

Prevalence of the Intestinal Flukes *Haplorchis taichui* and *H. yokogawai* in a Mountainous Area of Phongsaly Province, Lao PDR

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Abstract: Phongsaly Province, located in the northernmost area of Lao PDR, was previously suggested to be endemic for the liver fluke *Opisthorchis viverrini* infection. To confirm, or rule out, this suggestion, the Phonxay village in the Khoua District, Phongsaly Province, was selected for a survey. Ten volunteers (8 men and 2 women aged 31-57 years) who consumed raw freshwater fish and had gastrointestinal troubles were treated with a single dose of praziquantel (40 mg/kg) and pyrantel pamoate (10 mg/kg) and purged with magnesium sulfate to recover any worm parasites. Eight of the 10 volunteers expelled 1 or more species of trematodes, nematodes, or cestodes (worm positive rate; 80%). The worms were morphologically identified as *H. taichui* (861 worms from 8 people), *H. yokogawai* (59 from 6 people), *Phaneropsolus bonnei* (1 from 1 person), *Trichostrongylus* sp. (2 from 2 people), *Ascaris lumbricoides* (2 from 1 person), *Enterobius vermicularis* (11 from 3 people), and *Taenia saginata* (1 strobila with scolex from 1 person). The results indicate that the mountainous area of Phongsaly Province, Lao PDR, is not endemic for the liver fluke but endemic for intestinal flukes, in particular, *Haplorchis taichui* and *H. yokogawai*.

Key words: *Haplorchis taichui*, *Haplorchis yokogawai*, *Phaneropsolus bonnei*, *Trichostrongylus*, *Taenia saginata*, prevalence, Phongsaly Province, Lao PDR

The liver fluke *Opisthorchis viverrini*, an important fish-borne parasite [1], and soil-transmitted nematodes, including *Ascaris lumbricoides*, *Trichuris trichiura*, and hookworms, were the major helminth species prevalent among Laotian people [2-5]. However, in 1991, *Haplorchis taichui*, an intestinal fluke, was reported in Laotian students [6]. Since then, *H. taichui* and several other intestinal fluke species including *Haplorchis pumilio*, *Haplorchis yokogawai*, *Phaneropsolus bonnei*, and *Prosthodendrium molenkampi* have been added to the major helminths infecting Laotians, particularly among residents along the Mekong River [7-10].

There is strong locality specificity for helminth species. For example, soil-transmitted nematodes are highly prevalent in northern mountainous areas, including Luangprabang, Phongsaly, Huaphan, and Saysomboune Province, whereas foodborne trematodes, including *O. viverrini*, and *H. taichui* and other intestinal flukes, are prevalent along the Mekong River in the middle and southern areas, including Vientiane Municipality, Khammouane, Savannakhet, and Saravane Provinces [5,7-9]. In Vientiane Municipality, *O. viverrini* is dominant, whereas in Savannakhet Province, *O. viverrini* and *H. taichui* are half and half, and in Saravane and Khammouane Provinces, *H. taichui* is the dominant species [7].

When we [5] performed a national survey of intestinal parasites among primary schoolchildren in Lao PDR during 2000 and 2002, we found that the egg positive rate of *O. viverrini* or minute intestinal flukes (we designated these as small trematode eggs) were considerably higher in 4 villages (Phonxay, Tre-

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dsabane, Nathoune, and Phoxayneau) of the Khoua District, Phongsaly Province, the northernmost area of Lao PDR bordering southern China and northwest Vietnam (Fig. 1). The fecal examination results for 269 schoolchildren are shown in Table 1 (not shown in [5]). The prevalence of small trematode eggs was 27.6-57.4% (av. 48.7%) in addition to the high prevalence of soil-transmitted nematodes (Table 1). The egg positive rates were not significantly different ($P > 0.01$) between men and women, or between different age groups (data not shown). Unless adult worms are recovered, it is not possible to determine whether the eggs are of the liver fluke, intestinal fluke, or

