

Fishborne Trematode Metacercariae in Luang Prabang, Khammouane, and Saravane Province, Lao PDR

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Abstract: Fishborne trematode (FBT) metacercariae were investigated in fish from 3 Provinces of Lao PDR. Total 242 freshwater fish of 40 species were collected in local markets of Luang Prabang (59 fish of 16 species), Khammouane (81 fish of 19 species), and Saravane (97 fish of 14 species), and each of them was examined by artificial digestion method. Four species of metacercariae (*Opisthorchis viverrini*, *Haplorchis taichui*, *Haplorchis yokogawai*, and *Centrocestus formosanus*) were detected. *O. viverrini* was detected in 35 fish (14.5%), and their density was 252 per infected fish (Luang Prabang, 88 metacercariae in 5 fish; Khammouane, 187 in 6 fish; Saravane, 303 in 24 fish). *H. taichui* was found in 102 fish (42.1%), and their density was 485 per infected fish (Luang Prabang, 260 metacercariae in 38 fish; Khammouane, 1,084 in 23 fish; Saravane, 359 in 41 fish). *H. yokogawai* was detected in 92 fish (38.0%), and their density was 222 per infected fish (Luang Prabang, 362 metacercariae in 17 fish; Khammouane, 126 in 20 fish; Saravane, 214 in 55 fish). Metacercariae of *C. formosanus* were found in 8 fish (3.3%), and their density was 3 per infected fish. In the present study, it has been confirmed that FBT metacercariae, in particular, *H. taichui*, *H. yokogawai*, and *O. viverrini*, are highly prevalent in fish from Luang Prabang, Khammouane, and Saravane Province, Lao PDR.

Key words: *Opisthorchis viverrini*, *Haplorchis taichui*, *Haplorchis yokogawai*, *Centrocestus formosanus*, fishborne trematode, prevalence, density, metacercaria, Lao PDR

INTRODUCTION

Fishborne trematode (FBT) infection is a public health problem in some Asian countries, including Lao People's Democratic Republic (Lao PDR), Vietnam, Cambodia, and Thailand, where these flukes provoke remarkable morbidity and cause serious damages to aquaculture. Human infections by FBTs are almost entirely caused by habitual consumption of raw fish containing infective larvae, i.e., metacercariae. These infections are highly localized depending on the food habits of residents and on the presence of susceptible snail hosts.

Moreover, FBTs show low host-specificity, and many kinds of reservoir hosts can contribute to the maintenance of the life cycle, making it extremely difficult to control these parasite infections in the epidemiological points of view [1,2].

Lao PDR is located in the middle of Southeast Asia, and has the Mekong River flow through the whole country from north to south. Laotian people residing in the Mekong River basin, which occupy a quarter of the territory, have some unique food habits. Some of them like to eat dishes containing raw freshwater fish, and are easily infected with FBT [3,4].

It has been reported that many Laotian people are infected with trematodes such as *Opisthorchis viverrini*, heterophyids, echinostomatids, and lecithodendriids [5-7]. Some investigators have previously reported that FBTs, including *O. viverrini*, are prevalent in Lao PDR [8-14]. Metacercarial infections were also investigated in fish intermediate hosts from some local areas in Lao PDR by some workers. Schotz et al. [15] surveyed

•Received 6 May 2012, revised 20 June 2012, accepted 2 July 2012.

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freshwater fishes from rice fields around Vientiane Municipality and Nam Ngum water reservoir. Freshwater fish from Savannakhet Province and Vientiane Municipality were examined for their metacercarial infections by Rim et al. [16]. However, studies on the second intermediate hosts of FBT were performed in limited areas and, therefore, studies on more wide areas are needed to know the variety of fish intermediate hosts for FBT in Lao PDR. Therefore, we performed field surveys to determine the infection status of freshwater fish with FBT metacercariae caught in 3 Provinces, i.e., Luang Prabang, Khammouane, and Saravane, in Lao PDR.

