

Hyperendemicity of *Haplorchis taichui* Infection among Riparian People in Saravane and Champasak Province, Lao PDR

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Abstract: In this study, we found that *Haplorchis taichui*, a heterophyid intestinal fluke, is highly prevalent, with heavy worm loads, among riparian people in Saravane and Champasak province, Lao PDR. Fecal specimens were collected from 1,460 people (717 men and 743 women) in 12 riparian (Mekong river) districts and were examined by the Kato-Katz fecal smear technique. The overall helminth egg positive rate was 78.8% and 66.4% in Saravane and Champasak province, respectively. The positive rate for small trematode eggs (STE), which included *H. taichui* and other heterophyids, *Opisthorchis viverrini*, and lecithodendriids, was 69.9% and 46.3% in Saravane and Champasak province, respectively. To obtain adult flukes, 30 STE-positive people were treated with 40 mg/kg praziquantel and then purged. Whole diarrheic stools were collected 4-5 times for each person and searched for fluke specimens using a stereomicroscope. Mixed infections with various species of trematodes (*H. taichui*, *Haplorchis pumilio*, *O. viverrini*, *Prosthodendrium molenkampii*, *Centrocestus formosanus*, and *Echinochasmus japonicus*) and a species of cestode (*Taenia saginata*) were found. However, the worm load was exceptionally high for *H. taichui* compared with other trematode species, with an average of 21,565 and 12,079 specimens per infected person in Saravane and Champasak province, respectively, followed by *H. pumilio* (41.9 and 22.5, respectively) and *O. viverrini* (9.4 and 1.5, respectively). These results show that diverse species of intestinal and liver flukes are prevalent among riparian people in Saravane and Champasak province, Lao PDR, with *H. taichui* being the exceptionally dominant species.

Key words: *Haplorchis taichui*, *Haplorchis pumilio*, *Opisthorchis viverrini*, *Centrocestus formosanus*, hyperinfection, prevalence, riparian people, Saravane province, Champasak province, Lao PDR

INTRODUCTION

Foodborne liver and intestinal flukes are important groups of parasites from the public health point of view [1-4]. Lao Peoples Democratic Republic (Lao PDR) is located in the middle of the Indochina Peninsula, and the Mekong River runs through the whole length of the country from north to south. Until the 1990s, the liver fluke *Opisthorchis viverrini* and soil-transmitted nematodes, including *Ascaris*, *Trichuris*, and hook-

worms, were the major helminths prevalent among the Laotian people [5-8].

In 1991, *Haplorchis taichui* adult flukes were recovered for the first time from 5 Laotian students studying in Czechoslovakia [9]. Since then, other intestinal flukes, including *Haplorchis pumilio*, *Haplorchis yokogawai*, *Prosthodendrium molenkampii*, *Phanerocephalus bonnei*, *Centrocestus formosanus*, *Echinostoma revolutum*, *Artyfechinostomum malayanum*, *Echinochasmus japonicus*, and *Euparyphium* sp. have been recovered from infected residents of Vientiane municipality, Phongsaly, Saravane, Khammouane, and Savannakhet province [10-16]. The predominating species and the intensity of infection with each fluke species varied by locality. For example, *O. viverrini* infection was predominant over *H. taichui* in Vientiane municipality, whereas *H. taichui* infection was dominant over *O. viverrini* in

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Saravane province [10]. On the other hand, in Phongsaly Province, located in the northernmost area of Lao PDR, high prevalences of *H. taichui* and *H. yokogawai* were detected with no detection of *O. viverrini* [15].

After completion of the international collaboration project [10,11,14] between the Korea Association of Health Promotion and Ministry of Public Health (MOPH), Lao PDR on the control of intestinal helminthiasis in Lao PDR from 2000-2004, a new 5-year project plan was started in 2007 to control foodborne trematode infections by the Korea Foundation for International Healthcare and MOPH, Lao PDR [15,16]. In this new project, the primary goal was to control opisthorchiasis in endemic areas and to reduce the incidence of cholangiocarcinoma in the Mekong River basin.

For the successful implementation of this project, a desirable pre-requisite should be the elucidation of the actual status of infection with *O. viverrini* in each target village. The recovery

of eggs only in the feces is not sufficient for this purpose, because the eggs of *O. viverrini* and those of minute intestinal flukes are practically indistinguishable in routine fecal examinations. The present study was performed to determine the actual status of infection with foodborne trematodes by recovery of adult flukes from riparian people of Saravane and Champasak province, Lao PDR.

