

PRELIMINARY SURVEY OF TREMATODE METACERCARIAE IN CYPRINOID FISH FROM LAM PAO DAM, KALASIN, THAILAND

Nipawan Labbunruang, Jeerapaporn Insrichaengmai and Sasirin Chairach
Department of Biology, Faculty of Sciences, Udon Thani Rajabhat University,
Udon Thani, Thailand.
Email: nipawan7585@gmail.com

ABSTRACT Fish-borne trematode species have been associated with serious health problems in Thailand. The major causative trematode species are *Haplorchoides* sp. and *Opisthorchis viverrini*. Briefly, the infective stage of the parasite is transmitted to the human host by consumption of traditional dishes that contain raw cyprinoid fish, mainly in Northeast Thailand. In this study, we investigated the trematode metacercariae in cyprinoid fish from Lam Pao dam, Mueang District, Kalasin Province of Thailand during the time period of July–September 2014. A total of 534 cyprinoid fish samples consisting of six species namely; *Henicorhynchus siamensis*, *Hampala dispar*, *Cyclocheilichthys repasson*, *Puntioplites proctozysron*, *Osteochilus vittatus* and *Barbonymus gonionotus* were analyzed for infections. The fish samples were digested in 0.25% pepsin-HCl solution and the resulting mixture was then filtered through sieves with mesh sizes of 850, 300, 150 and 45 µm. The collected metacercariae were observed and identified under classical stereo and compound microscopes. *Cyclocheilichthys repasson* was found to be infected with the highest number of metacercariae (5.85 metacercariae/fish) while *Barbonymus gonionotus* had no metacercarial infection. The largest number of metacercariae was found for *Haplorchis pumilio* (58.89%), followed by *Haplorchoides* sp. (18.74%), *Haplorchis taichui* (17.40%), and *Opisthorchis viverrini* (2.20%). Therefore, this preliminary survey indicated that the prevalence of infection with *Haplorchoides* species is still high. Nevertheless, long term examination is needed to clarify the prevalence of trematode metacercariae in Lam Pao dam.

INTRODUCTION

Over 18 million people worldwide are infected with fish-borne trematodes, a serious public health problem,

especially in countries of South East Asia (WHO, 2004). The important causative parasites are liver and intestinal flukes including *Opisthorchis viverrini*, *Clonorchis sinensis* and members of the family Heterophyidae. Human infections with the parasite infective stage (metacercariae) take place by ingestion of raw or undercooked fish dishes (Sripa et al., 2010).

Campaigns in the endemic areas to avoid consumption of raw fish dish in order to prevent infection were not successful (Rangsin et al., 2009). The cyprinoid fish species are ingredients of traditional dishes of the local people in Northeast Thailand (Sripa et al., 2010).

Many studies investigated the liver flukes and intestinal flukes in cyprinoid fish. The cyprinoid fish species *Cyclocheilichthys repasson*, *C. armatus*, *Henicorhynchus lineatus*, *Puntioplites proctozysron*, *Hampala dispar*, and *Osteochilus waandersii* were reported to be positive for *Opisthorchis viverrini* metacercariae (Manivong et al., 2009). Metacercariae of Heterophyidae were found in *Puntius leiakanthus*, *Cyclocheilichthys armatus* and *Hampala dispar* (Nithiuthai et al., 2002). Northeast Thailand is the endemic area for opisthorchiasis (Parkin, 2006). Lam Pao dam is an important fresh water reservoir, located in Kalasin province that provides fresh water fish for residents who live along the dam. The observation of trematode metacercariae in cyprinoid fish from Lam Pao dam is the objective of this study.

MATERIALS AND METHODS

Collection site:

The study was conducted at the Lam Pao dam, Mueang district, Kalasin, northeastern Thailand during July–September 2014. A number of 534 fish samples was collected by local fishermen. Samples were kept on ice and transported to the laboratory.

