Discovery of Carcinogenic Liver Fluke Metacercariae in Second Intermediate Hosts and Surveillance on Fish-Borne Trematode Metacercariae Infections in Mekong Region of Myanmar

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Abstract: Countries of lower Mekong regions are highly alarmed by the spread of fish-borne trematode infections, i.e., small liver flukes and minute intestinal flukes especially in Thailand, Lao People’s Democratic Republic (Lao PDR), Vietnam, Cambodia and Myanmar. Moreover; the incidence of cholangiocarcinoma has also been increasing in the endemic area of liver fluke infections. Only a few reports have been published concerning the fish-borne trematodes infections in the central region of Myanmar. However; there is still a lack of information regarding the status of trematodes infections in second intermediate hosts in the Mekong region of Myanmar. Therefore, we conducted surveillance on the distribution of trematode metacercariae in small cyprinoid fishes collected from the Mekong region of Myanmar. A total of 689 fishes (12 different species of cyprinoid fishes) have been collected and examined by pepsin digestion methods. We discovered four species of fish-borne trematode metacercariae infections, i.e., carcinogenic liver fluke, Opisthorchis viverrini; minute intestinal flukes, Haplorchis taichui; Haplorchis pumilio and Haplorchoides sp. in Tachileik, the Mekong Region of Myanmar. The outcome of this study could be a useful index for the fish-borne zoonotic trematode epidemiology in the Mekong area. Besides, the results of our study contribute to filling the gap of information necessary for the control and prevention of fish-borne trematode zoonotic infections in the Mekong region.

Keywords: opisthorchiidae; opisthorchis viverrini; minute intestinal flukes (MIF); Heterophyidae

1. Introduction

The important public health problem of the spread of fish-borne trematodes (FBT) has highly alarmed the countries of the lower Mekong basin especially Thailand, Lao People’s Democratic Republic (Lao PDR), Cambodia, Vietnam and Myanmar [1–5]. Fish-borne zoonotic trematodes (FZT) especially small liver flukes (Opisthorchiidae) and minute intestinal flukes (Heterophyidae) are highly prevalent in those regions [6]. These two flukes have similar life cycles involving two intermediate hosts [7,8]. The first intermediate hosts are snails, and the second intermediate host are small freshwater fishes [9,10]. Humans are infected through ingestion of undercooked or raw freshwater fishes, which are contaminated with the infective stage of the parasite, i.e., metacercariae [11]. The traditional habit of eating raw or undercooked fish is a known risk factor for human trematode...
infections [7]. Opisthorchiasis caused by carcinogenic liver fluke, *Opisthorchis viverrini*, is an important public health problem in lower Mekong region countries [12–19]. Heterophyiasis is an infection of the small bowel, which is caused by minute intestinal flukes (Heterophyidae) [20–23]. The incidence of cholangiocarcinoma has also been increasing in the endemic area of liver fluke infections [24,25]. In Myanmar, there is one report in 2019 about a retrospective study of the cholangiocarcinoma cases admitted to the Hepatobiliary and Pancreatic surgery department, Yangon Specialty Hospital, Myanmar, which shows that the prevalence has notably been increasing since 2016, and the highest prevalence was in 2018 [26]. Myanmar people also have the traditional habit of eating raw small cyprinoid fishes pickled with rice, locally called (Nyar lay Chin) (Figure 1). Moreover, infection can also occur via poor personal hygiene and the contamination of food, hands and food preparation utensils that are contaminated with metacercariae [27–30]. For the increasing data of cholangiocarcinoma cases from Myanmar, the associated risk factors such as carcinogenic liver fluke infection and epidemiological data require more surveillance for the control and prevention. In the central region of Myanmar, seven species of minute intestinal flukes (members of the Heterophyidae) have been reported in Yangon, Myanmar [5]. However, there is still a lack of information about the fish-borne trematode infection status from the Mekong region of Myanmar. Tachileik is a town situated in the Mekong basin of Myanmar (Figure 2). Geographically, the city is also situated in the Golden Triangle that is the area where the borders of Thailand, Laos and Myanmar meet at the confluence of the Ruak and Mekong rivers. Therefore, we conducted a survey on trematode metacercariae infections in second intermediate hosts from Tachileik, Mekong region of Myanmar, and we believe that our data will provide required epidemiological information to fill the knowledge gap and provide valuable data to solve the public health problem of control and prevention of fish-borne trematode zoonotic infections in countries of the Mekong basin.