MATERIALS AND METHODS

Saravane province (capital city, Saravane) is located about 500 km south from Vientiane, and Champasak province (capital city, Pakse) is located nearby and just south of Saravane province (Fig. 1). Small riverside villages, each having 200-300 residents, located near the mainstream or tributaries of the Mekong River were selected for this study, which included 12 villages (in 6 districts) in both Saravane and Champasak prov-

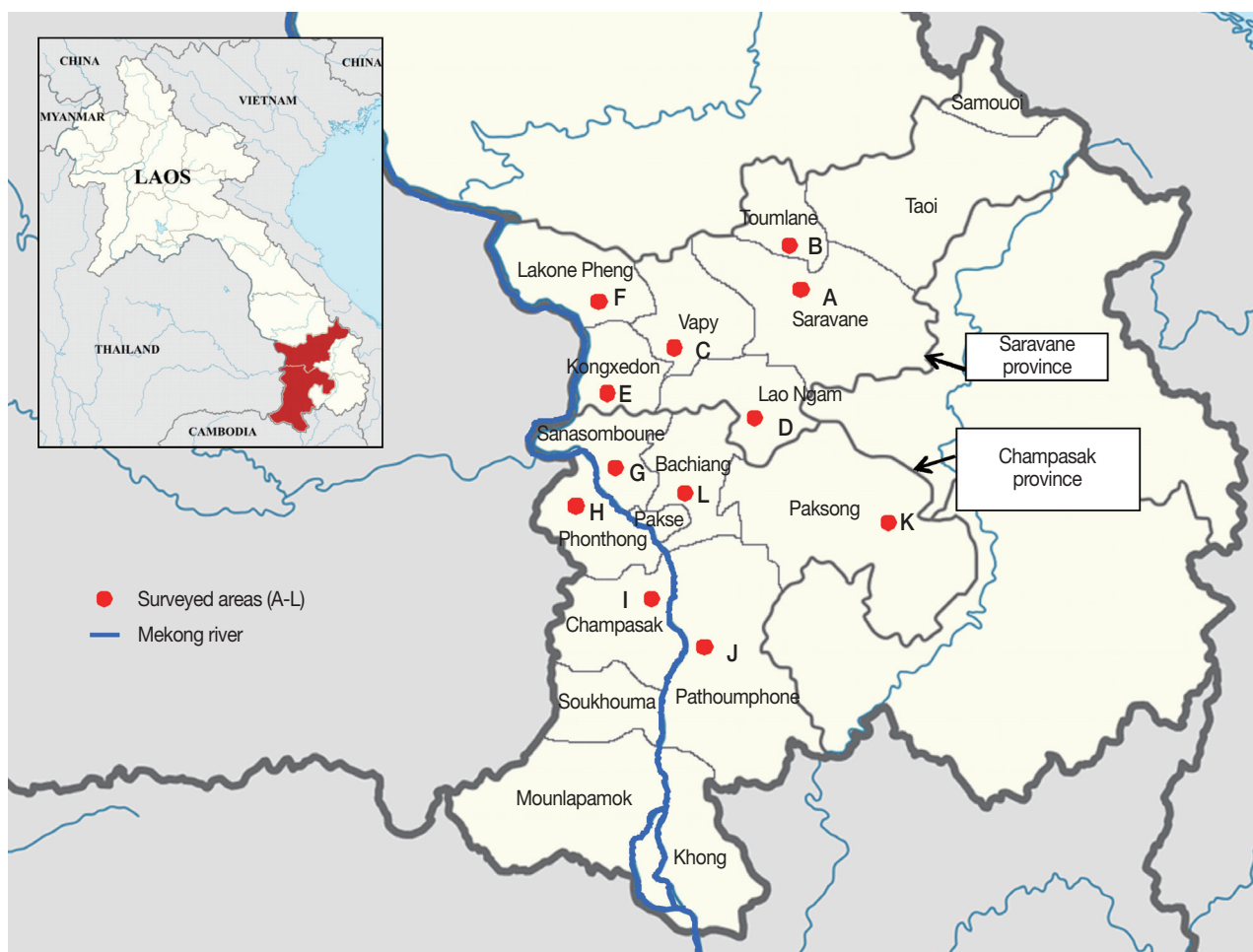


Fig. 1. Map showing the surveyed areas of Lao PDR, Saravane province (A-F districts) and Champasak province (G-L districts).

ince. Most villagers were agricultural workers, and some had caught freshwater fish from small streams and ponds and consumed them raw or improperly cooked.

