

# Zoonotic Trematode Metacercariae in Fish from Phnom Penh and Pursat, Cambodia

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**Abstract:** A survey was performed to investigate the infection status of freshwater fish with zoonotic trematode metacercariae in Phnom Penh and Pursat Province, Cambodia. All collected fish with ice were transferred to our laboratory and examined using the artificial digestion method. In fish from Phnom Penh, 2 kinds of metacercariae (*Opisthorchis viverrini* and *Haplorchis yokogawai*) were detected. *O. viverrini* metacercariae were positive in 37 (50.0%) of 74 fish in 11 species (average no. metacercariae/fish, 18.6). *H. yokogawai* metacercariae were detected in 23 (57.5%) of 40 fish in 5 species (average no. metacercariae/fish, 21.0). In fish from Pursat Province, 5 kinds of metacercariae (*O. viverrini*, *H. yokogawai*, *Haplorchis pumilio*, *Centrocestus formosanus*, and *Procerovum* sp.) were detected; *O. viverrini* metacercariae (n=3) in 2 fish species (*Henicorhynchus lineatus* and *Puntioplites falcifer*), *H. yokogawai* metacercariae (n=51) in 1 species (*P. falcifer*), *H. pumilio* metacercariae (n=476) in 2 species (*H. lineatus* and *Pristolepis fasciata*), *C. formosanus* metacercariae (n=1) in 1 species (*H. lineatus*), and *Procerovum* sp. metacercariae (n=63) in 1 species (*Anabas testudineus*). From the above results, it has been confirmed that various freshwater fish play the role of a second intermediate host for zoonotic trematodes (*O. viverrini*, *H. yokogawai*, *H. pumilio*, *C. formosanus*, and *Procerovum* sp.) in Cambodia.

**Key words:** *Opisthorchis viverrini*, *Haplorchis yokogawai*, *Haplorchis pumilio*, *Centrocestus formosanus*, *Procerovum* sp., zoonotic trematode, Cambodia

## INTRODUCTION

Zoonotic trematode infections are public health problems in Asian countries, including Lao People's Democratic Republic (Lao PDR), Vietnam, Thailand, China, and Korea. Especially, fishborne trematodes (FBT) provoke a remarkable morbidity among local people as well as a serious damage in aquaculture industry [1-3]. FBT infections in humans are almost entirely caused by habitual consumption of raw fish containing infective larvae (=metacercariae). These infections are highly localized in riverside areas, especially where riparian populations have the raw fish eating habit. It has been known that

riverside areas in Southeast Asia, especially the Mekong river basin in Vietnam, Lao PDR, and Thailand, are highly endemic with FBT infections [4-9].

Cambodia is located in the southern part of the Indochina Peninsula in Southeast Asia, and bordered by Thailand to the northwest, Lao PDR to the northeast, Vietnam to the east, and the Gulf of Thailand to the southwest. Administratively, it is divided into 24 provinces including Phnom Penh Municipality which is located in the central south region, and on the banks of the Tonle Sap Lake, Mekong, and Bassac rivers. Pursat Province is located in the western part of the country and between the Tonle Sap Lake and the northern end of the Cardamom Mountains [10].

It has been known that many Cambodian people are infected with helminth parasites, such as soil-transmitted nematodes and FBT including *Opisthorchis viverrini* [11-13]. This was re-confirmed by fecal examinations during the Korea-Cambodia International Collaboration Project on Intestinal Parasite Con-

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trol in Cambodia (2006-2011) [14,15]. With regard to FBT infections, several investigators have previously reported that *O. viverrini* are prevalent in some limited areas of Cambodia [14-17]. Recently, Touch et al. [17] reported infection of freshwater fish with *O. viverrini* metacercariae in areas adjacent to the Lake 500 near the border of Kandal and Takeo Provinces, southern Cambodia [17]. Subsequently, adult *O. viverrini* specimens were recovered from humans and experimental animals, and metacercariae were detected in fish from Takeo and Kratie Provinces [15,16]. However, in other localities of Cambodia, no studies have been available on FBT metacercarial infections in freshwater fish. Therefore, in this study, we investigated on the infection status of freshwater fish with FBT metacercariae in Phnom Penh and Pursat Province, Cambodia.