Identification of trematode metacercariae:

The species of a total of 534 cyprinoid fishes were identified by morphological identification. All samples were digested with pepsin solution (0.25% pepsin in 10 mM HCl) for 90 min at 37°C (modified from previous method Waikagul, 1998) and then filtered through a sieve with mesh size 850 µm and washed with 0.85% NaCl. Then the sample was allowed to settle in sedimentary jar and filtered through sieves with mesh sizes of 300, 150 and 45 µm. The sediment was observed under stereo microscope and compound microscope and metacercarial species were identified using morphological examination according to Scholz et al., 1991 and Sohn et al., 2009.

RESULTS

Six fish species were identified; 199 *Henicorhynchus siamensis*, 128 *Hampala dispar*, 113 *Cyclocheilichthys repasson*, 77 *Puntius proctozysron*, 12 *Osteochilus vittatus* and 5 *Barbonymus gonionotus*.

A total of 1,046 metacercariae was found, they were identified as *Opisthorchis viverrini*, *Haplorchis pumilio*, *Haplorchis taichui* and *Haplorchoides* sp. The number of metacercariae per fish was different in each month, the highest number was found in September (Table 1).

Table 1 Metacercarial burden per fish in July, August and September 2014.

Month	No. of fish	No. of MC	MC/fish
July	126	142	1.13
August	362	596	1.65
September	46	308	6.70

MC: metacercariae

Table 2 Identified fish species (1–6) and trematode species (A–E) collected in July–September 2014.

Fish	No. of fish	Trematode metacercariae					Total	MC /fish
		A	B	C	D	E		
1	199	21	112	36	64	7	240	1.21
2	128	1	35	23	14	5	78	0.61
3	113	0	445	103	96	17	661	5.85
4	77	1	24	19	22	0	66	0.86
5	12	0	0	1	0	0	1	0.08
6	5	0	0	0	0	0	0	0
Total	534	23	616	182	196	29	1046	

1: *Henicorhynchus siamensis*, 2: *Hampala dispar*, 3: *Cyclocheilichthys repasson*, 4: *Puntius proctozysron*, 5: *Osteochilus vittatus*, 6: *Barbonymus gonionotus*, A: *O. viverrini*, B: *H. pumilio*, C: *H. taichui* D: *Haplorchoides* sp., E: Unknown

The cyprinoid fish species *Cyclocheilichthys repasson* contained the highest number of metacercariae per fish (5.85) followed by *Henicorhynchus siamensis* (1.21), *Puntius proctozysron* (0.86), *Hampala dispar* (0.61), *Osteochilus vittatus* (0.08). Metacercariae were not found in *Barbonymus gonionotus*, details are shown in Table 2.

Among the metacercariae species, we found the highest percentage for *Haplorchis pumilio* metacercariae (58.89%), followed by *Haplorchoides* sp. (18.74%), *H. taichui* (17.40%), and *O. viverrini* (2.2%), respectively (shown in Figure 1).

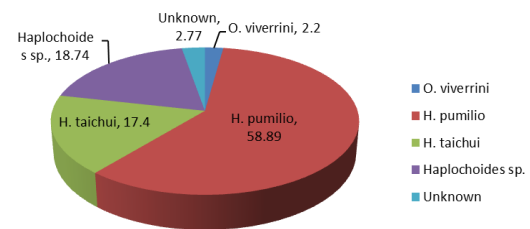


Fig. 1 Percentage of trematode metacercariae observed in cyprinoid fish from Lam Pao dam

Morphological analysis of the metacercariae showed that *O. viverrini* has brownish color, oral and ventral suckers have equal size. *Haplorchis pumilio* has a thin-wall, an O-shaped excretory bladder that fills a large portion of the metacercaria. *Haplorchis taichui* has a thin-wall, an S-shaped juvenile, a small excretory bladder, and a baseball glove-shaped ventrogenital sac. *Haplorchoides* sp. has a thin-wall and C- or S-shaped juvenile as present in Figure 2.

DISCUSSION

This preliminary survey was conducted during July–September 2014. In the three months, a number of 1,046 trematode metacercariae was detected in 534 fishes. The highest number of metacercariae per fish was found in September (6.69). This result is related to a previous study by Vichasri et al., 1982 that reported a peak of metacercariae in the late rainy season. The relationship between the prevalence of *O. viverrini* metacercariae and water levels was reported as significant negative correlation (Touch et al., 2013).