![Photos of local Myanmar food](image1.jpg)

**Figure 1.** Photos of local Myanmar food (a) Raw small cyprinoid fishes pickled with rice (Nyar lay Chin) (b) Nyar lay Chin prepared as an uncooked salad mixed with onion (Nyar lay Chin salad).

2. Material and Methods

2.1. Collection of Cyprinoid Fishes and Identification of Fish Species

A total of 689 fishes (12 different species of cyprinoid fishes) were purchased from local markets in Tachileik, lower Mekong region of Myanmar (Table 1). Tachileik is a town situated in the Mekong basin of Myanmar (Figure 2), which is also situated in the Golden Triangle that is the area where the borders of Thailand, Laos and Myanmar meet at the confluence of the Ruak and Mekong rivers. The cyprinoid fishes were collected from January 2018 to December 2019. All collected fishes were transferred on ice to the laboratory of the Department of Medicine, Mahasarakham University. The length and width of fishes were individually measured, and the species of all fishes were identified with the aid of the Fishbase website (http://www.fishbase.org/search.php) (Table 1).
were repeated 8 or 9 times until the supernatant became clear and a small quantity of the sediment was
placed in a 37 °C incubator for 2 h with occasional stirring and removed the larger particles by the
filtration of digested materials. Then 0.85% saline was added to the digested sample, and we let it
stand for a while and discarded the supernatant very carefully and kept the sediment. The procedures
were repeated 8 or 9 times until the supernatant became clear and a small quantity of the sediment was

detected metacercariae were isolated and were put into a small dish. Finally, the number of
metacercariae of each fluke species were counted for further analysis.

Table 1. Freshwater cyprinoids fishes purchased from the local market in Tachileik, Mekong region of Myanmar.

| Number | Fish Species               | Total Number | Average Length | Average Width |
|--------|----------------------------|--------------|----------------|---------------|
| 1      | Barbonymus gonionotus      | 20           | 9.5 cm ± 0.2236 | 3 cm ± 0.3244 |
| 2      | Puntioplites falcifer      | 30           | 9.5 cm ± 0.2170 | 3 cm ± 0.2110 |
| 3      | Systemus rubripinnis       | 28           | 9 cm ± 0.3502   | 3 cm ± 0.2377 |
| 4      | Labiobarbus siamensis      | 60           | 10 cm ± 0.219   | 3.3 cm ± 0.3188 |
| 5      | Henicorhynchus siamensis   | 50           | 12.5 cm ± 0.2175| 3.5 cm ± 0.3023|
| 6      | Mystacoleucus marginatus   | 100          | 9.5 cm ± 0.2564 | 3.3 cm ± 0.3128|
| 7      | Rasbora argyrotaenia       | 20           | 10.5 cm ± 0.2236| 3.5 cm ± 0.3244|
| 8      | Systemus orphoides         | 10           | 12.5 cm ± 0.9368| 3.5 cm ± 0.3496|
| 9      | Puntius brevis             | 100          | 10.5 cm ± 0.2422| 3.5 cm ± 0.2983|
| 10     | Trichogaster trichopterus  | 20           | 11.5 cm ± 0.2236| 3.5 cm ± 0.2511|
| 11     | Cyclocheilichthys repasson | 151          | 9.5 cm ± 0.2230 | 3.5 cm ± 0.2804|
| 12     | Anabas testudineus         | 100          | 9 cm ± 0.2240   | 3 cm ± 0.2876  |
| total  |                            | 689          |                |               |

Figure 2. Geographic mapping of freshwater cyprinid fishes collected area from Tachileik, Mekong region of Myanmar (20°27′ N 99°53′ E) by ArcGIS 10.5.

