

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

J.-Y. Chai<sup>1\*</sup>, J.-H. Park<sup>1</sup>, E.-T. Han<sup>2</sup>, S.-M. Guk<sup>1</sup>, E.-H. Shin<sup>1</sup>, A. Lin<sup>1</sup>, J.-L. Kim<sup>1</sup>, W.-M. Sohn<sup>3</sup>, T.-S. Yong<sup>4</sup>, K.S. Eom<sup>5</sup>, D.-Y. Min<sup>6</sup>, E.-H. Hwang<sup>7</sup>, B. Phommasack<sup>8</sup>, B. Insisiengmay<sup>8</sup> and H.-J. Rim<sup>9</sup>

<sup>1</sup>Department of Parasitology and Tropical Medicine, Seoul National University College of Medicine, and Institute of Endemic Diseases, Seoul National University Medical Research Center, Seoul 110-799, Korea:

<sup>2</sup>Department of Parasitology, College of Medicine, Kangwon National University, Chuncheon 200-701, Korea: <sup>3</sup>Department of Parasitology and Institute of Health Sciences, Gyeongsang National University College of Medicine, Jinju 660-751, Korea: <sup>4</sup>Department of Parasitology, College of Medicine, Yonsei University, Seoul 120-752, Korea: <sup>5</sup>Chungbuk National University, Chongju 361-763, Korea: <sup>6</sup>Hanyang University, Seoul 133-791, Korea: <sup>7</sup>Korea Association of Health Promotion, Seoul 157-704, Korea:

<sup>8</sup>Department of Hygiene and Prevention, Ministry of Public Health, Vientiane, Lao PDR: <sup>9</sup>Department of Parasitology, Korea University, Seoul 136-705, Korea

### 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<sup>-1</sup> 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 molenkampii*, and *Phanerocephalus 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 lecitichodendriids 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 (opisthorchiid or heterophyid/lecitichodendriid 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

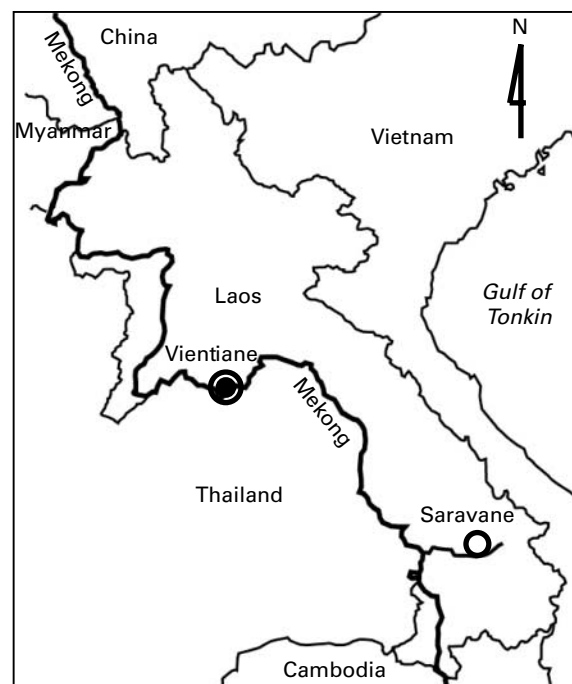


Fig. 1. Map showing the two surveyed localities (○) of Laos: Vientiane Municipality and Saravane Province.

with a single dose (20–30 mg kg<sup>-1</sup>) 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 clear layer was discarded, and the lower dark layer was fixed with 0.5–1.0% neutral buffered formalin and searched for worm parasites under a stereomicroscope. Some samples were also transported to the Department of Parasitology and Tropical Medicine, Seoul National University College of Medicine, Seoul, Korea for the same purpose. 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 under a light microscope.

