

High Prevalence of *Clonorchis sinensis* and Other Zoonotic Trematode Metacercariae in Fish from a Local Market in Yen Bai Province, Northern Vietnam

Fuhong Dai^{1,6}, Sung-Jong Hong¹, Jhang Ho Pak², Thanh Hoa Le³, Seung-Ho Choi⁴, Byoung-Kuk Na⁵, Woon-Mok Sohn^{5,*}

¹Department of Environmental Medical Biology, Chung-Ang University College of Medicine, Seoul 06974, Korea; ²Asan Institute for Life Sciences, University of Ulsan College of Medicine, Asan Medical Center, Seoul 05505, Korea; ³Department of Immunology, Institute of Biotechnology, Vietnam Academy of Science and Technology, Hanoi, Vietnam; ⁴Society of Korean Naturalist, Institute of Ecology and Conservation, Yangpyeong 12563, Korea; ⁵Department of Parasitology and Tropical Medicine, and Institute of Health Sciences, Gyeongsang National University College of Medicine, Jinju 52727, Korea, ⁶Department of Parasitology, School of Biology and Basic Medical Sciences, Medical College, Soochow University, Suzhou, Jiangsu 215123, P.R. China

Abstract: A small survey was performed to investigate the recent infection status of *Clonorchis sinensis* and other zoonotic trematode metacercariae in freshwater fish from a local market of Yen Bai city, Yen Bai province, northern Vietnam. A total of 118 fish in 7 species were examined by the artificial digestion method on March 2016. The metacercariae of 4 species of zoonotic trematodes, i.e., *C. sinensis*, *Haplorchis pumilio*, *Haplorchis taichui*, and *Centrocestus formosanus*, were detected. The metacercariae of *C. sinensis* were found in 62 (69.7%) out of 89 fish (5 species), and their intensity of infection was very high, 81.2 per fish infected. Prevalences of 3 intestinal flukes, *H. pumilio*, *H. taichui* and *C. formosanus*, were 75.0%, 47.6%, and 31.7% in positive fish species, respectively, with the metacercarial intensities of 15.5, 10.3, and 2.2 per fish infected. From the above results, it has been confirmed that various species of freshwater fish continue to play the role of the infection source of *C. sinensis* and other zoonotic trematodes in Yen Bai city, Yen Bai province, northern Vietnam. It is of particular note that the prevalence and intensity of *C. sinensis* metacercariae are much higher than those reported in previous studies in fish in northern Vietnam.

Key words: *Clonorchis sinensis*, *Haplorchis pumilio*, *Haplorchis taichui*, *Centrocestus formosanus*, zoonotic trematode metacercaria (ZTM), Yen Bai province, Vietnam

Fishborne zoonotic trematode (FZT) infections, including *Clonorchis sinensis*, affect the health of more than 30 million people throughout the world and are particularly prevalent in Asian countries, such as Lao PDR, Vietnam, Cambodia, Thailand, Philippines, Taiwan, China, and Korea [1-4]. Human infections with FZT are almost entirely caused by the habitual consumption of raw and/or undercooked fish meat containing infective larvae, the metacercariae [4-6]. Surveys on the infection status of fish with zoonotic trematode metacercariae (ZTM) have been conducted by many workers to obtain useful information on the epidemiology of zoonotic trematodes in particular areas [7-11]. In Vietnam, epidemiological surveys for ZTM were performed on fish intermediate hosts in local areas by many workers [8,12-22]. Especially, Chai et al. [8] surveyed

the infection status of ZTM in wild fish from 2 localities of northern Vietnam, Hanoi city and Nam Dinh province, and they also described the morphological characteristics of ZTM and their adults recovered from experimental animals. However, most of the studies were performed on fish from adjacent and/or lower latitude areas of Hanoi [8,12-22].

