

Prevalence and Intensity of *Opisthorchis viverrini* Metacercarial Infection in Fish from Phnom Penh, Takeo, and Kandal Provinces, Cambodia

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Abstract: The prevalence and intensity of *Opisthorchis viverrini* metacercariae (OvMc) were investigated in fish from 3 southern administrative regions along the Mekong River in Cambodia, i.e., Phnom Penh, Takeo, and Kandal Provinces from 2017 to 2020. A total of 295 freshwater fish (24 species) were transported to our laboratory with ice and examined using the artificial digestion method. In Phnom Penh, among 4 fish species positive for OvMc, 9 (23.7%) of 38 specimens examined were infected, and their intensity of infection averaged 4.3 metacercariae per infected fish. In Takeo Province, among 10 fish species positive for OvMc, 24 (38.1%) out of 63 fish examined were infected, and their intensity of infection was av. 14.4 metacercariae per infected fish. In particular, all of 3 *Osteochilus schlegelii* fish examined were infected, and their infection intensity was high, 34.7 metacercariae per fish. In Kandal Province, among 6 fish species positive for OvMc, 46 (90.2%) out of 51 specimens examined were infected, and their infection intensity was 24.0 metacercariae per infected fish. All fish of *Systomus orphoides* (n=17), *Barbonymus altus* (n=14), and *Rasbora aurotaenia* (n=2) were infected, and their intensity of infection averaged 37.7, 21.6, and 18.5 metacercariae per fish, respectively. Metacercariae of *Haplochis yokogawai*, *Haplorchis taichui*, and *Centrocestus formosanus* were detected in fish from Takeo and Kandal Provinces. From these results, it has been confirmed that a variety of fish species from Phnom Penh, Takeo, and Kandal Provinces are commonly infected with OvMc, and preventive measures to avoid human *O. viverrini* infection should be performed in Cambodia.

Key words: *Opisthorchis viverrini* metacercaria, freshwater fish, Phnom Penh, Takeo Province, Kandal Province, Cambodia

Fishborne zoonotic trematodes (FZT), including *Opisthorchis viverrini*, are public health problems in Southeast Asian countries, i.e., Thailand, Lao People's Democratic Republic (Lao PDR), Vietnam, and Cambodia. *O. viverrini* can provoke remarkable pathology and morbidity, such as cholangiocarcinoma, among residents of riverside endemic areas [1,2]. Infections with this liver fluke in humans are usually caused by habitual consumption of raw freshwater fish containing metacercariae. This infection is highly prevalent among riparian populations having the food habit of eating raw fish. Many riverside areas along

the Mekong river in Vietnam, Lao PDR, Thailand, and Cambodia are known to be highly endemic with *O. viverrini* infection [3–10].

Cambodia is administratively divided into 25 provinces, including the capital, i.e., Phnom Penh Municipality (= Phnom Penh). Among these administrative localities, Phnom Penh, Takeo, and Kandal Provinces are located along the Mekong River in the southern part of Cambodia. Kandal Province completely surrounds the capital city, Phnom Penh [11]. On the other hand, a lot of Cambodian people are infected with various species of helminths, especially, soil-transmitted nematodes and FZTs, including *O. viverrini* [12–14]. In the Project of Korea-Cambodia International Collaboration on Intestinal Parasite Control in Cambodia [15,16], it has been repeatedly reported that infections with *O. viverrini* are prevalent in several localities along the Mekong River in Cambodia [8–10,15].

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Khieu et al. [10] broadly analyzed the information regarding the epidemiological situation of *O. viverrini* infection in Cambodia.

Several workers previously reported the infection status of zoonotic trematode metacercariae (ZTM), in particular, *O. viverrini*, in freshwater fish purchased from Phnom Penh, Pursat, Takeo, and Kratie Provinces [8,9,17-20]. However, studies on the infection status of freshwater fish with ZTM need to be continuously performed in different localities of Cambodia. Therefore, in this study, we surveyed the prevalence and intensity of *O. viverrini* metacercarial infection in fish purchased from local markets in 3 southern administrative regions, i.e., Phnom Penh, Takeo, and Kandal Provinces.