MATERIALS AND METHODS

We collected a total of 242 freshwater fish of 40 species in local markets of Khammouane Province (81 fish of 14 species) in March 2003, Saravane Province (97 fish of 13 species) in November 2003, and Luang Prabang (59 fish of 16 species) in February 2004 (Fig. 1). All collected fish were transferred to the laboratories (on-the-spot local laboratories in Lao PDR and then to the Department of Parasitology, Gyeongsang National University School of Medicine) and identified the fish

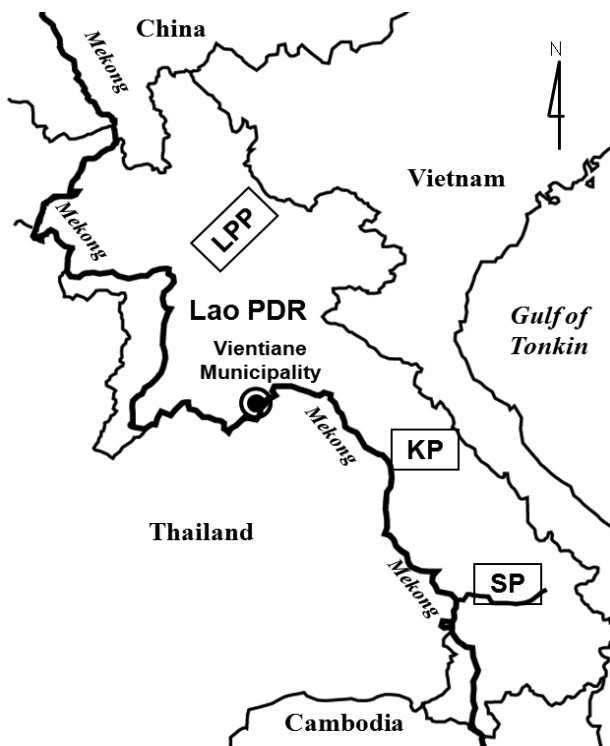


Fig. 1. Surveyed areas, Luang Prabang Province (LPP), Khammouane Province (KP), and Saravane Province (SP), in Lao PDR.

Table 1. Freshwater fish collected from 3 Provinces of Lao PDR

Species of fish	No. of fish ^a collected from			Total
	Luang Prabang	Khammouane	Saravane	
Cyprinidae				
<i>Barbonymus gonionotus</i>	1	5	3	9
<i>Cosmochilus harmondi</i>	-	5	-	5
<i>Crossocheilus oblongus</i>	-	6	-	6
<i>Cyclocheilichthys apogon</i>	4	-	-	4
<i>Cyclocheilichthys armatus</i>	-	4	-	4
<i>Cyclocheilichthys enoplos</i>	-	-	5	5
<i>Cyclocheilichthys furcatus</i>	4	-	-	4
<i>Cyclocheilichthys repasson</i>	2	4	-	6
<i>Esomus longimanus</i>	-	-	7	7
<i>Esomus metallicus</i>	-	-	10	10
<i>Hampala dispar</i>	-	2	19	21
<i>Henicorhynchus lineatus</i>	-	1	-	1
<i>Henicorhynchus siamensis</i>	4	-	-	4
<i>Hypsibarbus lagleri</i>	-	9	-	9
<i>Hypsibarbus malcolmi</i>	-	5	-	5
<i>Hypsibarbus pierrei</i>	3	-	1	4
<i>Hypsibarbus wetmorei</i>	-	3	1	4
<i>Labiobarbus leptocheilus</i>	3	-	-	3
<i>Labiobarbus siamensis</i>	4	-	-	4
<i>Lobocheilos rhabdoura</i>	1	-	1	2
<i>Mekongina erythrospila</i>	10	-	-	10
<i>Mystacoleucus ectypus</i>	1	-	-	1
<i>Mystacoleucus greenwayi</i>	-	-	16	16
<i>Neolissochilus stracheyi</i>	2	-	-	2
<i>Onychostoma fusiforme</i>	1	7	-	8
<i>Osteochilus vittatus</i>	-	7	-	7
<i>Oxygaster pointoni</i>	-	10	-	10
<i>Paralabuca barroni</i>	-	-	10	10
<i>Poropuntius dearatus</i>	-	-	6	6
<i>Puntioplites proctozysron</i>	-	-	7	7
<i>Puntius brevis</i>	-	3	10	13
<i>Puntius orphoides</i>	-	-	1	1
<i>Rasbora steineri</i>	-	5	-	5
<i>Scaphognathops bandanensis</i>	7	-	-	7
<i>Sinibrama melrosei</i>	7	-	-	7
Clupeidae				
<i>Tenualosa thibaudeaui</i>	-	4	-	4
Channidae				
<i>Channa striata</i>	-	4	-	4
Hemiramphidae				
<i>Dermogenys siamensis</i>	-	1	-	1
Balitoridae				
<i>Physoschistura meridionalis</i>	5	-	-	5
Siluridae				
<i>Kryptopterus bicirrhis</i>	-	1	-	1
Total	59	86	97	242