2.2. Pepsin Digestion Method

Examination of zoonotic trematode metacercariae in the second intermediate hosts was done by pepsin-HCl artificial digestion techniques. All the collected fishes were ground one by one in a mortar with pestle, and then, the ground samples were transferred into a beaker and mixed with artificial gastric juice. The artificial gastric juice is made by a mixture of 10 gm pepsin A, 10 ml of concentrated HCl and 0.85% NaCl or normal saline 1000 mL [31]. The grounded samples were mixed well and were placed in a 37 °C incubator for 2 h with occasional stirring and removed the larger particles by the filtration of digested materials. Then 0.85% saline was added to the digested sample, and we let it stand for a while and discarded the supernatant very carefully and kept the sediment. The procedures were repeated 8 or 9 times until the supernatant became clear and a small quantity of the sediment was
transferred into a Petri dish containing 6–7 ml physiological saline after which metacercariae were observed and identified using a stereomicroscope and light microscope. The detected metacercariae were isolated and were put into a small dish. Finally, the number of metacercariae of each fluke species were counted for further analysis.

2.3. Identification of Metacercariae

For the identification of metacercariae, first of all, similar-shaped metacercariae were collected separately based on the general morphological features in a small Petri dish. Secondly, they were moved with a spool onto a glass slide and were covered with a coverslip; then, detailed morphology was observed under a light microscope. Finally, the metacercariae were identified based on characteristic morphological features. As the characteristic features, the shape of cysts, size of suckers, shape and contents of the excretory bladder were identified [32,33].

2.4. Analysis of Findings

After collected metacercariae were categorized according to the size and morphological characteristics, their percentage prevalence was calculated as follows:

\[
\text{Prevalence (\%)} = \frac{\text{Number of infected fish}}{\text{Total number of fish examined}} \times 100
\]  
(1)

Intensity is the number of metacercariae per fish infected [5].

3. Results

3.1. Infection Status and Prevalence of Fish-Borne Trematode Infection in Freshwater Fishes from Tachileik, Mekong Region of Myanmar

A total of 689 fishes (12 different species of cyprinoid fishes) were collected from local markets in Tachileik, Mekong region of Myanmar. In the total of 689 fishes, 391 fishes were infected with the fish-borne trematode infections, and the overall prevalence was 46.29%. Among the 12 different species, 8 species of cyprinoid fishes, i.e., *B. gonionotus*, *P. falcifer*, *M. marginatus*, *C. repasson*, *S. Rubripinnis*, *l. siamensis*, *H. siamensis*, *R. argyrotaenia*, were contaminated with the infective stage of trematode parasites, metacercariae (Table 2). The metacercariae of *O. viverrini* were found in *C. repasson* (2.64%, n = 151). The metacercariae of *H. taichui* were found infecting 4 species among the total 12 species of fishes. The highest infection prevalence among the four species of fish was found in *R. argyrotaenia* (45%, n = 20), and then, in decreasing order, in *M. marginatus* (38%, n = 100), *C. repasson* (35%, n = 151) and *L. siamensis* (20%, n = 60). The metacercariae of *H. pumilio* were infected in 5 out of 12 species of fishes. The highest infection prevalence among the four species of fish was found in *B. gonionotus* (65%, n = 20) and then with decreasing order in *Systomus rubripinnis* (60.71%, n = 28), *P. falcifer* (53.33%, n = 30), *M. marginatus* (14%, n = 100) and *C. repasson* (7.94%, n = 151). The metacercariae *Haplorchoides* sp. were present in 5 out of 12 species of fishes. The highest prevalence was detected in *C. repasson* (82.78%, n = 151), and the lowest prevalence was occurred in *H. siamensis* (56%, n = 50) and the others were found in *L. siamensis* (63.33%, n = 60), *M. marginatus* (68%, n = 100) and *R. argyrotaenia* (70%, n = 20), respectively.

3.2. Fish-Borne Trematode Metacercarial Intensity in Cyprinid Fishes from Tachileik, Mekong Region of Myanmar

A total of 11 metacercariae of *O. viverrini* were detected from *C. repasson* with a mean intensity of 2.75 per fish infected (Table 3). The metacercariae of *H. taichui* were collected in 94 (28.39%) out of 331 fishes (4 species), i.e., *L. siamensis*, *M. marginatus*, *R. argyrotaenia*, *C. repasson*, with a mean intensity of 3.75 per fish infected (Table 4). The metacercariae of *H. pumilio* were detected in 72 (21.88%) out of 329 fishes (5 species), i.e., *B. gonionotus*, *P. falcifer*, *S. rubripinnis*, *M. marginatus*, *C. repasson*, with a mean intensity of 4.11 per fish infected (Table 5). The metacercariae of *Haplorchoides* sp. were detected in
273 (71.65%) out of 381 fishes (5 species), i.e., L. siamensis, H. siamensis, M. marginatus, R. argyrotaenia, C. repasson, with a mean intensity of 3.57 per fish infected (Table 6).