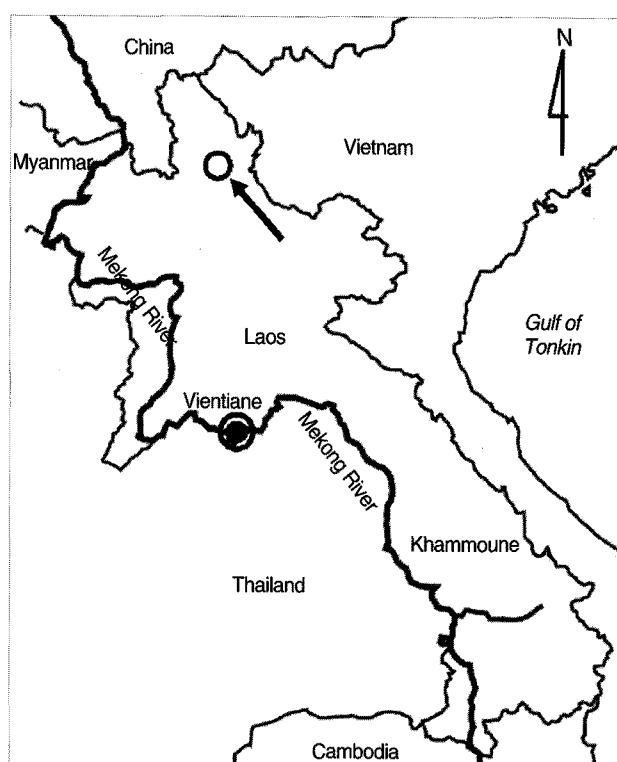


Fig. 1. Map showing the surveyed area of Lao PDR (arrow: Khoua District, Phongsaly Province).

both, because their eggs are very similar in size and shape. Therefore, in the present study, we attempted to determine the kinds of flukes through recovery of adult worms from residents of Phoxay village, a mountainous area of Khoua District, which is located in the southern part of Phongsaly Province, Lao PDR (Fig. 1).

We visited Phoxay village, Khoua District, in December 2008 and explained our purpose of visit to the village leader. Ten volunteers, consisting of 8 men and 2 women aged 31-57 years who frequently consumed raw freshwater fish and experienced at times gastrointestinal troubles such as diarrhea and abdominal discomfort, were recruited randomly. After obtaining informed consent from each person, without preliminary fecal examination for helminth eggs, they were treated with a single oral dose of 40 mg/kg praziquantel (Distocide®, Shinpoong Pharm. Co., Seoul, Korea) and 10 mg/kg pyrantel pamoate (Hangzhou Minsheng Pharm. Group, Hangzhou, China), and then purged with 30 g magnesium sulfate. Whole diarrhetic stools passed 4-5 times were pooled individually and processed as previously described [7]. Worms were collected using a glass pipette and then washed in water. Some were fixed in 10% formalin under cover slip pressure, acetocarmine-stained, and morphologically identified using a light microscope. This study was performed under the agreement of Korea-Laos International Collaboration Project on Parasite Control in Lao PDR (2007-2011).

Eight of the 10 treated people expelled 1 or more species of intestinal flukes with or without intestinal nematodes, and 2 were negative for any helminth worms (Table 2). The worms recovered included 861 specimens of *H. taichui* (from 8 people), 59 of *H. yokogawai* (6), 1 of *P. bonnei* (1), 2 of *Trichostrongylus* sp. (2), 2 of *A. lumbricoides* (1), 11 of *Enterobius vermicularis* (3), and 1 strobila with scolex of *Taenia saginata* (1).

These results suggest that mountainous areas of Phongsaly

Table 1. Helminth egg positive rates in the feces of residents in Khoua District, Phongsaly Province, Lao PDR (April-June 2002)

Helminth species	No. of residents examined (%) in each village of Khoua District				
	Phoxay	Tredsabane	Nathoune	Phoxayneau	Total
No. examined ^a	54	110	76	29	269
No. helminth egg positive cases (%)	54 (100.0)	110 (100.0)	76 (100.0)	29 (100.0)	269 (100.0)
<i>Ascaris lumbricoides</i>	49 (90.7)	100 (90.9)	69 (90.8)	27 (93.1)	245 (91.1)
Hookworms	0 (0.0)	12 (10.9)	28 (36.8)	10 (34.5)	50 (18.6)
<i>Trichuris trichiura</i>	45 (83.3)	92 (83.6)	54 (71.1)	28 (96.6)	219 (81.4)
Small trematode eggs ^b	31 (57.4)	59 (53.6)	33 (43.3)	8 (27.6)	131 (48.7)

^aFecal examination was performed by the Kato-Katz technique. One smear was examined for each person.

