

RESEARCH ARTICLE

Carcinogenic Liver Fluke and Others Contaminated in Pickled Fish of Northeastern Thailand

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Abstract

Twenty provinces in northeastern Thailand were investigated for fluke metacercariae contamination in pickled fish, or pla-som, during January–June 2016. A total of 129 pickled fish shops were randomly chosen. Samples were digested with acid-pepsin and those found to be infected with metacercariae were fed to hamsters to test for metacercariae infectivity. The results demonstrated that only 20.2% of the pla-som samples were infected with fluke metacercariae (mc), at various levels (1 to 268 mc/kg). All recovered fluke metacercariae were inactive, degenerated and could not develop to adults in the animal model. In conclusion, the fluke mc infection status in pla-som was correlated with the prevalence of fluke infection in this region known for high *O. viverrini* and cholangiocarcinoma development. Clearly, systematic control of the fluke life cycle is needed. Whether pickling is an effective preventive measure needs further assessment.

Keywords: Fluke- pickled fish- cholangiocarcinoma- Survey- metacercariae

Asian Pac J Cancer Prev, **18** (2), 529-533

Introduction

Fish-borne trematode (FBT) infection is a public health problem in Southeast Asia, including Laos, Cambodia, and especially northeastern Thailand (Chai et al., 2014). The transmission of disease is caused by eating raw cyprinid fish infected with metacercariae (mc) of parasites such as the intestinal flukes *Haplorchis taichui* and *Haplorchis pumilio* (Chai et al., 2005) and the human liver fluke, which classified by the World Health Organization as Group 1 carcinogens (IARC, 1994) which is the risk for developing cholangiocarcinoma.

The geographic environment of northeastern Thailand contains many bodies of water, including the main rivers Khong, Chi and Mun, dams, and local creeks and lakes, which are the natural habitat of several species of cyprinid fish are important food sources for people in this region (Pinlaor et al., 2013; Ziegler et al., 2013). Many of these fish are processed into fermented/pickled fish products such as pla-ra and pla-som that are sold in local markets.

In northeastern Thailand, pickled fish or pla-som is a popular fermented food. In Thai cuisine, pla-som consists of fish, fresh garlic, rice and salt, which is fermented for 3 days (Sriraj et al., 2016). In a recent study, pla-som was fermented under laboratory conditions; on day 1 to day 3, viable metacercariae were found that were able to infect hamsters (Onsurathum et al., 2016).

Currently there is insufficient data to make accurate assessments of metacercariae contamination in pickled fish from local markets, and also a few data on metacercariae distribution in natural freshwater fish. This survey investigated metacercariae infection in pickled fish sold in local markets in 20 provinces of northeastern Thailand. These findings should provide new data on parasite infection in fermented fish products, which could help people avoid consuming raw or uncooked pickled fish from endemic areas.

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Material and Methods

Area of observation

The 129 pickled fish shops were randomly selected in endemic areas of trematode infection in northeastern Thailand, comprising 98 districts in 20 provinces: Amnat Charoen, Bueng Kan, Buriram, Chaiyaphum, Kalasin, Khon Kaen, Loei, Maha Sarakham, Mukdahan, Nakhon Phanom, Nakhon Ratchasima, Nong Bua Lamphu, Nong Khai, Roi Et, Sakon Nakhon, Sisaket, Surin, Ubon Ratchathani, Udon Thani and Yasothon.

Pickled fish collection and digestion

Pickled fish (pla-som) were bought from 129 shops in markets located in 20 provinces of northeastern Thailand during January –June 2016. One kg of each sample of pickled fish was collected and transferred to the laboratory of the Department of Parasitology, Faculty of Medicine, Khon Kaen University, Khon Kaen, Thailand. Pickled fish were digested in acid-pepsin solution and the mixture was then incubated at 37 °C for 1 h. The solution was filtered through 1,000, 300, 200 and 106 µm mesh, and washed with 0.85% saline until the supernatant became clear. The sediment was examined for metacercariae under a microscope. All metacercariae from each infected site were counted and fed to hamsters (each site per hamster) to observe the infection rate at 1 month post-infection. Protocols were approved by animal ethics of Khon Kaen University (ACUC-KKU-20/2559).

