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Prevalence and intensity of metacercariae in cyprinoid fish from Lam Pao Dam reservoir, Northeastern Thailand

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Abstract - The northeast of Thailand is considered as an endemic area of trematode infection. The infective stage of trematode (metacercariae) is encysted in the cyprinoid fish which transmitted to human by raw fish ingestion. The aims of our study were to report the prevalence of the infected cyprinoid fish from Lam Pao Dam Reservoir and identify the metacercariae species. This research was conducted during May to August 2019. Fish species were identified by morphological method then an individual samples were minced and mixed in 0.25% pepsin solution in 1% HCl. The suspension was filtered with a series of sieves (850, 300 mm) and washed with 0.85% NaCl. All metacercariae were identified under a stereomicroscope and a compound light microscope. The total of 482 cyprinoid fish individuals were identified as seven fish species namely *Henicorhynchus siamensis*, *Puntioplites proctozystron, Barbonymus altus, Osteochilus vittatus, Osteochilus lini, Puntius brevis* and *Thynnichthys thynnoides.* Metacercariae were found in five of seven species which *H. siamensis* showed the highest percentage (8.18% of prevalence) with intensity was 13.61 metacercariae per fish. A total of 278 metacercariae were found and *Haplorchis pumilio* (88.13%) was the most abundant metacercariae. The infection of *Opisthorchis viverrini* and heterophyid metacercariae was also reported in this study. This research indicates the risk of human parasite infection when consuming raw fish.

Keywords: Prevalence, metacercariae, trematode, cyprinoid fish

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1. Introduction

Foodborne trematodiases are caused by trematode worms. A majority of human flukes are *Clonorchis sinensis, Opisthorchis viverrini, Fasciola spp.,* and *Paragonimus spp.* Foodborne Disease Burden Epidemiology Reference Group (FERG) estimates the foodborne illness from trematode infection in Southeast Asia were 3-12 per 100,000 and 11,059 Thai people were infected with *Opisthorchis* spp. (FERG, 2015). Human liver fluke, *Opisthorchis viverrini* (Ov), is a common infection in Southeast Asia; Thailand, Lao PDR, Cambodia, and Vietnam. Long lasting infection or severe infection would lead to a development of hepatobiliary disease and cholangiocarcinoma (CCA) (Sripa et al., 2007). The gold standard technique for diagnosis of liver fluke infection is a microscopic method with stool samples. However, the burden of parasite and the egg of intestinal fluke affect the accuracy of the test (Prueksapanich et al., 2018). The diagnosis can be made by examining the genetic material of liver flukes found in the stool. Detection can be achieved using a polymerase chain reaction (PCR)-based method. Ov eggs in human feces were detected by using PCR method. Wongratanecheewin et al., (2002) found the limit of our PCR detection was 200 eggs or 200 pg of genomic DNA. The expected size of PCR product was 330-bp and their doublets of 660 bp were in 32 of the 40 fecal specimens (80.0%) from patients with Ov.

In Thailand, the incidence of *Opisthorchis viverrini* in the northeast and the northern were investigated. Nakhon phanom was the highest prevalence (40.9%) among the other provinces in upper northern Thailand (Thaewnongiew et al., 2014) Mahasarakham province showed 15.0% prevalence of Ov infection in 2014 (Chaiputcha et al., 2015).

Regarding to trematode life cycle, cyprinoid fish are the second intermediate host in trematode life cycle. Human are infected by consuming raw or undercooked fish dish. Metacercariae will be excysted at duodenum and move to gall bladder, bile duct or liver.

Trematode metacercariae has been reported in Mekong Basin countries; Thailand, Lao PDR, Cambodia and Myanmar. The intensity of metacercariae from these countries was during 0.3 mc/fish to 2,440 mc/fish (Sukontason et al., 2001, Rim et al., 2008). The studies of metacercariae in Thailand were conducted in many provinces in Northern and Northeast regions for instance Lamphun province (164.2 and 252.5 mc/fish) (Butboonchoo1& Wongsawad, 2019), Yasothon province (267.23-0.5 Mc/fish) (Kaopiew et al., 2010). The cross-sectional study of Ov metacercariae in 20 provinces of northeastern, Thailand showed that metacercariae were found in 6 species of fish; *Cyclocheilichthys armatus*, *Puntius orphoides*, *Hampala dispar*, *Henicorhynchus siamensis*, *Osteochilus hasselti*, and *Puntioplites proctozysron* from 13 provinces. The fish samples from Kalasin province were negative (Pinlaor et al., 2013). However, the prevalence of liver fluke and intestinal fluke infection in humans was reported at 33.65% in this province (Yahom et al., 2013). Therefore, our study focused on the fresh water reservoir in Kalasin province.