#### 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 lecitodendriids, 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 1.5%), *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. bonnei* (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. bonnei* 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 lecitodendriid flukes, *P. molenkampi* was recovered in both localities, but

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

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

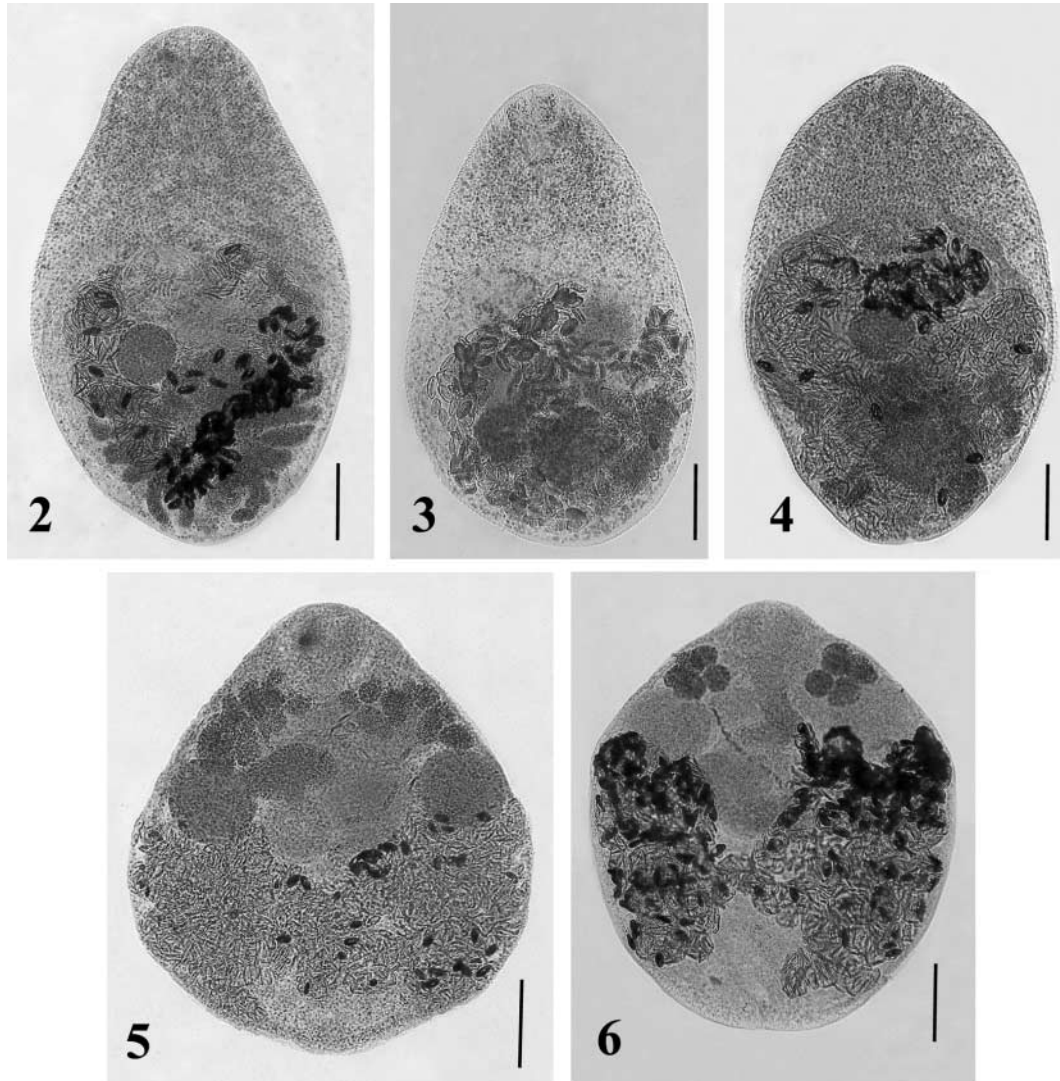
\* Faecal examination was performed by the Kato-Katz thick smear technique. One smear was examined for each person.

† Including the eggs of heterophyids (Heterophyidae) and lecitodendriids (Lecitodendriidae).

Table 2. The occurrence of intestinal flukes in villagers from Vientiane Municipality and Saravane Province, Laos, November 2002 to November 2003.