Results

Pickled fish infected with trematode metacercariae were found in 15 of the 20 provinces: Amnat Charoen, Bueng Kan, Buriram, Kalasin, Khon Kaen, Loei, Mukdahan, Nakhon Ratchasima, Nong Bua Lamphu, Nong Khai, Roi Et, Sakon Nakhon, Sisaket, Surin and Yasothon (Figure 1). Contamination with metacercariae was found in 20.2% of the pla-som samples (26/129 pickled fish shops) in 15 provinces. For the 129 pickled fish shops, fish and their water sources were grouped into two broad categories: 88.4% (114/129) came from fish markets in the central part of Thailand (Ang Thong, Ayutthaya, Chachoengsao, Nakhon Pathom and Samut Sakhon) and 11.6% (15/129) were local natural freshwater fish from nearby water sources (31.2% rivers, 25.0% dams and 43.7% creeks/lakes; Figure 2). Metacercaria infection can be found in 15 provinces: Amnat Charoen, Bueng Kan, Buriram, Kalasin, Khon Kaen, Loei, Mukdahan, Nakhon Ratchasima, Nong Bua Lamphu, Nong Khai, Roi Et, Sakon Nakhon, Sisaket, Surin and Yasothon (Table 1). Most of the pla-som samples (95.3%, 122/129) were fermented for 3 days, with pH 4 (0.8%), pH 5 (0.8%), pH 5.5 (0.8%), pH 6 (93.0%) and pH 6.5 (4.7%). The morphology of metacercariae tended to degrade, and large black granules. All inactive metacercariae could be observed, as shown in Figure 3. The 26 areas were infected samples were found and the number of metacercariae are shown in Table 2 and Figure 4. The three highest numbers of metacercariae (mc) found were: 268 mc (0.268 mc/g) in Buriram province, Lam Plai Mat district, in fishes brought

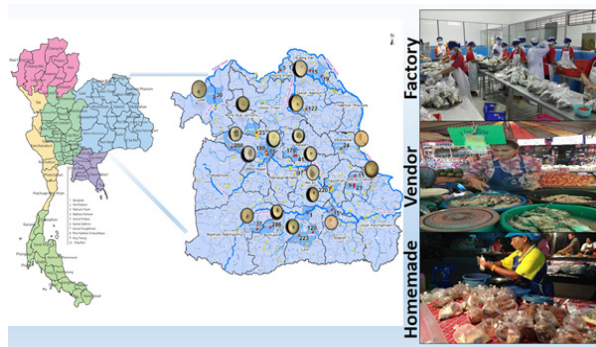


Figure 1. Geographical Location of the Study Area in Northeastern Thailand (Red Pins Indicate Mc-Positive Areas, Yellow Pins Indicate Observed Areas)

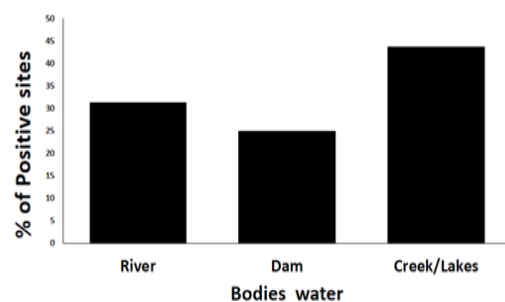


Figure 2. Local Water Sources of Fish Contaminated with Trematode Metacercariae: Rivers, Dams And Creeks/Lakes Located in 9 Provinces in Northeastern Thailand

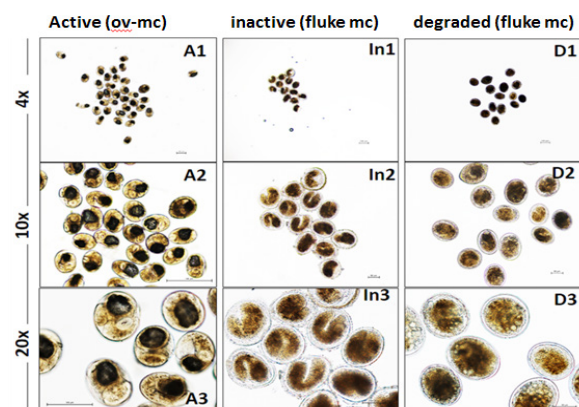
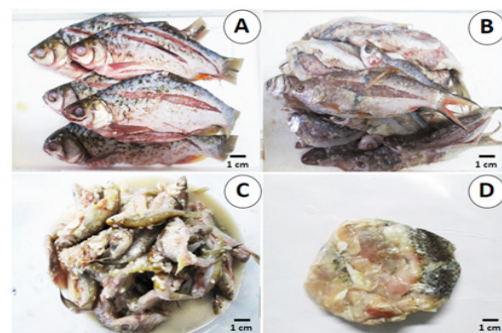