^aTotal 242 freshwater fish (40 species) were collected and examined.

species with the aid of ichthyologists in Lao PDR and FishBase site in internet (Table 1) [17]. Individual fish was finely ground with a mortar with pestle or a grinder, and the ground fish meat was mixed with artificial gastric juice. The mixture was incubated at 36°C for 2-3 hr. The digested material was filtered through 1 × 1 mm mesh, and washed with 0.85% saline until the supernatant became clear. The sediment was carefully examined using a stereomicroscope and then the metacercariae of each FBT species were separately collected based on their general features. The collected metacercariae were categorized according to their measurements and morphologic characters, and then the infection rate and the intensity of infection were calculated according to the fish species.

RESULTS

Opisthorchis viverrini metacercariae

O. viverrini metacercariae were detected in 4 fish species (25.0%), i.e., *Neolissochilus stracheyi*, *Cyclocheilichthys apogon*, *Hypsibarbus pierrei*, and *Cyclocheilichthys furcatus*, from Luang Prabang. Their infection rate was 38.5% and the average metacercarial density was 88 per infected fish. Total 6 (31.6%) out of 19 fish of 5 species (26.3%), i.e., *Onychostoma fusiforme*, *Puntius brevis*, *Cyclocheilichthys armatus*, *Hampala dispar*, and *Hypsibarbus wetmorei*, from Khammouane were infected with av. 187 metacercariae per fish. A total of 24 fish (42.1%) of 6 species (46.2%), i.e., *H. dispar*, *Puntioplites proctoysron*, *Poropuntius dearatus*, *P. brevis*, *Paralaubuca barroni*, and *Cyclocheilichthys enoplos*, from Saravane were infected with av. 303 metacercariae per fish. The status of metacercarial infection by surveyed areas and fish species are designated in Table 2.

Haplorchis taichui metacercariae

H. taichui metacercariae were detected in 10 fish species (62.5%) from Luang Prabang, and their infection rate was 86.4% and the average metacercarial density was 260 per infected fish. Total 23 (79.3%) out of 29 fish of 5 species (26.3%) from Khammouane were infected with av. 1,084 metacercariae per fish. A total of 41 fish (54.7%) of 9 species (64.3%) from Saravane were infected with av. 359 metacercariae per fish. The infection status by surveyed areas and fish species are shown in Table 3.

Haplorchis yokogawai metacercariae

H. yokogawai metacercariae were detected in 6 fish species

Table 2. Infection status of *Opisthorchis viverrini* metacercariae by species of fish in 3 Provinces of Lao PDR

