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-
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.

**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.

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

| Fish species         | No. of fish examined | No. (%) of fish infected | No. of metacercariae detected | Total | Range | Average |
|----------------------|----------------------|--------------------------|-------------------------------|-------|-------|---------|
| *Henicorhynchus siamensis* | 9                    | 3 (33.3)                 | 52                            | 4-43  | 17.3  |
| *Barbonymus schwanefeldi* | 5                    | 5 (100)                  | 373                           | 11-128| 74.6  |
| *Osteochilus melanopleurus* | 5                    | 4 (80.0)                 | 36                            | 1-18  | 9.0   |
| *Luciosoma bleekeri*   | 2                    | 1 (50.0)                 | 1                             |       | 1.0   |
| *Anabas testudineus*   | 22                   | 0                        | -                             |       | -     |
| *Catlocarpio siamensis* | 10                   | 0                        | -                             |       | -     |
| *Pristolepis fasciata*  | 8                    | 0                        | -                             |       | -     |
| *Hypsibarbus lagleri*   | 6                    | 0                        | -                             |       | -     |
| *Rhynogobio sp.*       | 5                    | 0                        | -                             |       | -     |
| *Thynnichthys thynnoides* | 4                    | 0                        | -                             |       | -     |
| *Cirrhinus jullieni*    | 2                    | 0                        | -                             |       | -     |
| *Henicorhynchus lineatus* | 2                    | 0                        | -                             |       | -     |

| Fish species         | No. of fish examined | No. (%) of fish infected | No. of metacercariae detected | Total | Range | Average |
|----------------------|----------------------|--------------------------|-------------------------------|-------|-------|---------|
| *Puntioplites proctozoneyn* | 12                   | 4 (33.3)                 | 5                             | 1-2   | 1.3   |
| *Cirrhinus jullieni*    | 10                   | 7 (70.0)                 | 60                            | 1-30  | 8.6   |
| *Labeo chrysophakian*   | 10                   | 6 (60.0)                 | 137                           | 2-110 | 22.8  |
| *Thynnichthys thynnoides* | 10                   | 4 (40.0)                 | 3                             | 1-2   | 1.5   |
| *Barbonymus altus*      | 7                    | 1 (14.3)                 | 1                             |       | 1.0   |
| *Henicorhynchus lobatus* | 1                    | 1 (100)                  | 1                             |       | 1.0   |
| *Henicorhynchus siamensis* | 15                   | 0                        | -                             |       | -     |
| *Paralaubuca typus*     | 9                    | 0                        | -                             |       | -     |
| *Syncrossus helodes*    | 9                    | 0                        | -                             |       | -     |
| *Lobocheilos rhabdoura* | 7                    | 0                        | -                             |       | -     |
| *Luciosoma bleekeri*    | 6                    | 0                        | -                             |       | -     |
| *Osteochilus melanopleurus* | 2                    | 0                        | -                             |       | -     |
| *Yasuhikotakiia modesta* | 2                    | 0                        | -                             |       | -     |
| *Yasuhikotakiia lecontei* | 1                    | 0                        | -                             |       | -     |

*Note: A total of 184 fish in 22 species were examined.*

**Table 2.** Infection status 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 |
|----------------------|----------------------|--------------------------|-------------------------------|-------|-------|---------|
| *Puntioplites proctozoneyn* | 10                   | 10 (100)                 | 47                            | 1-11  | 4.7   |
| *Cirrhinus jullieni*    | 10                   | 9 (90.0)                 | 315                           | 2-150 | 35.0  |
| *Labeo chrysophakian*   | 10                   | 2 (20.0)                 | 5                             | 2-3   | 2.5   |
| *Lobocheilos rhabdoura* | 7                    | 1 (14.3)                 | 11                            |       | 11.0  |
| *Barbonymus altus*      | 3                    | 1 (33.3)                 | 106                           |       | 106.0 |
| Total                 | 40                   | 23 (57.5)                | 484                           | 1-150 | 21.0  |

*Note: The study was done in November 2010.*

**Fig. 1.** Surveyed areas, Phnom Penh (1) and Pursat (2), Cambodia.
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, Barbounymys schuwanefeldi 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. viverrini metacercariae were found in 2 fish species (Haplorchis pumilio 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 proctozysron, 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. proctozysron (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.

