

## High prevalence of liver and intestinal fluke infections among residents of Savannakhet Province in Laos

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**Abstract:** The prevalence of liver and intestinal fluke infections was surveyed on residents of Savannakhet Province, Laos. Fecal specimens were collected from a total of 981 residents in 4 Mekong riverside villages and examined by the Kato-Katz thick smear technique. The results revealed that the overall helminth egg positive rate was 84.2%, and the positive rate for small trematode eggs, including *Opisthorchis viverrini*, heterophyids, or lecithodendriids, was 67.1%. To obtain adult flukes, 38 small trematode egg positive cases were treated with a 20-30 mg/kg single dose of praziquantel and purged. Diarrheic stools were then collected from 29 people and searched for helminth parasites using stereomicroscopes. Mixed infections with *O. viverrini* and 6 kinds of intestinal flukes were found, namely, *Haplorchis taichui*, *Haplorchis pumilio*, *Haplorchis yokogawai*, *Prosthodendrium molenkampi*, *Phaneropsolus bonnei*, and echinostomes. The total number of flukes collected was 7,693 specimens (av. no. per treated person; 265.3). The most common species was *O. viverrini*, followed by *H. taichui*, *P. molenkampi*, echinostomes, *H. pumilio*, *P. bonnei*, and *H. yokogawai*. The results indicate that foodborne liver and intestinal fluke infections are prevalent among residents of Savannakhet Province, Laos.

**Key words:** *Opisthorchis viverrini*, *Haplorchis taichui*, *Prosthodendrium molenkampi*, *Phaneropsolus bonnei*, prevalence, Savannakhet Province, Laos

### INTRODUCTION

The liver, lung and intestinal flukes are recognized as an important group of emerging and reemerging foodborne zoonotic parasites (Chai and Lee, 2002;

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Chai et al., 2005a). In Southeast Asia, *Clonorchis sinensis*, *Opisthorchis viverrini*, and *Paragonimus westermani* have been recognized as the 3 major foodborne trematode species. However, more than 50 species of intestinal flukes, including *Heterophyes nocens*, *Metagonimus yokogawai*, *Haplorchis* spp., *Centrocestus* spp., *Prosthodendrium molenkampi*, *Phaneropsolus bonnei*, and *Echinostoma* spp., are also included in the list of foodborne trematodes of public health importance (Yu and Mott, 1994; Chai and Lee, 2002; Chai et al., 2005a).

Laos is located in the middle of South Asia, and the Mekong River runs through Laos, from north to south. High prevalences of *O. viverrini* infection have been recorded among residents or schoolchildren in different localities of Laos (Sornmani et al., 1974; Pholsena et al., 1991; Chai and Hongvanthong, 1998; Kobayashi et al., 2000; Rim et al., 2003). However, with regard to intestinal flukes, only a small number of studies have been available (Ditrich et al., 1990; Giboda et al., 1991; Chai et al., 2005b). It is of note that mixed-infections with *O. viverrini*, heterophyids (*Haplorchis* spp.), and lecithodendriids (*P. molenkampi* and *P. bonnei*) are common, and the intensity of infection with each fluke species varies by locality along the Mekong River basin (Chai et al., 2005b). However, this tendency has been verified only in Vientiane Municipality and Saravane Province.

Therefore, the present study was performed to determine the status of *O. viverrini*, *H. taichui*, and other foodborne trematode infections among the residents of Savannakhet Province (Mekong riverside), which is located some distance between Vientiane Municipality and Saravane Province.

## MATERIALS AND METHODS

### Areas surveyed

Four villages (Bane Xok, Bane Phonsoun, Bane Houay, and Ban Nhangsoun) in Savannakhet Province were included in the present study (Fig. 1). These villages are located near the tributaries of the Mekong River. Most residents are agricultural workers, and some residents catch freshwater fish and aquatic insect larvae, such as the naiads of dragonflies

