PREVALENCE OF OPISTHORCHIS VIVERRINI INFECTION AMONG VILLAGERS HARBORING OPISTHORCHIS-LIKE EGGS

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Abstract. The precise occurrence of Opisthorchis viverrini infection in humans, who were positive for Opisthorchis-like eggs in a stool examination, was determined using the potassium permanganate staining method. In the 68 specimens examined, there were more individual O. viverrini eggs (38.24%) than singular Haplorchis taichui eggs (29.41%). One-fourth of the total specimens contained a mixed infection of O. viverrini and H. taichui eggs. The median ratio of O. viverrini: H. taichui eggs in mixed infection cases was 2.29 (min = 1, max = 17.5). It is suggested that chemotherapy with praziquantel treatment should be given to patients who are positive for O. viverrini-like eggs.

INTRODUCTION

Opisthorchis viverrini (liver fluke; Opisthorchiidae) and Haplorchis taichui (minute intestinal fluke; Heterophyidae) are the most common flukes found in humans as well as in fish that are the second intermediate host of the parasite in northern and northeastern Thailand (Pungpak et al., 1997; 1998; Radomyos et al., 1994; 1998; Srisawangwong et al., 1997; Waikagul, 1998; Sukontason et al., 1999a). The former species is a parasite of medical importance that causes a varied degree of such conditions as cholangitis, cholelithiasis and cholecystitis (Pungpak et al., 1985), and bile duct cancer (Parkin et al., 1991). There is no report of the latter species having any clinical importance. To date, the prevalence of H. taichui in fecal specimens after anthelmintic therapy has been reported as higher than that of O. viverrini in northern Thai people (Radomyos et al., 1994; 1998).

Humans can serve as the definitive host of both O. viverrini and H. taichui. Since the eggs of both parasites are passed in feces, stool examination was commonly used for detecting infections. The formalin-ether sedimentation method has been used routinely for the examinations. The eggs of O. viverrini and H. taichui, including other minute intestinal flukes, are very similar in both size and shape, and indistinguishable from one another when using a routine stool examination with light microscope (Ritchie, 1984). The potassium permanganate staining technique using potassium permanganate solution was developed to solve this problem (Sukontason et al., 1999b). The objective of this study was, therefore, to determine the existence of O. viverrini infection in humans positive for Opisthorchis-like eggs in their stool by using the potassium permanganate staining technique.

MATERIALS AND METHODS

Fecal specimens were obtained from people residing in two villages (Huay Hug and Mae Luang Nuer) of Doi Sa Ket district, Chiang Mai Province in northeastern Thailand, 700 km north of Bangkok. These villages are adjacent to Mae Guang Udomthara Dam, a man-made reservoir of the Mae Guang River. Both villages are about 30 km east of Chiang Mai city center. Sixty-eight specimens with O. viverrini-like eggs were found in stools by using the formalin-ether sedimentation method (Ritchie, 1984). The potassium permanganate staining method with slight modification (Sukontason et al., 1999b) was performed for an appearance of a distinct musk-melon ridge pattern on the O. viverrini egg surface. Briefly, the sediment resulting from the formalin-ether sedimentation method was added with 1 ml of diethyl ether and shaken vigorously for 1 minute. The upper layer of ether was then discarded. A few drops of sediment were transferred, using a transparent pipette, onto a glass slide before one drop of 1% potassium permanganate solution was added prior to covering with a coverslip. The eggs of the parasite were examined under a light microscope at a magnification of 100 x and 400 x.

Every egg that appeared was assessed regarding its individual length, width, morphology (O. viverrini-like or H. taichui-like), presence of knob, shoulder and

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Vol 32 (Suppl 2) 2001

23
distinct surface pattern. Eggs with a distinct muskmelon ridge pattern and those with a light striae pattern were diagnosed as *O. viverrini* and *H. taichui*, respectively.

As for the statistical analysis, the width and length of *O. viverrini* and *H. taichui* eggs were compared using the Student-\( t \)-test. The presence of knob, shoulder and unique bulb-like feature on *O. viverrini* and *H. taichui* eggs was analyzed using the Chi square test.

**RESULTS**

Eight hundred and sixty-nine *O. viverrini*-like eggs were detected from the 68 specimens collected. When observed under a magnification of 100 x, the eggs of *O. viverrini* and minute intestinal flukes were easily seen due to a dark brown eggshell (with a relatively green inner rim) and its light brown background. However, the differentiation of eggs species could not be determined. At the magnification of 400 x in conjunction with an alteration of the diaphragm, a musk-melon ridge pattern on the *O. viverrini* egg surface was clearly seen, while a light striae pattern was present on the *H. taichui* egg surface.