Species of fish	No. of fish examined	No. (%) of fish infected	No. of metacercariae detected		
			Total	Range	Average
Fish from Luang Prabang					
<i>Neolissochilus stracheyi</i>	2	2 (100)	4	1-3	2.0
<i>Cyclocheilichthys apogon</i>	4	1 (25.0)	430	-	430.0
<i>Hypsibarbus pierrei</i>	3	1 (33.3)	4	-	4.0
<i>Cyclocheilichthys furcatus</i>	4	1 (25.0)	3	-	3.0
Subtotal	13	5 (38.5)	441	1-430	88.2
Fish from Khammouane					
<i>Onychostoma fusiforme</i>	7	1 (14.3)	65	-	65.0
<i>Puntius brevis</i>	3	1 (33.3)	988	-	988
<i>Cyclocheilichthys armatus</i>	4	1 (25.0)	1	-	1.0
<i>Hampala dispar</i>	2	2 (100)	49	20-29	24.5
<i>Hypsibarbus wetmorei</i>	3	1 (33.3)	20	-	20.0
Subtotal	19	6 (31.6)	1,123	1-988	187.2
Fish from Saravane					
<i>Hampala dispar</i>	19	16 (84.2)	7,243	4-1,480	452.7
<i>Puntioplites proctoysron</i>	7	3 (42.9)	8	1-6	2.7
<i>Poropuntius dearatus</i>	6	2 (33.3)	10	3-7	5.0
<i>Puntius brevis</i>	10	1 (10.0)	1	-	1.0
<i>Paralaubuca barroni</i>	10	1 (10.0)	1	-	1.0
<i>Cyclocheilichthys enoplos</i>	5	1 (20.0)	6	-	6.0
Subtotal	57	24 (42.1)	7,269	1-1,480	302.9
Total	89	35 (39.3)	8,833	1-1,480	252.4

(37.5%) from Luang Prabang, and their infection rate was 70.8% and the average metacercarial density was 362 per infected fish. Total 20 (66.7%) out of 30 fish of 5 species (26.3%) from Khammouane were infected with av. 126 metacercariae. A total of 55 fish (74.3%) of 8 species (57.1%) from Saravane were infected with av. 214 metacercariae per fish. The infection status by surveyed areas and fish species are presented in Table 4.

Centrocestus formosanus metacercariae

Only 1 *C. formosanus* metacercaria was detected in 1 (25.0%) of 5 *Physoschistura meridionalis* from Luang Prabang. No metacercariae were detected in fish from Khammouane. Total 7 fish (29.2%) of 3 species (21.4%), i.e., *Mystacoleucus greenwayi*, *P. proctoysron*, and *Hypsibarbus pierrei*, from Saravane were infected with av. 3 metacercariae per fish. The infection status by surveyed areas and fish species are shown in Table 5.

DISCUSSION

In the present study, mainly 4 species of FBT metacercariae, i.e., *O. viverrini*, *H. taichui*, *H. yokogawai*, and *C. formosanus*,

Table 3. Infection status of *Haplorchis taichui* metacercariae by species of fish in 3 Provinces of Lao PDR

