Tropical Biomedicine 37(2): 443–451 (2020)

Fish-borne trematode metacercariae detected in fish commonly used for raw consumption in Ninh Binh Province, Vietnam

Khoa, D.V.[1], Hoa, D.T.[2], Anh, D.N.[3], Van, N.T.[3], Dung, D.T.[4], Huong, L.T.T.[5], Quyen, L.T.B.[6], Su, H.X.[7] and Tran-Anh, L.[3*]

[1]Phuc Yen 74 Central Hospital, Hung Vuong Ward, Phuc Yen City, Vinh Phuc Province, Vietnam
[2]Ha Noi Police Hospital, Ha Noi, Vietnam
[3]Department of Parasitology, Vietnam Military Medical University (VMMU), 160 Phung Hung, Ha Dong, Ha Noi, Vietnam
[4]Department of Parasitology, National Institute of Malariology, Parasitology and Entomology (NIMPE), 34 Trung Van St., Trung Van Ward, Nam Tu Liem district, Ha Noi, Vietnam
[5]Department of Microbiology and Biology, Ha Noi University of Pharmacy, 13 Le Thanh Tong, Ha Noi, Vietnam
[6]University of Science, National University of Ha Noi, Vietnam
[7]Department of Microbiology and Pathogens, Institute of Biomedicine and Pharmacy, VMMU, Ha Noi, Vietnam
[*]Corresponding author e-mail: anh_lt@vmmu.edu.vn
Received 22 April 2019; received in revised form 12 February 2020; accepted 13 February 2020

Abstracts. Raw or undercooked fish dishes are the major sources of human infection of fish-borne trematodes (FBT) and the situation of metacercarial infection in fish greatly affect the prevalence in humans, especially those fish that are commonly used for raw consumption. To investigate the situation of infection with metacercaria of FBT in fish often used to prepare raw fish dishes by local people to assess the risk of infection to humans in Ninh Binh province, Vietnam. 345 fish belonging to five species of freshwater and one species of brackish water fish were collected from fishermen or small-scale fish dealers in Kim Son and Yen Khanh districts, Ninh Binh province between May 2017 and May 2018. Metacercaria of FBT was discovered by pepsin and hydrochloric acid digestion techniques and identified by the morphological and molecular analysis. Among examined fish, 44.06% infected with FBT metacercaria and the highest prevalence was in *Cyprinus carpio* (86.54%), *Ctenopharyngodon idellus* (78.43%) and *Hypophthalmichthys molitrix* (66.67%) while *Konosirus punctatus* – the brackish water fish – were free from infection. Three species of FBT were found; namely *Haplorchis pumilio* (accounting for 99.84% of collected metacercariae), *Haplorchis taichui* and *Clonorchis sinensis*. The average density was 1.06 metacercariae per gram of freshwater fish and the highest number was of *C. idellus* (6.38 cysts/gram) followed by *Cirrhinus molitorella* and *C. carpio*. Results of the study show the high prevalence of infection of FBT metacercariae among freshwater fish often used to prepare raw fish dishes in Ninh Binh province. These findings suggest the need for greater awareness of the risk from raw fish dishes among public health authorities and people.

INTRODUCTION

Fish-borne trematodes (FBT) including small liver flukes (SLF) and minute intestinal flukes (MIF) are of medical importance and public health significance in Asia (WHO, 2002). Life cycles of FBT involve three types of hosts that are firstly aquatic snail hosts, secondary fish hosts and definitive hosts including a range of fish-eating mammals and birds (Chai, 2007). Raw or undercooked fish dishes are the major sources of human infection and the situation of metacercaria infection in fish greatly affect the situation of FBT in humans especially those fish that are commonly used for raw ingestion by

people (WHO, 1995). Some authors stress that the control of FBT is theoretically very simple by avoiding eating raw or not thoroughly cooked fish, but this can be extremely difficult in facing centuries-old traditions (Rim, 1997; Tang *et al.*, 2016), so supplying food safety/security and quality may be an important measure.

