Mixed infections with *Opisthorchis viverrini* and intestinal flukes in residents of Vientiane Municipality and Saravane Province in Laos


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Abstract

Faecal examinations for helminth eggs were performed on 1869 people from two riverside localities, Vientiane Municipality and Saravane Province, along the Mekong River, Laos. To obtain adult flukes, 42 people positive for small trematode eggs (*Opisthorchis viverrini*, heterophyid, or lecithodendriid eggs) were treated with a 20–30 mg kg$^{-1}$ single dose of praziquantel and purged. Diarrhoeic stools were then collected from 36 people (18 in each area) and searched for helminth parasites using stereomicroscopes. Faecal examinations revealed positive rates for small trematode eggs of 53.3% and 70.8% (average 65.2%) in Vientiane and Saravane Province, respectively. Infections with *O. viverrini* and six species of intestinal flukes were found, namely, *Haplorchis taichui*, *H. pumilio*, *H. yokogawai*, *Centrocestus caninus*, *Prosthodendrium molenkampi*, and *Phaneropsoleus bonnei*. The total number of flukes collected and the proportion of fluke species recovered were markedly different in the two localities; in Vientiane, 1041 *O. viverrini* (57.8 per person) and 615 others (34.2 per person), whereas in Saravane, 395 *O. viverrini* (21.9 per person) and 155207 others (8622.6 per person). Five people from Saravane harboured no *O. viverrini* but numerous heterophyid and/or lecithodendriid flukes. The results indicate that *O. viverrini* and several species of heterophyid and lecithodendriid flukes are endemic in these two riverside localities, and suggest that the intensity of
infection and the relative proportion of fluke species vary by locality along the Mekong River basin.

Introduction

Foodborne zoonotic parasites, including liver and intestinal flukes, are recognized as an important group of emerging and re-emerging human pathogens (WHO, 1995, 2004). In Southeast Asia, two species of liver flukes, Clonorchis sinensis and Opisthorchis viverrini, and more than 50 species of intestinal flukes, including Heterophyes nocens, Metagonimus yokogawai, Haplorchis spp., Centrocestus spp., Prosthodendrium molenkampi, Phaneropsolus bonnei and Echinostoma spp., are important representative examples of foodborne zoonotic parasites (Yu & Mott, 1994; Chai & Lee, 2002; Chai et al., 2005).

Lao People’s Democratic Republic (Lao PDR; Laos) is located in the middle of South Asia, and borders China, Myanmar, Thailand, Vietnam and Cambodia. The Mekong River runs from north to south in a southeasterly direction. As has been reported in northeast Thailand (Radomyos et al., 1994), foodborne zoonotic parasites are also prevalent in Laos. However, little information is available on the parasite species or the prevalence and intensity of parasitic infections. Previous studies have reported that Opisthorchis viverrini is the major foodborne parasite in the Vientiane Municipality (Sornmani et al., 1974; Pholsena et al., 1991), and in Khammouane (Kobayashi et al., 1996) and Champassak Provinces (Chai & Hongvanthong, 1998). With regard to intestinal flukes, a small number of studies (Ditrich et al., 1990; Giboda et al., 1991) have associated Haplorchis taichui with human infections in Laos. However, no data are available on the prevalence and intensity of H. taichui infection or of other intestinal fluke species. Therefore, the present study was performed to determine the status of infection of O. viverrini, H. taichui, and other species of foodborne parasites among the residents of two different localities along the Mekong River basin.

Materials and methods

Areas surveyed

Four villages (Haikham, Nakhouai Tai, Nakhouai Kang, and Thanaleng) in Vientiane Municipality and three villages (Mouang, Saphad, and Bunkang) in Saravane Province are involved in this study (fig. 1). The former four villages are located near the Mekong River, whereas the latter three are located along a tributary (fig. 1). Most residents are agricultural workers, and some residents used to catch freshwater fish and aquatic insect larvae such as the naiads of dragonflies from small streams and ponds, and consume them raw or improperly cooked. Traditional dishes called ‘Koi pla’ (contains raw fish) and ‘Som fak’ (contains fermented fish) are popular food items in these villages, as they are in northeast Thailand (Rim, 1982).

