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▣ ORIGINAL ARTICLE

Infection Status of Zoonotic Trematode Metacercariae in Fishes from Vientiane Municipality and Champasak Province in Lao PDR

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Abstract: The infection status of fishborne zoonotic trematode (FZT) metacercariae was investigated in fishes from 2 localities of Lao PDR. Total 157 freshwater fishes (17 species) were collected in local markets of Vientiane Municipality and Champasak Province in December 2010 and July 2011, and each fish was examined by the artificial digestion method. Total 6 species of FZT metacercariae, i.e., *Opisthorchis viverrini, Haplorchis taichui, Haplorchis yokogawai, Haplorchis pumilio, Centrocestus formosanus,* and *Procerovum varium*, were detected in fishes from Vientiane Municipality. The metacercariae of *O. viverrini* were detected in 50 (49.5%) out of 101 fishes (6 species), and their average number was 154 per fish infected. The remaining 5 species of heterophyid metacercariae were detected in 36.8%, 65.8%, 9.4%, 23.9%, and 5.1% fishes examined, and their average densities were 12, 1,038, 4, 15, and 13 per infected fish, respectively. In fishes from Champasak Province, 3 species of FZT metacercariae, i.e., *O. viverrini*, *H. taichui*, and *H. yokogawai*, were detected. Only 2 *O. viverrini* metacercariae were found in only 1 *Barbonymus schwanefeldi*. The metacercariae of *H. taichui* and *H. yokogawai* were detected in 60.0% and 50.0% of fishes examined, and their average densities were 47 and 28 per fish infected. By the present study, it has been confirmed that several species of FZT metacercariae are prevalent in fishes from Vientiane Municipality, with *P. varium* being a new member of FZT in Lao PDR. In comparison, FZT metacercariae are less prevalent in fishes from Champasak Province.

Key words: *Opisthorchis viverrini, Haplorchis taichui, Haplorchis yokogawai, Haplorchis pumilio, Centrocestus formosanus, Procerovum varium*, fishborne trematode (FBT) metacercaria, Lao PDR

INTRODUCTION

Mekong river basin in Lao People's Democratic Republic (Lao PDR) is known to be endemic areas of infections with fishborne zoonotic trematodes (FZT). Riparian Laotian peoples habitually consume raw or fermented freshwater fish, and thus they are easily infected with FZT [1,2]. Especially in stool examinations and worm recoveries, it has been reported that so many Laotian peoples are infected with FZT including *Opisthorchis viverrini* and heterophyid flukes [3-9].

Moreover, epidemiological studies on the second intermediate hosts (or the infection sources) of FZT were also performed in riverside areas of Lao PDR [9-13]. Schotz et al. [10] surveyed freshwater fishes from rice paddies and Nam Ngum water reservoir in Vientiane Province. Rim et al. [11,12] and Sohn et al. [9] investigated the infection status of FZT metacercariae in fishes from Vientiane Municipality, Savannakhet, Luang Prabang, Khammouane, and Saravane Provinces in Lao PDR. In the present study, to obtain further epidemiological information on FBT infections in Lao PDR, we additionally surveyed on the infection status of FBT metacercariae in freshwater fishes from Vientiane Municipality and Champasak Province (Fig. 1).

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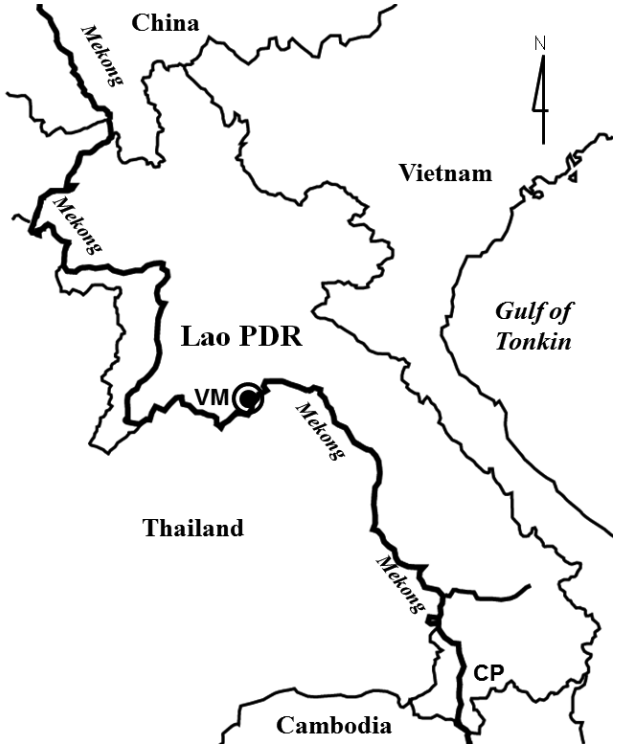