Of the 68 people examined, 69.12% (47/68) had *O. viverrini* egg, 38.24% (26/68) had pure *O. viverrini* eggs, and another 30.88% (21/68) had a mixture of *O. viverrini* and minute intestinal fluke eggs. Pure *H. taichui* eggs were found in only 29.41% (20/68) (Table 1). The eggs of unknown species, probably of those other minute intestinal flukes, were found in 5 specimens.

In the mixed infection cases, the median ratio of *O. viverrini* with *H. taichui* eggs was 2.29 (min = 1, max = 17.5; n = 19) (Table 2).

*O. viverrini* eggs were significantly longer and wider than *H. taichui* eggs (Student-\( t \)-test = 1.99 and 9.05; \( p = 0.048 \) and \( < 0.01 \), respectively) (Table 3). The presence of knob and shoulder were significantly higher in *O. viverrini* eggs than *H. taichui* eggs (\( \chi^2 = 26.05 \) and 398.25, respectively; \( p < 0.001 \)). The unique bulb-like feature of *O. viverrini* eggs was significantly higher in *O. viverrini* eggs (\( \chi^2 = 508.91; \ p < 0.001 \)).

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Table 1

<table>
<thead>
<tr>
<th>Species</th>
<th>No. infected case</th>
<th>(%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>O. viverrini</em></td>
<td>26</td>
<td>(38.24)</td>
</tr>
<tr>
<td><em>H. taichui</em></td>
<td>20</td>
<td>(29.41)</td>
</tr>
<tr>
<td>Unknown</td>
<td>1</td>
<td>(1.47)</td>
</tr>
<tr>
<td><em>O. viverrini</em> + <em>H. taichui</em></td>
<td>17</td>
<td>(25.00)</td>
</tr>
<tr>
<td><em>O. viverrini</em> + Unknown</td>
<td>2</td>
<td>(2.94)</td>
</tr>
<tr>
<td><em>O. viverrini</em> + <em>H. taichui</em> + Unknown</td>
<td>2</td>
<td>(2.94)</td>
</tr>
<tr>
<td>Total</td>
<td>68</td>
<td>(100.00)</td>
</tr>
</tbody>
</table>

Table 2

<table>
<thead>
<tr>
<th>Statistic features</th>
<th>OV:HT</th>
<th>OV:UNK</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of cases</td>
<td>19</td>
<td>4</td>
</tr>
<tr>
<td>Median</td>
<td>2.3</td>
<td>7.5</td>
</tr>
<tr>
<td>Minimum</td>
<td>1</td>
<td>1.5</td>
</tr>
<tr>
<td>Maximum</td>
<td>17.5</td>
<td>16.0</td>
</tr>
</tbody>
</table>

\( OV = O. viverrini, \ HT = H. taichui, \ UNK = Unknown \)
DISCUSSION

In northern Thailand, *H. taichui* was recently found in abundance as a primarily fluke in both humans (Wijit et al., 1998; Pungpak et al., 1998; Radomyos et al., 1998) and fish that are the second intermediate host (Sukontason et al., 1999a). This result, however, indicated that *O. viverrini* was still a major fluke that parasitized humans in some areas of Chiang Mai. Autopsies carried out in Chiang Mai revealed adult *O. viverrini* and was supportive evidence of the prevalence of infection, which was 52.7% (68/129) (Wijit et al., 1998).

In this study, although the number of cases with pure *O. viverrini* (n = 26) was close to those with pure *H. taichui* (n = 20), the former fluke was more common. Since praziquantel has been proved as effective against both *O. viverrini* and *H. taichui* at the same dosage (40 mg/kg body weight at a single dose) (Pungpak et al., 1998), chemotherapy should also be applied to kill both worm species.

The presence of *O. viverrini*-like eggs by stool examination suggested that 69.12% (47/68) were *O. viverrini* cases, and treatment with praziquantel was indicated. Some side effects of praziquantel may occur, but they can be minimized by changing the time of drug intake from the morning to after dinner (Sormmani et al., 1984). However, for the specific treatment of *H. taichui*, niclosamide is the drug of choice because of its high efficacy, very low side effects and low cost (Sukontason et al., 2000). Moreover, to obtain the precise prevalence of this minute intestinal fluke species only, potassium permanganate staining in stool examinations is recommended.

REFERENCES