Faecal examinations

A total of 1869 faecal samples, one sample from each person, were collected from residents (886 men and 983 women; aged 3–79 years), during November 2002 and November 2003. Samples were transported to a laboratory (a Malaria Station) in each locality within 2–3 days of collection and stored at 4°C until examined. The Kato-Katz thick smear technique was used to detect helminth eggs. It was difficult to differentiate eggs of O. viverrini from those of heterophyids such as H. taichui and lecithodendriids like Prosthodendrium molenkampi and Phaneropsolus bonnei (Tesana et al., 1991; Kaewkes et al., 1991; Ditrich et al., 1992; Chai et al., 2005) and therefore, these eggs were recorded as small trematode eggs.

Worm collection

Worm collection and faecal examinations of villagers were approved by the Ministry of Public Health, Lao PDR, under the terms of the Korea–Laos agreement on Parasite Control in Lao PDR (1999–2004). A total of 42 people who harboured small trematode eggs (opisthorchid or heterophyid/lecithodendriid eggs), 20–22 individuals from each area, were selected for adult fluke collection. These subjects, who all provided informed consent, visited field stations where they were treated

Fig. 1. Map showing the two surveyed localities (©) of Laos: Vientiane Municipality and Saravane Province.
with a single dose (20–30 mg kg
\(^{-1}\)) of praziquantel (Shinpoong Pharmaceutical Co., Seoul, Korea) and were then magnesium salt purged. Whole diarrhoeic stools passed successively 4–5 times were collected and pooled. Stools were then washed several times with 10 volumes of water, sieved through a 2 mm mesh and re-suspended in water. After 10 min, the upper clean layer was discarded, and the lower dark layer was fixed with 0.5–1.0% neutral buffered formalin under cover slip pressure, aceticarmine-stained, and morphologically identified under a light microscope.

Small trematode eggs, including those of
\(O.\) viverrini, were the most frequently encountered, with an egg positive rate of 75.7% in Vientiane and 57.3% in Saravane (table 1).

Overall helminth egg positive rates were significantly higher among residents of Saravane Province (75.7%) than among those of Vientiane (57.3%) (table 1). Small trematode eggs, including those of Opisthorchis viverrini, heterophyids and lecithodendriids, were the most frequently encountered, with an egg positive rate of 53.3% in Vientiane and 70.8% in Saravane (table 1). This difference between the two regions was statistically significant (\(P < 0.001\)). The eggs of other parasites included those of hookworms (4.0% and 10.6%, respectively), Trichuris trichiura (5.0% and 1.3%), Taenia spp. (1.8% and 5.7%), Ascaris lumbricoides (0.5% and 2.5%), and echinostomes (0.3% and 1.3%). Egg positive rates were not significantly (\(P > 0.01\)) different between men and women (data not shown), and the age-dependent prevalence was unremarkable after 20 years of age (data not shown).

### Statistical analysis

Faecal examination results for trematode eggs were analysed with respect to subject age, sex, and place of residence using the student’s t-test and the chi-square test.

### Results

#### Egg positive rates

Overall helminth egg positive rates were significantly (\(P < 0.001\)) higher among residents of Saravane Province (75.7%) than among those of Vientiane (57.3%) (table 1). Small trematode eggs, including those of Opisthorchis viverrini, heterophyids and lecithodendriids, were the most frequently encountered, with an egg positive rate of 53.3% in Vientiane and 70.8% in Saravane (table 1). This difference between the two regions was statistically significant (\(P < 0.001\)). The eggs of other parasites included those of hookworms (4.0% and 10.6%, respectively), Trichuris trichiura (5.0% and 1.3%), Taenia spp. (1.8% and 5.7%), Ascaris lumbricoides (0.5% and 2.5%), and echinostomes (0.3% and 1.3%). Egg positive rates were not significantly (\(P > 0.01\)) different between men and women (data not shown), and the age-dependent prevalence was unremarkable after 20 years of age (data not shown).