Fig. 1. Surveyed areas of Vientiane Municipality (VM) and Champasack Province (CP), Lao PDR.

MATERIALS AND METHODS

We collected total 157 freshwater fishes in 17 species in local markets of Vientiane Municipality (117 fishes in 10 species) on December 2010 and July 2011, and Champasack Province (40 fishes in 10 species) on December 2010 (Fig. 1). All fishes were transferred to the laboratories (on-the-spot local laboratories in Lao PDR and in Department of Parasitology and Tropical Medicine, Gyeongsang National University School of Medicine), and the fish species were identified with a book [14] and FishBase website (http://www.fishbase.org/search.php) (Tables 1, 3). Individual fish was finely ground with a mortar with pestle or a grinder. The ground fish meat was mixed with artificial gastric juice, and the mixture was incubated at 36℃ for 2 hr. The digested material was filtered with a sieve (1×1 mm of mesh), and washed with 0.85% saline until the supernatant became clear. The sediment was carefully examined under a stereomicroscope, and metacercariae were collected based on general features. These collected metacercariae were then categorized according to the measurements and morphological characteristics, and the intensity of infection and the infection rate were calculated for each fish species.

Identified metacercariae were experimentally infected to cats to obtain adult flukes. At day 7 after the infection, cats were killed by peritoneal injection with animal anesthetics. The small intestines of cats were extracted and longitudinally opened with a scissors in a beaker with 0.85% saline. Adult flukes were recovered in the sediment of intestinal contents which were diluted with 0.85% saline. Recovered worms were fixed with 10% formalin under a cover glass pressure, stained with Semichon's acetocarmine, and observed under a light microscope with a micrometer.

RESULTS

Infection status of FZT metacercariae in fishes from Vientiane Municipality

The metacercariae of *Haplorchis taichui* were found in 43 (36.8%) out of 117 fishes (in 10 species) examined, and their average density was 12 per fish infected. *Haplorchis yokogawai* metacercariae were detected in 77 (65.8%) fishes, and their average density was 1,038 per fish infected. *Haplorchis pumilio* metacercariae were found in 11 (9.4%) fishes, and their average density was 4 per fish infected. *Centrocestus formosanus* metacercariae were detected in 28 (23.9%) fishes, and their average number was 14.6 per fish infected. The detailed infection status of heterophyid fluke metacercariae is shown in Table 1. The metacercariae of *Procerovum varium* were found in 6 (37.5%) out of 16 climbing perch, *Anabas testudineus*, and their average density was 12.7 per fish infected.

The metacercariae of *Opisthorchis viverrini* were detected in 50 (49.5%) out of 101 fishes in 6 species (60.0%), i.e., *Cyclocheilichthys apogon*, *Cyclocheilichthys armatus*, *Puntius brevis*, *Cyclocheilichthys repasson*, *Anabas testudineus*, and *Henichorhynchus siamensis*. Their average density was 154 per fish infected. The detailed infection status is designated in Table 2.

Infection status of FZT metacercariae in fishes from Champasak Province

The metacercariae of *H. taichui* were detected in 24 (60.0%) out of 40 fishes (all 10 species), and their average density was 47 per fish infected. Total 20 (50.0%) fishes were infected with 28 *H. yokogawai* metacercariae in average. The infection status of heterophyid fluke metacercariae is shown in Table 3. Only 2 *O. viverrini* metacercariae were found in 1 fish (50.0%) of *Barbonymus schwanefeldi* from Champasak Province.