Species of fish	No. of fish examined	No. (%) of fish infected	No. of metacercariae detected		
			Total	Range	Average
Fish from Luang Prabang					
<i>Scaphognathops bandanensis</i>	7	7 (100)	7,095	22-3,965	1,013.6
<i>Henicorhynchus siamensis</i>	4	4 (100)	45	7-17	11.3
<i>Mekongina erythrospila</i>	10	9 (90.0)	1,317	57-308	146.3
<i>Physoschistura meridionalis</i>	5	4 (80.0)	7	1-4	1.8
<i>Cyclocheilichthys apogon</i>	4	3 (75.0)	828	92-526	276.0
<i>Cyclocheilichthys furcatus</i>	4	3 (75.0)	35	1-20	11.7
<i>Sinibrama melrosei</i>	7	5 (71.4)	62	1-41	12.4
<i>Mystacoleucus ectypus</i>	1	1 (100)	208	-	208.0
<i>Barbonymus gonionotus</i>	1	1 (100)	275	-	275.0
<i>Lobocheilos rhabdoura</i>	1	1 (100)	9	-	9.0
Subtotal	44	38 (86.4)	9,881	1-3,965	260.0
Fish from Khammouane					
<i>Hypsibarbus lagleri</i>	9	9 (100)	23,838	1,149-4,709	2,649
<i>Onychostoma fusiforme</i>	7	6 (85.7)	787	5-625	131.2
<i>Crossocheilus oblongus</i>	6	5 (83.3)	34	3-14	6.8
<i>Cyclocheilichthys repasson</i>	4	1 (25.0)	82	-	82.0
<i>Puntius brevis</i>	3	2 (66.7)	185	31-154	92.5
Subtotal	29	23 (79.3)	24,926	3-4,709	1,083.7
Fish from Saravane					
<i>Mystacoleucus greenwayi</i>	16	16 (100)	5,394	8-1,625	337.1
<i>Hampala dispar</i>	19	4 (21.1)	6,128	16-6,050	1,532
<i>Puntioplites proctozystron</i>	7	5 (71.4)	875	2-863	175.0
<i>Poropuntius dearatus</i>	6	5 (83.3)	1,113	82-608	222.6
<i>Paralaubuca barroni</i>	10	4 (40.0)	61	1-58	15.3
<i>Cyclocheilichthys enoplos</i>	5	3 (60.0)	1,038	14-986	346.0
<i>Esomus metallicus</i>	10	2 (20.0)	2	-	1.0
<i>Lobocheilos rhabdoura</i>	1	1 (100)	91	-	91.0
<i>Puntius orphoides</i>	1	1 (100)	3	-	3.0
Subtotal	75	41 (54.7)	14,705	1-6,050	358.7
Total	148	102 (68.9)	49,512	1-6,050	485.4

were detected in freshwater fish from 3 Provinces, i.e., Luang Prabang, Khammouane, and Saravane, in Lao PDR. Schotz et al. [15] found 5 species of trematode metacercariae, *O. viverrini*, *H. taichui*, *H. pumilio*, *Stellantchasmus falcatus*, and *C. formosanus*, in freshwater fishes from rice fields around Vientiane Municipality and Nam Ngum water reservoir, Lao PDR [15]. Rim et al. [16] also detected 2 (*O. viverrini* and *H. taichui*) and 4 (*O. viverrini*, *H. taichui*, *H. yokogawai* and *C. formosanus*) species of metacercariae in freshwater fish from Savannakhet Province and Vientiane Municipality, respectively [16]. On the other hand, Schotz et al. [15] examined 782 freshwater fish of 45 species by the flesh compression method [15]. Rim et al. [16] examined 156 freshwater fish of 17 species in Savannakhet and 177 fish of 12 species in Vientiane Municipality. They mainly used the artificial digestion method with the exception

of a few fish from Savannakhet which were examined by the flesh compression method [16]. In the present study, we examined a total of 242 fish of 40 species from Luang Prabang, Khammouane, and Saravane Province by the artificial digestion method.

As the second intermediate hosts for *O. viverrini*, 15 species of freshwater fish, i.e., *Barbonymus gonionotus*, *C. armatus*, *Cyclocheilichthys repasson*, *Esomus metallicus*, *H. dispar*, *Hampala macrolepidota*, *Hypsibarbus lagleri*, *Labiobarbus lineatus*, *Mystacoleucus marginatus*, *Onychostoma elongatum*, *Osteochilus hasseltii*, *Puntioplites falcifer*, *P. proctozystron*, *P. brevis*, and *Puntius orphoides*, have been reported in Thailand [18,19] and Lao PDR [15,16]. In the present study, *O. viverrini* metacercariae were detected in 4 fish species, *C. apogon*, *C. furcatus*, *H. pierrei*, and *N. stracheyi*, from Luang Prabang, in 5 fish species, *C. armatus*, *H. dispar*, *H.*

Table 4. Infection status of *Haplorchis yokogawai* metacercariae^a by species of fish in 3 Provinces of Lao PDR

