

TREMATODE METACERCARIAE INFECTION IN CYPRINOID FISH FROM UBOLRATANA RESERVOIR, NONG BUA LAM PHU PROVINCE, THAILAND

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ABSTRACT

Background: Fish-borne trematode species are associated with public health problems. Metacercariae is a parasitic infective stage that is transmitted to humans through consumption of traditional dishes containing raw cyprinoid fish. Liver fluke infection is one of the factors that can lead to cholangiocarcinoma or bile duct cancer.

Materials and Methods: In this study, trematode metacercariae in cyprinoid fish were identified. All fish were obtained from Ubolratana Reservoir in Nong Bua Lam Phu province, Thailand, from June to September 2019. A total of 521 cyprinoid fish samples were collected and analysed. The fish samples were digested in a 0.25% pepsin-HCl solution and filtered through various sized sieves. The metacercariae were observed under stereo and compound light microscope.

Results: 1,998 metacercariae in seven fish species were identified using stereo and compound microscopy. *Hampala dispar* was infected with the highest mean intensity of metacercariae (52 Mc/fish) whereas *Puntius* sp. showed the lowest mean intensity (0.24 Mc/fish). The trematode species, *Haplorchis pumilio*, presented the largest proportion in total fish infected with metacercariae (44.32%) followed by *Opisthorchis viverrini* (25.52%), *Haplorchis taichui* (12.44%) and *Haplorchoides* spp. (1.73%).

Conclusions: The results of our study indicate the presence of contaminated fish in Ubolratana Reservoir. This is a caution for villagers to avoid uncooked fish dishes. The mean intensities of metacercariae were calculated yielding interesting results. Therefore, a study of the prevalence of metacercariae infection will be conducted in 2020 by our research team.

Keywords: Metacercariae, Cyprinoid fish

1.0 Introduction

Human liver fluke is a carcinogenic agent for bile duct cancer or cholangiocarcinoma (CCA) (Sripa et al., 2007). This parasite is endemic in Southeast Asian countries, some European countries, Russia, China and South Korea (Suwannatrai, Saichua, & Haswell, 2018). There are three species of human liver fluke, *Opisthorchis viverrini*, *Clonorchis sinensis*, and *Opisthorchis felineus*. In Thailand, Opisthorchiasis, caused by *Opisthorchis viverrini*, has been studied. Its infection rate in Nakhon Ratchasima province was found to be 2.48% from a sample of 1,168 residents (Kaewpitoon et al., 2012). In Loei province, it was reported in 15.63% of 595 stool samples (Yospanya et al., 2015), while a study in Udon Thani province showed 31.5% of 5,347 participants infected (Prakobwong et al., 2017).

The life cycle of liver fluke requires two intermediate hosts, the *Bithynia* snail and a cyprinoid fish as the first and second intermediate hosts, respectively. The parasite is transmitted to humans when raw cyprinoid fish are consumed. The numbers of trematode metacercariae in cyprinoid fish have been reported in several areas of Thailand. These include Amnat Charoen (6.5 Mc/fish), Nakhon Phanom (4.3 Mc/fish), Mukdahan (4.1 Mc/fish), Khon Kaen (3.5 Mc/fish), Si Sa Ket (3.4 Mc/fish), Nakhon Ratchasima (51.9% prevalence of infected fish) and Chiang Mai (0.3-165.2 Mc/fish) (Pinlaor et al., 2013; Nithiuthai et al., 2002; Sukontason et al., 2001). The cyprinoid fish contaminated with metacercariae were *Cyclocheilichthys armatus*, *Puntius orphoides*, *Hampala dispar*, *Henicorhynchus siamensis*, *Osteochilus hasselti*, *Puntioplites proctozysron* and *Puntius leiakanthus* (Pinlaor et al., 2013; Nithiuthai et al., 2002).

Ubolratana Dam is located in Northeastern Thailand. It is a large fresh water reservoir situated between two provinces, Khon Kean and Nong Bua Lam Phu. Most of residents of the surrounding area are fisherman and fish is a major protein source for them. The aim of this study is to report the mean intensity of metacercariae in cyprinoid fish obtain from Ubolratana Reservoir. Moreover, the metacercariae species were determined. Knowledge of the incidence of metacercariae in cyprinoid fish will benefit the local people and public health organizations.

2.0 Materials and Methods

2.1 Sample collection

1-2 kg samples of cyprinoid fish were purchased from local fisherman at Khok-Yai subdistrict, Non Sang district, Nong Bua Lam Phu province. Fish were collected during the period of June to September 2019. All samples were kept in an ice-box and promptly brought to the laboratory. At the laboratory, fish species were identified morphologically (Saenjundaeng, 2014). The fish were counted and their lengths measured.