We purchased a total of 295 fish specimens (24 species) from local markets in 3 southern regions, i.e., Phnom Penh (86 fish in 8 species in May 2018), Takeo Province (99 fish in 16 species in November 2017), and Kandal Province (110 fish in 13 species in January-February 2020), Cambodia. The collected fish were transported to the Department of Parasitology and Tropical Medicine, Gyeongsang National University College of Medicine, Jinju, Korea with ice. The fish species were identified with the aid of the FishBase website (<http://www.fishbase.org/search.php>) and by the help of a Korean ichthyologist (Choi SH). Individual fish was finely ground in a mortar with a pestle, the ground fish meat was mixed with artificial gastric juice, and the mixture was incubated at 36°C for about 2 hr. The digested material was filtered through a 1×1 mm mesh, and washed with 0.85% saline until the supernatant became clear. The sediment was carefully examined for metacercariae under

a stereomicroscope. The metacercariae of each species (only ZTM) were collected viewing from the general features and were counted to get hold of the prevalence (%) and intensity of infection (no. of ZTM per fish infected) by fish species. The metacercariae of 175-210 (av. 195)×138-163 (150) 50 µm in size with characteristic morphologies of the oral and ventral suckers and the excretory bladder were regarded as the *O. viverrini* metacercaria (OvMc) (Fig. 1A).

A total of 86 fish specimens of 8 species were purchased from Phnom Penh in May 2018. The fish species (no. of fish) included *Anabas testudineus* (n=20), *Channa striata* (18), *Henicorhynchus lobatus* (13), *Hypsibarbus wetmorei* (10), *Monopterus albus* (10), *Barbonymus altus* (9), *Labeo chrysophekadion* (5), and *Puntius proctozysron* (1). The results revealed that OvMc were positive in 9 (23.7%) out of 38 fish, and their infection intensity was av. 4.3 metacercariae per infected fish (Table 1).

In Takeo Province, total 99 fish specimens of 16 species were examined in November 2017. The fish species (no. of fish) included *P. proctozysron* (n=18), *Cirrhinus julieni* (15), *A. testudineus* (12), *M. albus* (11), *Barbonymus gonionotus* (8), *Syncrossus helodes* (8), *Cirrhinus molitorella* (7), *C. striata* (7), *Osteochilus schlegelii* (3), *B. altus* (2), *Osteochilus microcephalus* (2), *Yasuhikotakia modesta* (2), *L. chrysophekadion* (1), *Systomus orphoides* (1), *Puntius* sp. (1), and *Pristolepis fasciata* (1). OvMc were detected in 24 (38.1%) out of 63 fish, and their infection intensity averaged 14.4 per fish infected. Especially, in the case of *O. schlegelii* fish, all 3 fish specimens examined were infected with OvMc, and their infection intensity was av. 34.7 metacercariae per infected fish (Table 2).