Vietnam is located in Southeast Asia, an endemic region of many food-borne trematodiasis (Sripa *et al.*, 2010). FBT infection is prevalent in many regions and the highest prevalences were recorded in Nam Dinh and Ninh Binh provinces (Doanh & Nawa, 2016). Metacercaria of SLF (*Clonorchis sinensis*) and MIF (*Haplorchis pumilio*, *H. taichui*, *H. yokogawai*, *Procerovum varium*, *Centrocestus formosanus*, *Stellantchasmus falcatus*, *Echinochasmus japonicus*) have been found in many species of fish in northern and central regions of Vietnam (Chi *et al.*, 2008; Van *et al.*, 2010b; Hung *et al.*, 2015). However, the examined fish in these surveys had been collected randomly so many species of fish not ingested raw have been examined and vice versa and the major source of infections for human has not been obvious yet. Moreover, most identification of metacercariae in fish only based on morphological characteristics which may result in some confusion or unidentification (Clausen *et al.*, 2012; Hung *et al.*, 2015). The present study was carried out to investigate the situation of infection with metacercaria of FBT in fish that are commonly used for raw ingestion by local people to assess the risk of infection to human in Ninh Binh province, Vietnam.

MATERIAL AND METHODS

The Site, Sampling, and Examination Procedure

The cross-sectional survey was conducted in Kim Son and Yen Khanh districts, Ninh Binh province (Fig. 1). The study site is located around 100 km southeast of the capital – Hanoi and in the Red River Delta region which has numerous rivers, lakes and ponds. Kim Tan commune (Kim Son district) is a coastal commune and local people usually collect brackishwater fish for raw consumption.

Based on the results of interviewing local health authorities, 6 species of fish commonly eaten raw by local people were selected for testing FBT metacercariae including five species of freshwater fish; common carp (*Cyprinus carpio)*, silver carp (*Hypophthalmichthys molitrix*), grass carp (*Ctenopharyngodon idellus*), mud carp (*Cirrhinus molitorella*), tilapia (*Orechromis niloticus*) and a species of brackish water fish; dotted (konoshiro) gizzard shad (*Konosirus punctatus*). A minimum of 50 fish of each species was collected from fishermen or small-scale fish dealers. Those fish were individually measured for length and weight, stored in a dry ice box and transported to the laboratory of Department of Parasitology, National Institute of Malariology, Parasitology and Entomology, Vietnam on the same day. Fish were maintained at 4°C in the laboratory for a maximum of 5 days until processed. Whole small fish (less than 50 gram in weight) or 50 gram of different parts of large fish (more than 50 gram in weight) were ground and analyzed using pepsin and hydrochloric acid digestion techniques according to WHO guideline (WHO 1995). The identification of metacercariae was based on morphological features as described elsewhere (Scholz, 1991; Sohn, 2009) (Fig. 2). Metacercariae of each type were counted, recorded and stored in sterile 1.5 ml tubes containing alcohol 70% for further analysis.

Time of the study: collection and examination of metacercariae were from May 2017 to May 2018. Molecular analysis was conducted in 2018.

Molecular analysis

DNA extraction from collected metacercariae

Nine representative tubes (four of *H. pumilio*, four of *H. taichui* and one of *C. sinensis*) were subjected to molecular analysis. Disruption of the metacercariae and liberation of the DNA was achieved by homogenization with a tissue homogenizer