**Table 1.** Infection status of freshwater fish from Phnom Penh Municipality, Cambodia, with *Opisthorchis viverrini* metacercariae

Fish species <sup>a</sup>	No. of fish examined	No. (%) of fish infected	No. of metacercariae detected		
			Total	Range	Average
June 2006 and December 2007 (total 80 fish in 12 species)					
<i>Henicorhynchus siamensis</i>	9	3 (33.3)	52	4-43	17.3
<i>Barbonymus schwanefeldi</i>	5	5 (100)	373	11-128	74.6
<i>Osteochilus melanopleurus</i>	5	4 (80.0)	36	1-18	9.0
<i>Luciosoma bleekeri</i>	2	1 (50.0)	1	-	1.0
<i>Anabas testudineus</i>	22	0	-	-	-
<i>Catlocarpio siamensis</i>	10	0	-	-	-
<i>Pristolepis fasciata</i>	8	0	-	-	-
<i>Hypsibarbus lagleri</i>	6	0	-	-	-
<i>Rhynogobio</i> sp.	5	0	-	-	-
<i>Thynnichthys thynnoides</i>	4	0	-	-	-
<i>Cirrhinus jullieni</i>	2	0	-	-	-
<i>Henicorhynchus lineatus</i>	2	0	-	-	-
November 2010 (total 104 fish in 15 species)					
<i>Cirrhinus microlepis</i>	12	4 (33.3)	5	1-2	1.3
<i>Puntiplites proctozyron</i>	10	7 (70.0)	60	1-30	8.6
<i>Cirrhinus jullieni</i>	10	6 (60.0)	137	2-110	22.8
<i>Labeo chrysophekadion</i>	10	4 (40.0)	6	1-2	1.5
<i>Thynnichthys thynnoides</i>	7	1 (14.3)	1	-	1.0
<i>Barbonymus altus</i>	3	1 (33.3)	18	-	18.0
<i>Henicorhynchus lobatus</i>	1	1 (100)	1	-	1.0
<i>Henicorhynchus siamensis</i>	15	0	-	-	-
<i>Paralabuca typus</i>	9	0	-	-	-
<i>Syncrossus helodes</i>	9	0	-	-	-
<i>Lobocheilos rhabdoura</i>	7	0	-	-	-
<i>Luciosoma bleekeri</i>	6	0	-	-	-
<i>Osteochilus melanopleurus</i>	2	0	-	-	-
<i>Yasuhikotakia modesta</i>	2	0	-	-	-
<i>Yasuhikotakia lecontei</i>	1	0	-	-	-

<sup>a</sup>A total of 184 fish in 22 species were examined.

## MATERIALS AND METHODS

We purchased a total of 184 freshwater fish (22 species) from local markets of Phnom Penh Municipality through 3 different times (in June 2006, December 2007, and November 2010) and also purchased 36 fish (5 species) from a local market in Pursat Province (in June 2007) (Fig. 1). The fish were transported to the Department of Parasitology and Institute of Health Sciences, Gyeongsang National University School of Medicine, Jinju, Korea under refrigeration. The fish species were identified with the aid of the FishBase website (<http://www.fishbase.org/search.php>) (Tables 1-3). Individual fish was finely ground with a mortar or a grinder, the ground fish meat was mixed with artificial gastric juice, and the mixture was incubated at 36°C for 2-3 hr. The digested material was filtered



**Fig. 1.** Surveyed areas, Phnom Penh (1) and Pursat (2), Cambodia.

**Table 2.** Infection status<sup>a</sup> of freshwater fish from Phnom Penh Municipality, Cambodia, with *Haplorchis yokogawai* metacercariae

Fish species	No. of fish examined	No. (%) of fish infected	No. of metacercariae detected		
			Total	Range	Average
<i>Puntiplites proctozyron</i>	10	10 (100)	47	1-11	4.7
<i>Cirrhinus jullieni</i>	10	9 (90.0)	315	2-150	35.0
<i>Labeo chrysophekadion</i>	10	2 (20.0)	5	2-3	2.5
<i>Lobocheilos rhabdoura</i>	7	1 (14.3)	11	-	11.0
<i>Barbonymus altus</i>	3	1 (33.3)	106	-	106.0
Total	40	23 (57.5)	484	1-150	21.0

<sup>a</sup>The study was done in November 2010.

