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High Prevalence of *Haplorchis taichui*, *Phaneropsolus molenkampi*, and Other Helminth Infections among People in Khammouane Province, Lao PDR

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Abstract: The prevalence of liver and intestinal helminth infections, including *Opisthorchis*, *Haplorchis*, *Phaneropsolus*, hookworms, *Enterobius*, and *Taenia*, was surveyed in Khammouane province, Lao PDR. Fecal specimens were collected from 1,242 people (590 men and 652 women) in 3 Mekong riverside villages and were examined by the Kato-Katz thick smear technique. The overall helminth egg positive rate was 81.1%. The positive rate for small trematode eggs, including *Opisthorchis viverrini*, heterophyids, and lecithodendriids, was 81.1% and the positive rate for hookworms was 6.7%. To obtain adult worms, 35 people who were positive for small trematode eggs were treated with 20-30 mg/kg praziquantel and 10-15 mg/kg pyrantel pamoate, and then purged. Diarrheic stools were collected from 33 of these people and searched for helminth parasites using a stereomicroscope. Mixed infections with various helminths (*Haplorchis taichui, Haplorchis yokogawai, Prosthodendrium molenkampi, Phaneropsolus bonnei,* echinostomes, hookworms, *Trichostrongylus* spp., *Trichuris trichiura, Enterobius vermicularis*, and/or *Taenia saginata*) were found. The total number of helminth specimens collected was 20,907 (approximately 634 per person). The most common species was *H. taichui*, followed by *P. molenkampi, O. viverrini, P. bonnei, E. vermicularis*, hookworms, and *Trichostrongylus* spp. These results show that diverse species of intestinal nematodes, trematodes, and cestodes are infecting humans in Khammouane province, Lao PDR.

Key words: *Opisthorchis viverrini, Haplorchis taichui, Haplorchis yokogawai, Prosthodendrium molenkampi, Phaneropsolus bonnei*, hookworm, *Trichostrongylus, Taenia saginata*, prevalence, Khammouane province, Laos

INTRODUCTION

Soil-transmitted and fecal-borne nematodes, and food-borne liver and intestinal trematodes and cestodes, are important parasites from public health points of view [1-3]. These parasites include *Ascaris*, hookworms, *Enterobius, Opisthorchis*, *Clonorchis*, echinostomes, heterophyids (*Heterophyes, Metagonimus, Haplorchis*, and *Centrocestus*), lecithodendriids (*Prosthodendrium* and *Phaneropsolus*), *Diphyllobothrium*, and *Taenia* [3-5]. To prevent and control these parasites, epidemiological studies are urgent and pre-requisite.

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In Lao PDR, which is located in the middle of the Indochina Peninsula, 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 lumbricoides*, *Trichuris trichiura*, and hookworms (*Ancylostoma duodenale* and *Necator americanus*), were the major helminth species known to be infecting Laotians [6-9]. However, in 1991, *Haplorchis taichui* worms were recovered from 5 Laotian students studying in Czechoslovakia [10]. Since then, mixed infections with *O. viverrini, H. taichui, Haplorchis pumilio, Haplorchis yokogawai, Centrocestus formosanus* (as *C. caninus*), *Prosthodendrium molenkampi, Phaneropsolus bonnei, Echinochasmus japonicus,* and other echinostomes have been recovered from residents of Vientiane Municipality, Saravane province, and Savan-

nakhet province [11-13]. In addition to these helminths, Sayasone et al. [13] also recovered *Taenia saginata* tapeworms. Interestingly, the intensity of infection of each parasite species varied with the locality surveyed. For example, in Vientiane Municipality, *O. viverrini* infection was dominant, whereas in Saravane province *H. taichui* infection was dominant [11].

Intestinal nematode recovery data after anthelmintic treatment and purgation has never been reported in Lao PDR. Therefore, the present study aimed to determine the infection status of foodborne intestinal trematodes and cestodes, including *O. viverrini*, *H. taichui*, and *Taenia* species, and soil-transmitted nematodes, including hookworms and *Trichostrongylus*, among the riverside residents of Khammouane province.

MATERIALS AND METHODS

Khammouane province, Lao PDR, is located about 300 km distance from Vientiane Municipality and about 120 km north of Savannakhet province (Fig. 1). Three small riverside villages (Bane Nong Bone, Bane Mahaxay, and Bane Phova) located near tributaries of the Mekong River were selected for this study. Most residents are agricultural workers. Some catch freshwater fish and aquatic insect larvae from small streams and ponds, and consume them improperly cooked.