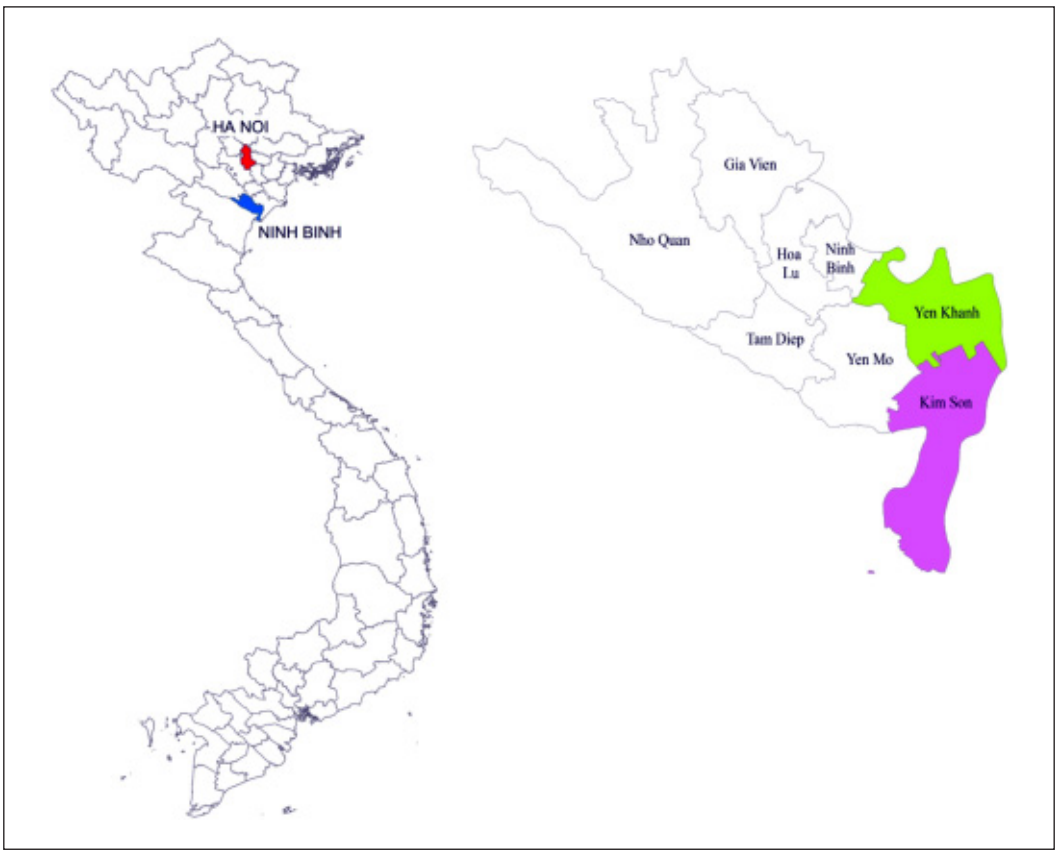

Figure 1. The study site in Ninh Binh province, Vietnam.

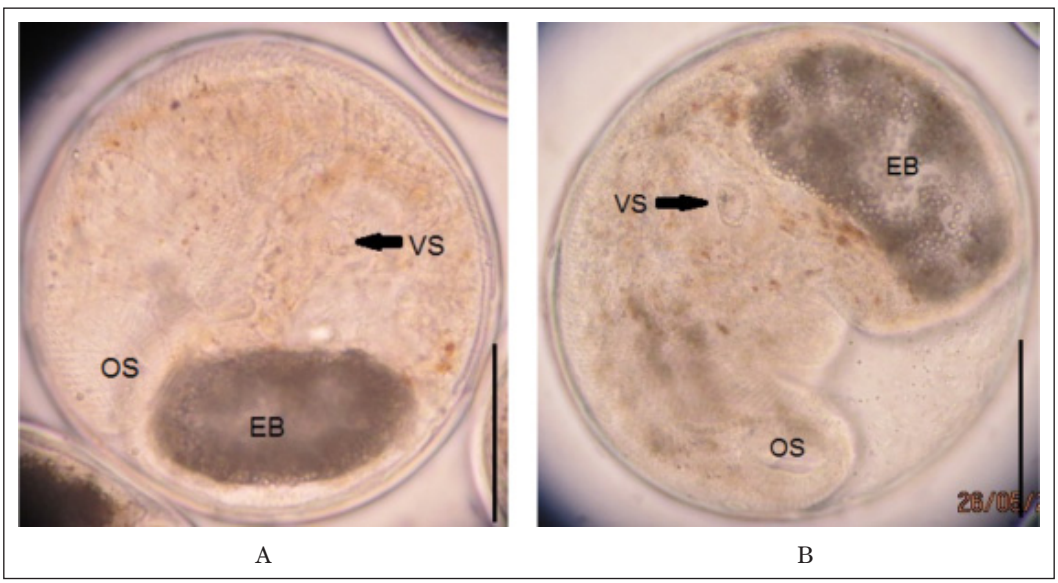

Figure 2. Metacercaria of *Haplorchis pumilio* (A) and *Haplorchis taichui* (B) detected in fish from Ninh Binh province, Vietnam.
(Scale bar = 75 µm; EB: excretory bladder, OS: oral sucker; VS: ventrogenital sac with numerous minute spines (arrow).

(Omni TH220, Omni International, Marietta, GA, USA) at 10,000 rpm for 1 min. After brief centrifugation, the supernatant was boiled in a water bath for 5 min to inactivate the pepsin and, subsequently, neutralized by the addition of 1 N potassium hydroxide. Total genomic DNA was extracted using DNA mini kit (QIAGEN, Hilden, Germany) according to the manufacturer's protocol except for ultrasound steps within 10 minutes before separation. The genomic DNA of each sample was eluted in 50 µl of the elution buffer provided in the kit and stored at -20°C until use.

