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Prevalence of trematode Metacercariae in Cyprinoid fish and food consumption behaviors of people from Chiang Rai Province, Northern Thailand

Jitkham, P. [1], Wongsawad, C.[2], Koychusakun, P.[3] and Phalaraksh, C.[1, 2*]

[1]Environmental Science Program, Environmental Science Research Center, Faculty of Science, Chiang Mai University, Chiang Mai 50200, Thailand; [2]Department of Biology, Faculty of Science, Chiang Mai University, Chiang Mai 50200, Thailand; [3]Department of Public Health, School of Health Sciences, Chiang Rai Rajabhat University, Chiang Rai 57100, Thailand.

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Abstract The prevalence of trematode metacercariae in cyprinoid fish and food consumption behaviors of local people from Nong Luang wetland area, Chiang Rai Province, Northern Thailand were investigated. The fish samples were collected from Nong Luang wetland between April and December 2016. The total number of 11 trematode species from 360 cyprinoid fish samples were identified. The prevalence rate of metacercaria in cyprinoid fishes was 39.17% which mostly found from *Henicorhynchus siamensis* 100% (7/7), *Systomus rubripinnis* 94.44% (17/18), *Barbonymus gonionotus* 76.74% (33/43), *Rasbora tornieri* 55.56% (5/9), *Osteochilus vittatus* 50% (1/2), *Puntius brevis* 30.39% (31/102), *Anematichthys repasson* 28.95% (33/114), *Luciosoma bleekeri* 25% (3/12), *Labiobarbus siamensis* 21.57% (11/51). The highest number of metacercaria identified from this study was *Haplorchoides spp*. followed by *Centrocestus formosanus*. Moreover the findings revealed that seasonal changes relatively affected to the prevalence of metacercaria in cyprinoid fishes. The highest prevalence of metacercaria can be found in winter season. The residents of Nong Luang Wetland had a moderate level of raw fish consumption behaviors (95.8%). However, the result shows that the residents of Nong Luang Wetland consume raw or half cooked fish according to easy cooking. The health education of appropriate fish consumption and the risk of liver fluke were recommened to transfer to the residents and local government of Nong Luang wetland area.

Keywords: Prevalence, Trematode, Metacercariae, Cyprinoid fish, Nong Luang Wetland

Introduction

Diseases were infected by parasite which directly affected on people's health and economic status. Fish-borne trematode has been one of significant public health issues in many countries worldwide including developed countries. Moreover, there are over 500 million people who are at risk of getting infected (Chai *et al*., 2005).

* **Corresponding Author:** Phalaraksh, C.; **Email:** chitchol.p@cmu.ac.th

In Thailand, trematode infections are mostly found in northeastern and northern regions (Kumchoo *et al*., 2005). There are several important kinds of trematode such as liver flukes and minute intestinal flukes. Moreover, the liver flukes stemmed from *Opisthorchis viverrini* can lead to liver cancer and Cholangiocarcinoma. There are approximately over 5.5 million people infected with the liver flukes in Thailand (Sripa *et al*., 2010). In addition, Radomyos (1998) also reported that there were more people infected by various intestinal flukes for instance, *Haplorhis taichui, Metagonimus yokogawai, H. pumillio, Centrocestus caninus, Stellantchasmus falcatus*.