Table 2. Infection status and prevalence of fish-borne trematode infections in freshwater fish from Tachileik, Mekong region of Myanmar.

| No | Fish Species               | No. of Fish Examined | No. (%) of Fish Infected with FBT | No. (%) of Fish Infected with Ov | No. (%) of Fish Infected with Ht | No. (%) of Fish Infected with Hp | No. (%) of Fish Infected with Hap |
|----|----------------------------|----------------------|----------------------------------|---------------------------------|--------------------------------|---------------------------------|----------------------------------|
| 1  | Barbonymus gonionotus      | 20                   | 13 (65)                          | _                               | 13 (65)                      | _                               | _                                |
| 2  | Puntioplites falcifer      | 30                   | 16 (53.33)                       | _                               | 16 (53.33)                   | _                               | _                                |
| 3  | Systemus rubripinnis       | 28                   | 17 (60.71)                       | _                               | 17 (60.71)                   | _                               | _                                |
| 4  | Labiobarbus siamensis      | 60                   | 38 (63.33)                       | 12 (20)                         | 38 (63.33)                   | _                               | _                                |
| 5  | Henicorhynchus siamensis   | 50                   | 28 (56)                          | _                               | 28 (56)                      | _                               | _                                |
| 6  | Mystacoleucus marginatus   | 100                  | 68 (68)                          | 38 (38)                         | 68 (68)                      | 14 (14)                         | _                                |
| 7  | Rasbora argyrotaenia       | 20                   | 14 (70)                          | 9 (45)                          | 14 (70)                      | _                               | _                                |
| 8  | Systemus orphoides         | 10                   | 0 (0)                            | _                               | _                            | _                               | _                                |
| 9  | Puntius brevis             | 100                  | 0 (0)                            | _                               | _                            | _                               | _                                |
| 10 | Trichogaster trichopterus  | 20                   | 0 (0)                            | _                               | _                            | _                               | _                                |
| 11 | Cyclocheilichthys repasson | 151                  | 125 (82.78)                      | 4 (2.64)                        | 125 (82.78)                  | _                               | _                                |
| 12 | Anabas testudineus         | 100                  | 0 (0)                            | _                               | _                            | _                               | _                                |
|    | Total                      | 689                  | 319 (46.29)                      | _                               | _                            | _                               | _                                |

FBT, Fish-borne trematode; Ov, Opisthorchis viverrini; Ht, Haplorchis taichui; Hp, Haplorchis pumilio; Hap, Haplorchoides sp.

Table 3. Intensity of O. viverrini metacercariae detected in freshwater fishes from Tachileik, Mekong region of Myanmar.

| No | Fish Species               | No. of Fish Examined | No. (%) of Fish Infected Ov | Total Metacercariae Detected | Range (Min–Max) | Intensity |
|----|----------------------------|----------------------|-----------------------------|-----------------------------|----------------|-----------|
| 1  | Cyclocheilichthys repasson | 151                  | 4 (2.64)                    | 11                          | (2–3)           | 2.75      |

Ov, O. viverrini.

