Detection of *Opisthorchis viverrini* in Human Stool Specimens by PCR

Opisthorchiasis is a liver fluke infection, found mainly in Southeast Asia (8) but increasingly in developed countries due to an influx of Asian immigrants (7). The diagnostic methods are based on the demonstration of eggs in stools, although there are still difficulties in distinguishing eggs from heterophyid and lecithodendriid parasites (2). We have successfully developed a PCR-based method for detection of *Opisthorchis viverrini* in experimentally infected hamsters (6). The aim of the present study was to determine the value of PCR for detection of *O. viverrini* eggs in human feces.

Stool specimens from 85 parasite-infected individuals, as confirmed by the formalin-ether technique, were collected from Srinagarind Hospital, Faculty of Medicine, Khon Kaen University. The specimens were composed of 40 *O. viverrini* parasites, 10 minute intestinal flukes, 10 hookworms, 10 echninostomes, 7 *Taenia* spp. parasites, 3 *Strongyloides stercoralis* parasites, 2 *Entamoeba coli* parasites, and 3 specimens from individuals with mixed infections. To evaluate the sensitivity of the method, normal stool specimens were spiked with either 1, 10, 25, 50, 100, 200, 500, and 1,000 *O. viverrini* eggs or 0.02, 0.2, 2, 20, and 200 pg and 2 ng of genomic DNA, respectively. The sensitivity of the detection for stool specimens was compared with that for Stoll's egg count technique (5).

To extract the DNA from stool specimens, 100 mg of feces in 1 ml of sterile distilled water was extracted with 200 µl of ether and centrifuged at 6,500 g for 2 min. The pellet was mixed with 250 µl of 3 M sodium acetate and 500 µl of absolute ethanol before being cleaned up with the QIAamp DNA Mini kit (Qiagen, Hilden, Germany), and 10 µl was used in the PCR (6).

The sensitivity test in the spiking experiment demonstrated that the limit of our PCR detection is 200 eggs or 200 pg of genomic DNA. The expected 330-bp product and its doublet of 660 bp were in 32 of the 40 fecal specimens (80.0%) from individuals with mixed infections. To evaluate the sensitivity of the method, normal stool specimens were spiked with either 1, 10, 25, 50, 100, 200, 500, and 1,000 *O. viverrini* eggs or 0.02, 0.2, 2, 20, and 200 pg and 2 ng of genomic DNA, respectively. The sensitivity of the detection for stool specimens was compared with that for Stoll's egg count technique (5).

<table>
<thead>
<tr>
<th>No. of specimens with indicated PCR result by egg count group&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Diagnosis for stool specimen&lt;sup&gt;b&lt;/sup&gt;</th>
<th>Positive</th>
<th>Negative</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;200</td>
<td>200–1,000</td>
<td>&gt;1,000</td>
<td>&lt;200</td>
</tr>
<tr>
<td>O. viverrini (40)</td>
<td>0</td>
<td>1</td>
<td>15</td>
</tr>
<tr>
<td>Other parasitic infections (45)</td>
<td>0</td>
<td>3&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0</td>
</tr>
</tbody>
</table>

<sup>a</sup>Diagnosis was performed based on the formalin-ether technique.

<sup>b</sup>Egg count was performed by Stoll's technique and expressed as eggs per gram of feces.

<sup>c</sup>One specimen was diagnosed as an echinostome, and the others were diagnosed as mixed infections with *Taenia* spp. and *O. viverrini*.

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REFERENCES


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