Age and sex of villager	No. of fluke specimens collected							Total
	<i>Opisthorchis viverrini</i>	<i>Haplorchis taichui</i>	<i>Haplorchis pumilio</i>	<i>Haplorchis yokogawai</i>	<i>Centrocestus caninus</i>	<i>Prosthodendrium molenkampi</i>	<i>Phaneroopsolus bonniei</i>	
Vientiane Municipality								
60M	40	12	0	0	0	0	0	52
47M	6	6	0	0	0	1	0	13
45M	3	24	0	0	0	0	0	27
42M	149	124	1	43	2	0	0	319
36M	4	4	0	0	1	1	0	10
30M	32	0	0	0	0	0	0	32
28M	140	13	0	0	0	0	0	153
27M	315	1	0	0	0	1	0	317
27M	7	75	0	0	0	0	0	82
23M	4	13	0	0	1	0	0	18
13M	28	0	0	0	0	0	0	28
12M	34	1	0	0	0	57	0	92
67F	42	6	0	0	0	0	0	48
60F	13	4	0	0	0	0	0	17
40F	72	4	0	0	0	0	0	76
27F	111	78	0	0	0	1	0	190
26F	28	1	0	0	0	0	0	29
10F	13	128	0	22	0	0	0	153
Subtotal	1041 (57.8)	484 (26.9)	1 (0.6)	65 (3.6)	4 (0.2)	61 (3.4)	0 (0.0)	1656 (92.0)
Saravane Province								
60M	0	22360	18	22	0	21	0	22421
55M	35	2298	69	0	0	0	0	2402
52M	8	7570	8	0	0	1	0	7587
50M	0	24159	101	0	0	0	0	24260
48M	4	466	17	0	0	32	0	519
48M	1	5220	40	0	0	0	0	5261
35M	35	36658	502	0	0	0	40	37235
30M	125	1218	6	0	0	0	0	1349
29M	6	1675	27	0	0	5	0	1713
28M	9	4814	8	0	0	155	0	4986
55F	0	14841	119	0	0	20	0	14980
50F	11	2744	1	0	0	0	0	2756
48F	17	401	0	0	0	0	57	475
45F	0	8743	0	0	0	0	0	8743
45F	26	14659	161	0	0	196	40	15082
27F	0	3948	43	0	0	0	229	4220
26F	1	1346	5	0	0	9	0	1361
21F	117	133	0	0	0	2	0	252
Subtotal	395 (21.9)	153253 (8514.1)	1125 (62.5)	22 (1.2)	0 (0.0)	441 (24.5)	366 (20.3)	155602 (8644.6)





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.

*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

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*, *H. pumilio*, *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*, *H. pumilio*, *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 Czechoslovakia (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 1000 specimens of *H. taichui* and that five 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.

## Acknowledgements

We are grateful to Dr Sithat Insiengmay and the staff of the Center for Laboratory and Epidemiology, Department of Hygiene and Prevention, Ministry of Public Health, Vientiane, and staff at the Station of Malariology and Parasitology, Saravane Provincial Health Department, Saravane, Lao PDR, for their help during the collection of faecal samples from villagers and for the preparation of Kato-Katz smears. We also thank the staff of the Korea Association of Health Promotion, Seoul, Republic of Korea, who participated in the Korea-Laos Cooperation Project on Parasite Control in Lao PDR (1999–2004). This study was supported by BK21 Human Life Sciences, Ministry of Education, Republic of Korea.

## References

- Chai, J.Y. & Hongvanthong, B. (1998) A small-scale survey of intestinal helminthic infections among the residents near Pakse, Laos. *Korean Journal of Parasitology* **36**, 55–58.
- Chai, J.Y. & Lee, S.H. (2002) Food-borne intestinal trematode infections in the Republic of Korea. *Parasitology International* **51**, 129–154.
- Chai, J.Y., Murrell, K.D. & Lymbry, A. (2005) Fishborne parasitic zoonoses: status and issues. *International Journal for Parasitology* (in press).
- Ditrich, O., Scholtz, T. & Giboda, M. (1990) Occurrence of some medically important flukes (Trematoda: Opisthorchiidae and Heterophyidae) in Nam Ngum water reservoir. *Southeast Asian Journal of Tropical Medicine and Public Health* **21**, 482–488.
- Ditrich, O., Giboda, M., Scholtz, T. & Beer, S.A. (1992) Comparative morphology of eggs of the Haplorchiinae (Trematoda: Heterophyidae) and some other medically important heterophyid and opisthorchiid flukes. *Folia Parasitologica* **39**, 123–132.
- Giboda, M., Ditrich, O., Scholtz, T., Viengsay, T. & Bouaphanh, S. (1991) Human *Opisthorchis* and *Haplorchis* infections in Laos. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **85**, 538–540.
- Kaewkes, S., Elkins, D.B., Sithithaworn, P. & Haswell-Elkins, M.R. (1991) Comparative studies on the morphology of the eggs of *Opisthorchis viverrini* and lecithodendriid trematodes. *Southeast Asian Journal of Tropical Medicine and Public Health* **22**, 623–630.
- Kobayashi, J., Vannachone, B., Xeutvongsa, A., Manivong, K., Ogawa, S., Sato, Y. & Pholsena, K. (1996) Prevalence of intestinal parasitic infection among children in two villages in Lao PDR. *Southeast Asian Journal of Tropical Medicine and Public Health* **27**, 562–565.
- Kobayashi, J., Vannachone, B., Sato, Y., Manivong, K., Nambanya, S. & Inthakone, S. (2000) An epidemiological study on *Opisthorchis viverrini* infection in Lao villages. *Southeast Asian Journal of Tropical Medicine and Public Health* **31**, 128–132.
- Manning, G.S. & Lertprasert, P. (1973) Studies on the life cycle of *Phaneropsolus bonnei* and *Prosthodendrium molenkampi* in Thailand. *Annals of Tropical Medicine and Parasitology* **67**, 361–365.