### Worm collection results

Worm collection was completed successfully in 36 persons (18 in each area; 22 men and 14 women; age range 10–67 years) (table 2). In addition to \(O.\) viverrini, six species of intestinal flukes were collected, including three species of Haplorchis (\(H.\) taichui, \(H.\) pumilio and \(H.\) yokogawai), and Centrocestus caninus, \(P.\) molenkampi and \(P.\) bonneti (table 2; figs. 2–6). The numbers of specimens of the fluke species collected were markedly different in the two areas (table 2). In the 18 subjects from Vientiane Municipality, 1041 \(O.\) viverrini specimens (3–315 individually; 57.8 per person) and 615 intestinal flukes (1–168 individually; 34.2 per person) were collected. Opisthorchis viverrini comprised 62.9% of flukes collected and intestinal flukes 37.1%. In contrast, in 18 persons from Saravane Province, 395 \(O.\) viverrini (0–125 individually; 21.9 per person) and 155,207 intestinal flukes (135–37,200 individually; 8622.6 per person) were collected, where \(O.\) viverrini comprised only 0.3% of the total flukes. These differences in the patterns of flukes collected from the two localities were statistically significant (\(P < 0.001\)).

Of the intestinal flukes, \(H.\) taichui, a heterophyid species, was the dominant species, followed by \(H.\) yokogawai (in Vientiane) or \(H.\) pumilio (in Saravane), \(P.\) molenkampi, \(P.\) bonneti and \(C.\) caninus (table 2). The highest number of \(H.\) taichui collected from a single person was 36,658 (in Saravane), and the lowest 0 (in Vientiane), with an average number of collected worms per person of 26.9 in Vientiane and 8514.1 in Saravane, demonstrating more than 300-fold higher worm burdens in the latter area. The number of \(H.\) pumilio collected per person ranged from 0 to 502, with an average of 0.6 in Vientiane and 62.5 in Saravane, which was decidedly smaller than the number of \(H.\) taichui. The number of \(H.\) yokogawai ranged from 0 to 43, with a mean of 3.6 in Vientiane and 1.2 in Saravane. Other heterophyid flukes collected included \(C.\) caninus (four specimens in Vientiane). With reference to lecithodendriid flukes, \(P.\) molenkampi was recovered in both localities, but

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**Table 1.** Helminth egg positive rates in the faeces of villagers in Vientiane Municipality and Saravane Province, the Mekong River basin, Laos.

<table>
<thead>
<tr>
<th>Helminth species</th>
<th>Vientiane</th>
<th>Saravane</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. examined*</td>
<td>599</td>
<td>1270</td>
<td>1869</td>
</tr>
<tr>
<td>No. helminth positive (%)</td>
<td>343 (57.3)</td>
<td>962 (75.7)</td>
<td>1305 (69.8)</td>
</tr>
<tr>
<td>Ascaris lumbricoides</td>
<td>3 (0.5)</td>
<td>32 (2.5)</td>
<td>35 (1.9)</td>
</tr>
<tr>
<td>Hookworms</td>
<td>24 (4.0)</td>
<td>135 (10.6)</td>
<td>159 (8.5)</td>
</tr>
<tr>
<td>Trichuris trichiura</td>
<td>30 (5.0)</td>
<td>19 (1.5)</td>
<td>49 (2.6)</td>
</tr>
<tr>
<td>Opisthorchis viverrini and small trematode eggs†</td>
<td>319 (53.3)</td>
<td>899 (70.8)</td>
<td>1218 (65.2)</td>
</tr>
<tr>
<td>Taenia spp.</td>
<td>11 (1.8)</td>
<td>73 (5.7)</td>
<td>84 (4.5)</td>
</tr>
<tr>
<td>Echinostomes</td>
<td>2 (0.3)</td>
<td>16 (1.3)</td>
<td>18 (1.0)</td>
</tr>
</tbody>
</table>