Primers, PCR amplification

Primers to amplify the entire second internal transcribed spacer region (ITS-2) were ITS2-F (5'-CTT GAA CGC ACA TTG CGG CCA TGG G-3') and ITS2-R (5'-GCG GGT AAT CAC GTC TGA GCC GAG G-3') (Sato *et al.*, 2009). PCR reaction was conducted on thermal cycler (Eppendorf Mastercycler Personal, Germany) in a total volume of 20 µl, including 5 µl template, 10 pmol of each primer and PCR master mix (PCR Master Mix from QIAGEN). The PCR was run by 35 cycles of 94°C for 10 seconds, 40°C for 30 seconds, and 72°C for 30 seconds with a final extension step of 15 minutes at 72°C. PCR products were separated by electrophoresis in 2% agarose gel for 1h and visualized under UV light after staining with ethidium bromide. The yielded PCR products were sequenced and deposited in GenBank. The produced sequences and some other ITS2 sequences of *H. pumilio*, *H. taichui*, *C. sinensis*, *Opisthorchis viverrini*, *Opisthorchis felineus*, *Fasciolopsis buski* retrieved from GenBank were aligned using Bioedi 7.0 (www.mbio.ncsu.edu/BioEdit/bioedit.html). A phylogenetic tree was constructed using MEGA7.0 software (www.megasoftware.net) by Neighbor-joining method based on the Kimura two-parameter model with 1 000 bootstrap replicates.

RESULTS

A total of 345 fish of 6 species were examined (Table 1). Among examined fish, 44.06% were infected with FBT metacercariae. All species of freshwater fish were infected with metacercariae of FBT but not for brackish water fish. Trematode metacercariae were more prevalent among *Cyprinus* sp., *Ctenopharyngodon* sp. and *Hypophthalmichthys* sp. Metacercariae of *H. pulmilio* presented in all species of freshwater fish while those of *C. sinensis* occurred only in grass carp. Grass carp were the only species infected with metacercariae of all three FBT species (Table 2). From the examined fish, 18 323 metacercariae were collected and most of them (99.84%) were of *H. pumilio*. The average intensity was 1.06 metacercariae per gram of freshwater fish and the highest number was of grass carp (6.38 cysts/gram) followed by mud carp and common carp (Table 3).

Molecular analysis

The PCR amplicons of *C. sinensis*, *H. pumilio* and *H. taichui* were 390, 380, and 530 bp for ITS2 as previously described (Sato *et al.*, 2009). The yielded PCR products were sequenced and some of them were deposited in GenBank under the code MK453254

Table 1. Freshwater and brackish water fish collected from the study site

Category	Number of fish examined	Length (cm)		Weight (g)	
		Range	Average	Range	Average
H. molitrix	87	24 – 41.5	30.58	106.15 – 808.08	309.38
C. molitorella	53	20 – 29	24.47	77.25 – 240.0	154.24
C. idellus	51	13.5 – 24.0	17.35	27.9 – 145.5	62.58
C. carpio	52	9 – 21	13.8	20.0 – 250.0	78.12
O. niloticus	52	12 – 22	17.43	40.0 – 250.0	136.0
K. punctatus	50	13 – 17.3	14.65	19.01 – 54.76	29.95
Total	345	9 – 41.5	32.5	19.01 – 808.08	147.58

Table 2. FBT diversity and prevalence in fish

Fish species	N	(%)	NFBT	(%)	NHp	(%)	NHt	(%)	NCs	(%)
C. idellus	51	14.78	40	78.43	40	78.43	5	9.80	2	3.92
C. carpio	52	15.07	45	86.54	45	86.54	0	0	0	0
H. molitrix	87	25.22	58	66.67	58	66.67	2	2.30	0	0
C. molitorella	53	15.36	8	15.09	8	15.09	0	0	0	0
O. niloticus	52	15.07	1	1.92	1	1.92	0	0	0	0
K. punctatus	50	14.49	0	0	0	0	0	0	0	0
Total	345	100	152	44.06	152	44.06	7	2.03	2	0.58

N: Number of specific fish species, %: percentage of each fish species. FBT: fish-borne trematode, Hp: *Haplochis pumilio*; Ht: *H. taichui*, Cs: *Clonorchis sinensis*.

Table 3. Number and intensity of metacercaria among different freshwater fish

	Number of metacercaria				Intensity (mertacercariae / gram fish)	
	Hp	Ht	Cs	Total	Average	Range
C. idellus	16 235	23	3	16 261	6.38	0 – 48.60
C. carpio	1 216			1 216	0.47	0 – 2.90
H. molitrix	687	3		690	0.15	0 – 3.19
C. molitorella	155			155	0.58	0 – 2.5

(sample 323664 2T43 2, *H. pumilio*), MK790157 (sample 3236649 33T1H, *H. taichui*) and MK780187 (sample 3236646 30T1C, *C. sinensis*). From the produced sequences in our study and some other ITS2 sequences of *H. pumilio*, *H. taichui*, *C. sinensis*, *O. viverrini*, *O. felineus*, *F. buski* retrieved from GenBank a phylogenetic tree was constructed (Fig. 3).