The infection is caused by consumption behavior of raw and undercooked cyprinoid fish, lack of preventive knowledge, manners, values and beliefs of food consumption of people in each region, and sources and types of available food, etc. Most of patients are infected by having the raw and undercooked food, especially fresh-water fish which are intermediate host of trematode. Generally, the trematode infection is stemmed from consumption of fish caught from natural water resources. Different raw or undercooked menus made from these fish like fermented fish, pickled fish, spicy raw fish, and spicy minced fish can lead to the risk of liver fluke and intestinal fluke diseases. According to the cost, belief, and food consumption behavior of local people, the raw or undercooked foods are more delicious than the well-cooked foods. It is also considered as special menu or honorable food that used for welcome guests. Additionally, they believe that this kind of food can provide more energy for working. The local people have the raw or undercooked foods in their daily lives. Traditional value has been succeeded from their ancestors. Eating the raw or undercooked foods is represent masculinity. Thus, they usually have consumed the raw or undercooked foods along with alcoholic beverages because they believe that liquor could make the foods cooked and kill the fluke. Most people also think that taking vermifuge can prevent the diseases. The misunderstanding causes chronic inflammation and lead to cholangiocarcinoma. It's difficult to notice early symptoms of infected patients due to less amount of fluke. The patients may have abdominal distension, stomachache, vomit, diarrhea, and anemia. Regarding the symptoms of liver fluke infection, the patients may feel burning pain around stomach and have a poor appetite, swollen abdomen and enlarged liver. Furthermore, additional symptoms are yellow skin and eyes, mild fever or high fever, and sensation of cold with convulsive shaking of the body. These ailments can lead to other complications such as biliary atresia due to the flukes, cholecystitis, and cholangiocarcinoma which are the most dangerous complication (Ministry of Public Health, 2013).

World Health Organization (WHO) indicates that the chronic liver fluke disease or repeated infection is one of the causes of cholangiocarcinoma. In Thailand, the diseases are mostly found in the northeastern and northern regions at 18.6% and 10% of population, respectively. Besides, almost 28,000 patients each year or at least 76 patients per day die due to cholangiocarcinoma. In 2011, 14,314 patients died because of liver cancer and cholangiocarcinoma. 7,593 patients lived in the northeastern area, followed by 2,638 patients in the northern part. According to these figures, a strategy to eliminate the liver fluke and reduce the occurrence of cholangiocarcinoma is defined by Ministry of Public Health. This project has been conducted in the northeastern and northern areas from 2013 to the present time (Ministry of Public Health, 2013). Furthermore, the Trematode metacercariae such as *Haplorchis taichui* were indicated as well. Most of them were in family heterophyidae family and could cause the disease in human in the northern part of Thailand (Sukontason *et al*., 1999; Wongsawad *et al*., 2000; Kumchoo *et al*., 2005). *Haplorchis taichui* was a minute intestinal fluke with various definitive hosts; for example, human, bird, cat, dog, and rat (Chai *et al*., 2005, Nithikathkul and Wongsawad, 2008).

Nong Luang wetland is the largest natural water resource in Chiang Rai Province, northern part of Thailand. It is located between 2 districts including Wiang Chai District and Mueng Chiang Rai District. Nong Luang Wetland is formed in an oval shape and has a surface area of 9,000 rais. The widest part is about 4 kilometers and the longest part is about 7 kilometers. There are diversities of fresh-water fish, aquatic animals, and various kinds of aquatic plants which are foods for the local people (Parnboon *et al*., 2007). There is a large number of northern people and northeastern people migrating from their hometowns to stay around Nong Luang wetland. Most of them earn their living by fishing to consume in their families and sell to neighboring communities. When the villagers eat unhygienic fish could lead to the risk of infection from the trematode metacercaria.

The study aimed to investigate the prevalence of trematode metacercariae in Cyprinoid fish and examine the food consumption behaviors of local people in Nong Luang Wetland, Wiang Chai District, Chiang Rai Province in order to bring the findings to be the basic information for public health agencies using as a guideline to prevent and solve infectious diseases in this area.

Materials and methods

Specimen collection and identification fish

Fish specimen was collected from April to December 2016 which was collected over three seasons; summer season was collected in April, rainy

season was collected in August and winter season was collected in December from 3 different sampling areas with at least 40 samples and one time in each season, a total of 360. The cyprinoid fish were caught and/or bought from local fishermen around Nong Luang wetland. Some of active alive fish were kept in water provided with oxygen; meanwhile, the dead fish were reserved in ice boxes and transported to the laboratory. The taxonomic identification of the fish was classified based on the guidelines and atlas of freshwater fish in Thailand by Smith (1945), Department of Fisheries, Ministry of Agriculture and Co-operative, and Wittayanon (2003).

