

INVESTIGATION OF TREMATODE METACERCARIAE IN FERMENTED FISH FROM UDON THANI AND KALASIN PROVINCES, THAILAND

Nipawan Labbunruang* and Jutharat Kulsantiwong

Department of Biology, Faculty of Science, Udon Thani Rajabhat University, Udon Thani, Thailand, 41000

*E-mail: nipawan7585@gmail.com

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ABSTRACT

Food-borne disease is an important public health problem in South East Asia including Opisthorchiasis which endemic in Thailand, Laos PDR and Cambodia. Infective stage of Trematode is the metacercariae which encysted in the muscles of Cyprinoid fish. The parasite transmission is caused by consuming of uncooked or raw infected fish. In this study, two products of fermented fish were collected from five districts of Udon Thani province and seven districts of Kalasin province. All samples were digested with 0.25% pepsin solution with 37°C for 1 hour, after that filtered through the various sizes of sieves. The trematode metacercariae were identified under a stereo microscope and a compound microscope. A total of 31 metacercariae (Mc) were found in *Pla-ra* (9 Mc) and *Pla-som* (22 Mc) with shorter fermentation period which collected from Kumphawapi Kumphawapi, Mueang Udon Thani, and Kut Chap districts. The average numbers of metacercariae were 0.038 Mc/g to 0.006 Mc/g. The percentage of positive sample sites was 9.1 % (1/11) for *Pla-ra* and 42.9% (3/7) for *Pla-som*. The infected fish species were *Barbodes aurotaeniatus* and *Barbomyrus gonionotus*. This observation is useful for the public health organization in terms of parasitic disease awareness and prevention.

Keywords: Trematode metacercariae, Fermented Fish, Cyprinoid fish

Introduction

Metacercariae are the infective stage of trematodes or flat worm parasites which cause a serious public health problem in many countries in South East Asia (WHO., 2004). The main species are *Opisthorchis viverrini*, *Clonorchis sinensis* and members of the family Heterophyidae. This encysted stage of the parasites is found in muscles of infected cyprinoid fish. Generally, people are infected by ingestion of raw fish or undercooked fish (Sripa et al., 2010).

Numbers of trematode metacercariae in cyprinoid fish have been reported from several provinces in Thailand, for example Chiang Mai, Nakhon Ratchasima, and Phitsanulok (Sukontason et al., 2001, Nithiuthai et al., 2002, Noikong et al., 2011).

O. viverrini metacercariae were detected in the cyprinoid fish species *Cyclocheilichthys repasson*, *C. armatus*, *Henicorhynchus lineatus*, *Puntioplites proctozysron*, *Hampala dispar*, and *Osteochilus waandersii* (Manivong et al., 2009). Metacercariae of Heterophyidae were positive in *Puntius leiacanthus*, *Cyclocheilichthys armatus*, and *Hampala dispar* (Nithiuthai et al., 2002). The popular traditional cyprinoid fish dishes among northeastern people are salt-fermented fish (*Pla-ra*), sticky rice-fermented fish (*Pla-som*), and raw fish salad (*Koi-pla*) (Prasongwatana et al., 2012). The infected cyprinoid fish will cause the parasitic disease of local people who consume the uncooked fish dishes. Opisthorchiasis has been reported in many regions of Northeast Thailand (Sithithaworn and Haswell-Elkins, 2003, Thaewnongiew et al., 2014).

Udon Thani and Kalasin provinces have large fresh water reservoirs and the local residents consume fish as a protein source. *Pla-ra* and *Pla-som* are the well-known local products of some districts in both provinces such as Nong Kung Si districts (Department of Cultural Promotion, 2019). Negative results were reported for metacercariae in cyprinoid fish from Udon Thani province and Kalasin province (Pinlaor et al., 2013). However, the prevalence of Opisthorchiasis remains a health problem in both areas (Thaewnongiew et al., 2014, Yahom et al., 2013). Therefore, the infection of fish products should be concerned. The aim of this study is to investigate the trematode metacercariae in fermented fish from Udon Thani province and Kalasin province. The demonstration of trematode infective metacercariae in fermented fish will support a campaign to consume only properly cooked fish in Northeast Thailand.

Methods

1. Sample collection

The study was conducted at 5 districts of Udon Thani province namely Sang Khom, Mueang Udon Thani, Kumphawapi, Nong Wua So, Kut Chap and 7 districts of Kalasin province including Mueang Kalasin, Tha Khantho, Nong Kung Si, Khong Chai, Yang Talat, Sahatsakhan and Kamalasai Northeast Thailand during July, 2017 to February, 2018 (Table 1). The samples consisted of two kinds of fermented fish namely *Pla-ra* and *Pla-som*. *Pla-ra* is produced from high salt-fermentation. This process needs more time of incubation (roughly 2 months to a year) whereas *Pla-som* is made from sticky rice-fermentation which consume less time of incubation (roughly 3 days to 12 days). Roughly half of kilogram to two kilograms of samples were randomly purchased from local markets, the sample quantities were different depend on the products were purchased from the villagers or vendors. Each sample was labelled with name of districts and time period of fermentation. The products were made from various fish species such as *Barbodes aurotaeniatus*, *Henicorhynchus siamensis* etc. All fish species are shown in table 2.