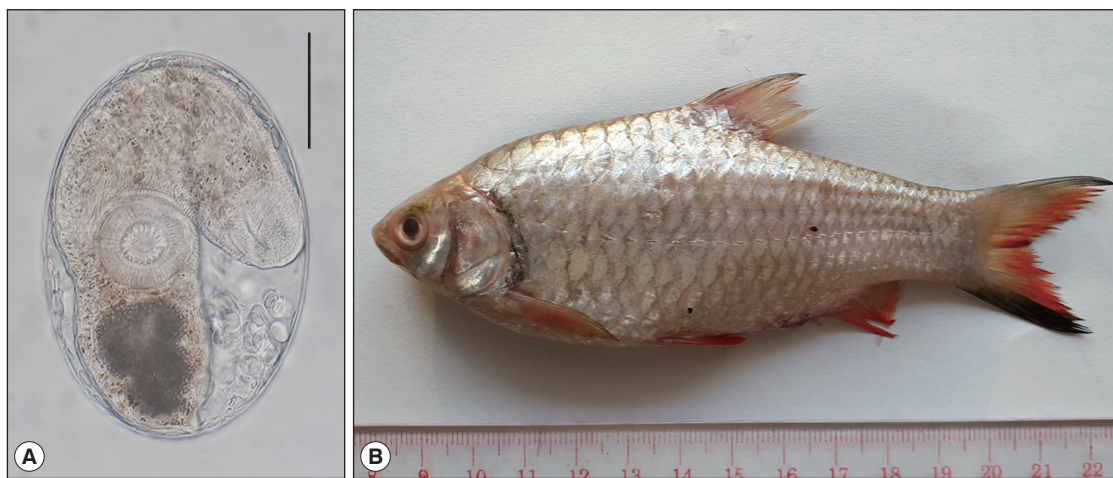


Fig. 1. (A) Metacercaria of *Opisthorchis viverrini* isolated from *Systomus orphoides* fish from Kandal Province, Cambodia. Scale bar = 50 µm. (B) *S. orphoides*, a susceptible fish host for *O. viverrini*, purchased from a local market in Kandal Province, Cambodia.

In Kandal Province, total 110 fish specimens of 13 species were purchased in January and February 2020. The fish species (no. of fish) included *Labiobarbus lineatus* (n=20), *S. orphoides* (17), *B. altus* (14), *Leptobarbus rubripinna* (11), *L. chrysophekadion* (10), *A. testudineus* (10), *Oxyeleotris marmorata* (8), *Cyclocheilichthys enoplus* (6), *O. schlegelii* (6), *B. gonionotus* (2), *P. procto-*
zysron (2), *Cyclocheilichthys apogon* (2), and *Rasbora aurotaenia* (2). OvMc were detected in 46 (90.2%) out of 51 fish, and their

infection intensity averaged 24.0 metacercariae per infected fish. Especially, in 3 fish species, *S. orphoides* (n=17) (Fig. 1B), *B. altus* (n=14), and *R. aurotaenia* (n=2), all examined specimens were infected with OvMc, and their infection intensity averaged 37.7, 21.6, and 18.5 metacercariae per infected fish, respectively (Table 3).

A few other ZTM that belong to the family Heterophyidae or the Echinostomatidae were also detected in this study. In Takeo

Table 1. Infection status of *Opisthorchis viverrini* metacercariae (OvMc) by the species of fish from Phnom Penh, Cambodia

Fish species examined	No. of fish examined	No. (%) of fish infected	No. of OvMc detected	
			Range	Average
<i>Channa striata</i>	18	4 (22.2)	2-13	6.8
<i>Hypsibarbus wetmorei</i>	10	2 (20.0)	-	1.0
<i>Barbonymus altus</i>	9	2 (22.2)	-	1.0
<i>Puntiplites procto-</i> <i>zysron</i>	1	1 (100)	-	8.0
Total	38	9 (23.7)	1-13	4.3

Other fish species examined, including *A. testudineus* (n=20), *Henicorhynchus lobatus* (13), *Monopterus albus* (10), and *L. chrysophekadion* (5), gave negative results.

Table 2. Infection status of *Opisthorchis viverrini* metacercariae (OvMc) by the species of fish from Takeo Province, Cambodia

Fish species examined	No. of fish examined	No. (%) of fish infected	No. of OvMc detected	
			Range	Average
<i>Puntiplites procto-</i> <i>zysron</i>	18	10 (55.6)	1-86	18.9
<i>Cirrhinus julieni</i>	15	1 (6.7)	-	1.0
<i>Barbonymus gonionotus</i>	8	1 (12.5)	-	2.0
<i>Cirrhinus molitorella</i>	7	4 (57.1)	1-6	2.8
<i>Channa striata</i>	7	1 (14.3)	-	1.0
<i>Osteochilus schlegelii</i>	3	3 (100)	15-69	34.7
<i>Barbonymus altus</i>	2	1 (50.0)	-	4.0
<i>Labeo chrysophekadion</i>	1	1 (100)	-	12.0
<i>Systemus orphoides</i>	1	1 (100)	-	15.0
<i>Puntius</i> sp.	1	1 (100)	-	7.0
Total	63	24 (38.1)	1-86	14.4