^bPossibly including the eggs of *Opisthorchis viverrini*, heterophyids (Heterophyidae), and lecitodendriids (Lecithodendriidae).

Table 2. Results of adult fluke collection from residents in Phonxay village, a mountainous area of Khoua District, Phongsaly Province, Lao PDR (December 2008)

Age and sex of residents	No. of helminth specimens collected ^a							Total
	<i>H. taichui</i>	<i>H. yokogawai</i>	<i>P. bonnei</i>	<i>Trichostrongylus</i> sp. ^b	<i>A. lumbricoides</i>	<i>E. vermicularis</i>	<i>T. saginata</i> ^c	
57M	305	28	0	1	0	1	0	335
42M	302	9	0	0	0	7	0	318
56M	95	15	0	0	0	3	0	113
48M	78	4	0	0	0	0	0	82
48F	33	0	1	0	0	0	0	34
42M	28	2	0	0	0	0	0	30
31F	14	1	0	1	2	0	0	18
54M	6	0	0	0	0	0	1	7
56M	0	0	0	0	0	0	0	0
53M	0	0	0	0	0	0	0	0
Total	861	59	1	2	2	11	1	937

^a*H. taichui*, *Haplorchis taichui*; *H. yokogawai*, *Haplorchis yokogawai*; *P. bonnei*, *Phaneropsolus bonnei*; *T. trichiura*, *Trichuris trichiura*; *A. lumbricoides*, *Ascaris lumbricoides*; *E. vermicularis*, *Enterobius vermicularis*; *T. saginata*, *Taenia saginata*.

^bSpecies not determined.

^cA part of strobila with scolex was recovered.

Province, Laos are not endemic for the liver fluke but are endemic for intestinal flukes, in particular *H. taichui* and *H. yokogawai*. The possibility for failure of praziquantel treatment and magnesium salt purgation to expel *O. viverrini* worms within a short time (5-6 hr) after treatment may be raised. However, we think that such possibility is low because, in our previous studies, the same protocol was applied and substantial numbers of *O. viverrini* worms were expelled in the diarrheic stools [4,7-9]. In the present study, we could not collect even a single specimen of *O. viverrini*.

We regret that we were unable to examine freshwater fish caught from the streams nearby the village. To confirm that there is no *O. viverrini* in this area, surveys of snails and fish are essential. In addition, surveys of *H. taichui* and *H. yokogawai* infections in intermediate hosts in this area (in particular, freshwater fish, the source of human infections) are urgently needed to establish preventive and control strategies against these intestinal fluke infections.

The results of the present study again clearly demonstrated that fecal examination is not enough to understand the real status of trematode infections or to determine the species of trematodes endemic in certain areas. This issue has been pointed out in Vientiane Municipality and Saravane Province [7], Savannakhet Province [8], and Khammouane Province [9], Laos. In Vientiane Municipality, where small trematode egg positive rate of some riverside people was 53.3% [7], the worms expelled after chemotherapy and purgation were a mixture of several different species of trematodes, including *O. viverrini*, *H. taichui*, *H.*

pumilio, *H. yokogawai*, *Centrocestus caninus*, *P. molenkampi*, and *P. bonnei*. Of the 1,656 fluke specimens recovered from 18 persons [7], *O. viverrini* comprised of 62.9% (1,041 worms) and 6 other species of trematodes comprised 37.1% (615 worms). Similar results were reported in Savannakhet Province, where people were mixed-infected with approximately 50% *O. viverrini* and 50% minute intestinal flukes [8,10].

In Saravane Province, where the small trematode egg positive rate of people was 70.8% [7], the worms expelled were predominantly intestinal flukes (155,207 worms). Of these, *H. taichui* (153,253 worms) and *H. pumilio* (1,125 worms) comprised over 99% of all flukes recovered. In Khammouane Province, over 90% (19,149 worms) of trematodes recovered were intestinal flukes and only a small proportion (1,377 worms) was *O. viverrini* [9].

The term 'small trematode eggs' should be used when trematode eggs of 20-30 μm in length are detected in fecal examinations of people in southeast Asia, including Laos. In Laos, eggs of *O. viverrini* ($27.1 \pm 2.6 \mu\text{m}$), *H. taichui* (27.0 ± 1.7), *H. pumilio* (30.8 ± 0.8), *H. yokogawai* (28.6 ± 0.7), *P. molenkampi* (25.3 ± 1.6), and *P. bonnei* (26.4 ± 2.4) [11-14] should be included among the list of differential diagnosis. Worm collection after chemotherapy and purgation is essential to draw a precise diagnosis. However, it is tedious, not feasible, and impracticable as a routine procedure. Therefore, other diagnostic techniques, using ultrastructural, molecular, or genetic characteristics, should be developed for differential diagnosis of small trematode eggs in fecal samples.