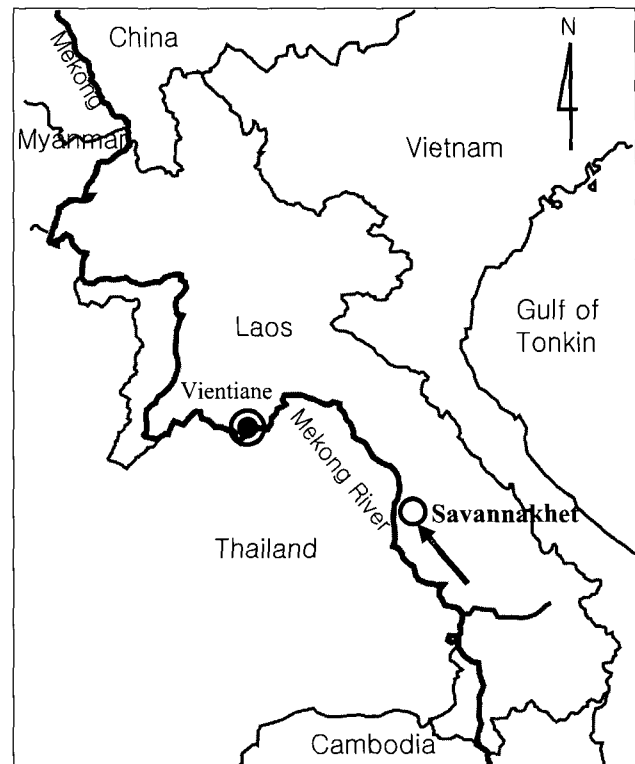


Fig. 1. Map showing the surveyed area (arrow: Savannakhet City) of Laos.

from small streams and ponds, and consume them raw or under improperly cooked conditions.

### Fecal examinations

A total of 981 fecal samples, one sample from each person, were collected from residents (494 men and 487 women; aged 3-76 years) of Savannakhet Province, in July 2002. Samples were transported to the Malaria Station in Savannakhet City 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 *P. molenkampi* and *P. bonnei* (Tesana et al., 1991; Kaewkes et al., 1991; Ditrich et al., 1992). Therefore, these eggs were recorded collectively as small trematode eggs.

### Worm collection

A total of 38 people who discharged small trematode eggs (opisthorchiid, heterophyid, or lecithoden-

**Table 1.** Helminth egg positive rates in the feces of residents in Savannakhet Province, Laos (July 2002)

Helminth species	No. of cases (%)
No. examined <sup>a)</sup>	981
No. helminth egg positive cases (%)	826 (84.2)
<i>Ascaris lumbricoides</i>	1 (0.1)
Hookworms	262 (26.7)
<i>Trichuris trichiura</i>	93 (9.5)
<i>Trichostrongylus orientalis</i>	1 (0.1)
<i>Opisthorchis viverrini</i> and small trematode eggs <sup>b)</sup>	658 (67.1)
Echinostomes	2 (0.2)
<i>Taenia</i> spp.	76 (7.7)
<i>Hymenolepis nana</i>	1 (0.1)

<sup>a)</sup>Fecal examination was performed by the Kato-Katz thick smear technique. One smear was examined for each person.

<sup>b)</sup>Including the eggs of heterophyids (Heterophyidae) and lecithodendriids (Lecithodendriidae).

driid eggs) were selected for adult fluke collection. After obtaining informed consent, these people who visited the Malaria Station were treated with a single dose (20-30 mg/kg) of praziquantel (Shinpoong Pharmaceutical Co., Seoul, Republic of Korea) and then purged with magnesium salt. Whole diarrheic stools passed successively 4-5 times were collected and pooled. Further processing of the diarrheic stools was done as previously described (Chai et al., 2005b). From the final sediment, worms were collected using a glass pipette and washed several times in water. The number of worms collected was counted, and some were fixed with 10% formalin under cover slip pressure, acetocarmine-stained, and morphologically identified using a light microscope. Fecal examination and worm collection from residents were approved by the Ministry of Public Health, Laos, under the terms of the Korea-Laos agreement on Parasite Control in Laos (1999-2004).

### Statistical analysis

Fecal examination results for trematode eggs were analyzed with respect to age and sex of subjects using the Student's *t*-test and the chi-square test. *P*-values < 0.05 were considered significant.

## RESULTS

### Helminth egg positive rates

The overall helminth egg positive rate was 84.2% (Table 1). Small trematode eggs, including those of *O. viverrini*, heterophyids and lecithodendriids, were the most frequently encountered, with an egg positive rate of 67.1% (Table 1). Eggs of other parasites included those of hookworms (26.7%), *Trichuris trichiura* (9.5%), *Taenia* spp. (7.7%), echinostomes (0.2%), *Ascaris lumbricoides* (0.1%), *Trichostrongylus orientalis* (0.1%), and *Hymenolepis nana* (0.1%). Egg positive rates were not significantly ( $P > 0.05$ ) different between men and women (data not shown), and the age-dependent prevalence was unremarkable (data not shown).