2.2 Examination of Metacercariae in Cyprinoid fish




A pool of samples were minced and mixed in a pepsin solution (0.25% in 1% HCl). The suspension was transferred to 50 ml centrifuge tubes, incubated at 37 °C for 1 hour with manual stirring every 10 minutes. After that, they were filtered through a sieve with a mesh size 850 µm. The solid material was washed through the sieve with 0.85% NaCl and allowed to precipitate in the sedimentation cone. Then, the sediment of the samples was filtered through a 300 µm sieve. After that, the flow through was precipitated and washed with 0.85% NaCl. The precipitate was observed under a stereo microscope. Metacercariae were prepared as a wet mount slide then examination under a compound light microscope. Morphological identification of trematode metacercariae, examining their suckers, shape of juveniles, shapes of their excretory bladders and ventrogenital sac were considered for classification of the trematode species according to Sohn et al. (2009).





3.0 Results

3.1 Sample collection

A total of 521 cyprinoid fish samples were examined, consisting of seven species, *Puntioplites proctozystron*, *Gymnostomus siamensis*, *Hampala dispar*, *Puntius* sp., *Cyclocheilichthys repasson*, *Barbonymus goinonotus* and *Labiobarbus leptocheilus*. The average length of the cyprinoid fish was 10.96 cm. (6 - 13 cm.) Examples of each cyprinoid fish species examined are shown in Table 1.

Table 1. Fish species and morphology of cyprinoid fish from Ubolratana Reservoir

Fish Species	Number of Fish	Morphology
<i>Puntioplites proctozystron</i>	121	
<i>Gymnostomus siamensis</i>	127	
<i>Hampala dispar</i>	2	

<i>Puntius sp.</i>	50	
<i>Barbonymus attus</i>	86	
<i>Cyclocheilichthys repasson</i>	114	
<i>Labiobarbus leptocheilus</i>	21	
Total	521	

3.2 Examination of Metacercariae in Cyprinoid fish

A total of 1,998 metacercariae were found. The metacercariae species were identified by morphological methods examining the specific characters of each species. The oral sucker, ventral sucker, ventroginital sac and excretory bladder of metacercariae are shown in Figure 1. The mean number of each metacercariae species was calculated and it is shown in Table 2. *H. dispar* exhibited the highest average numbers, 52 metacercariae per fish, followed by *G. siamensis* (6.26), *L. leptocheilus* (5.41), *B. goinonotus* (4.44), *C. repasson* (3.64), *P. proctozystron* (1.49), and *Puntius sp.* (0.24). The details of metacercariae findings are shown in Table 2.

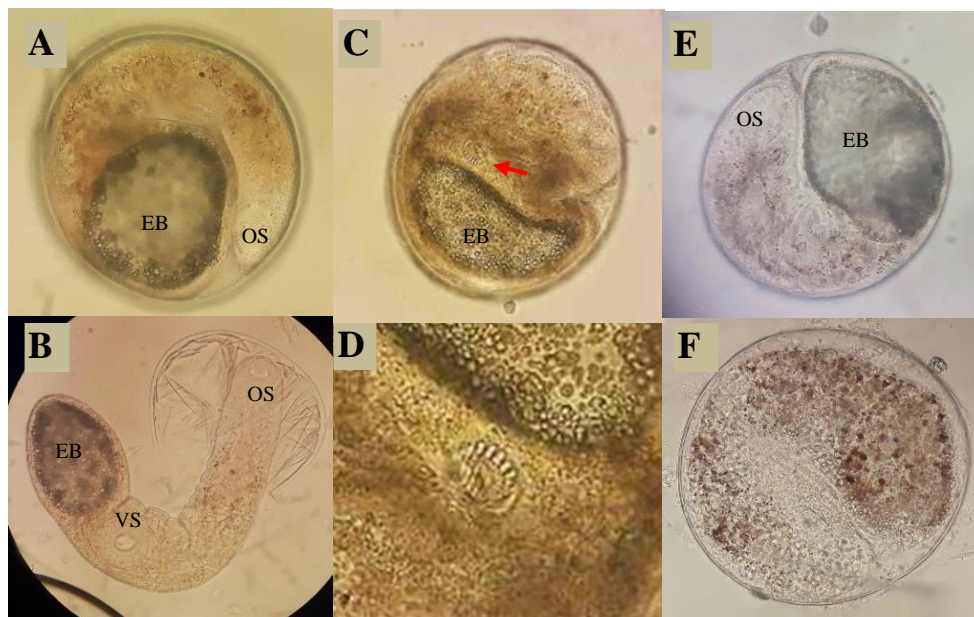


Figure 1. Panel A: *O. viverrini*, B: Juvenile of *O. viverrini*, C: *H. taichui*, D: Baseball glove shape of ventroginital sac from *H. taichui*, E: *H. pumilio*, F: Unidentified metacercariae
Abbreviation; OS: oral sucker, EB: excretory bladder, VS: ventral sucker