### 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 examined | No. (%) of fish infected | No. of metacercariae detected |
|-------------------------------|----------------------|--------------------------|------------------------------|
| Ophisthorchis viverrini        | 85                   | 100                      | 35                          |
| Henicorhynchus lineatus       | 10                   | 100                      | 3                            |
| Puntioplites falcifer         | 10                   | 100                      | 2                            |
| Haplorchis yokogawai          | 10                   | 100                      | 3                            |
| Pristolepis fasciata          | 2                    | 50.0                     | 1                            |
| Centrocestus formosanus       | 10                   | 100                      | 3                            |
| Henicorhynchus lineatus       | 10                   | 100                      | 3                            |
| Procerovum sp.                | 10                   | 100                      | 3                            |
| Anabas testudineus            | 9                    | 100                      | 3                            |
| Negative for metacercariae    | 5                    | 0                        | -                            |

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

As the second intermediate hosts of *O. viverrini*, 24 species of freshwater fish (*Barbonymus gonionotus*, *Cyclocheilichthys agopon*, *Cyclocheilichthys armatus*, *Cyclocheilichthys enoplos*, *Cyclocheilichthys fuscatus*, *Cyclocheilichthys repasson*, *Esomus metallicus*, *Hampala dispar*, *Hampala macrolepidota*, *Hypsibarbus lagleri*, *Hypsibarbus pirei*, *Hypsibarbus wetmorei*, *Labiobarbus lineatus*, *Mystacoleucus marginatus*, *Neoliscophis stracheyi*, *Onychostoma elongatum*, *Onychostoma fusiorme*, *Osteochilus hasseltii*, *Paralaubuca bannorni*, *Poropuntius dearatus*, *Puntioplites falciere*, *Puntioplites proctorzysron*, *Puntius brevis*, and *Puntius orphoides*) have been reported in Thailand and Lao PDR [18-22]. In Cambodia, Touch et al. [17] detected *O. viverrini* metacercaiae in 10 cyprinid fish species, i.e., *Barbodes altus* (= *Barbomyrus altus*), *C. agapen*, *C. enoplos*, *H. dispar*, *H. macrolepidota*, *Henicorhynchus siamensis*, *P. proctorzysron*, *P. brevis*, *Systomus orphoides* (= *Puntius orphoides*), and *Thynnichthys thynoides*, from Kandal and Takeo Provinces. Sohn et al. [16] reported *P. proctorzysron* as a fish host for *O. viverrini* in Takeo Province, and Sohn et al. [15] detected *O. viverrini* metacercaiae in *P. proctorzysron*, *P. orphoides*, and *Laboe chrysohekadion* from Kratie Province, Cambodia.

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

The prevalence and intensity of *O. viverrini* metacercaiae 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* metacercaiae 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 metacercaiae per fish). However, these results were somewhat higher than those of Touch et al. [17]. They detected total 789 *O. viverrini* metacercaiae (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* metacercaiae 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* metacercaiae were detected in 5 fish species (*P. proctorzysron*, *C. jullieni*, *L. chrysohekadion*, *L. rhabdoura*, and *B. altus*) from Phnom Penh Municipality, and 1 species (*P. falciere*) from Pursat Province. Sohn et al. [15] detected them in 3 fish species (*P. proctorzysron*, *P. orphoides*, and *H. siamensis*) from Kratie Province, Cambodia [15].

Metacercaiae 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 metacercaiae in Guangxi Zhuang Autonomous Region [29]. In Vietnam, the metacercaiae 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* metacercaiae 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 metacercaiae 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* metacercaiae were found in only 1 fish (*H. lineatus*) from Pursat Province.

*Procerovum* sp. (presumed to be *P. varium*) metacercaiae 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. rohitae*, *M. molitrix*, *C. mrigala*, *C. idella*, *S. curriculus*, *P. brachypomum*, *C. trachurus*, *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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