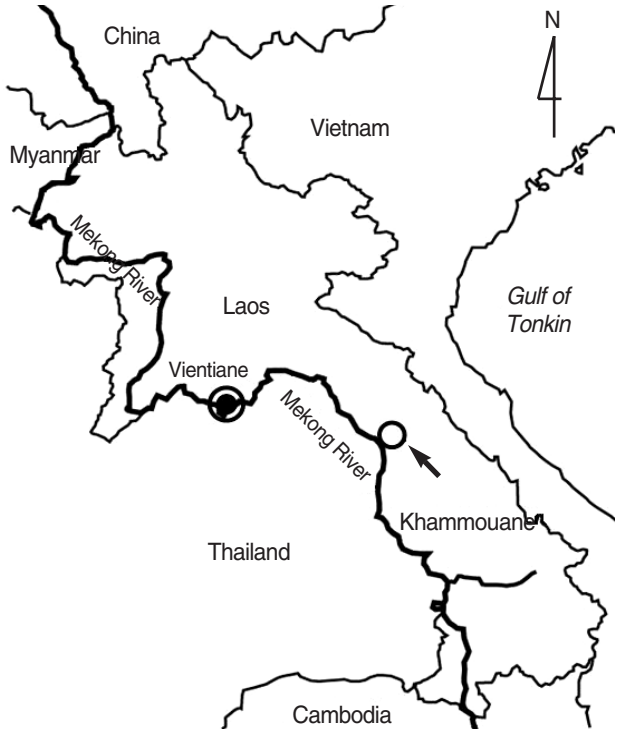

Fig. 1. Map showing the surveyed area of Lao PDR (arrow: Khammouane).

A total of 1,242 fecal samples, one sample from each person, were collected from residents (590 men and 652 women; aged 6-80 years) of the 3 villages, in March 2003. Samples were transported to the Malaria Station in Khammouane within 2-3 days of collection and were stored at 4℃ until examined. The Kato-Katz thick smear technique was used to detect helminth eggs. As it was not possible to differentiate eggs of *O. viverrini* from those of heterophyids and lecithodendriids, these eggs were collectively recorded as small trematode eggs. Fecal examinations of these residents and anthelmintic treatments were officially approved by the Ministry of Public Health, Laos, under the terms of the Korea-Laos Agreement on Parasite Control in Laos (1999-2004).

A total of 35 people who tested positive for small trematode eggs were selected for adult worm recovery at the Malaria Station. After obtaining informed consent, they were treated with a mixed single dose of 20-30 mg/kg praziquantel (Distocide@, Shinpoong Pharm. Co., Seoul, Republic of Korea) and 10-15 mg/kg of pyrantel pamoate (Combantrin@, Pfizer, New York, USA), and then purged with magnesium salt. Whole diarrheic stools passed successively 4-5 times were collected from 33 people. 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 with respect to age and sex of the subjects using the Student's *t*-test and the chi-

Table 1. Helminth eggs found in the feces of residents in Khammouane province, Laos (March 2003)

Helminth species	No. of residents (%)		
	Men	Women	Total
No. examined[a]	590	652	1,242
No. helminth egg positive cases (%)	490 (83.1)	517 (79.3)	1,007 (81.1)
Ascaris lumbricoides	5 (0.8)	5 (0.8)	10 (0.8)
Hookworms	49 (8.3)	34 (5.2)	83 (6.7)
Trichuris trichiura	39 (6.6)	36 (5.5)	75 (6.0)
Trichostrongylus spp.	1 (0.2)	0 (0.0)	1 (0.08)
Opisthorchis viverrini and small trematode eggs[b]	499 (84.6)	508 (77.9)	1,007 (81.1)
Echinostomes	3 (0.5)	4 (0.6)	7 (0.6)
Fasciolopsis buski	0 (0.0)	2 (0.3)	2 (0.2)
Taenia spp.	27 (4.6)	31 (4.8)	58 (4.7)

[a]Fecal examination was performed by the Kato-Katz smear technique. One smear was examined for each person.
[b]Including the eggs of Heterophyidae and Lecithodendriidae.

square test. *P*-values of < 0.05 were considered statistically significant.

RESULTS

Fecal examination

The overall helminth egg positive rate was 81.1% (Table 1), and there were mixed infections involving different kinds of trematodes and nematodes. The most frequently encountered were small trematode eggs, including those of *O. viverrini*, heterophyids, and lecithodendriids. The positive rate of small trematode eggs was 81.1% (Table 1). Other parasite eggs detected were hookworms (6.7%), *Trichuris trichiura* (6.0%), *Taenia* spp. (4.7 %), *Ascaris lumbricoides* (0.8%), echinostomes (0.6%), *Fasciolopsis buski* (0.2%), and *Trichostrongylus* spp. (0.08%). The egg positive rates were not significantly ($P > 0.01$) different between men