* Faecal examination was performed by the Kato-Katz thick smear technique. One smear was examined for each person.
† Including the eggs of heterophyids (Heterophyidae) and lecithodendriids (Lecithodendriidae).
<table>
<thead>
<tr>
<th>Age and sex of villager</th>
<th>Opisthorchis viverrini</th>
<th>Haplorchis taichui</th>
<th>Haplorchis pumilio</th>
<th>Haplorchis yokogawai</th>
<th>Centrocestus caninus</th>
<th>Prosthodendrium molenkampi</th>
<th>Phaneropsis bonnei</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vientiane Municipality</td>
<td>60M 40 12 0 0 0 0 0 52 0</td>
<td>47M 6 6 0 0 0 1 0 13 0</td>
<td>45M 3 24 0 0 0 0 0 27 0</td>
<td>42M 149 124 1 43 2 0 0 319 0</td>
<td>36M 4 4 0 0 0 1 0 10 0</td>
<td>30M 32 0 0 0 0 0 0 32 0</td>
<td>28M 140 13 0 0 0 0 0 153 0</td>
<td>27M 315 1 0 0 0 0 0 317 0</td>
</tr>
<tr>
<td>(Mean no. per person)</td>
<td>(57.8)</td>
<td>(26.9)</td>
<td>(0.6)</td>
<td>(3.6)</td>
<td>(0.2)</td>
<td>(3.4)</td>
<td>(0.0)</td>
<td>(92.0)</td>
</tr>
<tr>
<td>Saravane Province</td>
<td>60M 0 22360 18 22 0 0 0 22421 0</td>
<td>53M 35 2298 69 0 0 0 0 2402 0</td>
<td>52M 8 7570 8 0 0 0 0 7587 0</td>
<td>50M 0 24159 101 0 0 0 0 24260 0</td>
<td>48M 4 466 17 0 0 0 0 519 0</td>
<td>48M 1 5220 40 0 0 0 0 5261 0</td>
<td>35M 35 36658 502 0 0 0 0 37235 0</td>
<td>30M 125 1218 6 0 0 0 0 1349 0</td>
</tr>
<tr>
<td>(Mean no. per person)</td>
<td>(21.9)</td>
<td>(8514.1)</td>
<td>(62.5)</td>
<td>(12.0)</td>
<td>(0.0)</td>
<td>(24.5)</td>
<td>(20.3)</td>
<td>(8644.6)</td>
</tr>
</tbody>
</table>
P. bonnei was found only in Saravane residents (table 2). The number of *P. molenkampi* worms collected per person ranged from 0 to 57 in Vientiane (3.4 per person) and 0 to 196 in Saravane (24.5), and the number of *P. bonnei* ranged from 0 to 229 (20.3) in Saravane.

**Discussion**

The results of the present study are important from a parasitological and a public health point of view. Various species of intestinal flukes including *H. taichui*, *H. pumilio*, *H. yokogawai*, *P. molenkampi* and *P. bonnei* were found in mixed-infections with *O. viverrini* among the villagers of Vientiane Municipality and Saravane Province. Interestingly, the proportion of *O. viverrini* and intestinal flukes was markedly different in the two surveyed localities, i.e. *O. viverrini* composed two-thirds of all flukes recovered in Vientiane, whereas intestinal flukes accounted for over 99% of all recovered flukes in Saravane Province. Moreover, the recovery of adult worms of *H. pumilio*, *H. yokogawai*, *P. molenkampi*, *P. bonnei* and *C. caninus* are recorded for the first time in humans from Laos, whereas *H. taichui* from Laotians has been reported previously (Giboda et al., 1991).

