LIVER FLUKE-INFECTED CYPRINOID FISH IN NORTHEASTERN THAILAND (2016-2017)

Benjamabhorn Pumhirunroj[1,2,3], Ratchadawan Aukkanimart[4], Thidarut Boonmars[1,2], Pranee Sriraj[4], Parichart Boueroy[5], Atchara Artchayasawat[1,2], Jiraporn Songsri[1,2,6], Panupan Sripan[6], Kanpicha Chomphumee[2], Panaratana Ratanasuwan[7], Porntip Laummaunwai[1], Sukhonthip Khueangchaingkhwang[1,2], Apiporn Suwanatri[1], Surasit Aunpromma[8], Chatanun Eamudomkarn[1], Narong Khuntikeo[2,9], Watcharin Loilome[2,10], Nisana Namwat[2,10] and Puangrat Yongvanit[2]

[1]Department of Parasitology, Facullty of Medicine, [2]Cholangiocarcinoma Research Institute, Cholangiocarcinoma Screening and Care Program, Khon Kaen University; [3]Program in Animal Science, Faculty of Agriculture Technology, Sakon Nakhon Rajabhat University; [4]Department of Traditional Medicine, Faculty of Natural Resources, Rajamangala University of Technology ISAN Sakon Nakhon Campus; [5]Faculty of Public Health, Kasetsart University Chalermphrakiat Sakon Nakhon Province Campus, Sakon Nakhon; [6]College of Allied Health Science, Suan Sunandha Rajabhat University, Samut Songkhram; [7]Department of Anesthesiology, Faculty of Medicine, Khon Kaen University; [8]Department of Veterinary Pathobiology, Faculty of Veterinary Medicine, [9]Department of Surgery, [10]Department of Biochemistry, Faculty of Medicine, Khon Kaen University, Khon Kaen, Thailand

Abstract. The highest prevalence of liver fluke infection globally is found in northeastern Thailand, a region where bodies of water have been contaminated with liver fluke eggs from feces of infected subjects. An evaluation of liver fluke metacercaria in cyprinoid fish from 132 different water bodies in 59 districts of 20 provinces in northeastern Thailand was conducted from November 2016 to October 2017. Twelve of 28 cyprinoid fish species obtained from 14 out of 20 provinces were infected with *Opisthorchis viverrini* metacercariae (mc). Infection in fish was highest (12.1%) in canal/creek, followed by marsh/pond (6.1%), reservoir (3.8%), lake (3.0%), swamp (1.5%), dam/weir (0.8%), and river (0.8%). Intensity of *O. viverrini*-infected fish ranged from 0.04-2.47 mc per fish and 0.59-177.78 mc per kg fish, being highest in Sri Sa Ket Province. Six new *O. viverrini* fish hosts (*Barbonymus altus, B. gonionotus, Cyclocheilichthys lagleri, Henicorhynchus ornatipinnis, Puntius brevis,* and *P. spilopterus*) were discovered. The results should be useful for development of appropriate strategies to control human feces contamination and liver fluke infection in water bodies and cyprinoid fish in northeastern Thailand.

Keywords: cyprinoid fish, liver fluke, trematode, northeastern Thailand, water body

Correspondence: Thidarut Boonmars, Department of Parasitology, Faculty of Medicine, Khon Kaen University, Khon Kaen 40002, Thailand.
Tel: +66 4334 8387; Fax: +66 4320 2475; E-mail: bthida@kku.ac.th; boonmars@yahoo.com

INTRODUCTION

Liver fluke, *Opisthorchis viverrini*, is a food-borne trematode parasite from the Opisthorchiidae family, which infects human bile duct and is the major risk factor for cholangiocarcinoma in Cambodia, Lao PDR and northeastern Thailand (Khuntikeo *et al*, 2018). Humans become infected with *O. viverrini* by ingesting uncooked cyprinoid fish contaminated with *O. viverrini* metacercariae, in particular, raw cyprinoid spicy salad (*koi pla*) and pickled fish (*plasom*) (Prasongwatana *et al*, 2013; Onsurathum *et al*, 2016). Due to poor sanitation practices and inadequate sewerage infrastructure, people infected with *O. viverrini* pass the parasite eggs in their feces into natural water reservoirs, where *Bithynia* snail, the first intermediate host, ingests the eggs, and, following hatching, miracidia are released to develop sequentially into sporocysts, rediae and free-swimming cercariae. The latter in turn infect cyprinoid fish, the second intermediate hosts, by penetrating into the tissue or skin and become fully infective metacercariae (Sripa *et al*, 2007). When humans eat raw or undercooked infected fish, the life cycle is completed.

