santin et al., 2007), pigs (Langkjaer et al., 2007) and horses (Traub et al., 2005). In Australia, *Giardia* was found to be the most prevalent parasite in dogs (9.4%), while the prevalence reported in cats was 2.0% (Palmer et al., 2008). Furthermore, *Giardia duodenalis* classified as assemblage A and B was frequently found in feces of non-human primate that was recently reported in humans (Thompson et al., 2008, Thompson et al., 2010, Ye et al., 2012, Karim et al., 2014). In Thailand, there were a few reports such as the prevalence of 20.3% and 56.8% of *G. duodenalis* infections in humans and dogs, respectively (Traub et al., 2009). In addition, *Giardia* assemblage A and B were identified from fecal samples of children living in the West of Thailand (Kosuwin et al., 2010)

In Thailand, there are many areas where a number of monkeys have lived close to humans such as San Phra Kan shrine and Phra Prang Sam Yod of Lopburi province. Long-tailed macaques (*Macaca fascicularis*), the major species of monkey in this area, might act as a reservoir for several zoonotic diseases including giardiasis. There are still a limited information of this protozoa in long-tailed macaques. The objectives of this study were to determine the prevalence of *Giardia* infections and to characterize their assemblages from long-tailed macaques at San Phra Kan shrine, Lopburi, Thailand.

MATERIALS AND METHODS

1. Sample collection

The study was conducted in April 2014. A total of 200 fecal samples of long-tailed macaques (114 male and 86 female) located in San Phra Kan shrine area, Lopburi province were collected from 4 areas (Figure1) including San Phra Kan shrine (n=60), Manohra market (n=38), Malai Rama theater (n=52) and the abandoned buildings across from the shrine (n=50). The samples were collected from the ground after defecation by the macaques. The samples were kept cool during transportation and stored at -20°C until molecular analysis.
2. DNA extraction

Fecal samples were washed by distilled water and their DNAs were extracted from 200 µl of fecal suspension using the E.Z.N.A. stool DNA extraction kits (OMEGA Bio-tek, USA) according to the manufacturer's instructions. Extracted DNAs were stored at -20°C until use.

3. Amplification of Giardia DNA by nested PCR

The ssu-rDNA gene was amplified using primers RH11 and RH4 for primary PCR and GiarF and GiarR for secondary PCR as previously described by Hopkins et al. (1997) and Read et al. (2002). Briefly, the 25 µl reaction volume was consisted of 0.4 mM (1 µl) of each specific primer, 0.08 mM dNTP (1 µl), 4 mM MgCl₂ (2 µl), 1 U Taq DNA polymerase (0.2 µl), 1.25 µl of DMSO and 1 µl of DNA template. Amplification was performed under following conditions: initial denaturation at 94°C for 3 min, followed by 35 amplification cycles (94°C for 45 s, 54°C at primary PCR or 58°C at secondary PCR for 45 s and 72°C for 1 min) and the final extension at 72°C for 3 min. A negative control without template DNA was included in each PCR reaction.
The PCR product was recognized by electrophoresis on 1.5% agarose gel. The amplified band at 130 bp was considered as *Giardia* spp. (Figure 2) and the positive DNA was submitted for DNA purification and sequencing. The sequences of SSU-rDNA gene form *Giardia* spp. were compared with published sequences in the nucleotide database in GenBank by BLAST program of the National Center for Biotechnology Information (NCBI)

![DNA bands of Giardia spp. at 130 bp. (MK = DNA marker 100 bp; P = Giardia positive control; S1-S2 = positive samples; N = negative control)](image)

**Figure 2** DNA bands of *Giardia* spp. at 130 bp. (MK = DNA marker 100 bp; P = *Giardia* positive control; S1-S2 = positive samples; N = negative control)

4. Statistical analysis

Descriptive statistics were used to analyze the prevalence of *Giardia* infection. The variables were assessed using a Chi-square test and the *P* values < 0.05 were considered to be statistically significant.