Metacerriae examination and identification

The specimen of the fish was measured for its total length of the body and weigh, took its picture, then recording its detail. Each fish was examined by Dissection Techniques, which separated its dorsal fins, pectoral fins, caudal fins, pelvic fins, and scaly gum; then separated it into petri dish with distilled water. Metacerriae was separated by using the low stretchy microscope. Meanwhile, fish meat was cut into small pieces; then mixing it with 100% of pineapple juice (Sripakdee, 2005), instead of using pepsin. Then, it was mashed by grinding machine. After that it was incubated in a water bath shaker at 37 °C for 2 hours. Filtration process was done by using Sieve tube for separating the residue and debris of fish fillets. Rinsing it with NaCl 0.85% until clear. Metacercaria were identified under a stereoscope and compound light microscope, using the texts and papers of Yamaguti (1958), Pearson (1964), Pearson and Ow-Yang (1982) and Wongsawad *et al*. (1997). Data were analyzed for prevalence and density of pathogens in cyprinoid fish.

Screening for consumption behavior among villagers

Villagers who residing around Nong Luang wetlands between April and December 2016 and aged around 15-70 year were used as research samples. They were divided into 2 different grops: 1) Baan Samanmit Moo 1, Don Sila sub-district, Wiangchai district, Chiang Rai Province, where the villagers migrated from the Northeast of Thailand and 2) Ban Pong community, Moo 6, Wiangchai sub-district , Wiangchai sub-district, where villagers were the village hometown, total 798 people.

Sample

Sample size was determined using Yamane formula at 95%confidence level (Yamane 1973: 725).

$$n = \frac{N}{1 + Ne^2}$$

When n represents sample size
N represents population size.
e represents the error.

In this research allows error at 5%, at 95% confidence level.

$n = 738 /(1+ 738(0.05)^2)$

$= 259.40$

The total number is 260.

The study of fish consumption behaviors

The research tools used for collecting the data about fish consumption behaviors among people residing around Nong Luang wetland, Chiang Rai province was self-administered questionnaire which divided into 2 parts:

Part 1 Personal information

Part 2 Raw fish consumption behaviors, contributed by a researcher from literature reviews in raw fish consumption behaviors of people. The interpretation was divided into 3 types as follows:

Regularly	means	having to practice regularly at least once a week
Sometimes	means	having to perform at least 1-2 times a month
Never	means	never practice

Scoring criteria

Regularly	=	one point
Sometimes	=	two points
Never	=	three points

Interpretation (total 10 items)

21 – 30 points	means	Good practice of fish consumption
11 – 20 points	means	Moderated practice of fish consumption
1 – 10 point	means	Poor practice of fish consumption

Ethical consideration

The study was ethically approved by the Ethics Review Committee for Human experimentation committee research institute for health sciences (RIHES), Chiang Mai University, Thailand with COA No. 31/2560 (Project code : 7/59).