A total of 1,460 fecal samples (1 sample per person) were collected from the villagers (717 men and 743 women, 12-82 years-of-age) from December 2009 to June 2010. Samples were transported to the Malaria Stations in Saravane and Champasak province within 2-3 days of collection and were stored at 4°C until examined. The Kato-Katz thick smear technique was used to detect helminth eggs. Trematode eggs 23-32 µm in size were collectively recorded as small trematode eggs (STE). Fecal examinations and anthelmintic treatments were officially approved by the Ministry of Public Health, Laos, under the terms of the Korea-Laos Agreement on Foodborne Trematodiasis Control in Laos (2007-2011).

Thirty people who were positive for STE were selected for adult worm recovery at the Malaria Station. After obtaining informed consent, they were treated with a single oral dose of 40 mg/kg praziquantel (Distocide®; Shinpoong Pharm., Seoul, Korea) and then purged with 30 g magnesium salt. Whole diarrhetic stools passed successively 4-5 times were collected and pooled individually. Diarrhetic stools were processed as previously described [10,11]. Worms were collected using a glass pipette and were washed several times in water. Worms were counted and some were fixed with 10% formalin under cover slip pressure, acetocarmine-stained, and morphologically identified using a light microscope.

Fecal examination results were analyzed using the Student's *t*-test and the chi-square test. *P*-values < 0.05 were considered statistically significant.

RESULTS

Fecal examination

The overall helminth egg positive rate was 78.8% and 66.4% in Saravane and Champasak province, respectively (Table 1). There were some mixed infection cases involving different kinds of nematodes, trematodes, and cestodes. The most highly prevalent was STE, which included eggs of *O. viverrini*, heterophyids, and lecithodendriids, with a prevalence of 69.9% (46.5-87.2% by district) and 46.3% (7.5-73.9% by district) in Saravane and Champasak province, respectively (Table 1). The district showing the highest STE prevalence was Toumlane (87.2%) in Saravane province and Pathumphon (73.9%) in Champasak province. Paksong, an urban area of Champasak province, displayed the lowest STE prevalence (7.5%) among the 12 districts surveyed. Other parasite eggs detected in Saravane and Champasak province included hookworms (20.1% and 22.1%, respectively), *Ascaris lumbricoides* (5.7% and 4.0%, respectively), *Trichuris trichiura* (0.8% and 5.6%, respectively), *Taenia* spp. (2.3% and 4.3%, respectively), and *Trichostrongylus* sp. (7.6% and 2.5%, respectively). The egg positive rates were not significantly (*P* > 0.01) different between men and women (data not shown).

Table 1. Helminth eggs found in the feces of residents in Saravane and Champasak province, Laos from December 2009 to June 2010

Province & District	Area code in Fig. 1	No. people examined	No. positive for helminth eggs ^a (%)						
			Overall positive cases	Small trematode eggs ^b	<i>Ascaris lumbricoides</i>	<i>Trichuris trichiura</i>	Hookworms	<i>Trichostrongylus</i> sp.	<i>Taenia</i> sp.
Saravane									
Saravane	A	106	86 (81.1)	83 (78.3)	1 (0.9)	0 (0.0)	10 (9.4)	0 (0.0)	0 (0.0)
Toumlane	B	125	114 (91.2)	109 (87.2)	2 (1.6)	37 (29.6)	36 (28.8)	4 (3.2)	7 (5.6)
Vapy	C	170	152 (89.4)	144 (84.7)	1 (0.6)	4 (2.4)	53 (31.2)	2 (1.2)	10 (5.9)
Lao Ngam	D	101	65 (64.4)	47 (46.5)	6 (5.9)	1 (1.0)	35 (34.7)	1 (1.0)	3 (3.0)
Kongxedon	E	155	115 (74.2)	90 (58.1)	21 (13.5)	0 (0.0)	25 (16.1)	12 (7.7)	6 (3.9)
Lakone Pheng	F	118	79 (66.9)	69 (58.5)	0 (0.0)	2 (1.7)	13 (11.0)	1 (0.8)	1 (0.8)
Subtotal		775	611 (78.8)	542 (69.9)	31 (4.0)	44 (5.6)	172 (22.1)	20 (2.5)	34 (4.3)
Champasak									
Sanasomboune	G	161	110 (68.3)	87 (54.0)	1 (0.6)	1 (0.6)	45 (28.0)	13 (8.1)	2 (1.2)
Phonthong	H	139	68 (48.9)	46 (33.1)	1 (0.7)	0 (0.0)	18 (12.9)	3 (2.2)	3 (2.2)
Champasak	I	102	74 (72.5)	63 (61.8)	0 (0.0)	0 (0.0)	20 (19.6)	9 (8.8)	2 (2.0)
Pathoumphone	J	115	99 (86.1)	85 (73.9)	18 (15.7)	0 (0.0)	9 (7.8)	5 (4.3)	2 (1.7)
Paksong	K	107	53 (49.5)	8 (7.5)	16 (15.0)	5 (4.7)	26 (24.3)	5 (4.7)	3 (2.8)
Bachiang	L	61	51 (83.6)	28 (45.9)	3 (4.9)	0 (0.0)	20 (32.8)	17 (27.9)	4 (6.6)
Subtotal		685	455 (66.4)	317 (46.3)	39 (5.7)	6 (0.8)	138 (20.1)	52 (7.6)	16 (2.3)