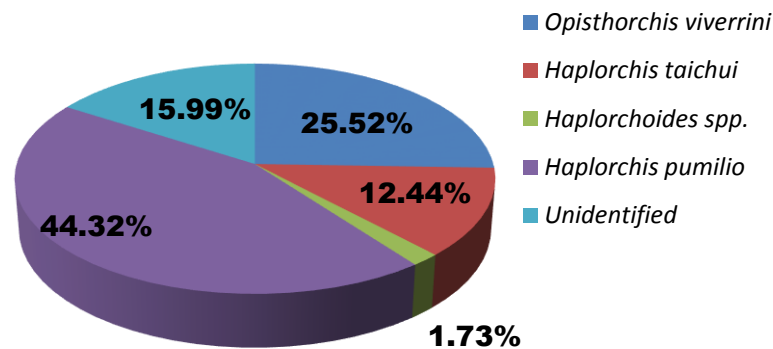
Table 2. Mean number of metacercariae in cyprinoid fish from Ubolratana Reservoir

Cyprinoid fish	No. of fish	No. of Metacercariae					Total of Mc	Mean (Mc/Fish)
		Ov	Ht	H	Hp	U		
<i>H. dispar</i>	2	20	20	-	50	14	104	52
<i>G. siamensis</i>	127	75	120	25	526	50	796	6.26
<i>L. leptocheilus</i>	21	-	-	-	93	15	108	5.14
<i>B. goinonotus</i>	86	238	40	1	80	23	382	4.44
<i>C. repasson</i>	114	252	42	12	110	-	416	3.64

<i>P. proctozystron</i>	121	72	52	-	21	35	180	1.49
<i>Puntius</i> sp.	50	-	-	-	12	-	12	0.24
Total	521	657	274	38	892	137	1,998	3.83

Ov= *O. viverrini* H= *Haplorchoides* spp. Ht= *H. taichui* Hp= *H. pumilio* U= Unidentified

Figure 2. Proportion of metacercariae species



Among the parasitic species, *H. pumilio* was the most prevalent (44.32%) followed by *O. viverrini* (25.52%), *H. taichui* (12.44%) and *Haplorchoides* spp. (1.73%) (Figure 2). There were some unidentified metacercariae in which oral and ventral suckers were hidden or otherwise obscured.

4.0 Discussion

The fish species, *H. dispar*, was infected with higher numbers of metacercariae. This result is similar to that of a previous study by Sithithaworn et al. (1997). They reported the intensity of metacercariae among three fish species, *Hampular dispar*, *Puntius leiacanthus* and *Cyclocheilichthys armatus*, during the period November 1991 to December 1992 at Mahasarakham Province. Also, Nithiuthai et al., (2002) found the least infected species in Nakhon Ratchasima Province was *H. dispar*. However, in our study, the samples were randomly collected and *H. dispar* was found in a small number of cases. This could affect the

mean intensity of infection (Jovani and Tella, 2006). Thus, a larger sample size is recommended.

This study was conducted during the rainy season in Thailand (June to September 2019) and revealed heavy infection in some species. This result agrees with Wiwanitkit (2005), who reported that rainfall during the season has a significant correlation with the prevalence of fresh water fish infection when the data was examined using least-squares regression ($r=0.858$, $p<0.05$). However, another research study showed higher infection rates in cyprinoid fish during the dry season (Prakobwong et al., 2017).

Minute intestinal flukes, *H. pumilio* and *H. taicui*, were the predominant species (44.32% and 12.44% respectively among the infections). Our results agree with Sukontason et al., (2001) reported that the *Haplorchis* spp. accounted for 75.45% of parasite infections in cyprinoid fish from Chiang Mai Province, Thailand. Additionally, Kamchoo et al., (2005) reported the most commonly found metacercariae was *H. taichui*. Interestingly, our research showed that the second most common metacercariae species was *O. viverrini*, accounting for 25.52% of the total number of infections. This is different from our previous results in 2018 (unpublished data) where we found a 6.4% rate of infection in cyprinoid fish due to *O. viverrini* in Ubolratana Reservoir. The difference is probably a consequence of a different sampling site at this location. Additionally, the numbers of first intermediate hosts (*Bithynia* snails) probably affects this result because snails are responsible for transmission to cyprinoid fish in the life cycle of *O. viverrini* (Kiatsopit et al., 2012, Piratae, 2015).

5.0 Conclusions and recommendations

The infection rate of cyprinoid fish in Ubolratana Reservoir at Non Sang district is valuable information for villagers who consume these fish as a protein source. Awareness of the unhealthy practice of consuming raw fish dishes will raise their concern. A long-term study of the prevalence of such infections is recommended.

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Declaration

Authors declare that there are no conflicts of interest in the conduct of the current study and publication of this article

Author contributions

N. Labunruang: Design of the study, preparing, writing and editing the manuscript

W. Kaensa: Fish species identification and manuscript review

S. Krommakot and O Buayairaksa: Data collection

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