Yen Bai province is an agricultural-based province located in the northern part of north-central Vietnam. The Red River (middle reach) and the Thac Ba Lake are ecologically important for the source of fish in inhabitants of this province [23]. Furthermore, there are no available information to know the infection status of ZTM in fish intermediate hosts, which is a kind of valuable protein source for the people in this region. Therefore, we performed a small survey to know the infection status of ZTM among wild fish purchased from a local market of Yen Bai city, Yen Bai province, northern Vietnam.

A total of 118 freshwater fish in 7 species were collected from a local market of Yen Bai city (latitude: 21.699533; longitude:104.879628), Yen Bai province, northern Vietnam on March 2016 (Fig. 1) [23]. All collected fish were transferred

•Received 24 March 2020, revised 19 May 2020, accepted 20 May 2020.

*Corresponding author (wmsohn@gnu.ac.kr)

© 2020, Korean Society for Parasitology and Tropical Medicine

This is an Open Access article distributed under the terms of the Creative Commons Attribution Non-Commercial License (<https://creativecommons.org/licenses/by-nc/4.0/>) which permits unrestricted non-commercial use, distribution, and reproduction in any medium, provided the original work is properly cited.



Fig. 1. The surveyed area (red region), Yen Bai province, northern Vietnam (from Wikipedia website [23]).

with ice to the laboratory of Department of Parasitology and Tropical Medicine, Gyeongsang National University College of Medicine, Jinju, Korea. Their length and weight were individually measured and the fish species were identified with the aid of a Korean ichthyologist and Search FishBase site in internet (Table 1) [24]. Individual fish was finely ground in a mortar with pestle, the ground fish meat was mixed with artificial gastric juice, and the mixture was incubated at 36°C for about 2 hr. The digested material was filtered through a 1 × 1 mm² of mesh, and washed with 0.85% saline until the supernatant became clear. The sediment was carefully examined under a stereomicroscope. The metacercariae of each species (only ZTM) were separately collected viewing from the general feature and counted to get hold of the prevalence (%) and intensity of infection (no. of ZTM per fish infected) by fish species [5].

Total 4 species of trematode metacercariae, i.e., *C. sinensis*, *Haplorchis pumilio*, *Haplorchis taichui*, and *Centrocestus formosanus*, were detected. The prevalence of *C. sinensis* metacercariae were very high to be positive in 62 (69.7%) out of 89 fish in 5 species, i.e., *Chanodichthys erythropterus* (Fig. 2A), *Toxabramis houdemeri* (Fig. 2B), *Saurogobio immaculatus*, *Opsariichthys bidens*, and *Squalidus atromaculatus*, with the average intensity of 81.2 per fish infected. The metacercariae of 3 intestinal flukes, i.e., *H. pumilio*, *H. taichui*, and *C. formosanus*, were detected in 75.0%, 47.6%, and 31.7% of positive fish groups, respectively, and their mean intensities were 15.5, 10.3, and 2.2 per fish infected. The infection status of each ZTM by fish species is designated in (Table 2).

The metacercariae (n = 20) of *C. sinensis* were elliptical and 140-175 (159 in average) by 120-160 (142) μm in size. They had nearly equal sized oral and ventral suckers, brownish pigment granules in the body, and an O-shaped excretory bladder in the posterior portion. The adult worms (40-day-old from

Table 1. Fish collected from a local market in Yen Bai city, Yen Bai province, Vietnam

Species of fish	No. of fish examined	Length		Weight	
		Range	Average	Range	Average
<i>Chanodichthys erythropterus</i>	30	9.6-13.2	11.2	10.9-31.3	19.3
<i>Toxabramis houdemeri</i>	30	7.5-9.4	8.1	5.4-10.2	6.6
<i>Carassius auratus</i>	27	8.1-12.9	9.9	12.3-50.6	23.9
<i>Saurogobio immaculatus</i>	10	6.4-11.2	9.3	3.9-17.2	10.8
<i>Opsariichthys bidens</i>	10	-	-	14.0-28.0	21.8
<i>Squalidus atromaculatus</i>	9	7.3-8.0	7.7	6.2-9.0	7.6
<i>Garra imbarbatus</i>	2	-	7.0	7.4-7.5	7.5

^aTotal 118 fish in 7 species were examined.