Table 1 Sample collection sites and fermented fish sample

Site	Province	Districts	Fermented Fish	
			<i>Pla-ra</i>	<i>Pla-som</i>
1	Udon Thani	Sang Khom	✓	✓
2		Mueang	✓	✓
3		Kumphawapi	✓	✓
4		Nong Wua So	✓	-
5		Kut Chap	✓	✓
6	Kalasin	Mueang	✓	-
7		Tha Khantho	✓	✓
8		Nong Kung Si	✓	-
9		Khong Chai	✓	✓
10		Yang Talat	✓	-
11		Sahatsakhan	-	✓
12		Kamalasai	✓	-

Table 2 Time period of fermentation of *Pla-ra* and *Pla-som*

Districts	<i>Pla-ra</i>		<i>Pla-som</i>	
	<3 months	>3 months	<1 week	>1 week
Sang Khom	-	✓	✓	✓
Mueang Udon Thani	-	✓	✓	-
Kumphawapi	✓	-	✓	-
Nong Wua So	-	✓	-	-
Kut Chap	-	✓	✓	-
Mueang Kalasin	-	✓	-	-
Sahatsakhan	-	-	-	✓
Tha Khantho	✓	-	-	✓
Nong Kung Si	-	✓	-	-
Khong Chai	-	✓	✓	-
Yangtalat	-	✓	-	-
Kamalasai	-	✓	-	-

2. Identification of trematode metacercariae

The species of fermented fishes were identified by morphological identification (Saenjundaeng, 2014). Fish were minced and weight on the electronic balance then thoroughly mixed with pepsin solution (0.25% pepsin in 1.0% HCl) and incubated at 37°C for 60 minutes, manually stir in every 10 minutes (modified from Waikagul, 1998). The digested samples were filtered through a sieve with mesh size 850 µm and washed with 0.85% NaCl. Afterwards, each sample was allowed to settle in a sedimentary jar and then filtered through a series of sieves with mesh sizes of 300, 150, and 45 µm. The sediment was observed under a stereo microscope. The wet mount slide was prepared for each metacercaria and observed under a compound microscope. Trematode metacercariae were identified using morphological examination according to Scholz et al., 1991 and Sohn et al., 2009.

Results

1. Fish species

Pla-ra and *Pla-som* were produced from five fish species namely, *Henicorhynchus siamensis*, *Osteochilus vittatus*, *Barbonymus schwanenfeldii*,

Barbodes aurotaeniatus and *Labibarbus leptolepis* (Tables 3). Only *Barbodes aurotaeniatus* and *Barbomyrus gonionotus* were contaminated with trematode metacercariae (Tables 4 and 5). Representative images of the fermented fish samples are shown in Figure 1.

Table 3 Fish species in Fermented fish samples

Districts	Fish species	
	Pla-ra	Pla-som
Sang Khom	<i>Osteochilus vittatus</i>	<i>Henicorhynchus siamensis</i>
Mueang Udon Thani	<i>Barbomyrus schwanenfeldii</i>	<i>Barbodes aurotaeniatus</i>
	<i>Barbodes aurotaeniatus</i>	
Kumphawapi	<i>Barbodes aurotaeniatus</i>	<i>Barbomyrus gonionotus</i>
Nong Wua So	<i>Henicorhynchus siamensis</i>	-
Kut Chap	<i>Barbodes aurotaeniatus</i>	<i>Barbodes aurotaeniatus</i>
Mueang Kalasin	<i>Henicorhynchus siamensis</i>	-
Sahatsakhan	-	<i>Henicorhynchus siamensis</i>
Tha Khantho	<i>Labibarbus leptolepis</i>	<i>Henicorhynchus siamensis</i>
Nong Kung Si	<i>Henicorhynchus siamensis</i>	-
Khong Chai	<i>Barbomyrus schwanenfeldii</i>	<i>Henicorhynchus siamensis</i>
Yangtalat	<i>Henicorhynchus siamensis</i>	-
Kamalasai	<i>Henicorhynchus siamensis</i>	