Results

The prevalence of trematode metacercariae in cyprinoid fishes

It was found that all 360 cyprinoid fishes sample can be classified into 11 species as follows: *Henicorhynchus siamensis*, *Systomus rubripinnis Barbonymus gonionotus*, *Rasbora tornieri*, *Osteochilus vittatus*, *Puntius brevis*, *Anematichthys repasson*, *Luciosoma bleekeri*, *Labiobarbus siamensis*, *Cirrhinus cirrhosus* and *Barbonymus schwanenfeldii*. From 360 cyprinoid fish samples, it was found that 141 of trematode metacercariae was in infection stage. Therefore, the prevalence of trematode metacercariae in infection stage was 39.17% (Table 1 and 2). In addition, the results revealed that 4,102 of trematode metacercariae was found from 360 cyprinoid fish samples; therefore, the average ratio of infection was 29.09 trematode metacercariae per one fish. There were nine species of fish found infection from trematode metacercariae larvar. The prevalence rate of infection in each fish as follows: *Henicorhynchus siamensis* 100% (7/7), *Systomus rubripinnis* 94.44% (17/18), *Barbonymus gonionotus* 76.74% (33/43), *Rasbora tornieri* 55.56% (5/9), *Osteochilus vittatus* 50% (1/2), *Puntius brevis* 30.39% (31/102), *Anematichthys repasson* 28.95% (33/114), *Luciosoma bleekeri* 25% (3/12), *Labiobarbus siamensis* 21.57% (11/51), *Cirrhinus cirrhosus* and *Barbonymus schwanenfeldii* the prevalence rate was not found, respectively (Figure 1). The metacercariae found from cyprinoid fishes can be classified into 2 species: *Haplorchoides* spp. and *Centrocestus formosanus* (Figure 2). The highest number of *Haplorchoides* spp. found in study was 3,951, with the average ratio of 28.02 per one fish followed by *Centrocestus formosanus* type which found 129, with the average ratio of 0.91 per one fish and unidentified metacercariae larvae species was 22, with the average ratio of 0.16 per one fish.

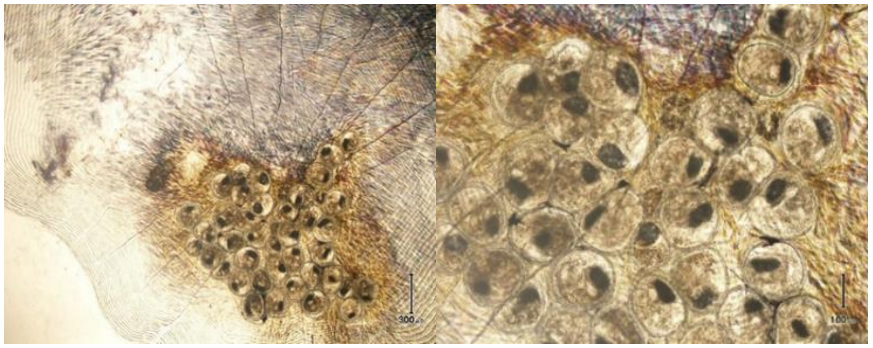

Figure 1. Metacercariae *of Haplorchoides* spp. found in cyprinoid fishes in Nong Luang, wetland Aaea, Chiang Rai Province, Thailand

Table 1. Prevalence of trematode metacercariae in cyprinoid fish from Chiang Rai province, Thailand

Fish species	No. of fish		No. metacercaria found				Intensity	Prevalence (%)
	Total	Infected	HP[1]	CF[2]	Us[3]	Total		
Henicorhynchus siamensis	7	7	232	0	0	232	33.14	100
Systomus rubripinnis	18	17	586	0	0	586	34.47	94.44
Barbodes gonionotus	43	33	576	0	0	576	17.45	76.74
Rasbora tornieri	9	5	0	12	7	19	3.8	55.56
Osteochilus vittatus	2	1	32	0	0	32	32	50
Puntius brevis	102	31	436	98	5	539	17.39	30.39
Anematichthys repasson	114	33	2017	13	6	2039	61.79	28.95
Luciosoma bleekeri	12	3	0	6	3	9	3	25.00
Labiobarbus siamensis	51	11	72	0	1	73	6.64	21.57
Cirrhinus cirrhosus	1	0	0	0	0	0	0	0
Barbonymus schwanenfeldii	1	0	0	0	0	0	0	0
Total	360	141	3,951	129	22	4,102	29.09	39.17

[1] *Haplorchoides* spp.
[2] *Centrocestus formosanus*
[3] Unidentified species

Table 2. Prevalence of trematode metacercariae in cyprinoid fish over three seasons from Chiang Rai province, Thailand