Figure 3. Types of Pla-Som (A-D) and Metacercariae From 26 Positive Sites in Northeastern Thailand, A1-3; Normal *O. viverrini* (Ov) Metacercaria (Mc), In1-3; Inactive Flake Metacercaria and D1-3; Degraded Flake Metacercaria

Table 1. Locations and Water Sources of Fish Contaminated with Trematode Metacercariae

No.	District	Province	Fish water source
1	Yang Talat 1	Kalasin	Ning Sim Creek*
2	Yang Talat 2	Kalasin	Chi River*
3	Chum Phae	Khon Kaen	Nakhon Pathom
4	Muang Khon Kaen 1	Khon Kaen	Nakhon Pathom
5	Muang Khon Kaen 2	Khon Kaen	Samut Sakhon
6	Chiang Khwan	Roi Et	Ubolratana Dam*
7	Tha Tum	Surin	Ra-wai Creek*
8	Sikhorphum	Surin	Huai Saneng Dam*
9	Lamduan	Surin	Tard Creek*
10	Muang Surin 1	Surin	Mun River*
11	Muang Surin 2	Surin	Mun River*
12	Waritchaphum	Sakon Nakhon	Chachoengsao
13	Porncharoen	Bueng Kan	Pak Chong Creek*
14	Seka	Bueng Kan	Samut Sakhon
15	Phon Phisai	Nong Khai	Luang Creek*
16	Muang Yasothon	Yasothon	Ang Thong
17	Muang Amnat Charoen 1	Amnat Charoen	Khong River*
18	Muang Amnat Charoen 2	Amnat Charoen	Lam Sae Bok River*
19	Senangkhanikhom	Amnat Charoen	Khong River*
20	Huai Thalaeng	Nakhon Ratchasima	Ayutthaya
21	Pak Chong	Nakhon Ratchasima	Chachoengsao
22	Muang Loei	Loei	–
23	Lam Plai Mat	Buriram	Lam Pao Dam*
24	Non Sang	Nong Bua Lamphu	Ubolratana Dam*
25	Rasi Salai	Sisaket	Nakhon Pathom
26	Khamcha-i	Mukdahan	Bang Sai Creek*

*Indicates water sources in each district

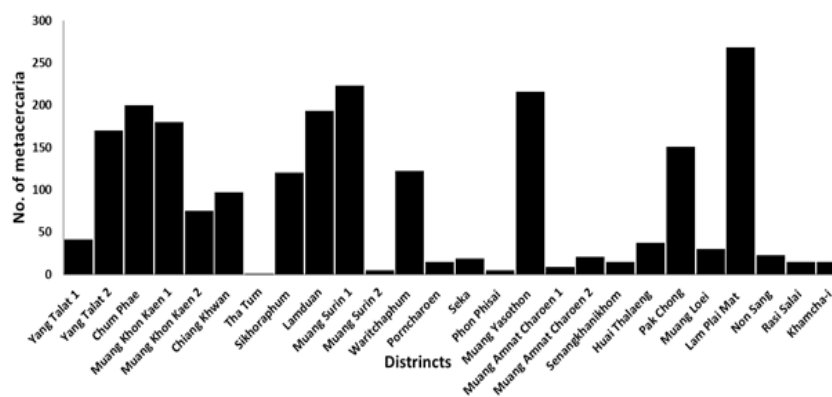


Figure 4. Number of Metacercariae Found in Pickled Fish, By District

from Lam Pao Dam in Kalasin province; 223 mc (0.223 mc/g) in Surin province that obtained fishes from the Mun River; and 216 mc (0.216 mc/g) in Yasothon province, in fishes originating in Ang Thong province (see Table 2 and Figure 4).

Discussion

Fish-borne trematodes such as intestinal and liver flukes are a major cause of morbidity and mortality

in northeast Thailand, largely because many of the inhabitants enjoy eating semi-cooked or raw fish, some of which are infected with metacercariae of the potentially carcinogenic liver fluke *Opisthorchis viverrini* (IARC, 1994). Liver flukes have important first and second intermediate hosts, Bithynia snails and cyprinid fish. The survey during 1992-1996, fish from markets in Thailand Contaminated with metacercariae of *O. viverrini* were found in fish from Udon Thani, Sa Kaeo and Prachin Buri Province. Cyprinoid fish such as Cyclocheilichthys,

Table 2. Data on Metacercariae Contamination of Pickled Fish by Districts.