- Manning, G.S., Lertprasert, P., Watanasirmit, K. & Chetty, C.** (1971) A description of newly discovered intestinal parasites endemic to northeastern Thailand. *Journal of the Medical Association of Thailand* **54**, 466–474.
- Pholsena, K., Sayaseng, B., Hongvanthong, B. & Vanisaveth, V.** (1991) The prevalence of helminth infection in Ban Nanin, Laos. *Southeast Asian Journal of Tropical Medicine and Public Health* **22**, 137–138.
- Radomyos, P., Bunnag, D. & Harinasuta, T.** (1984) Worms recovered in stool following praziquantel treatment. *Arzneimittel-Forschung/Drug Research* **34**, 1215–1217.
- Radomyos, P., Radomyos, B. & Tungtrongchitr, A.** (1994) Multi-infection with helminths in adults from north-east Thailand as determined by post-treatment fecal examination of adult worms. *Tropical Medicine and Parasitology* **45**, 133–135.
- Rim, H.J.** (1982) Opisthorchiasis. pp. 109–121 in Steele, J.H. (Ed.) *CRC handbook series in zoonoses, Section C: Parasitic zoonoses, Vol. III (trematode zoonoses)*. Boca Raton, Florida, CRC Press.
- Rim, H.J., Chai, J.Y., Min, D.Y., Cho, S.Y., Eom, K.S., Hong, S.J., Sohn, W.M., Yong, T.S., Deodato, G., Standgaard, H., Phommasack, B., Yun, C.Y. & Hoang, E.H.** (2003) Prevalence of intestinal parasite infections on a national scale among primary schoolchildren in Laos. *Parasitology Research* **91**, 267–272.
- Segal, D.B., Humphrey, J.M., Edwards, S.J. & Kirby, M.D.** (1968) Parasites of man and domestic animals in Vietnam, Thailand, Laos, and Cambodia. *Experimental Parasitology* **23**, 412–464.
- Scholtz, T., Ditrich, O. & Giboda, M.** (1990) Larval stages of medically important flukes (Trematoda) from Vientiane Province, Laos. Part I. metacercariae. *Annales de Parasitologie Humaine et Comparée* **65**, 238–243.
- Sornmani, S., Pathammavong, O., Bunnag, T., Impand, P., Intarakhao, C. & Thirachantra, S.** (1974) An epidemiological survey of human intestinal parasites in Vientiane, Laos. *Southeast Asian Journal of Tropical Medicine and Public Health* **5**, 541–546.
- Tesana, S., Srisawangwonk, T., Kaewkes, S., Sithithaworn, P., Kanla, P. & Arunyanart, C.** (1991) Eggshell morphology of the small eggs of human trematodes in Thailand. *Southeast Asian Journal of Tropical Medicine and Public Health* **22**, 631–636.
- Yu, S.H. & Mott, K.E.** (1994) Epidemiology and morbidity of food-borne intestinal trematode infections. *Tropical Diseases Bulletin* **91**, R125–R152.
- Waikagul, J.** (1991) Intestinal fluke infections in southeast Asia. *Southeast Asian Journal of Tropical Medicine and Public Health* **22** (Suppl.), 158–162.
- World Health Organization** (1995) Control of foodborne trematode infections. *WHO Technical Report Series No. 849*, pp. 1–157.
- World Health Organization** (2004) Report of Joint WHO/FAO Workshop on Food-borne trematode infections in Asia, Ha Noi, Vietnam, 26–28 November, 2002. WHO, WPRO, pp. 1–58.