The percentage of *Haplorchis pumilio* and *Haplorchis taichui* in this study was higher than *O. viverrini* which is consistent with the report from Nithiuthai et al., 2002 in which *O. viverrini* metacercariae were not found in the cyprinoid fish species *Puntius leiakanthus*, *Cyclocheilichthys armatus* and *Hampala dispar*. Moreover, Nithiuthai et al., 2002 also found that *Cyclocheilichthys* spp. harbored a large number of metacercariae which is consistent with our study that shows that *Cyclocheilichthys repasson* has the

highest burden of metacercariae per fish (5.85). In addition, almost all *O. viverrini* were detected in *Henicorhynchus siamensis* whereas a previous study reported almost all of *O. viverrini* metacercariae was found in *Cyclocheilichthys armatus* (Pinlaor et al., 2013).

Although this is a preliminary survey the results indicate that cyprinoid fish in Lam Pao dam are positive for trematode metacercariae. This data benefits local public health centers, raises awareness of fish borne parasitic infection since heavy infections of parasite will cause a serious health problem, for instance chronic liver fluke infection leads to cholangiocarcinoma development (Sripa & Pairojkul, 2008). However, further studies of trematode metacercariae in cyprinoid fish from Lam Pao dam is needed to obtain better statistics on the prevalence and density of metacercariae.

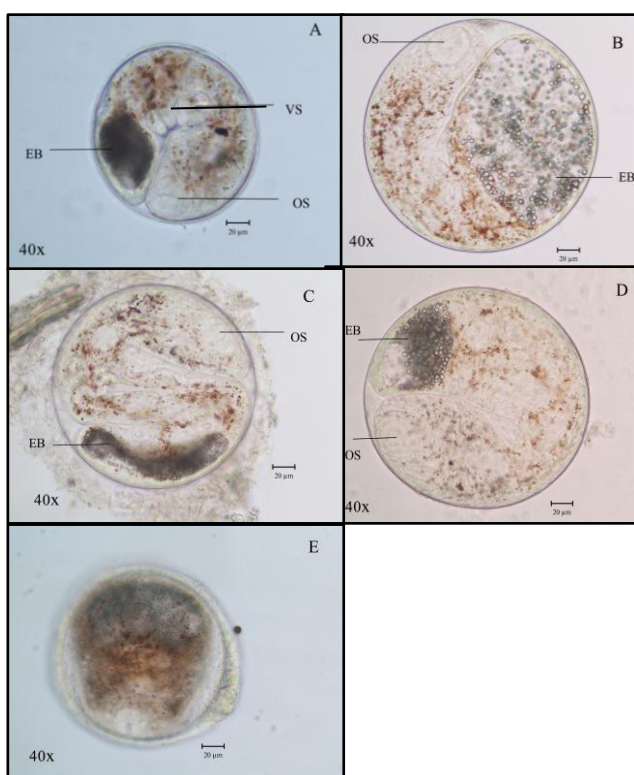


Fig. 2 Morphology of trematode metacercariae.
(Scale bar = 20 μm)

A: *Opisthorchis viverrini*, B: *Haplorchis pumilio*,
C: *Haplorchis taichui*, D: *Haplorchoides* sp.,
E: Unknown, OS: Oral sucker, VS: ventral sucker,
EB: excretory bladder

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Nipawan Labbunruang received the B.Sc. in Genetics (2003) from Kasetsart University, the M.Sc. in Biology (2007) from Khon Kaen University, Ph.D candidate in Biomedical Sciences program at Thammasat University.

She is a lecture in Department of Biology, Udon-Thani Rajabhat University. Her Current interests include trematode metacercariae and molecular parasitology.



Jeerapapor Insrichaengmai received the B. Sc. in Biology (2015) from Udon Thani Rajabhat University.



Sasirin Chairach received the B. Sc. in Biology (2015) from Udon Thani Rajabhat University.