**RESULTS AND DISCUSSION**

1. Prevalence of *Giardia*

The fecal samples obtained from 200 long-tailed macaques were examined and showed that 7% (14/200) of fecal samples were positive for *Giardia duodenalis* using nested PCR. The prevalence in male macaques was 7.9% (9/114), higher than in female which exhibited a prevalence of 5.8% (5/86). The prevalence of infection from San Phra Kan shrine, Manohra market, Malai Rama theater and the buildings across from the shrine was 6.7% (4/60), 0% (0/38), 11.5% (6/52) and 8% (4/50), respectively (Table 1). No significant associations were found between positive *Giardia*, sex and location of macaques (*P* > 0.05) (Table 2). The prevalence of this study was quite close to the previous study in rhesus monkey at Qianling Park, Guiyang, China (8.5%, 35/411) (Ye et
al., 2012). However, this prevalence was higher than the other prevalence in China (2.2%; 30/1,336) and Western Australian (4.8%; 17/351) (Thompson et al., 2010 and Karim et al., 2014).

2. Sequence analysis

Sequence analysis of the 14 PCR positive samples using the ssu-rDNA gene revealed the presence of G. duodenalis assemblages B from 11 samples and unidentified assemblages from 3 samples. Two of the 14 sequences showed 100% homology to the G. duodenalis Hungary isolate that found in dogs and humans (accession no: DQ890189). Five sequences revealed that they shared 99% identity with the sequence of the G. duodenalis assemblage B which isolated from rhesus macaque in China (accession no: KF679735), while 6 sequences showed 99% similarity to G. duodenalis assemblage B Australia human isolate (accession no: LN611617) and the last sequence showed 99% similarity to G. duodenalis Portugal human isolate (accession no: FR751209). This study demonstrated that G. duodenalis majorly found in macaques was assemblage B correlated with the previous study in China (Ye et al., 2012, Ryan and Caccio, 2013, Karim et al., 2014).

Table 1 The prevalence (%) of Giardia spp. in long-tailed macaques at different location and varied by sex.

<table>
<thead>
<tr>
<th>Location</th>
<th>Male</th>
<th>Female</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Sample (n)</td>
<td>Positive (%)</td>
<td>Sample (n)</td>
</tr>
<tr>
<td>San Phra Kan shrine</td>
<td>34</td>
<td>3 (8.82)</td>
<td>26</td>
</tr>
<tr>
<td>Manohra market</td>
<td>25</td>
<td>0 (0)</td>
<td>13</td>
</tr>
<tr>
<td>Malai Rama theater</td>
<td>28</td>
<td>4 (14.29)</td>
<td>24</td>
</tr>
<tr>
<td>The buildings across from the</td>
<td>27</td>
<td>2 (7.41)</td>
<td>23</td>
</tr>
<tr>
<td>shrine</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>


Table 2 Factors related to Giardia infection in long-tailed macaques, $\chi^2$ and the $P$-value

<table>
<thead>
<tr>
<th>Variable</th>
<th>No. of infected macaques</th>
<th>No. of non-infected macaques</th>
<th>$\chi^2$</th>
<th>$P$-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>9</td>
<td>105</td>
<td>0.33</td>
<td>0.57</td>
</tr>
<tr>
<td>Female</td>
<td>5</td>
<td>81</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Location</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>San Phra Kan shrine</td>
<td>4</td>
<td>56</td>
<td>4.59</td>
<td>0.20</td>
</tr>
<tr>
<td>Manohra market</td>
<td>0</td>
<td>38</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Malai Rama theater</td>
<td>6</td>
<td>46</td>
<td></td>
<td></td>
</tr>
<tr>
<td>The buildings across</td>
<td>4</td>
<td>46</td>
<td></td>
<td></td>
</tr>
<tr>
<td>from the shrine</td>
<td></td>
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</table>

CONCLUSION

The present study indicated that long-tailed macaques at San Phra Kan shrine, Lopburi might be the potential source of G. duodenalis assemblage B which can cause giardiasis in humans. A good personal hygiene should be restricted and warned for people who have involved with these macaques particularly for the tourists or out of towner. Further studies including the investigation of giardiasis in macaques or other type of monkeys in the other locations of Thailand should be performed. Furthermore, to detect Giardia from macaques, pet animals and humans in the same environment might help understanding the transmission of Giardia to humans.

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