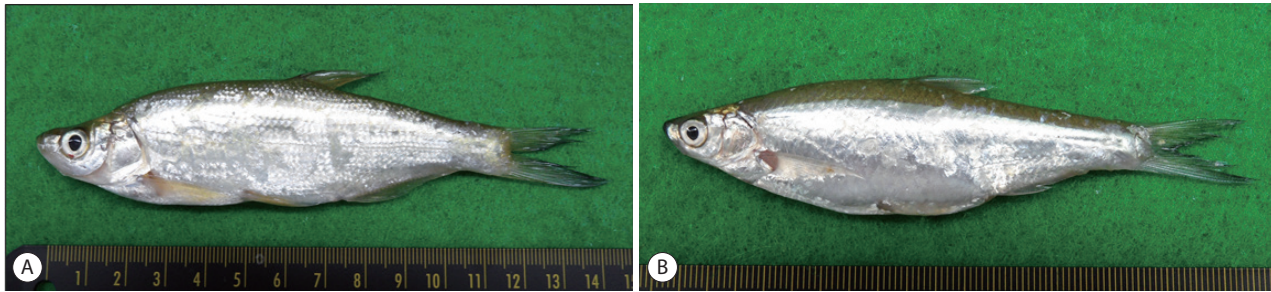


Fig. 2. Susceptible fish hosts, *Chanodichthys erythropterus* (A), *Toxabramis houdemeri* (B), to *C. sinensis* metacercariae in the surveyed area, Yen Bai city, Yen Bai province, northern Vietnam.

Table 2. Infection status of zoonotic trematode metacercariae (ZTM) by the fish species from a local market in Yen Bai city, Yen Bai province, Vietnam

ZTM and fish species infected	No. of fish examined	Infection rate (%)	No. of ZTM detected	
			Range	Average
<i>Clonorchis sinensis</i>				
<i>Chanodichthys erythropterus</i>	30	93.3	2-521	105.5
<i>Toxabramis houdemeri</i>	30	100	8-179	69.1
<i>Saurogobio immaculatus</i>	10	2.0	-	1.0
<i>Opsariichthys bidens</i>	10	10.0	-	1.0
<i>Squalidus atromaculatus</i>	9	11.1	-	1.0
Subtotal	89	69.7	1-521	81.2
<i>Haplorchis pumilio</i>				
<i>Chanodichthys erythropterus</i>	30	100	2-152	28.6
<i>Carassius auratus</i>	27	70.4	1-14	4.9
<i>Toxabramis houdemeri</i>	20	75.0	1-19	4.5
<i>Squalidus atromaculatus</i>	9	11.1	-	1.0
<i>Garra imbarbatus</i>	2	50.0	-	3.0
Subtotal	88	75.0	1-152	15.5
<i>Haplorchis taichui</i>				
<i>Chanodichthys erythropterus</i>	30	36.7	1-8	3.3
<i>Saurogobio immaculatus</i>	10	70.0	1-44	9.4
<i>Garra imbarbatus</i>	2	100	16-90	53.0
Subtotal	42	47.6	1-90	10.3
<i>Centrocestus formosanus</i>				
<i>Chanodichthys erythropterus</i>	30	100	1-4	1.8
<i>Toxabramis houdemeri</i>	20	5.0	-	1.0
<i>Saurogobio immaculatus</i>	10	50.0	1-9	3.4
Subtotal	60	31.7	1-9	2.2

an experimental hamster) were flat, elongate leaf-like, tapering anteriorly and somewhat pointed posteriorly, and 5.75-12.0 (av. 8.98) mm long and 1.55-2.15 (1.84) mm wide. Eggs were small, yellowish, mature, operculate, and 25-28 (26) by 13-14 (13) μm in size.

