Molecular detection of *Toxoplasma gondii* in North American river otters (*Lontra canadensis*) from Michigan

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INTRODUCTION

*Toxoplasma gondii* is a common zoonotic parasite capable of infecting warm-blooded animals. Wild animals contribute to the maintenance and persistence of *T. gondii*. However, there is little reported on the exposure or infection with this parasite in North American river otters (*Lontra canadensis*). A 1997 serologic survey of *T. gondii* in river otters from eastern North Carolina reported 45% positive titers among 103 otters. Another study reported 17.5% seroprevalence for *T. gondii* out of 40 marine-foraging river otters from several states. The purpose of our work was to determine the prevalence of *T. gondii* in river otters from the upper peninsula of Michigan. Because only tissue samples were available, we sought to directly amplify *T. gondii* DNA from tissues by PCR amplification of the B1 gene.

METHODS

**Sampling**
- 148 samples were harvested by trappers from all 15 counties of the upper peninsula of Michigan (Fig. 1), including 131 tongue tissues and 17 temporalis muscle tissues.

**Molecular Detection**
- DNA extractions were performed using the Quick-DNA Miniprep kit, followed by a cleanup step using the OneStep PCR Inhibitor Removal Kit following manufacturer’s recommendations (Zymo Research, Irvine, CA, USA).
- A nested PCR was performed using a total of 50 ng of genomic DNA with specific oligonucleotide primers to amplify a 531 bp fragment of the B1 gene as previously described (Table 1).
- Amplification products were analyzed on 1% agarose gels and visualized under UV imaging system (Fig. 2).

**DNA Sequencing**
- PCR products were excised and extracted using QIAquick Gel Extraction Kit (Qiagen, Hilden, Germany) following manufacturer’s recommendations.
- Purified products were sequenced and analyzed with Unipro Ugene Software.

RESULTS

**Figure 1. Geographic distribution of otter samples**

**Table 1. B1 Gene Primer Sequences**

<table>
<thead>
<tr>
<th>Primer Type</th>
<th>Sequence</th>
<th>Product Size (bp)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Outer primer forward</td>
<td>5′-TGTTCTGCTCATGCAACG-3′</td>
<td>580</td>
</tr>
<tr>
<td>Outer primer reverse</td>
<td>5′-ACGGATGCGTTCCCTTCTG-3′</td>
<td>531</td>
</tr>
<tr>
<td>Inner primer forward</td>
<td>5′-TCTCCCCAGACGTGGATTTC-3′</td>
<td></td>
</tr>
<tr>
<td>Inner primer reverse</td>
<td>5′-CTCGAAADCGCTGTCTGGA-3′</td>
<td></td>
</tr>
</tbody>
</table>

**Figure 2. Evaluation of PCR products**

**DISCUSSION**

Detection of *Toxoplasma gondii* directly from tissue samples have been previously described. Here, we report a preliminary finding on the prevalence of *T. gondii* among 148 North American river otters from the upper peninsula of Michigan. PCR amplification of the B1 gene, detected *T. gondii* DNA in 7 of the 148 otters with a 4.7% prevalence (Fig. 3). Positive samples were confirmed by sequencing.

Compared to previous studies, we found a lower percentage of positive samples. A possible explanation is the presence of PCR inhibitors for *T. gondii* DNA in the samples. We were able to improve detection after purification of the previously extracted genomic DNA, which led us to this possibility. Moreover, the methods of detection between previous analyses and the present one were different, suggesting a difference between exposure and infection.

Due to the inherent challenges in obtaining wildlife samples, we were limited in the type of tissue available; however, we were able to detect *T. gondii* DNA in tissue samples from river otters from the upper peninsula of Michigan. This finding suggests a risk of exposure to toxoplasmosis for trappers in the area. We believe that the prevalence of *T. gondii* in the samples tested could be higher than the preliminary data presented. Therefore, analysis of serum from river otters in this region is recommended. Further genotyping analysis of the positive samples will provide valuable insight in identifying the distribution of various strains and contribute to a better understanding of the epidemiology of *T. gondii* in the area.

REFERENCES


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