Other species of fish examined, including *Anabas testudineus* (12), *Monopterus albus* (11), *Syncrossus helodes* (8), *Osteochilus microcephalus* (2), *Yasuhikotakia modesta* (2), and *Pristolepis fasciata* (1), gave negative results.

Table 3. Infection status of *Opisthorchis viverrini* metacercariae (OvMc) by the species of fish from Kandal Province, Cambodia

Fish species examined	No. of fish examined	No. (%) of fish infected	No. of OvMc detected	
			Range	Average
<i>Systemus orphoides</i>	17	17 (100)	2-92	37.7
<i>Barbonymus altus</i>	14	14 (100)	1-90	21.6
<i>Labeo chrysophekadion</i>	10	7 (70.0)	1-21	6.1
<i>Osteochilus schlegelii</i>	6	5 (83.3)	2-30	15.2
<i>Puntiplites procto-</i> <i>zysron</i>	2	1 (50.0)	-	7.0
<i>Rasbora aurotaenia</i>	2	2 (100)	3-34	18.5
Total	51	46 (90.2)	1-92	24.0

Other species of fish examined, including *Labiobarbus lineatus* (n=20), *Leptobarbus rubripinna* (11), *A. testudineus* (10), *Oxyeleotris marmorata* (8), *Cyclocheilichthys enoplus* (6), *B. gonionotus* (2), and *Cyclocheilichthys apogon* (2), gave negative results.

Province, the metacercariae of *H. yokogawai* (HyMc) were detected in 2 fish species, i.e., *P. proctozyron* (7/18; 38.9%) and *C. julieni* (3/15; 20.0%) with their infection intensity of 102.6 and 11.3 per fish, respectively. The HyMc were also found in Kandal Province in 48 (57.1%) out of 84 fish (9 species) examined, and their infection intensity averaged 65.9 per infected fish. In fish from Kandal Province, 2 other species of heterophyid metacercariae were detected; *Haplorchis taichui* metacercariae (n=30) in 1 (5.0%) of 20 *L. lineatus*, and *Centrocestus formosanus* metacercariae (n=6) in 2 (11.8%) of 17 *S. orphoides*. An unidentified species of echinostome metacercariae was detected in 6 (75.0%) out of 8 *O. marmorata* (the marble goby), and their infection intensity averaged 8 (1-21) metacercariae per fish.

In our study, the prevalence and intensity of OvMc in fish were relatively low compared with our previous studies (by the Korean team) in Cambodia [8,9,20], with the exception of Kandal Province which revealed higher values. The endemicity indices of OvMc (mean no. of metacercariae per fish \times prevalence/100) in this study were 1.0 (Phnom Penh), 5.5 (Takeo), and 21.6 (Kandal). Chai et al. [20] detected a total of 690 OvMc (18.6 metacercariae per fish) in 37 (50.0%) out of 74 fish examined in Phnom Penh, where the endemicity index was 9.3. Sohn et al. [9] detected a total of 50 OvMc (10.0/fish) in 5 (100%) of 5 *P. proctozyron* from Takeo province, and the endemicity index was 10.0. Sohn et al. [8] also detected a total of 367 OvMc (19.3/fish) in 19 (63.3%) out of 30 fish examined in Kratie Province, and the endemicity index was 12.2.