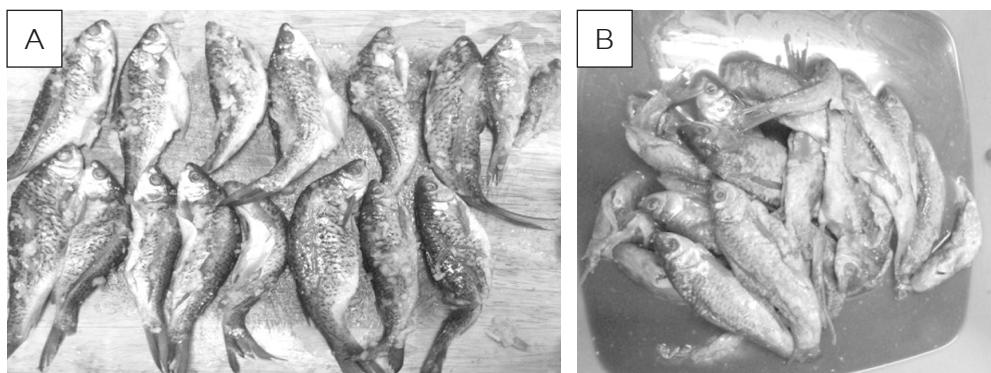


Figure 1 Fermented fish made from Cycrinoid fish. *Pla-som* from Sang Khom, Udon Thani province (A) and *Pla-ra* from Mueang Kalasin (B)

2. Identification of Trematode Metacercariae

A total of 6997 grams *Pla-ra* and 4194 grams *Pla-som* were analysed. There were various quantities (104 grams to 1,242 grams) of minced *Pla-ra* and *Pla-som* in each sampling site which revealed in table 4 and 5. A total of 31 inactive metacercariae were found, 9 metacercariae were discovered in *Pla-ra* (Table 4) and 22 metacercariae were detected in *Pla-som* (Table 5). The average number of metacercariae in *Pla-ra* from Kumphawapi was 0.013 metacercariae per gram (Mc/g) and *Pla-som* were 0.038, 0.016, 0.006 Mc/g from Kut Chap, Kumphawapi, Mueang Udon Thani districts, respectively. Trematode metacercariae were found in the shorter incubation process of both products which *Pla-ra* less than 3 months and *Pla-som* less than 1 week. (Tables 4 and 5). Metacercariae from *Pla-ra* and *Pla-som* were inactive metacercariae which no movement of juvenile inside the cyst. However, the oral sucker and excretory bladder of metacercariae were seen (Fig. 2). The percentage of positive sample sites was 9.1 % (1/11) for *Pla-ra* and 42.9% (3/7) for *Pla-som*. All positive samples were collected from Udon Thani province which totally 3704 grams of *Pla-ra* and 2345 grams of *Pla-som*. The percentage of positive sample sites in Udon Thani province was 20% (1/5) for *Pla-ra* and 80% (3/4) for *Pla-som*.

Table 4 The number of metacercariae determined in *Pla-ra*.

Districts	Fish Species Fermented fish : <i>Pla-ra</i>			
	Species	Weight (grams)	Fermented Period	Number of Metacercariae (Mc/g)
Sang Khom	<i>O. vittatus</i>	442	>3 months	-
Mueang Udon	<i>B. schwanenfeldii</i> and	301	>3 months	-
Thani	<i>B. aurotaeniatus</i>			
Kumphawapi	<i>B. aurotaeniatus</i>	689	<3 months	9 (0.013)
Nong Wua So	<i>H. siamensis</i>	1,242	>3 months	-
Kut Chap	<i>B. aurotaeniatus</i>	1,030	>3 months	-
Mueang Kalasin	<i>H. siamensis</i>	442	>3 months	-
Tha Khantho	<i>L. leptocheilus</i>	701	<3 months	-
Nong Kung Si	<i>H. siamensis</i>	403	>3 months	-
Khong Chai	<i>B. schwanenfeldii</i>	610	>3 months	-
Yangtalat	<i>H. siamensis</i>	664	>3 months	-
Kamalasai	<i>H. siamensis</i>	473	>3 months	-
Total		6,997		9 (0.0013)

Table 5 Number of metacercariae found in *Pla-som* samples.