In order to evaluate water bodies contaminated with human feces, different techniques are available to detect *Escherichia coli*, such as culture, DNA detection or a DipTest (Gunda *et al*, 2017). However, the low concentrations of fluke eggs in the water bodies makes direct detection very difficult, and the best surrogate is to determine the prevalence of *O. viverrini* in the intermediate hosts. Few *O. viverrini*-infected snails are found in a number of water sources, which do not correlate with prevalence of human opisthorchiasis in the test regions (Wang *et al*, 2015; Kim *et al*, 2016). An alternative approach is to survey infection in cyprinoid fish.

Hence, a large scale year-long large-scale evaluation of *O. viverrini* infection in cyprinoid fish was conducted in 20 provinces of northeastern Thailand. This project constitutes one part of a strategic plan for eradication of liver fluke and cholangiocarcinoma in Thailand through coordination among the Ministries of Public Health, of Agriculture, of Social Development and Human Security, and of Education, together with Khon Kaen University.

MATERIALS AND METHODS

Sites and samples collection

Cyprinoid fish were collected from 132 water bodies (creek/canal, dam/weir, lake, marsh/pond, reservoir, river, and swamp) in 73 sub-districts across 59 districts in 20 provinces of northeast Thailand (Fig 1A), known for its high prevalence of *O.viverrini* infection and cholangiocarcinoma (Sripa and Pairojkul, 2008). Cyprinoid fish was collected during November 2016 to October 2017 covering the rainy and dry seasons.

Fish identification and determination of infection intensity

At least one kg of cyprinoid fish was collected from each site (GPS coordinates recorded) by a village health volunteer and kept on ice until transportation to the Department of Parasitology, Faculty of Medicine, Khon Kaen University, Khon Kaen. Each fish species was photographed, weighed and identified using a freshwater fish key (http://www.fishbase.org/home.html). Each fish was then digested with a 0.85% NaCl solution containing 0.25% pepsin (Sigma-Aldrich Co, St Louise, MO, USA) and 0.15% HCl for metacercariae recovery and *O. viverrini* metacercariae were identified as previously described