In Kandal and Takeo Provinces, Touch et al. [18] reported lower prevalence and lower intensity of OvMc; they detected a total of 789 OvMc (4.8/fish) in 163 (17.5%) out of 929 fish, and the endemicity index was only 0.8. From the present and previous studies, we could confirm that the endemicity of OvMc has been continuously maintained in southern localities of Cambodia along the Mekong River, i.e., Phnom Penh, Takeo, Kratie, and Kandal Provinces. However, the endemicity was more or less different by survey localities. The endemicity index of OvMc was the highest (21.6) in Kandal Province as depicted in this study.

A lot of fish species have been found to be infected with OvMc in Cambodia. In this study, OvMc were detected in 12 fish species, namely, *B. altus*, *B. gonionotus*, *C. striata*, *C. julieni*, *C. molitorella*, *H. wetmorei*, *L. chrysophekadion*, *O. schlegelii*, *P. proctozyron*, *Puntius* sp., *R. aurotaenia*, and *S. orphoides*. Touch et al. [18] reported 10 infected fish species, i.e., *Barbodes altus* (= *Barbo-*

nymus altus), *Cyclocheilichthys apagon*, *Cyclocheilichthys enoplos*, *Hampala dispar*, *Hampala macrolepidota*, *Henicorhynchus siamensis*, *P. proctozyron*, *Puntius brevis*, *S. orphoides*, and *Thynnichthys thynnoides*, from the border of Kandal and Takeo Provinces. Sohn et al. [9] obtained OvMc in *P. proctozyron* from Takeo Province, and Sohn et al. [8] found OvMc in 3 fish species, i.e., *P. proctozyron*, *Puntius orphoides* (= *Systemus orphoides*) and *L. chrysophekadion*, from Kratie Province. Chai et al. [20] detected OvMc in 11 fish species from Phnom Penh, i.e., *B. altus*, *Barbonymus schwanefeldi*, *C. julieni*, *Cirrhinus microlepis*, *Henicorhynchus lobatus*, *H. siamensis*, *L. chrysophekadion*, *Luciosoma bleekeri*, *Osteochilus melanopleurus*, *P. proctozyron*, and *T. thynnoides*, and 2 fish species from Pursat Province, i.e., *P. falcifer* and *Henicorhynchus lineatus*. Summarizing these results, a total of 20 fish species, including *B. altus*, *B. schwanefeldi*, *C. julieni*, *C. microlepis*, *C. apagon*, *C. enoplos*, *H. dispar*, *H. macrolepidota*, *H. lineatus*, *H. lobatus*, *H. siamensis*, *L. chrysophekadion*, *L. bleekeri*, *O. melanopleurus*, *O. schlegelii*, *R. aurotaenia*, *P. proctozyron*, *P. brevis*, *S. orphoides*, and *T. thynnoides*, could be listed as the second intermediate hosts for *O. viverrini* in Cambodia [8,9,18-20].

In our study, 4 species of ZTM, including *O. viverrini*, *H. yokogawai*, *H. taichui*, and *C. formosanus*, were detected. In previous studies [8,9,17-20], total 5 species, including *O. viverrini*, *H. yokogawai*, *H. pumilio*, *C. formosanus*, and *Procerovum* sp., were detected in several localities of Cambodia. For example, all 5 species of ZTM were detected in Pursat Province [20], and 2 species, *O. viverrini* and *H. yokogawai* metacercariae, were found in Phnom Penh, Takeo, and Kratie Provinces [8,9,20]. In addition, in Phnom Penh, 2 other heterophyid species metacercariae were detected in largescale mullets, *Chelon macrolepis*; their adult flukes recovered from experimental hamsters were identified as *Stellantchasmus falcatus* and *Pygidioopsis cambodien-sis* (Digenea: Heterophyidae) [21,22].

Conclusively, it has been confirmed in this study that OvMc are more or less prevalent in a variety of fish species commercially available in Phnom Penh, Takeo, and Kandal Provinces, Cambodia along the Mekong River. However, with the exception of Kandal Province, their endemicity was not so high compared with our previous reports in Cambodia. There were some limitations in this study, such as a small number of fish specimens and a small number of fish species examined.

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interest related with this article.

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