DISCUSSION

Prevalence of metacercarial infection in fish

Based on the results of interviewing local health authorities, 6 species of fish commonly eaten raw by local people were selected for testing FBT larvae including five species of freshwater fish and one species of brackish water fish (gizzard shad). The situation of FBT metacercariae in fish may be influenced by many factors such as species of fish as well as seasons, types of water bodies where fish collected (Kim *et al.*, 2016). The present study focused on species of fish that are commonly used for raw ingestion by local people so we intentionally collected those fish in warm months (from May to August), coinciding with high transmission of FBT in fish in northern Vietnam (Hung *et al.*, 2007; Madsen *et al.*, 2015).

Nearly half of the examined fish (44.06%) were infected with FBT metacercariae and all species of freshwater fish were infected. The high rate of infection in fish was concordant with some other surveys in the north and centre of Vietnam (Chi *et al.*, 2008; Hung *et al.*, 2015) which made people who had a habit of eating raw fish at high risk of getting the infection because they were likely to eat fish infected with FBT metacercariae. The highest prevalence of infection was among fish belonging to Cyprinidae family such as grass carp, common carp and silver carp which was in agreement with other reports (Van *et al.*, 2010a; Hung *et al.*, 2015).

The intensity of metacercarial infection in fish

The intensity of infection in the present study was from 0.0004 to 6.38 with a mean intensity of 1.06 metacercariae per gram of fish that

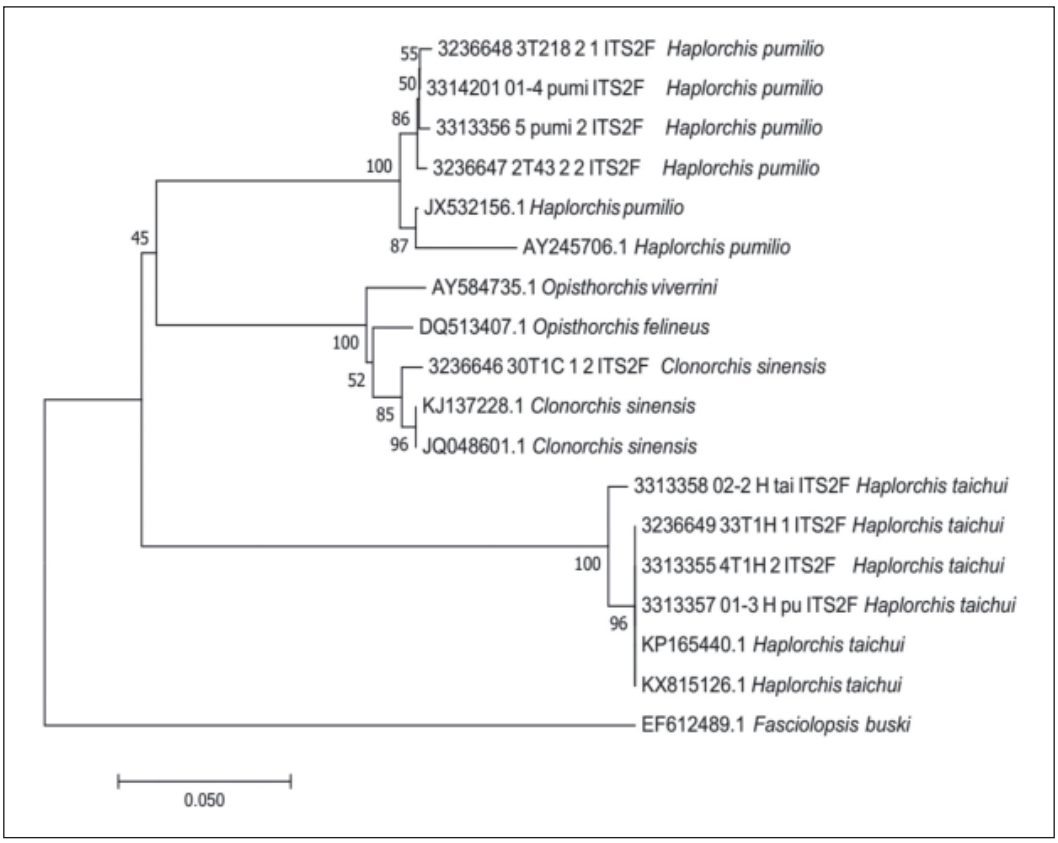

Figure 3. The phylogenetic tree of sequenced strains to identify trematode species.

was comparable to some other reports in Vietnam (Chi *et al.*, 2008; Van *et al.*, 2010b; Hung *et al.*, 2015). This number was very low comparing to some very high intensity reported elsewhere (more than 6,000 metacercariae per gram of a small fish, *Pseudorasbora parva*, in China) (Chen *et al.*, 1994). The highest density was noted in grass carp (6.38 metacercaria/ g fish) and was similar to findings from a report in Nghe An, a central province of Vietnam (7.1 metacercariae/ g fish) (Chi *et al.*, 2008). Tilapia had a very low prevalence of infection (1.92%) which was consistent with some other report in Thailand (Wiriya *et al.*, 2013).