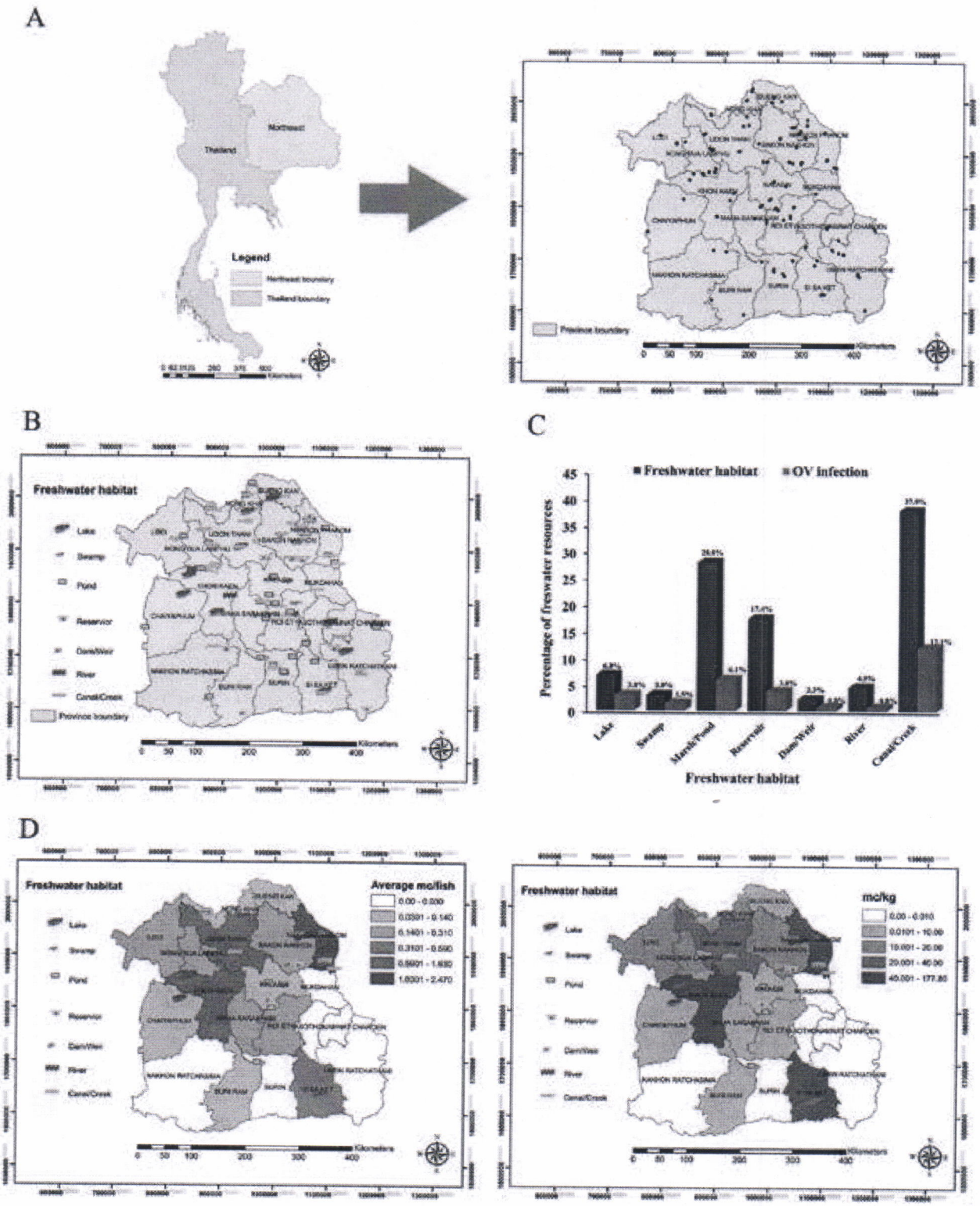

Fig 1-(A) Location of study area and cyprinoid fish sampling sites (black dots), (B) types of water habitat, (C) percent type and *Opisthorchis viverrini*-infected water habitat, (D) intensity of *O. viverrini* infection in each province of northeastern Thailand defined as number of *O. viverrini* metacercariae (mc)/(number of fish or kg of fish). OV: *O. viverrini*.

(Sriraj *et al*, 2016). Intensity of *O. viverrini* infection in fish is defined as number of *O. viverrini* metacercariae (mc) divided by number of fish or kg of fish).

RESULTS

Among 132 freshwater habitats surveyed, 1,088 *O. viverrini*-infected fish and 578 metacercariae were found in 37 localities distributed across 28 sub-districts of 14 provinces in northeastern Thailand. These localities constituted 1 dam/weir, 1 river, 2 swamps, 4 lakes. 5 reservoirs, 8 marshes/ponds, and 16 canals/creeks (Fig 1B, C). Average intensity of *O. viverrini*-infected fish in each province was 0.53 mc/fish (ranging from 0.04-2.47 mc/fish) or 31.2 mc/kg fish (ranging from 0.59-177.8 mc/kg fish). The highest intensity of *O. viverrini* infection was found in Sri Sa Ket Province (Fig 1D). The intensity of *O. viverrini* metacercariae in fish was not different between the dry (265 mc/479 fish or 0.55 mc/fish) and rainy (313 mc/609 fish or 0.51 mc/fish) seasons. *O. viverrini* metacercariae-infected fish were found in 9 and 12 provinces in the dry and rainy season, respectively.

Twenty-eight species of cyprinoid fishes were collected, of which 12 were infected with *O. viverrini* metacercariae (Table1), with six new fish hosts (*Barbonymus altus, B. gonionotus, Cyclocheilichthys lagleri, Henicorhynchus ornatipinnis, Puntius brevis,* and *P. spilopterus*) discovered (Fig 2). *Henicorhynchus ornatipinnis* was the smallest *O. viverrini*-infected fish species and had the highest intensity of *O. viverrini* metacercaria infection (128 mc/25 fish or 5.1 mc/fish).