Table 1. Infection status of heterophyid fluke metacercariae in fishes from Vientiane Municipality, Lao PDR

Fish species examined	No. of fish examined	*Haplorchis taichui*		*Haplorchis yokogawai*		Haplorchis pumilio		*Centrocestus formosanus*	
		No (%) of fish infected	Average (range)[a]	No (%) of fish infected	Average (range)[a]	No (%) of fish infected	Average (range)[a]	No (%) of fish infected	Average (range)[a]
Cyclocheilichthys repasson	25	6 (24.0)	2.5 (1-4)	20 (80.0)	22 (1-80)	0	-	0	-
Cyclocheichthys armatus	20	6 (30.0)	2.3 (1-5)	20 (100)	2,450 (860-5,500)	0	-	4 (20.0)	6.3 (1-9)
Anabas testudineus	16	0	-	3 (18.8)	4 (1-7)	7 (43.8)	5.6 (1-21)	10 (62.5)	5.3 (1-18)
Henicorhynchus siamensis	15	15 (100)	25 (5-47)	0	-	3 (20.0)	1.3 (1-2)	4 (26.7)	1.3 (1-2)
Cyclocheilichthys apogon	15	0	-	15 (100)	1,667 (187-3,835)	0	-	0	-
Puntius brevis	10	5 (50.0)	1.2 (1-2)	10 (100)	93 (7-275)	0	-	10 (100)	32.8 (5-80)
Cirrhinus molitorella	10	8 (80.0)	13 (5-28)	4 (40.0)	52 (1-195)	0	-	0	-
Osteochilus hasseltii	2	1 (50.0)	5	1 (50.0)	3	1 (50.0)	1	0	-
Osteochilus lini	2	2 (100)	3.0 (1-5)	2 (100)	1,940 (260-3,620)	0	-	0	-
Puntius orpoides	2	0	-	2 (100)	226 (5-446)	0	-	0	-
Total	117	43 (36.8)	12 (1-47)	77 (65.8)	1,038 (1-5,500)	11 (9.4)	4.0 (1-21)	28 (23.9)	14.6 (1-80)

[a]No. of metacercariae detected.

Table 2. Infection status of *Opisthorchis viverrini* metacercariae in fishes from Vientiane Municipality in Lao PDR

Fish species examined	No. of fish examined	No. (%) of fish infected	No. of metacercariae detected		
			Total	Range	Average
Cyclocheilichthys apogon	15	15 (100)	4,562	26-1,509	304.1
Cyclocheilichthys armatus	20	20 (100)	2,901	1-359	145.1
Puntius brevis	10	10 (100)	239	1-85	23.9
Cyclocheilichthys repasson	25	3 (12.0)	16	2-10	5.3
Anabas testudineus	16	1 (6.3)	2	-	2
Henichorhynchus siamensis	15	1 (6.7)	1	-	1
Total	101	50 (49.5)	7,721	1-1,509	154.4

Morphology of metacercariae and adults of *Procerovum varium* (unit; μm)

Metacercariae (n = 15; Fig. 2): Cyst elliptical, 150-193 (175) × 118-148 (136) in size, having yellow brownish pigment granules scattering in body areas of intestinal bifurcation, a pair of eyespots lateral to pharynx, a ventral sucker deflectively located from median, a long thick-walled expulser, and a D-shaped (half-moon-shaped) excretory bladder with grouped granules.

Adults (n = 10; Fig. 3): Body small, pear-shaped, 280-325 (304) long, and 165-215 (186) wide, with greatest width at posterior 1/3. Oral sucker subterminal, 25-38 (32) by 38-43 (41). Pharynx subglobular or elliptical, 20-28 (25) by 15-25 (19). Esophagus short, 35-50 (43) in length. Ventral sucker very small, 13-20 (17) by 18-25 (21), embedded in ventrogenital sac. Expulsor long and thick-walled, 88-150 (123) by 15-23 (20). Ovary spherical or subspherical, 30-45 (38) by 35-53 (43), slightly dextral to midline. Seminal receptacle long saccular, 70-105 (75) by 18-38 (28), lying between expulsor and the left-side of testis. One testis globular or subglobular, 75-103 (87) by 88-115 (99), situated in middle of hind-body. Uterus with eggs occufying from anterior 1/3 to posterior end, most of hind-body. Vitellaria follicular, distributing from posterior border of ovary to posterior extremity. Eggs small, yellow, and 25-28 (27) by 13-15 (14).