Table 4. Intensity of H. taichui metacercariae detected in freshwater fishes from Tachileik, Mekong region of Myanmar.

| No | Fish Species               | No. of Fish Examined | No. (%) of Fish Infected Ht | Total Metacercariae Detected | Range (Min–Max) | Intensity |
|----|----------------------------|----------------------|------------------------------|------------------------------|----------------|-----------|
| 1  | Labiobarbus siamensis      | 60                   | 12 (20)                      | 102                          | (3–12)          | 8.50      |
| 2  | Mystacoleucus marginatus   | 100                  | 38 (38)                      | 120                          | (2–10)          | 3.15      |
| 3  | Rasbora argyrotaenia       | 20                   | 9 (45)                       | 10                           | (1–2)           | 1.11      |
| 4  | Cyclocheilichthys repasson | 151                  | 35 (23.17)                   | 121                          | (1–9)           | 3.45      |
|    | Total                      | 331                  | 94 (28.39)                   | 353                          | (1–12)          | 3.75      |

Ht, H. taichui.
Table 5. Intensity of *H. pumilio* metacercariae detected in freshwater fishes from Tachileik, Mekong region of Myanmar.

| No | Fish Species                | No. of Fish Examined | No. (%) of Fish Infected Hp | Total Metacercariae Detected | Range (Min–Max) | Intensity |
|----|-----------------------------|----------------------|------------------------------|------------------------------|-----------------|-----------|
| 1  | *Barbonymus gonionotus*     | 20                   | 13 (65)                      | 24                           | (1–4)           | 1.84      |
| 2  | *Puntioplites falcifer*     | 30                   | 16 (53.33)                   | 16                           | (1)             | 1.00      |
| 3  | *Systomus rubripinnis*      | 28                   | 17 (60.71)                   | 59                           | (1–7)           | 3.47      |
| 4  | *Mystacoleucus marginatus*  | 100                  | 14 (14)                      | 67                           | (1–6)           | 4.78      |
| 5  | *Cyclocheilichthys repasson*| 151                  | 12 (7.94)                    | 130                          | (1–21)          | 10.83     |
|    | Total                       | 329                  | 72 (21.88)                   | 296                          | (1–21)          | 4.11      |

Hp, *Haplorchis pumilio*.

Table 6. Intensity of *Haplorchoides* sp. metacercariae detected in freshwater fishes from Tachileik, Mekong region of Myanmar.

| No | Fish Species                | No. of Fish Examined | No. (%) of Fish Infected Hap | Total Metacercariae Detected | Range (Min–Max) | Intensity |
|----|-----------------------------|----------------------|------------------------------|------------------------------|-----------------|-----------|
| 1  | *Labiobarbus siamensis*     | 60                   | 38 (63.33)                   | 102                          | (1–7)           | 2.68      |
| 2  | *Henicorhynchus siamensis*  | 50                   | 28 (56)                      | 43                           | (1–3)           | 1.53      |
| 3  | *Mystacoleucus marginatus*  | 100                  | 68 (68)                      | 120                          | (1–5)           | 1.76      |
| 4  | *Rasbora argyrotaenia*      | 20                   | 14 (70)                      | 16                           | (1–2)           | 1.14      |
| 5  | *Cyclocheilichthys repasson*| 151                  | 125 (82.78)                  | 695                          | (1–21)          | 5.56      |
|    | Total                       | 381                  | 273 (71.65)                  | 976                          | (1–21)          | 3.57      |

Hap, *Haplorchoides* sp.

3.3. Co-infections of Trematode Metacercariae in Fresh Water Cyprinoid Fishes Collected from Tachileik, Mekong Region of Myanmar

Simultaneous infections of one fish with two or more trematode species were recorded in some fish species, i.e., *L. siamensis*, *M. marginatus*, *R. argyrotaenia*, *C. repasson* (Table 2). Four species of fish-borne trematode metacercariae, i.e., *O. viverrini*, *H. taichui*, *H. pumilio*, *Haplorchoides* sp. simultaneously infected *Cyclocheilichthys repasson* (Table 2). The fish species *M. marginatus* was infected with *H. taichui*, *H. pumilio*, *Haplorchoides* sp. (Table 2). Co-infection of trematode metacercariae *H. taichui*, *Haplorchoides* sp. was observed in *L. siamensis* and *R. argyrotaenia* (Table 2).