Table 2. Adult flukes colleced from residents in Khammouane province, Laos, after treatment with praziquantel and pyrantel pamoate (March 2003)

Age and sex of resident	No. of helminth specimens collected[a]											
	O. viverrini	*H. taichui*	*H. yokogawai*	*P. molenkampi*	*P. bonnei*	Echinostomes[b]	Hookworms[b]	*T. trichiura*	*Trichostrongylus* spp.	*E. vermicularis*	*Taenia*[c]	Total
80M	2	469	8	1	0	0	1	0	0	1	0	482
65M	1	96	13	7	2	0	0	0	1	8	1	129
61M	21	23	0	0	53	0	0	0	1	13	0	111
60F	19	115	0	5	1	0	0	0	0	6	0	146
59M	0	222	0	10	0	0	0	0	0	27	0	259
58M	27	208	0	69	0	0	0	0	0	4	0	308
56M	41	241	0	2	0	0	0	0	2	0	1	287
55M	0	204	0	0	4	0	0	0	0	2	1	211
50M	4	399	0	76	145	0	0	0	1	11	0	636
50F	0	7	0	0	0	0	0	0	0	0	0	7
48M	205	110	0	256	29	3	3	1	0	1	0	608
43M	1	36	0	6	47	1	8	0	0	6	1	106
42M	7	730	0	96	0	4	0	0	0	0	0	837
42F	26	197	0	190	48	25	1	0	0	1	1	489
41F	22	479	0	0	0	0	0	0	0	1	1	503
40M	11	102	0	3,967	129	0	8	0	0	1	0	4,218
40F	51	111	0	304	5	1	0	0	7	7	1	487
37M	23	0	0	0	0	0	0	0	0	0	0	23
35F	12	13	17	9	5	0	1	0	2	3	0	62
34M	0	137	0	40	125	1	0	0	6	0	1	310
32M	41	32	0	42	9	2	2	0	1	12	0	141
32F	369	2,246	0	5	3	0	12	0	0	20	0	2,655
30F	68	28	0	6	197	14	0	0	0	2	2	317
28M	94	11	0	4	1	0	34	0	0	0	2	146
25M	4	3,045	0	2	31	1	2	0	0	5	0	3,090
23M	60	144	0	2,225	58	0	2	0	35	14	2	2,540
18M	46	136	0	162	16	0	4	0	0	0	1	365
17M	49	170	0	6	0	0	1	0	0	24	0	250
16M	0	770	0	0	0	0	1	0	0	0	0	771
16F	132	116	0	4	2	0	1	0	0	5	0	260
13M	0	32	0	6	0	0	2	0	0	0	0	40
9M	21	62	0	10	0	0	0	0	0	0	0	93
6M	20	0	0	0	0	0	0	0	0	0	0	20
Total (Mean No./ person)	1,377 (50)	10,691 (324)	38 (1)	7,510 (228)	910 (28)	52 (2)	83 (3)	1 (0.03)	56 (2)	174 (5)	15 (1)	20,907 (634)

[a]*O. viverrini, Opisthorchis viverrini; H. taichui, Haplorchis taichui; H. yokogawai, Haplorchis yokogawai; P. molenkampi, Prosthodendrium molenkampi; P. bonnei, Phaneropsolus bonnei; T. trichiura, Trichuris trichiura; E. vermicularis, Enterobius vermicularis*; [b]Species not determined; [c]The whole or a part of strobilae with or without a scolex were recovered. All were morphologically identified as *Taenia saginata*.

and women (Table 1), or between different age groups (data not shown).

Worm collection

The adult worm collection was completed in 33 people (24 men and 9 women; age range 6-80 years) (Table 2) and a total of 20,907 helminth specimens were recovered. Between 7 and 4,218 were collected per person, with an average of 634. Intestinal flukes were recovered from 31 cases (93.9%), and *O. viverrini* was from 27 cases (81.8%). The numbers of trematode specimens were 10,691 *H. taichui*, 7,510 *P. molenkampi*, 1,377 *O. viverrini*, 910 *P. bonnei*, 52 echinostomes (species to be determined), and 38 *H. yokogawai* (Table 2). Intestinal trematodes, including *Haplorchis*, lecithodendriids, and echinostomes, comprised 91.8% of all helminth specimens recovered, whereas *O. viverrini* and intestinal nematodes comprised only 6.6% and 1.5 %, respectively (Table 2).

The highest worm load of *H. taichui* collected from a single person was 3,045, and the average number of *H. taichui* recovered per treated person was 324. Specimens of *H. yokogawai* were collected only from 3 persons. As for lecithodendriids, the number of *P. molenkampi* worms ranged from 0 to 3,967 (av. 228 worms per person), and the number of *P. bonnei* ranged from 0 to 197 (av. 28 worms per person). Data on echinostomes will be published separately.