It is important to mention that in Vietnam most researches have focused on freshwater fish and little is known about the situation of FBT metacercaria infection among brackish water fish, though some species of them contain trematode metacercariae (Kim *et al.*, 2006; Cho *et al.*, 2012; El Assal & Mohamed 2018). The current study investigated metacercarial infection in dotted (konoshiro) gizzard shad (*Konosirus punctatus*) – a species of brackish water commonly used for raw consumption by local people especially those who live in coastal areas. In South Korea, *K. punctatus* was found to contain the minute intestinal fluke *Heterophyopsis continua* with the prevalence of 58.3% (Cho *et al.*, 2012). Metacercariae of *H. continua* was discovered in marine fish (*Epinephelus coioides*, *Epinephelus bleekeri*, *Mugil cephalus*) in Khanh Hoa, a central province of Vietnam (Dung *et al.*, 2008) and in *Coilia lindmani* – a brackish water fish from Nam Dinh province (Chai *et al.*, 2012). Adults worms of *H. continua* have been found in seagulls (*Larus genus*) of Vietnam seas (Dung *et al.*, 2008). These results suggest that *H. continua*, a MIF species has been found in Vietnam. Although no case of *H.*

continua infection has been detected in humans in Vietnam, these findings imply that people who eat raw or uncooked gizzard shad are at risk of getting FBT infection. Our results showed that *K. punctatus* in that area were not infected with metacercariae of FBT. In our opinion, while the goal of complete abandonment of human consumption of raw fish is very difficult to get, the propagation of the choice of species with low infection or non-infected fish like *K. punctatus* was a reasonable approach.

Distribution of different FBT

The identification of metacercariae in the present study was based on morphological features and confirmed by molecular analysis. In the present study, the ITS2 region was subjected to analysis as recommended by other authors (Sato *et al.*, 2009; Kim *et al.*, 2009). The size of PCR products and phylogenetic tree resulting from ITS2 data (using 9 sequences in our study and 9 other sequences retrieved from Genbank) in Figure 3 clearly showed that the FBT metacercariae isolates of Ninh Binh were *C. sinensis*, *H. pumilio* and *H. taichui*.

Almost all (99.84%) collected metacercariae from fish were *H. pumilio*. The very low prevalence of metacercariae of SLF and the predominance of MIF among fish in our study followed the trend described previously (Chi *et al.*, 2008; Van *et al.*, 2010b; Clausen *et al.*, 2012). The very low rate of infection with metacercariae of SLF among fish in this area may result from the different affinity of metacercariae of FBT in fish. In endemic areas of *C. sinensis* infection, small sized-fish are more frequently and more intensively infected with the metacercariae of *C. sinensis* than large fish; however, large fish are preferred as a source of raw fish (Rim, 2005). The present study focused more on the risk of human infection and status of FBT metacercarial infection among fish that more commonly used by local as raw fish, mostly large fish so that the prevalence of SLF in fish would likely be very low. Metacercariae of *H. pumilio* presented in all species of freshwater fish which agreed with previous reports (Chi *et al.*, 2008). Grass carps were infected with all three species of FBT which was consistent with previous reports (Van *et al.*, 2010a).

The absence of some other MIF such as *P. varium*, *C. formosanus*, *H. yokogawai* *S. falcatus* and *E. japonicus* which have been found in fish from Vietnam in the present study may be explained by the different species of fish examined or very low prevalence of that metacercaria. Hung *et al.* (2015) found *P. varium*, *C. formosanus* in rohu (*Labeo rohita*), snakehead murrel (*Channa striata*), crucian carp (*Carassius auratus*) and ray-finned fishes (*Rasborinus hautus*) collected from Gia Vien district, Ninh Binh province (Hung *et al.*, 2015), however, those kinds of fish were not included in our study. Metacercaria of *H. yokogawai*, *S. falcatus* and *E. japonicus* were found in fish from the north and centre of Vietnam but with very low prevalences (0.1% for *H. yokogawai*, 0.5% for *S. falcatus* and *E. japonicus*) (Chi *et al.*, 2008; Van *et al.*, 2010b).