**Table 3.** Infection status of freshwater fish from Pursat Province, near the Tonle Sap Lake, Cambodia, with trematode metacercariae

Trematode species fish species	No. of fish <sup>a</sup> ex- amined	No. (%) of fish infected	No. of metacercariae detected		
			Total	Range	Average
<i>Opisthorchis viverrini</i>					
<i>Henicorhynchus lineatus</i>	10	1 (10.0)	1	-	1.0
<i>Puntioplites falcifer</i>	10	1 (10.0)	2	-	2.0
<i>Haplorchis yokogawai</i>					
<i>Puntioplites falcifer</i>	10	8 (80.0)	51	1-27	6.4
<i>Haplorchis pumilio</i>					
<i>Henicorhynchus lineatus</i>	10	9 (90.0)	381	1-190	42.3
<i>Pristolepis fasciata</i>	2	1 (50.0)	95	-	95.0
<i>Centrocestus formosanus</i>					
<i>Henicorhynchus lineatus</i>	10	1 (10.0)	1	-	1.0
<i>Procerovum</i> sp.					
<i>Anabas testudineus</i>	9	9 (100)	63	1-20	7.0
Negative for metacercariae					
<i>Boesemania microlepis</i>	5	0	-	-	-

<sup>a</sup>Total 36 fish were examined in June 2007. *H. lineatus* was positive for 3 kinds of metacercariae, and *P. falcifer* was positive for 2 kinds of metacercariae.

with 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 separately collected by their general morphological features. The collected metacercariae were categorized according to the size and morphological characteristics, and then the intensity of infection and the infection rate were calculated according to the species of fish positive for metacercariae.

## RESULTS

In fish from Phnom Penh Municipality, 2 species of metacercariae (*O. viverrini* and *H. yokogawai*) were found. The metacercariae of *O. viverrini* were collected from 37 (50.0%) of 74 fish (11 of 27 species), and the average number of metacercariae per fish was 18.6. Among the 11 fish species positive for *O. viverrini* metacercariae, *Barbonymus schwanefeldi* was the most heavily infected, with the average intensity of infection per fish of 74.6 metacercariae (Table 1). The metacercariae of *H. yokogawai* were detected from 23 (57.5%) of 40 fish (5 of 27 species), and the average number of metacercariae per fish was 21.0 (Table 2).

In fish from Pursat Province, 5 species of metacercariae (*O. viverrini*, *H. yokogawai*, *Haplorchis pumilio*, *Centrocestus formosanus*, and *Procerovum* sp.) were detected (Table 3). Total 3 *O. vi-*

*verrini* metacercariae were found in 2 fish species (*Henicorhynchus lineatus* and *Puntioplites falcifer*). Total 51 *H. yokogawai* metacercariae were detected in 8 (80%) of 10 *P. falcifer* examined. A total of 476 *H. pumilio* metacercariae were collected from 9 (90%) of 10 *H. lineatus* and 1 (50%) of 2 *Pristolepis fasciata*. Only 1 *C. formosanus* metacercaria was detected in 1 (10%) of 10 *H. lineatus*. Total 63 *Procerovum* sp. metacercariae were detected in all 9 (100%) *Anabas testudineus* examined (Table 3).