Before the 1970s, only two species of liver flukes, namely *O. viverrini* and *Fasciolopsis buski*, were recorded among the list of Laotian trematodes infecting man (Segal et al., 1968). In the case of *O. viverrini*, high prevalences have been reported among residents in different localities of Laos (Sornmani et al., 1974; Giboda et al., 1991; Pholsena et al., 1991; Kobayashi et al., 1996, 2000; Chai & Hongvanthong, 1998). A nationwide survey in 18 administrative districts also reported a high

Figs. 2–6. Adult specimens of *Haplorchis taichui* (2), *H. pumilio* (3), *H. yokogawai* (4), *P. molenkampi* (5) and *P. bonnei* (6) recovered from the villagers of Saravane Province, Laos, after praziquantel treatment and purgation. Bars = 0.2 mm.
prevalence of opisthorchiasis among primary school-children, particularly in middle and southern Laos, along the Mekong River (Rim et al., 2003). Heterophyid flukes were first reported in Laos in 1990 when metacercariae of *H. taichui*, *P. molenkampi*, *Centrocestus formosanus* and *Stellantchasmus falcatus* were discovered in fish caught in the Vientiane Municipality and in the Nam Ngum water reservoir (Scholtz et al., 1990), and adult flukes of *H. taichui*, *P. molenkampi*, *H. yokogawai* and *S. falcatus* were recovered from the intestines of domestic cats (Ditrich et al., 1990). Subsequently, adult *H. taichui* flukes were identified in five Laotian students studying in fish caught in the Vientiane Municipality and in the Nam Ngum water reservoir (Scholtz et al., 1990), and adult *H. taichui* flukes were identified in five Laotian students studying in fish from Saravane (Giboda et al., 1991). In the present study, *H. taichui* was found to be the most common human-infecting foodborne trematode in Saravane Province but it takes second place to *O. viverrini* in Vientiane Municipality. It was also surprising to find that 15 of 18 persons from Saravane were infected with more than 10,000 worms. The sources of *Haplorchis* spp. infection are known to be various species of freshwater or brackish water fish (Scholtz et al., 1990; Yu & Mott, 1994; Chai et al., 2005). All the people infected with *Haplorchis* spp. in this study had eaten raw freshwater fish.

With reference to the lecithodendriid flukes, the presence of human *P. molenkampi* and *P. bonnei* infections was first reported after autopsying 24 people who resided in Udornthani and Nonkhai Provinces, Thailand (Manning et al., 1971). Later, high prevalences of these fluke infections were reported in Thailand (Radomyos et al., 1984, 1994). The prevalence values for *P. molenkampi* and *P. bonnei* were 19.4% and 15.0%, respectively, among 681 egg positive individuals (small trematode eggs including *O. viverrini*) treated with praziquantel and purged in northeast Thailand (Radomyos et al., 1994). As northeast Thailand is geographically close to Laos, and in particular, Nonkhai is near the border between the two countries, these two lecithodendriid flukes were presumed to be distributed in Laos although they had never been previously reported (Waikagul, 1991). Thus the present study proves their presence in Laos. Metacercariae of *P. molenkampi* and *P. bonnei* were discovered in naiads and adult dragonflies and damselflies in Thailand (Manning & Lertprasert, 1973). Local people in northeast Thailand are known to eat naiads but not the adults of these insects (Manning & Lertprasert, 1973). However, reservoir hosts such as monkeys are known to catch and eat both adults and naiads. In the present study, those found to be infected with *P. molenkampi* and *P. bonnei* admitted that they had eaten dragonfly naiads.

The two localities surveyed in this study were found to be endemic areas of *O. viverrini*, heterophyids and lecithodendriids. In Saravane, *H. taichui* was the most dominant species, whereas in Vientiane, *O. viverrini* predominated. These differences seem to be attributed to remarkable differences in the metacercarial infection in local fish, i.e. in fish from Saravane, the metacercariae of *H. taichui* prevailed, whereas in fish from Vientiane, those of *O. viverrini* predominated (to be published). We recommend that the small trematode eggs found in the faeces of riverside inhabitants in Laos warrant further investigation.

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**References**


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