CONCLUSION

In conclusion, the high prevalence of infection of fish-borne trematode among species often used to prepare raw fish dishes in Ninh Binh province may cause a serious zoonotic risk to local inhabitants. Greater awareness of the risk from raw fish dishes among public health authorities and people are needed. People should avoid consuming insufficiently cooked or raw fish to prevent and control the hazard associated with fish-borne infections. Better aquaculture management practices to control FBT or inspection for the presence of metacercariae in fish, especially freshwater fish are needed to supply safe fish for human consumption.

List of abbreviations

DNA: Deoxyribonucleic acid; dNTP: Deoxynucleotide; FBT: Fish-borne trematodes; ITS: internal transcribed spacer region, MIF: Minute intestinal flukes; PCR: Polymerase chain reaction; SLF: Small liver flukes, WHO: World Health Organization.

Declarations

Ethics Approval
Not applicable.

Consent for publication
The authors agreed to publish this article.

Availability of data and material
The datasets used and analyzed during the current study are available from the corresponding author on reasonable request.

Disclosure of interest
The authors declare that they have no conflicts of interest concerning this article.

Funding
The data collection process was partly funded by the Department of Science and Technology of Ninh Binh Province. The funder does not have any role in data analysis and writing of the manuscript.

Acknowledgement. This research is part of thesis work for the fulfilment of Doctor of Literature and Philosophy in Health at National Institute of Malaria, Parasitology and Entomology of Vietnam. We thank Dr Bui Ngoc Thanh, Research Institute for Aquaculture No1, Vietnam for his help in the collection and identification of fish.

REFERENCES

El Assal, F.M. & Mohamed, N.M. 2018. Impact of Fish Infected with Encysted Metacercariae on the Public Health, at Cairo District, Egypt. *International Journal of Agricultural Policy and Research* **5**(6): 72-82.

Chai, J.Y. 2007. Intestinal Flukes. In: Food-Borne Parasitic Zoonoses Fish and Plant-Borne Parasites Series: World Class Parasites, Murrell D., K., Fried B. (editors), New York: Springer, pp. 53-116.

Chai, J.Y., De, N.V. & Sohn, W.M. 2012. Foodborne Trematode Metacercariae in Fish from Northern Vietnam and Their Adults Recovered from Experimental Hamsters. *The Korean Journal of Parasitology* **50**(4): 317-25.

Chen, M., Lu, Y., Hua, X., Mott, K.E. & World Health Organization, Schistosomiasis Control Unit. 1994. Progress in Assessment of Morbidity Due to Clonorchis Sinensis Infection/: A Review of Recent Literature. *Tropical Diseases Bulletin* **91**: 7-65.

Cho, S.H., Kim, I.S., Hwang, E.J., Kim, T.S., Na, B.K. & Sohn, W.M. 2012. Infection Status of Estuarine Fish and Oysters with Intestinal Fluke Metacercariae in Muan-Gun, Jeollanam-Do, Korea. *The Korean Journal of Parasitology* **50**(3): 215-20.

Clausen, H.J., Madsen, H., Murrell, K.D., Van, P.T., Ha, N.T.D., Dung, D.T., Anh, N.T.L., Hung, N.M. & Dalsgaard, A. 2012. Prevention and Control of Fish-Borne Zoonotic Trematodes in Fish Nurseries, Vietnam. *Emerging Infectious Diseases* **18**(9): 1438-45.

Doanh, P.N. & Nawa, Y. 2016. Clonorchis Sinensis and Opisthorchis spp. in Vietnam: Current Status and Prospects. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **110**(1): 13-20.

Dung, V.T., Murrell, D., Dalsgaard, A., Bristow, G., Dung, N.H., Thanh, B.N. & Dung, V.T. 2008. Prevalence of Zoonotic Metacercariae in Two Species of Grouper, *Epinephelus Coioides* and *Epinephelus Bleekeri*, and Flathead Mullet, *Mugil Cephalus*, in Vietnam. *The Korean Journal of Parasitology* **46**(2): 77-82.

Hung, N.M., Dung, D.T., Anh, N.T.L., Van, P.T., Thanh, B.N., Ha, N.V., Hien, H.V. & Canh, L.X. 2015. Current Status of Fish-Borne Zoonotic Trematode Infections in Gia Vien District, Ninh Binh Province, Vietnam. *Parasites & Vectors* **8**(21).