No.	District	Province	Number of metacercariae	Mc/g
1	Yang Talat ¹	Kalasin	41	0.041
2	Yang Talat ²	Kalasin	170	0.17
3	Chum Phae	Khon Kaen	200	0.2
4	Muang Khon Kaen ¹	Khon Kaen	180	0.18
5	Muang Khon Kaen ²	Khon Kaen	75	0.075
6	Chiang Khwan	Roi Et	97	0.097
7	Tha Tum	Surin	1	0.001
8	Sikhoraphum	Surin	120	0.12
9	Lamduan	Surin	193	0.193
10	Muang Surin ¹	Surin	223	0.223
11	Muang Surin ²	Surin	5	0.005
12	Waritchaphum	Sakon Nakhon	122	0.244
13	Porncharoen	Bueng Kan	15	0.015
14	Seka	Bueng Kan	19	0.019
15	Phon Phisai	Nong Khai	5	0.005
16	Muang Yasothon	Yasothon	216	0.216
17	Muang Amnat Charoen ¹	Amnat Charoen	9	0.009
18	Muang Amnat Charoen ²	Amnat Charoen	21	0.021
19	Senangkhanikhom	Amnat Charoen	15	0.015
20	Huai Thalaeng	Nakhon Ratchasima	37	0.037
21	Pak Chong	Nakhon Ratchasima	151	0.151
22	Muang Loei	Loei	30	0.06
23	Lam Plai Mat	Buriram	268	0.268
24	Non Sang	Nong Bua Lamphu	23	0.023
25	Rasi Salai	Sisaket	15	0.015
26	Khamcha-i	Mukdahan	15	0.015

Mc/g, metacercariae per gram; 1, shop no. 1; 2, shop no. 2

Table 3. Data on Sources of Pickled Fish, Time for Fermentation and Number of Metacercaria

Sources of food preparation		Infected site/total	Duration for fermentation		
			3 day	4 days	5 days
Plasom	Vendor	9/72 (12.5%)	1	0	68
	Factory	15/49 (30.61%)	0	3	45
	Homemade	2/8 (25%)	0	1	8
	Total	26/129 (20.15%)	1	4	121
		No. of metacercaria (mean/site/kg)	223	209.4	44.5

Hampala and *Puntius* can be infected with metacercariae from different trematode species, depending on the region. (Waikagul, 1998; Nithiuthai et al., 2002). The relationship between natural water sources (rivers, creeks and lakes), dam building, fish ecology and food security pose a public health problem (Guerrier et al., 2011). A previous study, metacercariae of the parasite in fish is found in markets located in five provinces (Amnat Charoen, Nakhon Phanom, Mukdahan, Khon Kaen and Sisaket) and previous survey of fermented fish collected were infected were found only 9.58% (7/73) of sites in 20 provinces of northeastern Thailand whereas the prevalence of Ov infection in human remains high (Pinlaor et al., 2013; Onsurathum et al., 2016). In

comparison, in a present survey was found higher in positive areas because we increased area of observation, 15 different positive areas (provinces) of metacercariae in pickled fish were correlated with natural fish sources and water bodies (rivers, dams and creeks/lakes). Dam building could increase the incidence of many tropical diseases and may increase the habitat necessary for the survival and reproduction of vectors (Dugan et al., 2010). For *O. viverrini* infection can be found in small bodies water related with its intermediate hosts (snail and fish), associated with the outbreak of the disease in endemic area, disposing of feces method and sanitation. In our study, the highest numbers of metacercariae in pickled fish were found in Buriram, Surin and Yasothon provinces.

Buriram, which had the highest number of metacercariae (268 mc, 0.268 mc/g) used fresh fish from Lam Pao Dam. The intensity of metacercariae infection may differ depending on the location and time of year of fish collection. The density of infection was 1–15 per infected fish in southern Cambodia (Touch et al., 2009) and 252 per infected fish in Laos (Rim et al., 2013). In a previous study in Thailand, the intensity of infection with liver fluke metacercariae was found to be 8–88 metacercariae per fish in the northeast and 1.4 metacercariae per fish in the Northern area (Sukontason et al., 1999). The density of metacercariae in pickled fish is lower than in fresh fish, resulting from the fish production process and fermentation time. In present study, the intensity of mc in pickled fish was 1-268 metacercaria per kilogram with abnormal morphology (inactive or degenerated) as show in Figure 3. Duration of fermentation for 3,4 and 5 days associated with number of metacercaria discovery were 223, 209.5 and 44.5 mc, respectively (Table 3). Interviews indicate that they send pickled fish product for sale after finished pickled fish process in factory immediately may risk of *O. viverrini* infection for consumers. Some of pickled fish shop found inactive or degenerated *O. viverrini* metacercaria can identified by size of metacercaria and then infected in hamster. Animals infection with pickled fish indicated that inactive or degenerated *O. viverrini* metacercaria collected from pickled fish unable to infect in hamster. However, in general people in northeastern part like to eat pla-som in 0-3 days of fermented time which has different level of acidic therefore, people who eat fermented fish less than 3 days may have a high risk of liver fluke infection more than who like to eat the fermented fish more than 3 days.