## DISCUSSION

Metacercariae of 5 FBT species, namely, *O. viverrini*, *H. yokogawai*, *H. pumilio*, *C. formosanus*, and *Procerovum* sp., were detected in freshwater fish from Phnom Penh Municipality and Pursat Province, Cambodia. In Phnom Penh Municipality, 2 FBT species (*O. viverrini* and *H. yokogawai*) were found, whereas, in Pursat Province, 5 FBT species were detected. From previous studies in Cambodia, only 2 species of FBT metacercariae, *O. viverrini* and *H. yokogawai*, were reported [15-17]. Touch et al. [17] detected *O. viverrini* metacercariae in various species of freshwater fish from areas adjacent to the Lake 500 near the border of Kandal and Takeo Provinces, southern Cambodia [17]. Sohn et al. [16] found *O. viverrini* metacercariae in 1 fish species, *Puntioplites proctozystron*, from Takeo Province. Sohn et al. [15] also detected *O. viverrini* and *H. yokogawai* metacercariae in freshwater fish from Kratie Province. Therefore, by the present study, it has been confirmed for the first time that the 3 additional species of FBT, namely, *H. pumilio*, *C. formosanus*, and *Procerovum* sp., are distributed in Cambodia.

The number and species of fish examined in this study were not much compared with the study of Touch et al. [17]. They examined a total of 1,479 fish in 30 species by the compression method [17]. On the other hand, Sohn et al. [16] examined 2 fish species, *P. proctozystron* (n=5) and *Cyclocheilichthys apagon* (n=10), from Takeo Province, and Sohn et al. [15] examined 9 fish species (n=85) from Kratie Province. In the present study, we examined a total of 184 freshwater fish (22 species) from several local markets of Phnom Penh Municipality and 36 fish (5 species) from a market of Pursat Province by means of the artificial digestion method. Anyhow, there may be some points to be considered which include the exact origin of the fish examined in this study. For example, fish purchased from a local market of Phnom Penh Municipality may not have been caught from Phnom Penh area. Those from Pursat Province are not enough in the number and species of fish

to show the epidemiological trend of FBT metacercariae infections in the subjected area and fish species.

As the second intermediate hosts of *O. viverrini*, 24 species of freshwater fish (*Barbonymus gonionotus*, *Cyclocheilichthys apogon*, *Cyclocheilichthys armatus*, *Cyclocheilichthys enoplos*, *Cyclocheilichthys furcatus*, *Cyclocheilichthys repasson*, *Esomus metallicus*, *Hampala dispar*, *Hampala macrolepidota*, *Hypsibarbus lagleri*, *Hypsibarbus pierrei*, *Hypsibarbus wetmorei*, *Labiobarbus lineatus*, *Mystacoleucus marginatus*, *Neolissochilus stracheyi*, *Onychostoma elongatum*, *Onychostoma fusiforme*, *Osteochilus hasseltii*, *Paralaubuca barroni*, *Poropuntius dearatus*, *Puntioplites falcifer*, *Puntioplites proctozystron*, *Puntius brevis*, and *Puntius orphoides*) have been reported in Thailand and Lao PDR [18-22]. In Cambodia, Touch et al. [17] detected *O. viverrini* metacercariae in 10 cyprinoid fish species, i.e., *Barbodes altus* (= *Barbonymus altus*), *C. apogon*, *C. enoplos*, *H. dispar*, *H. macrolepidota*, *Henicorhynchus siamensis*, *P. proctozystron*, *P. brevis*, *Systomus orphoides* (= *Puntius orphoides*), and *Thynnichthys thynnoides*, from Kandal and Takeo Provinces. Sohn et al. [16] reported *P. proctozystron* as a fish host for *O. viverrini* in Takeo Province, and Sohn et al. [15] detected *O. viverrini* metacercariae in *P. proctozystron*, *P. orphoides*, and *Labeo chrysophekadion* from Kratie Province, Cambodia.