DISCUSSION

In the present study, 6 species of FZT metacercariae, i.e., *O. viverrini*, *H. taichui*, *H. yokogawai*, *H. pumilio*, *C. formosanus*, and *P. varium*, were detected in fishes from Vientiane Municipality. Schotz et al. [10] found 5 species (*O. viverrini*, *H. taichui*,

Table 3. Infection status of heterophyid fluke metacercariae in fishes from Champasak Province, Lao PDR

Fish species examined	No. of fish examined	*Haplorchis taichui*		*Haplorchis yokogawai*	
		No (%) of fish infected	Average (range)[a]	No (%) of fish infected	Average (range)[a]
Anabas testudineus	10	1 (10.0)	1	2 (20.0)	1
Mystacoleucus marginatus	10	10 (100)	68.1 (6-218)	8 (80.0)	8.6 (2-23)
Barbonymus gonionotus	8	4 (50.0)	29.0 (2-62)	4 (50.0)	4.0 (1-11)
Puntioplites proctozysron	3	2 (66.7)	22.5 (11-34)	1 (33.3)	3
Henicorhynchus siamensis	2	1 (50.0)	2	0	-
Barbonymus schwanefeldi	2	2 (100)	45.0 (3-87)	2 (100)	12.0 (1-23)
Cyclocheilichthys repasson	2	2 (100)	22.5 (11-34)	2 (100)	217 (9-425)
Puntioplites falciper	1	1 (100)	18	0	-
Hampala dispar	1	1 (100)	130	1 (100)	3
Probarbus labeaminor	1	1 (100)	1	0	-
Total	40	24 (60.0)	47.0 (1-218)	20 (50.0)	27.6 (1-425)

[a]No. of metacercariae detected .

H. pumilio, *Stellantchasmus falcatus*, and *C. formosanus*) of FZT metacercariae in the fishes from Vientiane Province. Rim et al. [11] detected 4 species (*O. viverrini*, *H. taichui*, *H. yokogawai and C. formosanus*) of FZT metacercariae in fishes from Vientiane Municipality. In Rim et al. [12], 4 species (*O. viverrini*, *H. taichui*, *H. yokogawai*, and *C. formosanus*) of FZT metacercariae were also detected in fishes from 3 Provinces, i.e., Luang Prabang, Khammouane, and Saravane. Sohn et al. [9] detected 3 *Haplorchis species*, *H. taichui*, *H. yokogawai*, and *H. pumilio*, metacercariae in fishes from Luang Prabang Province. However, *P. varium* metacercariae were detected for the first time in fishes from Lao PDR in the present study, although these metacercariae were detected in only 1 fish species, *Anabas testudineus*. Meanwhile, *P. varium* metacercariae were detected in more than 10 fish species including *A. testudineus* from an adjacent country, Vietnam [15-17].

The metacercariae of *P. varium* (175×136 μm) detected in the present study were slightly smaller than those (187×147) of the previous study performed in Vietnam [17]. However, their general morphologies, i.e., elliptical in shape, presence of brownish pigment granules in the worm body, a pair of eyespots lateral to the pharynx, a thick-walled expulsor, and a D-shaped excretory bladder, were almost identical to those of the previous studies [17,18].