Sites	Fermented fish : <i>Pla-som</i>			
	Species	Weight (grams)	Fermented Period	Number of Metacercariae (Mc/g)
Sang Khom	<i>H. siamensis</i>	607	>1 week	-
Mueang Udon Thani	<i>B. aurotaeniatus</i>	811	<1 week	5 (0.006)
Kumphawapi	<i>B. gonionotus</i>	823	<1 week	13 (0.016)
Kut Chap	<i>B. aurotaeniatus</i>	104	<1 week	4 (0.038)
Sahatsakhan	<i>H. siamensis</i>	270	>1 week	-
Tha Khantho	<i>H. siamensis</i>	850	>1 week	-
Khong Chai	<i>H. siamensis</i>	729	<1 week	-
Total		4,194		22 (0.0052)

Table 6 Number of metacercariae in *Pla-ra* and *Pla-som* from Udon Thani province

Sites	Districts	Fermented Fish (grams)		Number of Metacercariae
		<i>Pla-ra</i>	<i>Pla-som</i>	
1	Sang Khom	442	607	-
2	Mueang	301	811	5 ^a
3	Kumphawapi	689	823	22 ^b
4	Nong Wua So	1,242	-	-
5	Kut Chap	1,030	104	4 ^a
Total		3,704	2,345	31^b

a : Metacercariae obtained from *Pla-som*

b : Metacecercariae obtained from *Pla-ra* and *Pla-som*

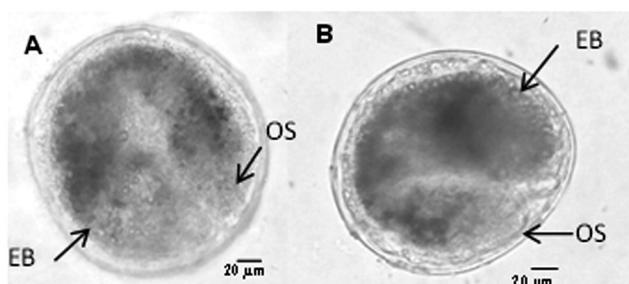


Figure 2 Inactive Trematode Metacercariae from *Pla-ra* (A) and *Pla-som* (B) OS: Oral sucker, EB: Excretory Bladder

Conclusion and Discussion

In this study, we investigated trematode metacercariae in fermented fish which is the favorite dish for local people in Northeast Thailand. We found a total of 31 metacercariae in the fermented fish from Udon Thani province. This result is consistent with a previous study (^bOnsurathum et al., 2016) which had shown positive results in *Pla-som* from Udon Thani but had not detected metacercariae in fermented fish from Kalasin.

The highest average number of metacercariae was 0.038 in *Pla-som* from Kut Chap and the lowest number was 0.006 in *Pla-som* from Mueang Udon Thani. The small quantities of samples per sites, is a possible cause of this low number of metacercariae. The acidity and salt concentration of preservation process also effects to the *O. viverrini* metacercariae (Sriraj et., 2013, Onsurathum et al.,2016). Moreover, the infected fish species of this study were *B. aurotaeniatus* and *B. Gonionotus* which dissimilar to another study that discovered the metacercariae in *H. siamensis*, *P. bimaculatus*, *H. dispar* and *P. orphoides*. (Onsurathum et al.,2016). Finally, the cyprinoid fish from Udon Thani and Kalasin provinces were reported for absence of metacercariae (Pinlaor et al., 2013).

Trematode metacercariae shown in *Pla-ra* with 9.09% (1/11) of sampling sites whereas *Pla-som* were contaminated with metacercariae with 42.86% (3/7) of sampling sites. Only Udon Thani province, metacercariae were detected. The higher percentage of positive sampling site as 20% (1/5) for *Pla-ra* and 80% (3/4) of *Pla-som* were calculated. *Pla-som* was more often found contaminated with metacercariae than *Pla-ra*. This result is different from previous study which reported higher prevalence in *Pla-ra* than *Pla-som* (Onsurathum et al.,2016). The metacercariae in both fish products were inactive by morphological observation. In order to investigate the metacercariae infectivity, the animal infection is required (Aukkanimart et al.,2017). The shorter fermentation provided the positive of parasite in *Pla-ra* and *Pla-som* in current study which related to previous experiment. They indicated that *Pla-ra* can kill *O. viverrini* metacercariae within 3 days and *Pla-som* with sticky rice fermentation will destroy metacercariae after 69 hours of fermentation (Prasongwatana et al., 2012).

Positive metacercariae infection occurred in Kumphawapi and Kut chap where located nearby the large fresh water reservoir which are Lam Pao dam and

Huai Luang Dam. The fish material for *Pla-ra* and *Pla-som* were caught from both dams. This result is in agreement with another study that had shown *O. viverrini* metacercariae were found in dams more than lakes and rivers (Pinlaor et al., 2010).

The samples of this study were obtained from some districts of Udon thani and Kalasin provinces with small amount of samples (totally 11,191 grams of minced fish). This small scale study revealed only 31 metacercariae in fermented fish. Therefore, a large scale of areas and sample sizes investigation is advised. Although our study provides the low percentage of infected fish products, it represents a chance of parasite infection in people who usually consume uncooked fish. The patients who infected with small amount of *O. viverrini* but long lasting infection, it also leads to development of cholangiocarcinoma (CCA) or bile duct cancer (Sripa et al., 2010).

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