Species of fish	No. of fish examined	No. (%) of fish infected	No. of metacercariae detected		
			Total	Range	Average
Fish from Luang Prabang					
<i>Scaphognathops bandanensis</i>	7	6 (85.7)	1,525	4-788	254.2
<i>Cyclocheilichthys apogon</i>	4	3 (75.0)	4,037	183-2,874	1,345.7
<i>Cyclocheilichthys furcatus</i>	4	3 (75.0)	443	19-312	147.7
<i>Sinibrama melrosei</i>	7	3 (42.9)	21	1-17	7.0
<i>Mystacoleucus ectypus</i>	1	1 (100)	128	-	128.0
<i>Lobocheilos rhabdoura</i>	1	1 (100)	4	-	4.0
Subtotal	24	17 (70.8)	6,158	1-2,874	362.2
Fish from Khammuan					
<i>Hypsibarbus lagleri</i>	9	9 (100)	1,387	67-274	154.1
<i>Onychostoma fusiforme</i>	7	6 (85.7)	898	18-553	149.7
<i>Crossocheilus oblongus</i>	6	1 (16.7)	2	-	2.0
<i>Cyclocheilichthys armatus</i>	4	3 (75.0)	201	-	201.0
<i>Cyclocheilichthys repasson</i>	4	1 (25.0)	29 5	-	17 9.7
Subtotal	30	20 (66.7)	2,517	2-553	125.9
Fish from Salavan					
<i>Mystacoleucus greenwayi</i>	16	16 (100)	6,002	6-975	375.1
<i>Hampala dispar</i>	19	8 (42.1)	1,249	5-1,011	156.1
<i>Puntiplites proctozyrson</i>	7	6 (85.7)	210	5-120	35.0
<i>Poropuntius dearatus</i>	6	6 (100)	1,507	173-408	251.2
<i>Paralaubuca barroni</i>	10	9 (90.0)	143	1-75	15.9
<i>Cyclocheilichthys enoplos</i>	5	5 (100)	2,516	47-2,210	503.2
<i>Puntius brevis</i>	10	4 (40.0)	102	1-92	25.5
<i>Hypsibarbus pierrei</i>	1	1 (100)	13	-	13.0
Subtotal	74	55 (74.3)	11,742	1-2,210	213.5
Total	128	92 (71.9)	20,417	1-2,874	221.9

^aThese were encysted chiefly in the fin and scale of fish.

Table 5. Infection status of *Centrocestus formosanus* metacercariae by species of fish in 2 Provinces of Lao PDR

Species of fish	No. of fish examined	No. (%) of fish infected	No. of metacercariae detected		
			Total	Range	Average
Fish from Luang Prabang					
<i>Physoschistura meridionalis</i>	5	1 (25.0)	1	-	1.0
Fish from Saravane					
<i>Mystacoleucus greenwayi</i>	16	4 (25.0)	17	1-8	4.3
<i>Puntiplites proctozyrson</i>	7	2 (28.6)	4	1-3	2.0
<i>Hypsibarbus pierrei</i>	1	1 (100)	1	-	1.0
Subtotal	24	7 (29.2)	22	1-8	3.1
Total	29	8 (27.6)	23	1-8	2.9

wetmorei, *O. fusiforme* and *P. brevis* from Khammouane, and 6 fish species, *C. enoplos*, *H. dispar*, *P. barroni*, *P. dearatus*, *P. proctozyrson*, and *P. brevis* from Saravane Province. Accordingly, 9 fish species, *C. apogon*, *C. enoplos*, *C. furcatus*, *H. pierrei*, *H. wetmorei*,

N. stracheyi, *O. fusiforme*, *P. barroni*, and *P. dearatus*, are newly recorded as the second intermediate hosts for *O. viverrini* by the present study.