Trematodes in the genus *Procerovum* (Heterophyidae) are characterized by possessing a single testis and a long prominent seminal vesicle modified into an expulsor. Only 3 species, i.e., *P. varium*, *P. calderoni*, and *P. cheni*, have been certified for their validity based on the morphology of the seminal vesicle. Our specimens (304×186 μm) were somewhat smaller than those of Chai et al. [17] (434×223 μm), which were recovered from a hamster experimentally infected with the metacercariae from fish of Vietnam. The length of expulsor

Fig. 2. A metacercaria of *Procerovum varium* detected in a climbing perch, *Anabas testudineus*, from a local fish market of Vientiane Municipality, Lao PDR. It is elliptical and 175×136 μm in average size, and has brownish pigment granules, a muscular oral sucker (OS), a pair of eyespots (arrow) lateral to pharynx, a ventral sucker deflectively located from median, a thick-walled expulsor, and a D-shaped excretory bladder (EB) with grouped granules. Scale bar is 50 μm.

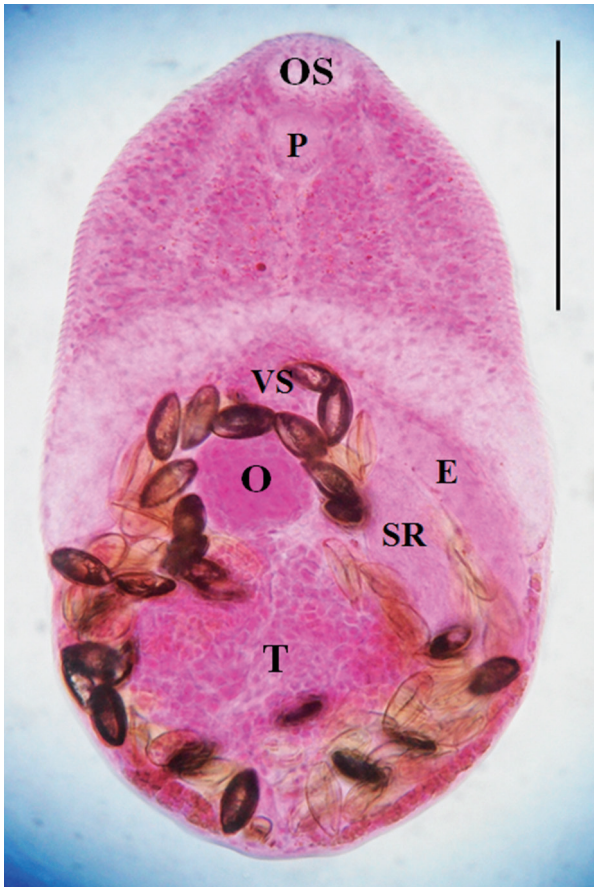

Fig. 3. An adult of *P. varium* recovered in the small intestine of cat experimentally infected with metacercariae at day 7 after infection. Its body is small, 304×186 μm in average size, has a muscular oral sucker (OS) and pharynx (P), a small ventral sucker (VS), a long and thick-walled expulsor (E), a long saccular seminal receptacle (SR), a spherical ovary (O), single globular testis (T), and follicular vitellaria distributing in post-ovarian fields. Scale bar is 100 μm.

(123 μm) is more or less longer than that in Chai et al. [17] (115 μm). In spite that there were small differences between the 2 studies, we were able to identify our samples as *P. varium* by the presence of a saccular seminal vesicle with thick-walled chambers, an expulsor, less than 160 μm long. By comparison, *P. calderoni* has a very long expulsor measuring more than 200 μm, and *P. cheni* has a bipartite seminal vesicle with thin-walled chambers less than 100 μm long [17,18].

Schotz et al. [10] surveyed total 782 freshwater fishes (45 spp.) from Vientiane Province. Rim et al. [11] examined 156 fishes (17 spp.) from Savannakhet and 177 fishes (12 spp.) from Vientiane Municipality. Rim et al. [12] investigated total 242 freshwater fishes (40 spp.) from 3 Provinces, i.e., Luang Prabang, Khammuane, and Saravane. Sohn et al. [9] examined 207 freshwater fish (17 spp.) purchased in a market in Luang Prabang Province. In the present study, we also examined total 157 fishes (17 spp.) from Vientiane Municipality and Champasack Province. However, the number of fish (40 fishes) from Champasack Province was not enough to evaluate the endemicity of trematode infections.