Season	No. of fish studied		No.metacercaria found				Intensity	Prevalence (%)
	Total	Infected	HP[1]	CF[2]	Us[3]	Total		
Summer	120	20	0	89	18	107	5.35	16.66
Rainy	120	54	335	40	1	375	6.96	45.00
Winter	120	67	3616	0	3	3619	54.01	55.83
Total	360	141	3951	129	22	4102	29.09	39.17

[1] *Haplorchoides* spp.
[2] *Centrocestus formosanus*
[3] Unidentified species

Figure 2. Metacercariae found in this study (scale bar = 30 µm), A=*Haplorchoides* spp.; B= *Centrocestus formosanus*

Food consumption behaviors among people residing around Nong Luang wetland

The study of food consumption behaviors among people residing around Nong Luang wetlands, by collecting data from 260 participants (Table 3). They were aged between 15 -70 years and lived in 2 communities: 1) Samanmit village, Moo 1, Donsila subdistrict, Wiang Chai District, Chiang Rai Province, 2) Pong Luang village, Moo 6 Wiang Chai District, Chiang Rai Province. Self-administered questionnaire was used to conduct data. The study found that majority of participants, with 249 people (95.8%), had a moderate level of raw fish consumption behaviors.

Table 3. Number and percentage of raw fish consumption behaviors

Level of raw fish consumption behaviors	Score range	Number	Percent
Good	21 – 30 points	11	4.2
Moderate	11 – 20 points	249	95.8
Poor	1 – 10 points	0	0
Total		**260**	**100.0**

Discussion

According to the study, prevalence of trematode metacercariae in cyprinoid fish collected from Chiang Rai Province, Thailand from April to December 2016. It was found that all 360 cyprinoid fishes sample can be classified into 11 species as follows: 1) *Henicorhynchus siamensis*, 2) *Systomus rubripinnis, 3) Barbodes gonionotus, 4) Rasbora tornieri, 5) Osteochilus*

vittatus, 6) Puntius brevis, 7) Anematichthys repasson, 8) Luciosoma bleekeri Puntius orphoides, 9) Labiobarbus siamensis, 10) Cirrhinus cirrhosus and 11) Barbonymus schwanenfeldii. From 360 cyprinoid fish samples, it was found that 141 of trematode metacercariae was in infection stage. Therefore, the prevalence of trematode metacercariae in infection stage was valued at 39.17 (141/360). The results revealed that 4,102 of trematode metacercariae was found from 360 cyprinoid fish samples; therefore, the average ratio of infection was 29.09 trematode metacercariae per one fish. Hence, the prevalence rate of infection in each fish as follows: *Henicorhynchus siamensis was* 100% (7/7), *Systomus rubripinnis* was 94.44% (17/18), *Barbodes gonionotus was* 76.74% (33/43), *Rasbora tornieri* was 55.56 (5/9), *Osteochilus vittatus* was 50% (1/2), *Puntius brevis* was 30.39% (31/102), *Anematichthys repasson* was 28.95% (33/114), *Luciosoma bleekeri* was 25% (3/12), and *Labiobarbus siamensis* was 21.57% (11/51) respectively (Table 1).

The metacercariae found from cyprinoid fishes can be classified into 2 species: *Haplorchoides* spp. and *Centrocestus formosanus.* The highest number of *Haplorchoides* spp. found in study was 3,951, with the average ratio of 28.02 per one fish followed by *Centrocestus formosanus* type which found 129, with the average ratio of 0.91 per one fish which is in line with Nithikathkul and Wongsawad (2008) reported that the *Haplorchoides spp*. of metacercariae was found in cyprinoid fishes in Chiangrai area. Moreover, the study results was relevant with many research reported that the metacercariae was found from *Barbodes gonionotus* (Kumchoo *et al*., 2005; Nithikathkul and Wongsawad, 2008b; Saenphet *et al*., 2008; Noikong *et at*., 2011; Suntaravitun and Dokmaikaw, 2014a,b; Nikong *et al*., 2011), *Labiobarbus siamensis* (Kumchoo *et al*., 2005; Nithikathkul and Wongsawad, 2008a,b; Saenphet *et al*., 2008; Noikong *et at*., 2011; Suntaravitun and Dokmaikaw, 2014) *Rasbora tornieri* (Kumchoo *et al*., 2005; Purivirojkul, 2011) and *Osteochilus vittatus* (Saenphet *et al*., 2008).