Our result suggests that pickled fish had the fluke metacercariae infection eventhrough those metacercariae were degraded and had no the infectivity. So far, it means that this region remains the contaminated fluke in the environment and high risk for cholangiocarcinoma which should be monitor.

Acknowledgments

This study was supported by the Cholangiocarcinoma Screening and Care Program (CASCAP), Faculty of Medicine, Khon Kaen University (Thailand Grand Challenges: “Fluke-Free Thailand”).

References

- Chai JY, Sohn WM, Na BK, et al (2014). Zoonotic trematode metacercariae in fish from Phnom Penh and Pursat, Cambodia. *Korean J Parasitol*, **52**, 35-40.
- Chai JY, Darwin Murrell K, Lymbery AJ (2005). Fish-borne parasitic zoonoses: status and issues. *Int J Parasitol*, **35**, 1233-54.
- Dugan PJ, Barlow C, Agostinho AA, et al (2010). Fish migration, dams, and loss of ecosystem services in the Mekong basin. *Ambio*, **39**, 344-8.
- Guerrier G, Paul R, Sananikhom P, et al (2011). Strategic success for hydropower in Laos. *Science*, **334**, 38.
- IARC (1994). Infection with liver flukes (*Opisthorchis viverrini*, *Opisthorchis felinus* and *Clonorchis sinensis*). *IARC Monogr Eval Carcinog Risks Hum*, **61**, 121-75.
- Nithiuthai S, Suwansaksri J, Wiwanitkit V, Chaengphukeaw P (2002). A survey of metacercariae in cyprinoid fish in Nakhon Ratchasima, northeast Thailand. *Southeast Asian J Trop Med Public Health*, **33**, 103-5.
- Onsurathum S, Pinlaor P, Haonon O, et al (2016). Effects of fermentation time and Low temperature during the production process of Thai pickled fish (pla-som) on the viability and infectivity of *Opisthorchis viverrini* metacercariae. *Int J Food Microbiol*, **218**, 1-5.
- Onsurathum S, Pinlaor P, Charoensuk L, et al (2016). Contamination of *Opisthorchis viverrini* and *Haplorchis taichui* metacercariae in fermented fish products in northeastern Thailand markets. *Food Control*, **59**, 493-98.
- Pinlaor S, Onsurathum S, Boonmars T, et al (2013). Distribution and abundance of *Opisthorchis viverrini* metacercariae in cyprinid fish in Northeastern Thailand. *Korean J Parasitol*, **51**, 703-10.
- Rim HJ, Sohn WM, Yong TS, et al (2013). Fishborne trematode metacercariae in Luang Prabang, Khammouane, and Saravane Province, Lao PDR. *Korean J Parasitol*, **51**, 107-14.
- Sriraj P, Boonmars T, Aukkanimart R, et al (2016). A combination of liver fluke infection and traditional northeastern Thai foods associated with cholangiocarcinoma development. *Parasitol Res*, **115**, 3843-52.
- Sukontason K, Piangjai S, Muangyimpong Y, Sukontason K, Methanitikom R, Chaithong U (1999). Prevalence of trematode metacercariae in cyprinoid fish of Ban Pao district, Chiang Mai Province, northern Thailand. *Southeast Asian J Trop Med Public Health*, **30**, 365-70.
- Touch S, Komalamisra C, Radomyos P, Waikagul J (2009). Discovery of *Opisthorchis viverrini* metacercariae in freshwater fish in southern Cambodia. *Acta Trop*, **111**, 108-13.
- Waikagul J (1998). *Opisthorchis viverrini* metacercaria in Thai freshwater fish. *Southeast Asian J Trop Med Public Health*, **29**, 324-6.
- Ziegler AD, Petney TN, Grundy-Warr C, et al (2013). Dams and disease triggers on the lower Mekong river. *PLoS Negl Trop Dis*, **7**, e2166.