As the second intermediate hosts for *O. viverrini*, about 35 species of freshwater fish have been reported in Thailand, Cambodia, and Lao PDR [10-12,19-21]. In the present study, *O. viverrini* metacercariae were detected in 6 fish species, *A. testudineus, C. apogon, C. armatus, C. repasson, H. siamensis*, and *P. brevis*, from Vientiane Municipality, and in only 1 species, *B. schwanefeldi*, from Champasack Province. Among these fish hosts, *A. testudineus* is newly reported as the second intermediate host for *O. viverrini* by the present study.

The infection rates and intensities of *O. viverrini* metacercariae in fishes from Vientiane Municipality were much higher than those in fishes from Champasack Province. However, they were relatively low in the level when we compared these with those of the previous studies performed in Vientiane Municipality, Savannakhet, Khammuane, and Saravane Provinces [11,12]. On the other hand, it has been known that 5 fish species, i.e., *Onychostoma elongatum, C. armatus, C. apogon, P. brevis,* and *Hampala dispar*, are highly susceptible fish hosts for *O. viverrini* metacercariae in Lao PDR [11,12]. In the present study, 3 fish species, i.e., *C. apogon, C. armatus*, and *P. brevis*, from Vientiane Municipality were highly and heavily infected with *O. viverrini* metacercariae.

About 48 fish species have been recorded as the second intermediate hosts for *H. taichui* in endemic Asian countries, i.e., India, China, the Philippines, Tailand, Vietnam, and Lao PDR [9-12,22-26]. In the present study, *H. taichui* metacercariae were detected in total 15 fish species. Among them, 6 species, *Osteochilus hasseltii, Osteochilus lini, A. testudineus, B. schwanefeldi, Puntioplites proctozysron*, and *Probarbus labeaminor*, have not been reported as the fish hosts of *H. taichui* yet. However, the infection rates and densities were relatively low when they were compared with those of previous studies, especially in fishes from 3 Provinces, Luang Prabang, Khammuane, and Saravane, in Lao PDR [12]. In addition, it is known that the worm burden of *H. taichui* is remarkably high among the residents of aforementioned 3 Provinces by the results of worm recovery after praziquantel treatment and $MgSO_4$ purgation [5-7,9].

The metacercariae of *H. yokogawai* have been detected in 47

fish species from some Asian and Middle East countries such as India, Egypt, Thailand, Cambodia, and Lao PDR [11,23,25,27-31]. In the present study, they were found in 9 and 7 fish species from Vientiane Municipality and Champasack Province, respectively. Among these fish hosts, 3 species, *A. testudineus, B. schwanefeldi,* and *Mystacoleucus marginatus*, are to be recorded as the new second intermediate hosts of *H. yokogawai.*

As the second intermediate hosts of *C. formosanus*, 12 species of freshwater fishes, i.e., *Acheilognathus tonkinensis, Cirrhinus molitorella, C. armatus, C. repasson, Esomus longimanus, Hypsibarbus pierrei, Mystacoleucus greenwayi, O. hasseltii, Opsariichthys bidens, P. proctozysron,* and *P. brevis,* have been reported in Lao PDR [10-13,32]. In the present study, the metacercariae of *C. formosanus* were found in 4 fish species, *A. testudineus, C. armatus, H. siamensis,* and *P. brevis*, from Vientiane Municipality. Therefore, the 2 species, *A. testudineus* and *H. siamensis,* are added among the list of fish intermediate hosts for *C. formosanus* in Lao PDR.

It was collectively proved that *O. viverrini* is more endemic in fish intermediate hosts in Vientiane Municipality, but it is less endemic in Champasack Province although Sayasone et al. [33] reported a high prevalence (64.3%) of opisthorchiasis among 669 residents of Champasack Province. Four heterophyid species, i.e., *H. taichui, H. pumilio, C. formosanus,* and *P. varium*, except for *H. yokogawai*, are not prevalent in fishes from Vientiane Municipality, and 2 species, i.e., *H. taichui* and *H. yokogawai*, are less prevalent in fishes from Champasack Province. However, to reveal the exact infection status of FZT in humans and fishes from Champasack Province, the more profound studies should be performed at near future.

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CONFLICT OF INTEREST

The authors have no conflicts of interest concerning the work reported in this paper.

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