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REFERENCES

1. Chai JY, Murrell KD, Lymbry A. Fishborne parasitic zoonoses: status and issues. *Int J Parasitol* 2005; 35: 1233-1254.
2. Sommani S, Pathammavong O, Bunnag T, Impand P, Intarakhao C, Thirachantra S. An epidemiological survey of human intestinal parasites in Vientiane, Laos. *Southeast Asian J Trop Med Public Health* 1974; 5: 541-546.
3. Kobayashi J, Vannachone B, Xeutvongsa A, Manivong K, Ogawa S, Sato Y, Pholsena K. Prevalence of intestinal parasitic infection among children in two villages in Lao PDR. *Southeast Asian J Trop Med Public Health* 1996; 27: 562-565.
4. Chai JY, Hongvanthong B. A small-scale survey of intestinal helminthic infections among the residents near Pakse, Laos. *Korean J Parasitol* 1998; 36: 55-58.
5. Rim HJ, Chai JY, Min DY, Cho SY, Eom KS, Hong SJ, Sohn WM, Yong TS, Deodato G, Standgaard H, Phommasack B, Yun CY, Hoang EH. Prevalence of intestinal parasite infections on a national scale among primary schoolchildren in Laos. *Parasitol Res* 2003; 91: 267-272.
6. Giboda M, Ditrich O, Scholz T, Viengsay T, Bouaphanh S. Human *Opisthorchis* and *Haplorchis* infections in Laos. *Trans R Soc Trop Med Hyg* 1991; 85: 538-540.
7. Chai JY, Park JH, Han ET, Guk SM, Shin EH, Lin A, Kim JL, Sohn WM, Yong TS, Eom KS, Min DY, Hwang EH, Phommasack B, Insiengmay B, Rim HJ. Mixed infections with *Opisthorchis viverrini* and intestinal flukes in residents of Vientiane Municipality and Saravane Province in Laos. *J Helminthol* 2005; 79: 283-289.
8. Chai JY, Han ET, Guk SM, Shin EH, Sohn WM, Yong TS, Eom KS, Lee KH, Jeong HG, Ryang YS, Hoang EH, Phommasack B, Insiengmay B, Lee SH, Rim HJ. High prevalence of liver and intestinal fluke infections among residents of Savannakhet Province in Laos. *Korean J Parasitol* 2007; 45: 213-218.
9. Chai JY, Han ET, Shin EH, Sohn WM, Yong TS, Eom KS, Min DY, Um JY, Park MS, Hoang EH, Phommasack B, Insiengmay B, Lee SH, Rim HJ. High prevalence of *Haplorchis taichui*, *Prosthodendrium molenkampi*, and other helminth infections among people in Khammouane Province in Laos. *Korean J Parasitol* 2009; 47: 243-247.
10. Sayasone S, Vonghajack Y, Vanmany M, Rasphone O, Tesana S, Utzinger J, Akkhavong K, Odermatt P. Diversity of human intestinal helminthiasis in Lao PDR. *Trans R Soc Trop Med Hyg* 2009; 103: 247-254.
11. Ditrich O, Giboda M, Scholz T, Beer SA. Comparative morphology of eggs of the Haplorchiinae (Trematoda: Heterophyidae) and some other medically important heterophyid and opisthorchiid flukes. *Folia Parasitol* 1992; 39: 123-132.
12. Tesana S, Srisawangonk T, Kaewkes S, Sithithaworn P, Kanla P, Arunyanart C. Eggshell morphology of the small eggs of human trematodes in Thailand. *Southeast Asian J Trop Med Public Health* 1991; 22: 631-636.
13. Chai JY, Lee SH. Food-borne intestinal trematode infections in the Republic of Korea. *Parasitol Int* 2002; 51: 129-154.
14. Chai JY, Shin EH, Lee SH, Rim HJ. Foodborne intestinal flukes in southeast Asia. *Korean J Parasitol* 2009; 47: S69-S102.