The most common metacercaria found in this study was *Haplorchoides spp*. which in line with the study of Suntaravitun and Dokmaikaw, (2014), reported that they found metacercaria, *Haplorchoides* spp., in cyprinoid fishes from Chiangrai province area. It is also in line with the study reported by Kumchoo *et al*. (2005), Nithikathkul and Wongsawad, (2008) that the metacercaria, *Haplorchoides spp.,* was found in cyprinoid fishes in Chiang Mai province.

There is no report of diseases in humans as the parasite of this species is a parasite that lives in the intestines of catfish (Shameem and Madhavi, 1988) which always found this parasite in *Anematichthys repasson* species. However, this finding was different from previous study which reported that the

metacercariae, *Haplorchoides* spp. and *Haplorchis taichui* species were found in Chiang Rai Province; but in this study, one species of metacercaria, *Centrocestus formosanus*, was found.

From 11 species of fish, the most common species of cyprinoid fishes were *Puntius brevis, Anematichthys repasson, Rasbora tornieri, and Luciosoma bleekeri, respectively*, which is in with the study of Phalee *et al*. (2008) reported that the metacercaria, *Centrocestus formosanus,* found in Puntius brevis fish since this parasite was a small intestinal fluke caused of diseases in humans (Radomyos, 1998).

The prevalence of trematode metacercariae in cyprinoid fishes collected from Nong Luang wetland, Wiang Chai District, Chiang Rai Province over three 3 seasons (summer, rainy, winter). 360 cyprinoid fish samples (120 samples per season) was examined. The prevalence rate of trematode metacercariae was 39.17 percent (141/360). The highest prevalence rate of trematode metacercariae found in winter time was 55.83 percent (76/120), followed by rainy season, 45 percent (54/120) and summer time 16.66 percent (20/120) respectively. The most common species of metacercariae was *Haplorchoides* spp., followed by the *Centrocestus formosanus*, respectively.

The findings of metacercarial infection in this study was consistent with Sukontason *et al*. (1999) and Noikong *et al*. (2011) who reported that the prevalence of metacercarial infection, *Haplorchoides* spp. *and Centrocestus formosanus species, were* the highest type found during winter season and late rainy season (Vichasri *et al*.,1982; Sithithaworn, 1997). As there was a large amount of water in rainy season, water would sweep the eggs of liver flukes in the feces to flow down to the water sources. When freshwater mussels eat fluke eggs, the parasite larvae will grow into cercariae stage which takes about 6 weeks. Then, a cercariae comes out of freshwater shellfish into freshwater fish which takes about 2 weeks for growing to metacercariae. Overall, it takes total 2 months to grow into the metacercaria stage which corresponds to the winter time; consequently, we can detect a large number of metacercaria spreading in water (Sukontason *et al*., 1999). In addition, it was found that the prevalence of infection in freshwater fish may depend on the amount of rainfall (Wiwanitkit, 2005a) since during this time with a large amount of rainfall which can be detected a lot of the metacercaria.

In addition, the spread of the metacercaria may depend on the type and number of freshwater shells and freshwater fish species that live together in the water sources since both types are important intermediate hosts that complete the life cycle of liver fluke. However, in each area may be different situation as well as it may depend on the consumption behaviors of raw fish and stool excretion in water sources, which all are a risky behaviors that causes the

spread of fluke pathogens (Suntaravitun, 2014). Therefore, the study of the prevalence of metacercaria in different regions across Thailand will help to estimate the risk of liver fluke disease of people in that area. In addition, the findings can be used as the important basic information for planning the prevention of liver fluke infection in the future.

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