### Worm collection results

Worm collection was completed successfully in 29 persons (19 men and 10 women; age range 25-68 years) (Table 2). A total of 3,347 *O. viverrini* specimens (0-1,350 individually; av. 115.4 per person) and a total of 4,346 intestinal flukes (0-2,561; av. 149.9 per person) were collected (Table 2). *O. viverrini* comprised 43.5% of flukes collected and intestinal flukes 56.5%. Of the intestinal flukes, *H. taichui* was the dominant species, followed by *P. molenkampi*, echinostomes (species undetermined), *H. pumilio*, *P. bonnei* and *H. yokogawai* (Table 2). The highest number of *H. taichui* collected from a single person was 2,561, with an average number of collected worms per person of 102.7. The number of *H. pumilio* collected per person ranged from 0 to 60, with an average of 2.8 worms, which was much smaller than the number of *H. taichui*. The number of *H. yokogawai* was only 3. With regard to lecithodendriid flukes, the number of *P. molenkampi* ranged from 0 to 404 worms per person (av. 61.2 worms), and the number of *P. bonnei* ranged from 5 to 12 (av. 0.9 worms). Data on echinostomes will be published separately.

**Table 2.** Results of adult fluke collection from residents in Savannakhet Province, Laos (July 2002)

Age and sex of resident	No. of fluke specimens collected <sup>a)</sup>							Total
	<i>O. viverrini</i>	<i>H. taichui</i>	<i>H. pumilio</i>	<i>H. yokogawai</i>	<i>P. molenkampi</i>	<i>P. bonnei</i>	echinostomes <sup>b)</sup>	
68M	0	10	0	0	0	0	0	10
60M	12	0	0	0	0	0	0	12
58F	0	71	0	0	1	0	0	72
55F	12	79	10	0	404	12	0	517
54M	23	1	0	0	8	0	0	32
54F	42	3	0	0	0	0	0	45
53M	0	21	0	0	1	0	0	22
53M	16	20	0	0	0	0	0	36
53M	120	1	0	0	0	0	335	456
52F	134	32	0	0	0	0	36	202
50M	1	0	0	0	16	10	0	27
50F	2	40	0	0	3	0	0	45
49F	3	0	0	0	0	0	0	3
47M	1	5	0	0	15	0	0	21
45M	0	0	0	0	5	0	0	5
43M	393	2,561	60	2	4	0	0	3,020
42M	1,350	1	0	0	1	0	0	1,352
40M	0	10	0	0	0	0	0	10
39M	31	0	0	0	0	0	0	31
37M	0	69	10	1	397	5	0	482
36M	4	0	0	0	0	0	0	4
36M	340	0	0	0	0	0	0	340
33F	76	1	0	0	0	0	0	77
32M	410	3	0	0	0	0	0	413
32F	0	0	0	0	32	0	0	32
29M	71	0	0	0	0	0	0	71
29F	91	1	0	0	0	0	0	92
27M	154	40	0	0	0	0	0	194
25F	61	8	0	0	0	0	1	70
Total	3,347	2,977	80	3	887	27	372	7,693
(Mean No./ person)	(115.4)	(102.7)	(2.8)	(0.1)	(61.2)	(0.9)	(12.8)	(265.3)

<sup>a)</sup>*O. viverrini*, *Opisthorchis viverrini*; *H. taichui*, *Haplorchis taichui*; *H. pumilio*, *Haplorchis pumilio*; *H. yokogawai*, *Haplorchis yokogawai*; *P. molenkampi*, *Prosthodendrium molenkampi*; *P. bonnei*, *Phaneropsolus bonnei*.

<sup>b)</sup>Species not determined.