Kim, C.S., Echaubard, P., Suwannatrai, A., Kaewkes, S., Wilcox, B.A. & Sripa, B. 2016. Seasonal and Spatial Environmental Influence on Opisthorchis Viverrini Intermediate Hosts, Abundance, and Distribution: Insights on Transmission Dynamics and Sustainable Control. *PLOS Neglected Tropical Diseases* **10**(11): e0005121.

Kim, D.G., Kim, T.S., Cho, S.H., Song, H.J. & Sohn, W.M. 2006. Heterophyid Metacercarial Infections in Brackish Water Fishes from Jinju-Man (Bay), Kyongsangnam-Do, Korea. *The Korean Journal of Parasitology* **44**(1): 7-13.

Madsen, H., Dung, B.T., The, D.T., Viet, N.K., Dalsgaard, A. & Van, P.T. 2015. The Role of Rice Fields, Fish Ponds and Water Canals for Transmission of Fish-Borne Zoonotic Trematodes in Aquaculture Ponds in Nam Dinh Province, Vietnam. *Parasites & Vectors* **8**(625).

Hop, N.T., De, N.V., Murrell, D. & Dalsgaard, A. 2007. Occurrence and Species Distribution of Fishborne Zoonotic Trematodes in Wastewater-Fed Aquaculture in Northern Vietnam. *Tropical Medicine & International Health* **12**: 66-72.

Rim, H.J. 1997. Epidemiology and Control of Clonorchiasis in Korea. *The Southeast Asian Journal of Tropical Medicine and Public Health* **28**: 47-50.

Rim, H.J. 2005. Clonorchiasis: An Update. *Journal of Helminthology* **79**: 269-81.

Sato, M.U., Thaenkham, P.D. & Waikagul, J. 2009. Discrimination of O. viverrini, C. sinensis, H. pumilio and H. taichui Using Nuclear DNA-Based PCR Targeting Ribosomal DNA ITS Regions. *Acta Tropica* **109**(1): 81-83.

Scholz, T., Ditrich, O. & Giboda, M. 1991. Differential Diagnosis of Opisthorchiid and Heterophyid Metacercariae (Trematoda) Infecting Flesh of Cyprinid Fish from Nam Ngum Dam Lake in Laos. *The Southeast Asian Journal of Tropical Medicine and Public Health* **22**: 171-73.

Sohn, W.M. 2009. Fish-Borne Zoonotic Trematode Metacercariae in the Republic of Korea. *The Korean Journal of Parasitology* **47**: 103-13.

Sripa, B., Kaewkes, S., Intapan, P.M., Maleewong, W. & Brindley, P.J. 2010. Food-Borne Trematodiases in Southeast Asia Epidemiology, Pathology, Clinical Manifestation and Control. *Advances in Parasitology* **72**: 305-50.

Tang, Z.L., Huang, Y. & Yu, X.B. 2016. Current Status and Perspectives of Clonorchis Sinensis and Clonorchiasis: Epidemiology, Pathogenesis, Omics, Prevention and Control. *Infectious Diseases of Poverty* **5**: 71.

Tran, T.K.C., Dalsgaard, A., Turnbull, J.F., Tuan, P.A. & Murrell, K.D. 2008. Prevalence of Zoonotic Trematodes in Fish from a Vietnamese Fish-Farming Community. *The Journal of Parasitology* **94**(2): 423-28.

Van, P.T., Ersboll, A.K., Khue, N.V., Madsen, H. & Dalsgaard, A. 2010a. Farm-Level Risk Factors for Fish-Borne Zoonotic Trematode Infection in Integrated Small-Scale Fish Farms in Northern Vietnam. *PLoS Neglected Tropical Diseases* **4**(7): e742.

Van, P.T., Ersboll, A.K., Khue, N.V., Madsen, H., Hang, N.T. & Dalsgaard, A. 2010b. Fish-Borne Zoonotic Trematodes in Cultured and Wild-Caught Freshwater Fish from the Red River Delta, Vietnam. *Vector Borne and Zoonotic Diseases* **10**(9): 861-66.

WHO. 1995. *Control of Foodborne Trematode Infections Reports of a WHO Study Group*. Geneva.

WHO. 2002. *Food-Borne Trematode Infections in Asia*. Report of a joint WHO/FAO workshop, Ha Noi, Vietnam.

Wiriya, B., Clausen, J.H., Inpankaew, T., Thaenkham, U., Jittapalapong, S., Satapornvanit, K. & Dalsgaard, A. 2013. Fish-Borne Trematodes in Cultured Nile Tilapia (Oreochromis Niloticus) and Wild-Caught Fish from Thailand. *Veterinary Parasitology* **198**(1-2): 230-34.