^aFecal examination was performed by the Kato-Katz smear technique; 1 smear was examined for each person. Data not shown for echinostome eggs (*n* = 1), *Hymenolepis nana* (1), *H. diminuta* (2), and *Enterobius vermicularis* (1) for Saravane, and echinostome eggs (1) for Champasak.

^bInclude the eggs of *Opisthorchis viverrini*, Heterophyidae, and Lecithodendriidae.

Worm collection

The adult worm collection was completed in 30 people (15 men and 15 women; 20-65 years; 19 in Saravane and 11 in Champasak) (Table 2). A total of 543,852 helminth specimens were recovered (average of 18,128 per person; range 6-129,238 worms per person). Intestinal flukes were recovered in all 30 cases (100.0%), and *O. viverrini* was recovered in 17 cases (56.7%). The total number of *H. taichui* specimens recovered in Saravane and Champasak province were 409,738 (n=19; average 21,562 per person) and 132,865 (n=11; average 12,078 per person), respectively, which was markedly higher than the other helminth parasite species (Table 2). The next most frequent species was *H. pumilio*, with 796 (n=19; average 41.9

per person) and 247 (n=11; average 22.5 per person) in Saravane and Champasak province, respectively (Table 2), followed by *O. viverrini*, with 179 (n=19; average 9.4 per person) and 17 (n=11; average 1.5 per person) in Saravane and Champasak province, respectively (Table 2). Small numbers of specimens were collected for *Taenia saginata*, *C. formosanus*, *P. molenkampi*, and *E. japonicus* from a small number of people (Table 2). Two *Haplorchis* spp. comprised 99.96% (99.95% and 99.98% in Saravane and Champasak province, respectively) of the total helminth specimens recovered, whereas *O. viverrini* comprised only 0.04% (0.04% and 0.01% in Saravane and Champasak province, respectively). The highest worm load of *H. taichui* collected from a single person was 129,159 in Cham-

Table 2. Adult flukes collected from residents in Champasak and Saravane Province, Laos after treatment with praziquantel from December 2009 to June 2010