The Lam Pao Dam reservoir is situated between Kalasin and Udon Thani provinces. It is surrounding with several villages. Fish are the main source of

protein for the local people. The consumption of uncooked fish is a risk of the parasite infection. Therefore, our research aim is an investigation of metacercariae in cyprinoid fish from the Lam Pao Dam reservoir.

2. Materials and methods

2.1 Sample collection

Sample size calculation (Cochran, 1977)

$$n = Z^2/4\ e^2$$

n = 384 samples

P = 0.5

Confident level = 95%

Precision = 5%

Cyprinoid fish were purchased from the local market at Dong Som Boon village, Tha Khan Tho district, Kalasin province (16°54'09.2"N 103°22'40.8"E). This market is located next to Lam Pao Dam Reservoir. Fish were collected four times during the period of May to August 2019 (one time per month). All samples were kept in an ice-box and promptly brought to the laboratory, Faculty of Sciences, Udon Thani Rajabhat University. At the laboratory, fish species were identified morphologically following a handbook of Saenjundaeng (2014). The fish were counted and their lengths measured.

2.2 Examination of metacercariae in cyprinoid fish

An individual samples were minced and mixed in a 0.25% pepsin solution in 1% HCl. The suspension was transferred to 50 ml centrifuge tubes then incubated at 37 °C for 1 hour with manual stirring every 10 minutes. The samples were filtered through a sieve with a mesh size 850 µm. The sieve was washed with 0.85% NaCl and allowed the flow through to precipitate in 50 ml centrifuge tubes. The sediment of the samples was filtered through a 300 µm sieve. After that, the suspension was precipitated and washed with 0.85% NaCl. The sediment was observed under a stereo microscope. A wet mount slides with metacercariae were prepared and examined under a compound light microscope. Morphological identification of trematode metacercariae was considered for classification of the trematode species following to Sohn et al. (2009). Their specific characters were the size of suckers, a shape of juveniles, a shape of excretory bladders and ventrogenital sac.

2.3 Calculation of prevalence and intensity

The infected fish was counted and calculate the percentage of prevalence and intensity

$$\text{Prevalence} = \frac{(\text{Number of infected fish x } 100)}{\text{Number of examine}}$$

$$\text{Intensity} = \frac{\text{Number of metacercariae}}{\text{Number of infected fish}}$$

Range = Minimum number of metacercariae per fish - Maximum number of metacercariae per fish

3. Results & discussion

3.1 Sample collection

A total of 482 fresh water fish (n = 482) were collected. The fish species were identified by their morphology. There are seven species including *Henicorhynchus siamensis, Puntioplites proctozystron*, *Barbonymus altus*, *Osteochilus vittatus*, *Osteochilus lini*, *Puntius brevis* and *Thynnichthys thynnoides*. Their average lengths were varied from 18.00 to 10.70 centimeter Morphological classification is shown in Table 1.

Table 1. Classification of Cyprinoid fish species and average length

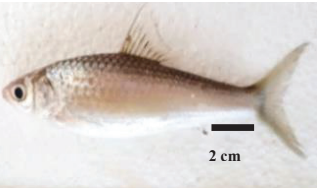
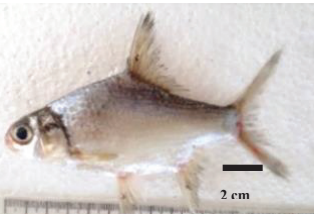
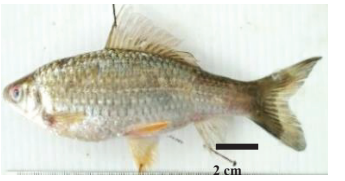
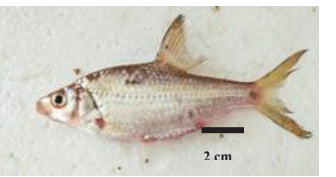
Fish species (numbers)	Average length (cm.)	Morphology
Henicorhynchus siamensis (220)	12.67	
Puntioplites proctozystron (101)	11.98	
Barbonymus altus (93)	10.9	
Osteochilus vittatus(44)	14.24	
Osteochilus lini (18)	13.36	

Table 1. Classification of Cyprinoid fish species and average length (cont.)