The number of nematode and cestode specimens was 174 *E. vermicularis*, 83 hookworms (*A. duodenale* and *N. americanus*), 56 *Trichostronglylus* spp., 1 *T. trichiura*, and 15 strobilae of *T. saginata* (Table 2). With regard to *E. vermicularis*, the worms were collected from 22 of 33 treated persons and the worm load averaged 5 worms (mostly females) per treated person. The worm load was higher among 17-32 and 59-65 year age groups. Hookworms were collected from 16 of 33 treated persons, averaged 3 worms per treated person, and consisted of *A. duodenale* and *N. americanus* in a 1 : 1 ratio. *Trichostrongylus* spp. specimens consisted of male and female worms (about 0.8 : 1), and were recovered from a total of 9 persons (Table 2).

DISCUSSION

Our results suggested that most of the people who were positive for small trematode eggs had mixed-infections with several species of intestinal flukes, including heterophyids and lecithodendriids, and the liver fluke, *O. viverrini*. Fecal examinations to detect helminth eggs have considerable limitations for understanding the prevalence and intensity of liver and intestinal flukes in Lao PDR, which was already known in the Republic of Korea [3] and Thailand [14,15]. In Lao PDR, this kind of limitation was reported in Vientiane Municipality and Saravane province [11] and also in Savannakhet province [12].

The relative prevalence of each fluke species varied remarkably by locality surveyed. For example, in Savannakhet and Vientiane, *O. viverrini* comprised up to 43.5% and 62.9% of all fluke specimens recovered, whereas in Saravane, intestinal flukes accounted for over 99.0% of all recovered flukes [11,12]. By comparison, in a recent study performed in Vientiane, Savannakhet, and Saravane, the worm loads of *O. viverrini* (av. 186 worms per worm-positive patient) and *H. taichui* (av. 207 worms) were quite similar in an analysis of 97 worm-recovered cases [13]. In our study, intestinal fluke infections predominated in Khammouane, although the degree of predominance (91.8%) was a little less than in Saravane [11].

There is a possibility that not all of the flukes (in particular, *O. viverrini*) were collected from the praziquantel treated and purged patients, since the diarrheic stools were collected only 4-5 times (for 5-6 hr) [11,12] or 6-8 times (for 24 hr) [13] after praziquantel administration. It may take longer for all *O. viverrini* to be expelled from the bile duct and gall bladder into the stools. However, data can be compared between surveyed areas, if the same protocol was applied. Thus, the individual worm loads of *O. viverrini* could be compared between different provinces. In Savannakhet province, the number of expelled worms averaged 115 worms per treated person [12], followed by an average of 58 worms in Vientiane Municipality and an average of 22 worms in Saravane province [11]. In our study, an average of 50 worms was recovered in Khammouane, which is quite similar to Vientiane [11].

The average worm load of *H. taichui* per treated person was remarkably high (8,514) in Saravane [11] compared to Khammouane (324; this study), Savannakhet (103) [12], and Vientiane (27) [11]. The highest worm load of lecithodendriid flukes was found in Khammouane (an average of 228 for *P. molenkampi* and an average of 28 for *P. bonnei*; this study), followed by Savannakhet (an average of 61 and 1, respectively) [12], Saravane (an average of 25 and 20, respectively), and Vientiane (an average of 3 and 0, respectively) [11]. These results suggest that the relative predominance of trematode species varies greatly by locality in Lao PDR.

It is of note that a total of 4 nematode species and a cestode species were recovered from the residents of Khammouane. The

higher worm loads of *E. vermicularis* among the persons at the ages of 17-32 and 59-65 year could be explained as that these people may have more contact with children, since they may have young children or grand children. Despite the relatively low prevalence of hookworm eggs (6.7%), hookworm specimens were collected from 16 of 33 treated persons. This may indicate that the actual prevalence of hookworms may be higher than the egg positive rate. Only 1 specimen of *T. trichiura* was recovered, which seems to have been due to the negligible efficacy of pyrantel pamoate and praziquantel against this nematode. A least expected result, because of a low egg positive rate (0.08%) in the fecal examination, was that a total of 56 specimens of *Trichostrongylus* spp. were recovered from 9 persons. A specimen collected from a Laotian was identified morphologically as *T. colubriformis* and its ITS-1 sequence was most similar to that of *T. colubriformis* [16]. However, the presence of *T. orientalis*, a common species in Asian countries [17], should not be ruled out in Lao PDR. The egg positive rate of *Taenia* spp. was 4.7%, but 12 of 33 treated persons expelled *Taenia* tapeworms after praziquantel treatment, which suggests a considerably higher prevalence of this tapeworm infection in this area.

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