DISCUSSION

This is the first broad survey of fish-borne liver fluke, an indirect indicator of human liver fluke egg contamination in bodies of water, across all 20 provinces of northeastern Thailand. *O. viverrini* is the most common fish-borne trematode identified in the region (Sithithaworn *et al*, 2012, Pinlaor *et al*, 2013). Over a one-year period (2016 -2017), 43% of cyprinoid fish species collected from various freshwater habitats were infected with *O. viverrini* metacercariae and 50% of the infected fish species were identified as new hosts. High infection intensities of *O.viverrini* metacercariae were found in 14 provinces. These findings were consistent with earlier studies that reported *O. viverrini* metacercaria infection in four cyprinid fish species from Khon Kaen Province (*Cirrhinus jullieni, Cyclocheilichthys armatus, Mystacoleucus atridorsalis,* and *Puntius leiacanthus*) (Srisawangwong *et al*, 1997), in eight fish species from Prachin Buri, Sa Kaeo, and Udon Thani Provinces (*Ci. jullieni, Cyclocheilichthys* spp, *Esomus metallicus, Hampala dispar, Labiobarbus spilopleura, Osteochilus vittatus, Puntius* spp., and *Trichogaster* spp) (Waikagul, 1998), in five fish species from Nakhon Ratchasima Province (*Cy. armatus, Cy. repasson, Ha. dispar, Ha. macrolepidota,* and *Puntioplites proctozysron*) (Kaewpitoon *et al*, 2012), and six fish species from Amnat Charoen, Khon Kaen, Mukdahan, Nakhon Phanom, and Si Sa Ket Provinces (*Cy. armatus, Ha. dispar, Henicorhynchus siamensis, Pu. Orphoides* (not found in present study), *Pu. proctozysron,* and *Os. hasseltii*) (Pinlaor *et al*, 2013). The highest *O. viverrini* metacercaria infection intensity was found in *Henicorhynchus ornatipinnis,* one of the new host species. Cyprinid fish of genera *Puntius, Cyclocheilichthys* and *Hampala* were reported to harbor high *O. viverrini* metacercariae infection intensity (Wykoff *et al*, 1965).

Table 1
Cyprinoid fish species collected in northeastern Thailand (November 2016 to October 2017) and intensity of *Opisthorchis viverrini* metacercaria infection.

No.	Fish species	Size (width × length, cm)	No.of *O. viverrini* metacercariae found (mc) / No. of infected fish	Intensity of metacercariae (mc/infected fish)
1	*Barbonymus altus*	5.7-9.0 × 13.1-17.0	1/2	0.50
2	*Barbonymus gonionotus*	4.2-8.8 × 9.0-19.5	20/295	0.07
3	*Barbodes schwanenfeldii*	2.5-13.6 × 4.8-28.5	0/0	0
4	*Cirrhinus jullieni*	2.0-3.5 × 7.8-10.0	0/0	0
5	*Cirrhinus microlepis*	4.0-7.5 × 17.0-26.5	0/0	0
6	*Cirrhinus molitorella*	2.6-5.7 × 8.2-18.4	0/0	0
7	*Cyclocheilichthys armatus*	2.2-5.0 × 6.1-13.5	43/79	0.54
8	*Cyclocheilos enoplos*	1.8-5.0 × 5.3-16.2	0/0	0
9	*Cyclocheilichthys lagleri*	4.0-5.2 × 15.0-25.0	157/231	0.68
10	*Discherodontus ashmeadi*	2.0-3.0 × 6.0-7.5	0/0	0
11	*Hampala dispar*	2.2-7.4 × 5.7-23.6	100/124	0.81
12	*Hampala macrolepidota*	4.2-5.2 × 12.5-17.0	0/0	0
13	*Henicorhynchus ornatipinnis*	2.4-2.6 × 7.5-9.5	128/25	5.12
14	*Henicorhynchus siamensis*	1.6-6.2 × 6.51-8.2	8/16	0.5
15	*Labiobarbus siamensis*	2.2-6.0 × 7.0-18.5	11/40	0.28
16	*Morulius chrysophekadian*	4.5-7.5 × 15.2-20.0	0/0	0
17	*Mystacoleucus marginatus*	2.4-3.6 × 6.6-8.5	0/0	0
18	*Osteochilus hasseltii*	2.7-8.0 × 8.2-16.5	9/19	0.47
19	*Osteochilus lini*	2.2-8.2 × 7.2-16.0	0/0	0
20	*Osteochilus melanopleurus*	3.0-4.7 × 9.5-13.5	0/0	0
21	*Puntius brevis*	5.0-7.0 × 11.0-15.0	71/36	1.97
22	*Puntioplitus falcifer*	2.6-6.0 × 6.5-12.5	0/0	0
23	*Puntius proctozysron*	2.2-11.0 × 5.0-20.5	10/42	0.24
24	*Puntius spilopterus*	1.7-4.7 × 5.0-14.1	20/179	0.11
25	*Rasbora dorsinotata*	1.0-3.6 × 4.1-8.8	0/0	0
26	*Sikukia stejnegeri*	3.8-7.0 × 10.0-24.0	0/0	0
27	*Systomus orphoides*	3.2-5.5 × 8.5-15.5	0/0	0
28	*Thynnichthys thynnoides*	2.5-4.5 × 6.5-13.6	0/0	0

A survey in 2009 conducted by the Department of Disease Control, Ministry of Public Health, Thailand reported all 20 provinces in northeastern Thailand have high prevalence of human opisthorchiasis (Sithithaworn *et al*, 2012) and later surveys reported high prevalence in Yasothon Province (Saengsawang *et al*, 2013) and Surin Province (Rujirakul *et al*, 2015), which are inconsistent with the present results of *O. viverrini*-infected cyprinid fish being detected in 14 provinces. One possible expalanation is that not all raw *O. viverrini*-infected fish meat enter into the human food chain in a particular region.