In the present study, 4 species of zoonotic trematode metacercariae, i.e., *C. sinensis*, *H. pumilio*, *H. taichui*, and *C. formosanus*, were detected in fish from a local market of Yen Bai city, Yen Bai province, northern Vietnam. Among these, *C. sinensis*

metacercaria (CsMc) was the dominant species with regard to the intensity of infection in fish hosts (81.2 CsMc per fish infected). Chai et al. [8] found 6 species of ZTM, i.e., *H. taichui*, *H. pumilio*, *C. formosanus*, *Procerovum varium*, *Stellantchasmus falcatus*, and *Heterophyopsis continua*, in fish from Hanoi and Nam Dinh province, Vietnam. They were all intestinal flukes belonging to the family Heterophyidae, and *H. pumilio* was the dominant species. Phan et al. [19] also detected 6 species of ZTM, i.e., *H. pumilio*, *H. taichui*, *H. yokogawai*, *C. formosanus*, *P.*

varium, and *C. sinensis*, in cultured and wild-caught freshwater fish from the Red River delta, in Nam Dinh province. *H. pumilio* was also the dominant species, and CsMc were found in only 1 out of 1,543 fish examined. Hung et al. [22] also detected 6 species of ZTM, i.e., *H. pumilio*, *H. taichui*, *H. yokogawai*, *C. formosanus*, *P. varium*, and *C. sinensis*, in fish from Ninh Binh province. Among them, *Haplorchis* spp. metacercariae were dominant, and CsMc were found in only 1 (12.5%) out of 8 *Hemiculter leucisculus* examined. In our study, CsMc were highly prevalent in several species of fish from Yen Bai city, Yen Bai province unlike in previous studies performed in adjacent localities of the other northern parts of Vietnam, i.e., Hanoi, Nam Dinh, and Ninh Binh province.

Two species of human-infecting liver flukes, *C. sinensis* and *Opisthorchis viverrini*, are known to be distributed in Vietnam. The former is prevalent in northern provinces, whereas the latter is mainly found in central and southern provinces [25]. In previous studies, the metacercariae of liver flukes were very rarely detected in fish from Vietnam. Only 19 *O. viverrini* metacercariae in total were found in 1.9% of fish from An Giang province, in the Mekong Delta of Vietnam [12]. In addition, Thien et al. [13] could not find CsMc at all in 13 species of cultured fish from the Mekong Delta area. Also, in Nghe An province, no liver fluke metacercariae were detected among farmed fish [15].

With regard to *C. sinensis*, the metacercariae were found in 1 of 1,185 silver carps, *Hypophthalmichthys molitrix*, from small-scale farms in northern parts, and in 1 out of 1,543 freshwater fish from Red River delta in Nam Dinh province [18,19]. Hung et al. [22] also detected CsMc in 1 (12.5%) of 8 *H. leucisculus* examined. In other northern provinces, prevalences of CsMc were very low, 0.1-0.4% [21]. However, in the present study, we detected a very high intensity of CsMc (81.2 per fish infected) from 69.7% freshwater fish in 5 species, i.e., *C. erythropterus*, *T. houdemeri*, *S. immaculatus*, *O. bidens*, and *S. atromaculatus*. In addition, it has been first confirmed in this study that 2 fish species, *C. erythropterus* and *T. houdemeri*, are highly susceptible for CsMc.

Clonorchiasis is known to be endemic among the residents of northern provinces of Vietnam. Especially, this disease was known to be prevalent in Nam Dinh and Ninh Binh provinces [25]. However, since the FIBOZOPA (Fish-Borne Zoonotic Parasite) project (2004-2012), it became known that various species of minute intestinal trematodes, i.e., *H. pumilio*, *H. taichui*, *H. yokogawai*, *C. formosanus*, *S. falcatus*, and *P. varium*, co-