Fish species (numbers)	Average length (cm.)	Morphology
Puntius brevis (5)	10.70	
Thynnichthys thynnoides (1)	18.00	

3.2 Examination of metacercariae in cyprinoid fish

Five of seven fish species contained metacercariae which the highest prevalence was in *Henicorhynchus siamensis* (8.18%) followed by *Osteochilus lini* (5.56%), *Osteochilus vittatus* (4.54%), *Puntioplites proctozystron* (3.96%), *Barbonymus altus* (2.16%). This result is agreed with Charoensuk et al. (2022) the high prevalence of *H. siamensis* was reported in the upper part of northeast of Thailand. *H. siamensis* was infected by *O.viverrini* as 13.7% prevalence. Besides, Jitkham et al. (2020) were reported *H. siamensis* in Chiang Rai province was found with high prevalence (100%) and the high intensity (33.14 metacercariae per infected fish (mc/fish)). Interestingly, in the past 16 years *H. siamensis* showed 100 % of prevalence with 120.4 mc/fish which is greater than our study (Nithikathkul & Wongsawad, 2008). The other fish species; *M. marginatus* and *O. vittatus,* from Li river, Lamphun province were infected with a large number of metacercariae with 100% and 69.2% prevalence and 164.2 and 252.5 of intensity, respectively (Chaiwang et al., 2019). *O. vittatus* was our sample which found 4.54% prevalence and intensity was 1.5 mc/fish. This different prevalence and intensity would relate to the geography and the season of fish collection.

A total of 278 metacercariae were found. Morphology of metacercariae species shows in figure 1. Morphological classification showed the largest portion of trematode species was *H. pumilio* with 88.13% (245/278), and then, in decreasing order, *Haplorchoides* spp. (5.76%), *O. viverrini* (2.88) and *H. taichui* (1.44%). Our result disagrees with the result of Jitkham et al. (2020) regarding the metacercariae of *Haplorchoides* spp., which was found to have the biggest portion (95.32% of total metacercariae) in cyprinoid fish from Chiang Rai province. In addition, the metacercariae from cyprinoid fish from Lampang province showed *Haplorchoides* spp. was the majority of metacercariae numbers as well (Kaewchanta & Wijit, 2021).

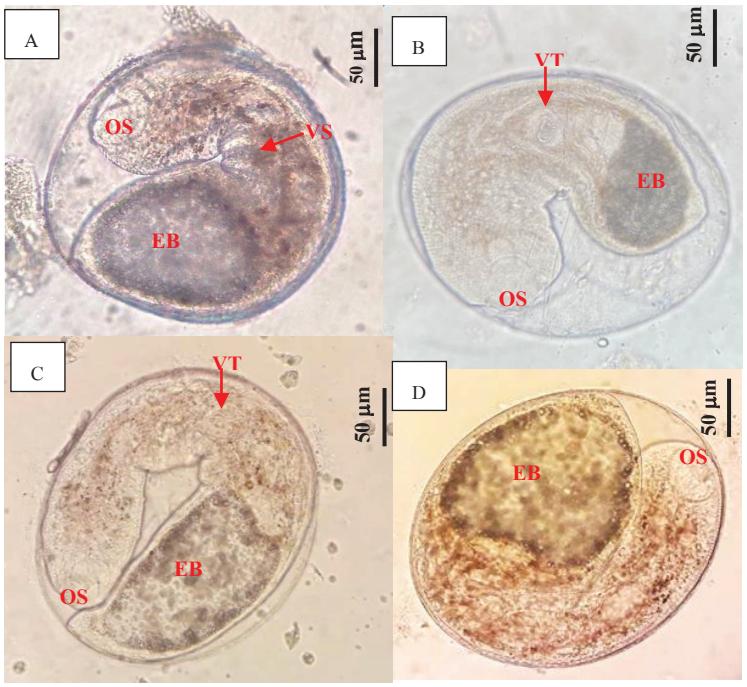


Figure 1. Morphology of metacercariae. Panel (A) *Opisthorchis viverrini*, (B) *Haplochis taichui* (C) *Haplorchis pumilio* (D) *Haplorchoides* spp.