## DISCUSSION

The results of the present study indicate that the liver fluke *O. viverrini* and intestinal flukes, including *H. taichui*, *H. pumilio*, *H. yokogawai*, *P. molenkampi*, *P. bonnei*, and echinostomes, are mixed-infected among the residents of Savannakhet Province, Laos. *O. viverrini* and *H. taichui* appeared to be the 2 most common species. Mixed infections with various fluke species

were similar to our previous study performed in Vientiane Municipality and Saravane Province, Laos (Chai et al., 2005b). However, the intensity of infection with each fluke species varied by locality surveyed, i.e., in Vientiane, *O. viverrini* composed 62.9% of all flukes recovered, whereas, in Saravane, intestinal flukes accounted for over 99.0% of all recovered flukes (Chai et al., 2005b). In the present study, 43.5% of flukes collected were *O. viverrini* and 56.5% were

intestinal flukes. Therefore, Savannakhet Province was intermediate between Vientiane Municipality and Saravane Province with respect to the predominance of *O. viverrini* and intestinal flukes infected among the riverside residents.

However, the intensity of *O. viverrini* infection was the highest in Savannakhet Province (av. 115.4 worms per treated person), followed by Vientiane Municipality (av. 57.8) and Saravane Province (av. 21.9) (Chai et al., 2005b). In the case of *H. taichui*, the average number of worms recovered per person was remarkably high (8,514) in Saravane Province, whereas it was comparatively low in Savannakhet Province (102.7) and Vientiane Municipality (26.9) (Chai et al., 2005b). In the case of lecithodendriid flukes, the highest average number of worms was found in Savannakhet Province (61.2 for *P. molenkampi* and 0.9 for *P. bonnei*), followed by Saravane Province (24.5 and 20.4, respectively), and Vientiane Municipality (3.4 and 0.0) (Chai et al., 2005b). These results together indicate that the relative predominance of trematode species vary by locality in Laos. However, Savannakhet Province was found to be generally more endemic for *O. viverrini*, *H. taichui*, and lecithodendriids than Vientiane Municipality, and less endemic for *H. taichui* than Saravane Province.

Human *O. viverrini* infection has been reported to be prevalent in northeast Thailand (Kaewkes, 2003), Mekong riverside areas of Laos (Sornmani et al., 1974; Chai and Hongvanthong, 1998), and southern areas of Vietnam (De et al., 2003). In Thailand, the infection sources of *O. viverrini* are various species of freshwater fish, including *Cyclocheilichthys siaja*, *Hampala dispar*, *Puntius orphoides*, *Puntius gonionotus*, *Puntius proctozysron*, *Puntius viehovei*, *Labiobarbus lineatus*, *Esomus metallicus*, and *Osteochilus* sp. (Rim, 1982; Kaewkes, 2003). In Laos, freshwater fish species, including *Hampala macrolepidota*, *H. dispar*, *Puntius brevis*, *P. gonionotus*, *Barbodes gonionotus*, and *Cyclocheilichthys repasson* (Scholz et al., 1990; Dittrich et al., 1990) were verified to be the second intermediate hosts. Dishes containing raw freshwater fish, such as 'Koi pla', 'Pla som', and 'Som fak', are popular foods among the residents of Savannakhet Province, Laos and considered to be the main sources of infection.

Human *H. taichui*, *H. pumilio* and *H. yokogawai* infections have been reported in Southeast Asia, including the Philippines, Thailand, Laos, Taiwan, and South China (Giboda et al., 1991; Yu and Mott, 1994, Chai et al., 2005b; Belizario et al., 2005). Various species of freshwater fish, including *Cyprinus* spp. and *Puntius* spp., have been reported to be the second intermediate hosts (Velasquez, 1982). In Laos, *H. macrolepidota*, *H. dispar*, *P. gonionotus*, *Mystacoleucus marginatus* and *Esomus longimana* were verified to be the fish hosts for *H. taichui* and *H. pumilio* (Scholz et al., 1990; Dittrich et al., 1990).

Human *P. molenkampi* and *P. bonnei* infections were first discovered at autopsy of 24 people who resided in Udornthani and Nonkhai Provinces, Thailand, which are geographically close to Laos (Manning et al., 1971), and now known to prevalent in Thailand (Radomyos et al., 1994) and Laos (Chai et al., 2005b). In Thailand, the infection sources with these flukes were verified to be naiads, i.e., the juvenile form in aquatic environments, of dragon- and damselflies (Manning and Lertprasert, 1973). Also in Laos, people found to be infected with *P. molenkampi* and *P. bonnei* admitted that they had eaten dragonfly naiads (Chai et al., 2005b). However, in Laos, no larval stages of these flukes have been demonstrated in naiads of dragonflies. Studies are required on this point.

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