Province (District) Age and sex of resident	No. of fluke specimens collected ^a							Total
	<i>Haplorchis taichui</i>	<i>Haplorchis pumilio</i>	<i>Opisthorchis viverrini</i>	<i>Prosthodendrium molenkampi</i>	<i>Centrocestus formosanus</i>	<i>Echinochasmus japonicus</i>	<i>Taenia saginata</i>	
Saravane (Saravane)								
45F	97,791	1	0	0	0	0	0	97,792
46M	71,647	448	0	1	0	0	0	72,096
30M	59,096	2	2	0	0	0	0	59,100
45F	32,764	3	5	0	0	0	0	32,772
45M	16,785	0	0	0	0	0	0	16,785
48M	14,660	0	4	0	0	0	0	14,664
34F	6,559	0	0	0	0	0	0	6,559
30M	2,952	15	1	0	0	0	0	2,968
25F	1,981	0	5	0	0	0	0	1,986
30F	940	0	2	0	0	0	0	942
Saravane (Toumlane)								
25M	39,467	153	88	0	2	0	0	39,710
32M	21,172	21	4	0	0	0	0	21,197
34M	19,435	0	1	0	0	0	0	19,436
30F	17,592	0	4	0	0	0	1	17,597
30F	2,724	11	0	0	0	0	1	2,736
29F	1,636	0	0	0	0	0	0	1,636
20F	1,436	0	11	0	0	0	0	1,447
24M	1,059	142	35	0	0	0	1	1,237
45M	42	0	17	0	0	0	0	59
Subtotal (Mean no./person)	409,738 (21565.2)	796 (41.9)	179 (9.4)	1 (0.1)	2 (0.1)	0 (0.0)	3 (0.2)	410,719 (21616.8)
Champasak (Sanasomboune)								
35M	129,159	77	0	0	1	1	0	129,238
58F	925	42	0	0	0	0	0	967
54M	866	0	1	0	0	0	0	867
33F	822	0	2	0	0	0	0	824
40M	614	9	1	0	0	0	1	625
35F	331	0	0	0	0	0	1	332
50M	20	107	0	0	0	0	0	127
65F	6	0	13	0	0	0	0	19
Champasak (Paksong)								
30F	63	0	0	0	0	0	0	63
57M	53	12	0	0	0	0	0	65
39F	6	0	0	0	0	0	0	6
Subtotal (Mean no./person)	132,865 (12078.6)	247 (22.5)	17 (1.5)	0	1 (0.1)	1 (0.1)	2 (0.2)	133,133 (12103.0)

pasak province, followed by 97,791 and 71,647 in Saravane province.

DISCUSSION

An extremely high predominance of intestinal flukes, in particular, *H. taichui*, was evident in Saravane and Champasak province. The liver fluke, *O. viverrini*, constituted only a very small portion of the helminths infected among the riparian people in the surveyed localities. Therefore, we could confirm that the real *O. viverrini* heavy endemic areas in Lao PDR include Vientiane municipality and Savannakhet province, based on the previous results from our research group [10,11]. *Haplorchis* spp. dominate in Khammouane, Saravane, and Champasak provinces with only a low-grade endemicity of opisthorchiasis, based on our previous observations [10,14] and those of the present study. Phongsaly province [15] and Luang Prabang and Xiengkhouang provinces are also *Haplorchis*-dominant areas without *O. viverrini* endemicity (to be published).

The individual worm load of *H. taichui* was extremely high in this study, particularly in Saravane province. It was 67 times higher than in Khammouane province (324 vs. 21,565) [14], 209 times that of Savannakhet province (103 vs. 21,565) [11], and 799 times that of Vientiane municipality (27 vs. 21,565) [10]. Indeed, it was even 3-times higher than that of our previous study in Saravane province [10]. This may have been due to the fact that different districts were surveyed in our earlier and present studies. The present report of intestinal flukes, including *H. taichui* and *H. pumilio*, from people in Champasak province is novel. However, recovery of *O. viverrini* was previously reported in 3 people (5, 10, and 395 specimens) residing near Pakse, the capital of Champasak province [7]. No intestinal flukes were recovered from these 3 people [7]. Therefore, in the present study, it was surprising to see that the worm load of *H. taichui* and *H. pumilio* was markedly higher than that of *O. viverrini* in Champasak province. One patient, a 35-year-old man, expelled as many as 129,159 specimens of *H. taichui*, and thus he represents the most heavily infected case with *H. taichui* ever recorded in the literature. Details of other intestinal fluke infections and *T. saginata* will be published separately.

The hyperendemicity of *H. taichui* infection among riparian people in Saravane Province was closely correlated with the report of metacercarial prevalence in fish in Saravane Province [17]. *H. taichui* metacercariae were detected in various freshwater fish species from Saravane that included *Hampala dispar*,

Cyclocheilichthys enoplos, *Mystacoleucus greenwayi*, and *Puntioptiles proctozysron* with the average metacercarial density per fish of 1,532 (range 16-6,050), 346 (14-986), 337 (8-1,625), and 175 (2-863), respectively [17]. However, the metacercariae of *O. viverrini* were also detected heavily in *H. dispar* with the average number per fish of 453 [17]. No or very few *O. viverrini* metacercariae were detected in other species of fish examined in Saravane Province [17]. It is strongly suggested that *H. dispar* is an important source of infection both for *H. taichui* and *O. viverrini* in this area.