O. viverrini infection intensity of

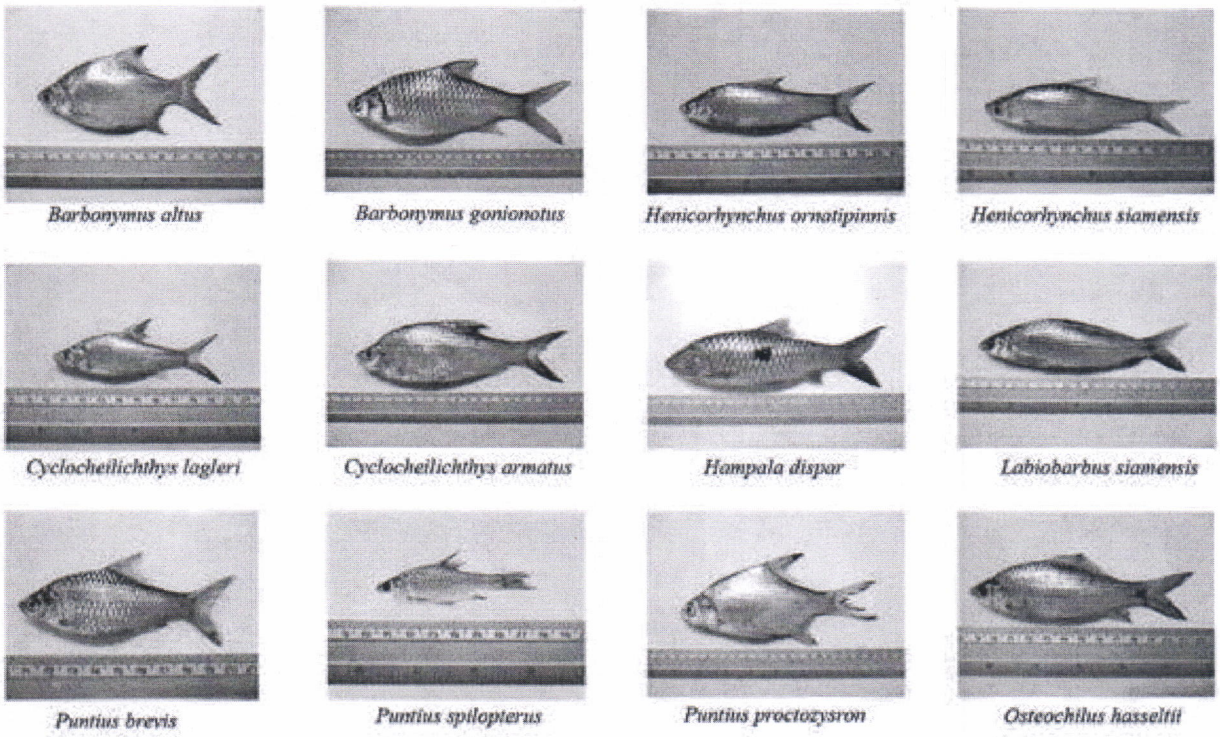

Fig 2-The twelve species of cyprinoid fish with *Opisthorchis viverrini* infection collected from north-eastern Thailand (November 2016 to October 2017). Cyprinoid fish species was identified using a freshwater fish key (http://www.fishbase.org/home.html).

cyprinoid fish in the present study was lower than that reported in 2011-2012, which demonstrated infection intensity ranging from 0.01 to 6.5 mc/fish or 1.3-287.5 mc/kg fish (Pinlaor *et al*, 2013). During the earlier collection period, 10 kg of cyprinoid fish collected from natural freshwater habitats yielded >5,000 *O. viverrini* metacercariae (unpublished data) compared to 31 metacercariae/kg fish in the present survey. This might reflect the (partial) success of the Thai Government campaign to eradicate the liver fluke infection and reduce cholangiocarcinoma in this region of the country (Khuntikeo *et al*, 2018).

In conclusion, *O. viverrini* metacercariae-infected cyprinoid fish collected from bodies of freshwater were found in 14 provinces of northeastern Thailand, reflecting contamination by feces from infected individuals. These findings should assist in focussing liver fluke eradication programs to regions of highest risk for human opisthorchiasis.

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CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

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