Table 2. Prevalence and numbers of metacercariae in infected fish

Fish species	Infected fish / Examined fish	% Prevalence	Metacercariae species					Total metacercariae (Range = Min-Max)
			O. viverrini	*H. pumilio*	*H. taichui*	*Haplorchoides* spp.	Unknown	
H. siamensis	18/220	8.18	5	222	1	16	1	245 (1-54)
P. proctozystron	4/101	3.96	0	11	0	0	4	15 (1-10)
B. altus	2/93	2.16	3	6	0	0	0	9 (3-6)
O. vittatus	2/44	4.54	0	0	3	0	0	3 (1-2)
O. lini	1/18	5.56	0	6	0	0	0	6 (6)
P. brevis	0/5	0.00	0	0	0	0	0	0
T. thynnoides	0/1	0.00	0	0	0	0	0	0
Total	**27/482**	**5.60**	**8**	**245**	**4**	**16**	**5**	**278 (1-54)**
Percentage of metacercariae			**2.88**	**88.13**	**1.44**	**5.76**	**1.80**	

The intensity of metacerariae varied from 13.16 to 1.5 metacercariae per infected fish (mc/fish), as shown in table 3. *H. siamensis* showed the highest intensity (13.6), which is different species from the study They reported that *B. gonionotus* from Surat Thani province showed the highest mean intensity of 25.6 and 28.9 mc/fish in the summer and rainy seasons for *H. taichui* metacercariae. Fish species *Labiobarbus siamensis* present a mean intensity of 105.4 and 119.3 mc/fish in the summer and rainy seasons for *Haplorchoides mehrai* metacercariae. In northeastern Thailand, the intensity of *O. viverrini* metacercariae was reported in a variety of fish species from 20 provinces. The intensity ranged from 0.07 to 5.20 mc/fish, and *H. siamensis* was 0.50. The average intensity of the metacercariae of *O. viverrini* was 0.53 mc/fish, ranging from 0.04 to 2.47 mc/fish Moreover, the metacercariae of *O. viverrini* were investigated in nine provinces in the upper part of northeast Thailand. *B. gonionotus* showed the highest intensity at 11.5 mc/fish (Charoensuk et al., 2022). The intensity of metacercariae in Southeast Asia was measured in Myanmar. They presented the intensity of *Haplorchoides* sp. in *H. siamensis* as 1.5 mc/fish (Myint et al., 2020).

The season of sampling would be the explanation for the difference in prevalence and intensity in these studies. The seasonal variation of metacercariae infection rates has been reported. The prevalence of heterophyid metacercariae in the summer time of Thailand (February-May, 2007) showed 83.9% and 74.2% in the Mae Ngad and Mae Kuang Udomtara reservoirs, Chiang Mai province, Thailand (Nithikathkul & Wongsawad, 2008). Nevertheless, our research was conducted in the rainy season, which caused a different percentage of parasite infection in our result (5.60% total prevalence).

The metacercariae infection rate in Cambodia was low during the rainy season (June-September), which is similar to our result (Touch et al., 2013). Interestingly, Kamchoo and Chai (2023) show that metacercariae infection rates were higher in the rainy season (41.4%) than the summer season (23.6%). The quantity of metacercariae varies seasonally. It depends on the rainfall, which has an effect on fish behavior and the chance that cercariae from snails will penetrate into the fish.

Table 3. Prevalence and intensity of metacercariae in infected fish

Fish species	Infected fish/ Examined fish	% Prevalence	Total metacercariae (Range=Min-Max)	Intensity
H. siamensis	18/220	8.18	245 (1-54)	13.61
P. proctozystron	4/101	3.96	15 (1-10)	3.75
B. altus	2/93	2.16	9 (3-6)	4.5
O. vittatus	2/44	4.54	3 (1-2)	1.5
O. lini	1/18	5.56	6 (6)	6.0
P. brevis	0/5	0.00	0	0
T. thynnoides	0/1	0.00	0	0
Total	**27/482**	**5.60**	**278 (1-54)**	**10.30**

4. Conclusion

The fish samples from the Lam Pao Dam reservoir were identified as seven species. Five of them were infected with trematode. *Henicorhynchus siamensis* showed the highest prevalence with 18/220 (8.18%) and *Barbonymus altus* was the lowest prevalence with 2/93 (2.16%). The intensity ranged from 1.5 to 13.6 mc/fish. The largest portion of metacercariae was *Haplorchis pumilio* with 88.13% of total metacercariae. There were *O. viverrini* infected fishes which can cause the Opisthorchiasis and possibly develop cholangiocarcinoma in human. The outcome of this study is useful for the residents in term of raw fish consumption awareness. A long period study is recommended to obtain the statistical data on trematode infection in cyprinoid fish from the Lam Pao Dam reservoir.

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