The present study indicated that the sole use of fecal examinations to detect helminth eggs does not fully allow the determination of the actual prevalence and intensity of liver and intestinal flukes in Lao PDR. For example, 2 recent studies performed in Champasak province reported the results of fecal examinations on the residents [18-20]; the STE were interpreted only as *O. viverrini* eggs, and the possibility for them to be eggs of intestinal flukes, in particular, *Haplorchis* spp., was overlooked. The adult worm recovery after chemotherapy and purgation, like our studies [10,11,14-16] and others [13], is helpful to determine the actual and relative predominance of liver and intestinal flukes in each area, although it is a tedious work and needs experts who have much experience and skill. It would be preferable to have a proper technique to distinguish the species of STE in fecal specimens.

Several diagnostic techniques have been reported to be useful for morphologic discrimination of STE in fecal samples [21-23]. Iodine staining was helpful for discriminating lecithodendriid eggs (*P. molenkampi* and *P. bonnei*) from those of *O. viverrini* [21]. Potassium permanganate temporary staining, using 1% (w/v) solution for 1 min, was also applied to discriminate the eggs of *O. viverrini*, *H. taichui*, and *P. bonnei* [23].

Molecular techniques have been applied to detect *O. viverrini* DNA in fecal samples [24-26]. In Khon Kaen, Thailand, a 98% specificity and a 100% sensitivity of the PCR technique were obtained in cases of moderate to severe infections (> 1,000 EPG) but only a 68% sensitivity was shown in light infections (< 200 EPG) [24]. In Lao PDR, the PCR sensitivity was lower; only around 50% in cases even with high EPG counts > 1,000 [25]. The possible presence of PCR inhibitors in the feces was suggested [25]. Cetyltrimethyl-ammoniumbromide was used to remove such inhibitors, which resulted in an enhanced sensitivity of PCR technique to detect fecal *O. viverrini* DNA, from 44.8% to 79.3% [26]. It was stated that the primers used for PCR did not cross-react with *Haplorchis* spp. [25]. In this re-

spect, the possibility of mixed infections with *O. viverrini* with minute intestinal flukes, including *Haplorchis* spp., was raised as a reason for low sensitivity of PCR technique to detect *O. viverrini* DNA [25,26]. Subsequently, PCR assays to discriminate *O. viverrini* from those of *H. taichui* and *H. pumilio* were developed using the internal transcribed spacer (ITS) regions, ITS1 and ITS2 [27]. The PCR amplicons of *O. viverrini*, *H. taichui*, and *H. pumilio* were 800, 930, and 1,250 bp, respectively, for ITS1, which successfully differentiated the 3 species, whereas those for ITS2 were 380, 530, and 380, respectively, which could not discriminate *O. viverrini* and *H. pumilio* [27]. The sensitivity of ITS1 PCR using fecal samples, however, was relatively low (76.2%). Further studies are needed before they can be used practically as a coprodiagnostic tool [27].

It may be argued that not all of the *O. viverrini* worms were expelled from the subjected cases by a 40 mg/kg single dose treatment with praziquantel. This possibility cannot be completely ruled out. However, in our previous studies in Vientiane municipality and Savannakhet province, the same protocol as in the present study was applied, and 3-315 *O. viverrini* specimens in Vientiane and 1-1,350 *O. viverrini* specimens in Savannakhet were recovered individually in the diarrheic stools of the subjects within 5-6 hr (4-5 successive stools) after praziquantel administration [10,11]. These observations indicate that if a person is infected with *O. viverrini*, a majority, if not all, of the worms are expelled by this procedure. A previous study in Thailand supports this hypothesis; in 9 patients treated with 40 mg/kg praziquantel in a single dose followed by purging and whose diarrheal feces were collected at least 24 hr after purgation, 95% of all worms were expelled within the first 4 hr [28]. A study performed in Khon Kaen, Thailand, a hyperendemic area of opisthorchiasis, reported that the worm load of *O. viverrini* among 181 human autopsy cases averaged 182.4 and ranged from 1-2,946 per individual [29]. In our previous studies in Savannakhet and Vientiane [10,11], the average worm load measured by worm recovery after praziquantel treatment and purging was 115.4 ($n=29$; 0-1,350 per individual) and 57.8 ($n=18$; 3-315 per individual), respectively. Therefore, it is suggested that the procedure we used is a reliable method for recovery of both liver and intestinal flukes from humans.

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