3.4. Morphology of Detected Metacercariae of Fish-Borne Trematode Infections in Freshwater Fishes from Tachileik, Mekong Region of Myanmar

Metacercariae of *O. viverrini* were elliptical, had nearly equal sized oral sucker and ventral sucker, brownish pigment granules scattered within the body and an O-shaped excretory bladder occupying the greater part of the posterior body (Figure 3a). *H. taichui* metacercariae were elliptical and had a baseball glove-shaped ventrogenital sac with rodlets and an O-shaped excretory bladder occupying large portion of posterior body (Figure 3b). *H. pumilio* metacercariae were elliptical and had deer horn-like minute spines arranged in 1–2 rows around the ventrogenital complex and an O-shaped excretory bladder occupying large portion of posterior body (Figure 3c). *Haplorchoides* sp. are nearly spherical, with a double layered cystic wall and have lance-shaped bodies, with a scale like spine on the body surface. Acetabulum with spines present and excretory bladder is saccular (Figure 3d).
We discovered four species of fish-borne trematode metacercariae infections, i.e., carcinogenic liver fluke, *O. viverrini* (Heterophyidae). There are many reports of zoonotic trematode infected fishes that are found in lower Mekong basin countries especially in Thailand [34–37], Laos [38,39], Cambodia [40–43], Vietnam [44–47] and the central region of Myanmar [48–50]. Nevertheless, the contaminated fish species are different in each region. In our study, a total of twelve species of cyprinoid fishes, i.e., *B. gonionotus*, *P. falcifer*, *S. rubripinnis*, *L. siamensis*, *H. siamensis*, *M. marginatus*, *R. argyrotaenia*, *S. Orphoides*, *P. Brevis*, *T. trichopterus*, *C. repasson*, *A. testudineus* were collected and examined by pepsin digestion methods. We discovered four species of fish-borne trematode metacercariae infections, i.e., carcinogenic liver fluke, *O. viverrini*; minute intestinal flukes, *H. taichui*, *H. pumilio* and *Haplorchoides* sp. in Tachileik, Mekong Region of Myanmar.

Chronic infection caused by the Southeast Asian liver fluke (*O. viverrini*) is a critical risk factor for the development of the bile duct cancer cholangiocarcinoma (CCA), which is a major public health concern in Mekong region countries [12–16]. *O. viverrini* infection in humans occurs via the consumption of raw or uncooked fish, which contains metacercariae. A better understanding of the epidemiology of these fish-borne parasites is important for the prevention of CCA in the community. In our study, we discovered the presence of *O. viverrini* metacercariae in small freshwater cyprinoid fish, *C. repasson*, from Tachileik, Mekong region of Myanmar. In addition, this finding is the first report of carcinogenic liver fluke, *O. viverrini* metacercariae infection from Mekong region of Myanmar. The spread of *O.

Figure 3. Photos of fish-borne trematode metacercariae detected in freshwater cyprinoid fishes from Tachileik, Mekong region of Myanmar. (a) *O. viverrini* (b) *H. taichui* (c) *H. pumilio* (d) *Haplorchoides* sp. (EB, excretory bladder; OS, oral sucker; VS, ventral sucker).

4. Discussion

Small freshwater cyprinid fishes are the second intermediate host of trematode infections. Two major agents of fish-borne infections are liver flukes (Opisthorchiidae) and intestinal flukes (Heterophyidae). There are many reports of zoonotic trematode infected fishes that are found in lower Mekong basin countries especially in Thailand [34–37], Laos [38,39], Cambodia [40–43], Vietnam [44–47] and the central region of Myanmar [48–50]. Nevertheless, the contaminated fish species are different in each region. In our study, a total of twelve species of cyprinoid fishes, i.e., *B. gonionotus*, *P. falcifer*, *S. rubripinnis*, *L. siamensis*, *H. siamensis*, *M. marginatus*, *R. argyrotaenia*, *S. Orphoides*, *P. Brevis*, *T. trichopterus*, *C. repasson*, *A. testudineus*, were collected and examined by pepsin digestion methods. We discovered four species of fish-borne trematode metacercariae infections, i.e., carcinogenic liver fluke, *O. viverrini*; minute intestinal flukes, *H. taichui*, *H. pumilio* and *Haplorchoides* sp. in Tachileik, Mekong Region of Myanmar.