The infection rates and intensities of *O. viverrini* metacercariae were relatively low in fish from 3 Provinces in the present study. However, 3 particular fish species, *C. apogon* from Luang Prabang, *P. brevis* from Khammouane, and *H. dispar* from Saravane Province, were heavily infected with *O. viverrini* metacercariae. Among them, *P. brevis* and *H. dispar* were already known as highly suitable fish hosts for *O. viverrini* in a previous study [16]. These 3 fish species together with another 2, *C. armatus* and *O. elongatum*, are highly dangerous due to the high intensity of metacercariae when they were eaten raw by humans. The liver flukes, like *O. viverrini*, provoke severe pathological changes in the bile duct, such as dilatation, wall thickening, inflammation and mucosal hyperplasia, and the cirrhotic changes of the liver [18]. Moreover, they act as a risk factor for development of cholangiocarcinoma in humans [20,21].

H. taichui metacercariae have been recorded in fish from

some Asian countries, i.e., India, China, Thailand, the Philippine, and Lao PDR. They have been detected in 35 fish species, i.e., *Abbottina rivularis*, *Amblypharyngodon mola*, *B. gonionotus*, *Carasius auratus*, *Channa striata*, *Chanodichthys dabryi*, *Cirrhinus molitorella*, *Culter recurviceps*, *C. armatus*, *C. repasson*, *Cyprinus carpio*, *H. dispar*, *H. macrolepidota*, *Hemibarbus maculatus*, *Hemiculter leucisculus*, *Hypophthalmichthys molitrix*, *L. leptocheila*, *Labeo ariza*, *Labeo bata*, *Metzia lineata*, *M. marginatus*, *O. elongatum*, *Opsariichthys bidens*, *Pseudohemiculter dispar*, *P. falcifer*, *Puntius binotatus*, *P. brevis*, *P. orphoides*, *Puntius semifasciolatus*, *Puntius sophore*, *Saugogobio dabryi*, *Spratellicypris palata*, *Squalidus argentatus*, and *Toxabramis houdemeri*, in endemic countries [15,16,22-26]. In the present study, *H. taichui* metacercariae were found in 9 fish species, *B. gonionotus*, *C. apogon*, *C. furcatus*, *Henicorhynchus siamensis*, *L. rhabdoura*, *Mekongina erythrospila*, *Physoschistura meridionalis*, *Scaphognathops bandanensis*, and *Sinibrama melrosei*, from Luang Prabang, in 5 fish species, *Crossocheilus oblongus*, *C. repasson*, *H. lagleri*, *O. fusiforme*, and *P. brevis*, from Khammouane, and 9 fish species, *C. enoplos*, *E. metallicus*, *H. dispar*, *L. rhabdoura*, *Mystacoleucus greenwayi*, *P. barroni*, *P. dearatus*, *P. proctozyrson*, and *P. orphoides*, from Saravane Province. Therefore, 13 fish species, *C. oblongus*, *C. apogon*, *C. enoplos*, *C. furcatus*, *E. metallicus*, *H. lagleri*, *H. siamensis*, *L. rhabdoura*, *M. erythrospila*, *M. greenwayi*, *P. meridionalis*, *S. bandanensis*, and *S. melrosei*, are added as new second intermediate hosts for *H. taichui* by the present study.

The infection status of *H. taichui* metacercariae was relatively high in fish from all 3 Provinces. Infection rates of fish from Luang Prabang (86.4%) and Khammouane (79.3%) were very high, and that of fish from Saravane (54.7%) was moderate. Intensities of *H. taichui* metacercariae per infected fish were 260, 1,084, and 359 in fish from Luang Prabang, Khammouane, and Saravane Province, respectively. Particularly 3 fish species, *S. bandanensis* from Luang Prabang, *H. lagleri* from Khammouane, and *H. dispar* from Saravane Province, were heavily infected with *H. taichui* metacercariae, more than 1,000 in number. On the other hand, Chai et al. [6] demonstrated that the worm burden of *H. taichui* was remarkably high in residents of Saravane Province [6]. Based on these findings and high infection rates and intensities of *H. taichui* metacercariae in the present study, we could speculate that residents in Khammouane and Luang Prabang may be heavily infected with *H. taichui* like Saravane.