In the present study, *O. viverrini* metacercariae were detected in 11 fish species (*B. altus*, *B. schwanefeldi*, *Cirrhinus jullieni*, *Cirrhinus microlepis*, *Henicorhynchus lobatus*, *H. siamensis*, *L. chrysophekadion*, *Luciosoma bleekeri*, *Osteochilus melanopleurus*, *P. proctozystron*, and *T. thynnoides*) from Phnom Penh Municipality and 2 fish species (*Henicorhynchus lineatus* and *P. falcifer*) from Pursat Province. Therefore, in Cambodia, total 18 fish species (*B. altus*, *B. schwanefeldi*, *C. jullieni*, *C. microlepis*, *C. apogon*, *C. enoplos*, *H. dispar*, *H. macrolepidota*, *H. lineatus*, *H. lobatus*, *H. siamensis*, *L. chrysophekadion*, *L. bleekeri*, *O. melanopleurus*, *P. proctozystron*, *P. brevis*, *P. orphoides*, and *T. thynnoides*) have been confirmed to be the second intermediate hosts for *O. viverrini* [15-17].

The prevalence and intensity of *O. viverrini* metacercariae were relatively low in fish examined in the present study compared to Lao PDR [21,22]. In Phnom Penh Municipality, the average number of *O. viverrini* metacercariae per fish was 18.6 in 37 positive fish (11 species). Among the positive fish species, *B. schwanefeldi* revealed the highest prevalence (100%) and intensity (av. 74.6 metacercariae per fish). However, these results were somewhat higher than those of Touch et al. [17]. They detected total 789 *O. viverrini* metacercariae (4.8 per fish) in 163 positive fish (10 species) collected from Kandal and Takeo Provinces, Cambodia. Some people in Phnom Penh like to eat

raw fish, as has been reported in Takeo Province [14,16,17], and there may be a substantial number of opisthorchiasis patients in Phnom Penh areas of Cambodia.

Distribution of *H. yokogawai* metacercariae has been reported in Asian and Middle East countries such as India, Thailand, Lao PDR, and Egypt, involving 43 fish species [21-28]. In the present study, *H. yokogawai* metacercariae were detected in 5 fish species (*P. proctozystron*, *C. jullieni*, *L. chrysophekadion*, *L. rhabdoura*, and *B. altus*) from Phnom Penh Municipality, and 1 species (*P. falcifer*) from Pursat Province. Sohn et al. [15] detected them in 3 fish species (*P. proctozystron*, *P. orphoides*, and *H. siamensis*) from Kratie Province, Cambodia [15].

Metacercariae of *H. pumilio* have also been found in Asian and Middle East countries [21-28]. In China, 18 fish species were found to harbour the metacercariae in Guangxi Zhuang Autonomous Region [29]. In Vietnam, the metacercariae were detected in 17 fish species from Nam Dinh Province [8] and in 13 fish species from Hanoi City and Nam Dinh Province [30]. In the present study, *H. pumilio* metacercariae were detected in only 1 fish species (*H. lineatus*) from Pursat Province.

*C. formosanus* is known to be distributed in China, Taiwan, Japan, the Philippines, India, Lao PDR, and Vietnam [8,20-22, 30-33]. Their metacercariae were detected in 9 fish species from Lao PDR [20-22], and in 16 fish species from Vietnam [8,30]. In China, Sohn et al. [29] reported 10 fish species as the second intermediate host in Guangxi Zhuang Autonomous Region. In the present study, *C. formosanus* metacercariae were found in only 1 fish (*H. lineatus*) from Pursat Province.

*Procerovum* sp. (presumed to be *P. varium*) metacercariae were detected in all of 9 *A. testudineus* from Pursat Province in this study. This fish species was previously reported to be a second intermediate host for *P. varium* in Vietnam, together with 9 more fish species (*L. rohita*, *H. molitrix*, *C. mrigala*, *C. idella*, *S. curriculum*, *P. brachypomum*, *C. batrachus*, *B. gonionotus*, and *M. cephalus*) [8,30].

In conclusion, the present study confirmed that various species of freshwater fish play the role of a second intermediate host for various kinds of FBT (*O. viverrini*, *H. pumilio*, *H. yokogawai*, *C. formosanus*, and *Procerovum* sp.) in Cambodia. Epidemiological studies to detect endemic areas of human FBT infections remain to be undertaken in Cambodia.

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## CONFLICT OF INTEREST

We declare that we have no conflict of interest related with this article.

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