Chronic infection caused by the Southeast Asian liver fluke (*O. viverrini*) is a critical risk factor for the development of the bile duct cancer cholangiocarcinoma (CCA), which is a major public health concern in Mekong region countries [12–16]. *O. viverrini* infection in humans occurs via the consumption of raw or uncooked fish, which contains metacercariae. A better understanding of the epidemiology of these fish-borne parasites is important for the prevention of CCA in the community. In our study, we discovered the presence of *O. viverrini* metacercariae in small freshwater cyprinoid fish, *C. repasson*, from Tachileik, Mekong region of Myanmar. In addition, this finding is the first report of carcinogenic liver fluke, *O. viverrini* metacercariae infection from Mekong region of Myanmar. The spread of *O.
viverrini infections relies on the presence of intermediate hosts, i.e., first intermediate host (snails), the second intermediate hosts (freshwater fishes) and dietary preferences of local populations for uncooked or undercooked fish. Traditional dishes prepared from freshwater cyprinoid fishes are the source of fish-borne trematode infections [36]. Therefore, local people should be educated about the danger of ingestion of undercooked fish dishes, in order to increase awareness for the prevention of fish-borne trematode transmission.

In Myanmar, seven species of minute intestinal flukes, i.e., H. taichui, H. pumilio, H. yokogawai, Centrocestus sp., Stellantchasmus falcatus, Pygidioopsis cambodiensis, and Procerovum sp., were reported in fishes from local market of Yangon, Myanmar in 2017 by J. Chai et al. [5]. They found that H. taichui metacercariae were detected in 5 species of fishes, T. thynnoides, P. aurotaeniatus, E. altus, Mystacoleucus sp. and Labeo sp. [5]. In our present study, 4 out of 12 species of freshwater fishes were found positive for H. taichui metacercariae, i.e., M. marginatus, C. repasson, L. siamensis and R. argyropaenia. Therefore, a total of 9 species of freshwater fishes from Myanmar are confirmed to be second intermediate hosts of H. taichui metacercariae. Currently, H. taichui metacercariae have been identified as occurring in 48 species of freshwater cyprinoid fishes in southeast Asia countries [51–54].

In addition, we found that H. pumilio metacercariae infected 5 out of 12 species, i.e., B. gonionotus, P. falcifer, M. marginatus, C. repasson and S. rubripinnis. In the study from J. Chai et al. 2017, H. pumilio metacercariae were detected in 9 species of freshwater fishes, T. thynnoides, P. aurotaeniatus, E. altus, C. striata, A. testudineus, Rhynogobius sp., T. pectoralis, Mystacoleucus sp. and Labeo sp. [5]. Hence, a total of 14 species of freshwater fishes from Myanmar are the second intermediate hosts of H. pumilio metacercariae. Currently, a total of 39 freshwater cyprinoid fish species have been recorded as the second intermediate host of H. pumilio in southeast Asia countries [55–58].

The metacercariae Haplorchoides sp. were infected in 5 species among the total 12 species, i.e., L. siamensis, H. siamensis, M. marginatus, R. argyropaenia and C. repasson. The finding of Haplorchoides sp. from our study is the first reported from Myanmar. In 2018, K. Apiwong et al. reported finding Haplorchoides sp. in B. schwanenfeldii and C. repasson from Chiang Mai province, Thailand [59]. Our study confirms that the life cycle of Haplorchoides sp. happens around Tachileik, the Mekong Region of Myanmar.

5. Conclusions

In this study, four species of trematode metacercariae (carcinogenic liver flukes, O. viverrini, and minute intestinal flukes, members of the Heterophyidae, H. taichui, H. pumilio and Haplorchoides sp.) were detected in freshwater fishes from a local market of Tachileik, the Mekong region of Myanmar. This study demonstrates the existence of the life cycles of four species of fish-borne trematode infections around Tachileik, the Mekong region of Myanmar. The study of the distribution and infection status of the fish-borne trematode metacercariae could contribute to solving the important public health problem posed by these trematodes and may provide valuable information for prevention and control programs of human liver fluke and intestinal fluke infections for the community. Furthermore, the outcome of this study could be a useful index in the trematode epidemiology in the Mekong area. In addition, the results of our study contribute important information for collaborative prevention of carcinogenic liver fluke infection among the lower Mekong region countries. Further research studies should be considered for public health interventions using health-education and sanitation-improvement approaches in the control program. The findings of our present study would be important and supportive not only from a parasitological but also from a public-health point of view.

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