Distribution of *H. yokogawai* metacercariae has been reported in some Asian countries such as India, Thailand, and Lao

PDR. They were found in 28 fish species, *A. mola*, *B. gonionotus*, *Channa punctatus*, *C. molitorella*, *C. armatus*, *C. repasson*, *Cyprinus carpio*, *Glossogobius giuris*, *H. dispar*, *Hypsibarbus wetmorei*, *H. lagleri*, *Labeo ariza*, *L. bata*, *L. leptocheila*, *Mystus vittatus*, *Nandus nandus*, *Ompok bimaculatus*, *Oreochromis niloticus niloticus*, *O. elongatum*, *O. hasseltii*, *Osteochilus lini*, *P. binotatus*, *Puntius chola*, *Puntius leiacanthus*, *P. orphoides*, *Puntius sarana*, *P. sophore*, and *Tilapia zillii* [16,23,25,27-31]. In the present study, *H. yokogawai* metacercariae were detected in 6 fish species, *C. apogon*, *C. furcatus*, *L. rhabdoura*, *M. ectypus*, *S. bandanensis*, and *S. melrosei*, from Luang Prabang, 5 fish species, *C. oblongus*, *C. armatus*, *C. repasson*, *H. lagleri* and *O. fusiforme*, from Khammouane, and 8 fish species, *C. enoplos*, *H. dispar*, *H. pierrei*, *M. greenwayi*, *P. barroni*, *P. dearatus*, *P. proctozyrson*, and *P. brevis*, from Saravane. Among them, 15 fish species, i.e., *C. oblongus*, *C. apogon*, *C. enoplos*, *C. furcatus*, *H. pierrei*, *L. rhabdoura*, *M. ectypus*, *M. greenwayi*, *O. fusiforme*, *P. barroni*, *P. dearatus*, *P. proctozyrson*, and *P. brevis*, *S. bandanensis* and *S. melrosei*, have never been reported as the second intermediate hosts of *H. yokogawai* in the available literature. Therefore, we record aforementioned 15 fish species as new second intermediate hosts for *H. yokogawai*.

C. formosanus is distributed in China, Taiwan, Japan, the Philippines, India, and Lao PDR [15,16,32-34]. Schotz et al. [15] detected *C. formosanus* metacercariae in only 1 fish species, *Esomus longimanus*, among 45 fish species examined. Rim et al. [16] found them in 4 fish species, *C. repasson*, *P. brevis*, *O. hasseltii*, and *C. molitorella*, in Vientiane Municipality. In the present study, *C. formosanus* metacercariae were detected in 1 fish species, *P. meridionalis*, from Luang Prabang, and 3 fish species, *M. greenwayi*, *P. proctozyrson*, and *H. pierrei*, from Saravane. Collectively, total 9 fish species, *C. molitorella*, *C. repasson*, *E. longimanus*, *H. pierrei*, *M. greenwayi*, *O. hasseltii*, *P. proctozyrson*, and *P. brevis*, are listed as the second intermediate hosts for *C. formosanus* in Lao PDR.

It has been proved that *O. viverrini* is more endemic in humans and fish in Vientiane Municipality and Savannakhet Province, and *H. taichui* is highly endemic in humans and fish in Saravane Province [6,7,16]. From this study, it is suggested that Luang Prabang and Khammouane are highly endemic areas of intestinal flukes, *H. taichui* and *H. yokogawai*, rather than *O. viverrini*.

ACKNOWLEDGMENTS

We thank Dr. Bounnaloth Insisiengmay, a staff of Depart-

ment of Hygiene and Prevention, Ministry of Public Health, Vientiane, Ms. Souvanny Phommakone, a technical staff of LAPRec, Ministry of Agriculture and Irrigation, Vientiane, and Mr. Bounthong Sengvilaykham, deputy head in Livestock and Fisheries Section, Division of Agriculture and Forestry of Savannakhet Province, Lao PDR, for their help in collection and identification of fish. We also thank the staff of the Korea Association of Health Promotion, Seoul, the Republic of Korea, who participated in the Korea-Laos Cooperation Project on Parasite Control in Lao PDR (1999-2004).

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