existed in these areas [3,8,18,19,21,22]. Dung et al. [3] reported 64.9% positive rate of minute trematode eggs from 615 residents of Nam Dinh province, and they recovered 6 species of trematode adults, i.e., *C. sinensis*, *H. pumilio*, *H. taichui*, *H. yokogawai*, *S. falcatus*, and *Fasciolopsis buski*, from 33 residents positive for small trematode eggs after treatment with praziquantel and purging with MgSO₄. Adult worms of *C. sinensis* were recovered from 17 (51.5%) residents, and the intensity of infection was 4 worms in average, whereas adults of *H. pumilio* and *H. taichui* were recovered from all 33 (100%) and 23 (69.7%) residents, with the infection intensities of 416 and 40 in average, respectively. Likewise, in endemic areas of clonorchiasis in northern Vietnam, the coexistence of minute intestinal trematodes has been confirmed by ZTM studies performed in the same survey areas [8,18,19,21,22].

Several working groups reported the morphological characteristics of ZTM with their infection status in fish hosts from the specific regions in Southeast Asian countries [8-11]. Especially in Vietnam, Chai et al. [8] described the morphological characteristics of 6 species of ZTM, i.e., *H. taichui*, *H. pumilio*, *C. formosanus*, *P. varium*, *S. falcatus*, and *H. continua*, from fish in Hanoi city and Nam Dinh province, and they also described the morphologies of adult flukes, i.e., *H. taichui*, *H. pumilio*, *C. formosanus*, *P. varium*, and *S. falcatus*, recovered from experimentally infected animals. In our study, *C. sinensis* metacercariae were nearly the same size (140-175 × 120-160 μm) as those of the Korean-origin (150-170 × 130-150 μm), but they are, more or less, smaller than those of *O. viverrini* (190-250 × 150-220 μm) [5,6,11]. Because of the general morphology of the metacercariae, including the elliptical shape, nearly same sized 2 suckers, brownish pigment granules in the body, and O-shaped excretory bladder, of 2 species of liver flukes is very similar except for the cyst size, it is difficult to distinguish one from the other if they are mixed-infected. Therefore, when the metacercariae of liver flukes are detected in fish from somewhere of Vietnam, it would be better to infect them to experimental animals to obtain adult flukes for species identification. Adult worms of *C. sinensis* in this study were more slender compared to those of the Korean-origin. The ratio of the body length to body width was higher (4.87-5.01 in this study) than that of the Korean-origin (4.36) (Table 3). Other morphological characters of *C. sinensis* adults were almost the same in both origins. However, the egg size was more or less smaller in this study (25-28 × 13-14 μm) than those of the Korean-origin (27-33 × 15-19 μm).

Conclusively, it has been confirmed in this study that several species of freshwater fish continue to play the role of the second intermediate host and infection source of zoonotic trematodes, including *C. sinensis* and 3 species of intestinal flukes in Yen Bai province, Vietnam. In particular, the prevalence and intensity of CsMc were much higher in this study than those of previous studies. To reveal the detailed epidemiological situation of zoonotic trematode infections in Yen Bai province, Vietnam, more profound studies on intermediate (snail and fish) and definitive hosts (humans and reservoir host animals) should be performed in the near future.

ACKNOWLEDGMENTS

We thank Jung-A Kim and Hee-ju Kim, Department of Parasitology and Tropical Medicine, Gyeongsang National University College of Medicine, Jinju, Korea, for their help in fish examinations.

CONFLICT OF INTEREST

The authors declare no conflict of interest related to this study.

REFERENCES

1. WHO. Food-Borne Trematode Infections in Asia. Report Joint WHO/FAO Workshop, Hanoi, Vietnam, 2002.
2. Chai JY, Murrell KD, Lymbery A. Fishborne parasitic zoonoses: status and issues. *Int J Parasitol* 2005; 35: 1233-1254.
3. Trung Dung D, Van De N, Waikagul J, Dalsgaard A, Chai JY, Sohn WM, Murrell KD. Fishborne intestinal zoonotic trematodiasis, Vietnam. *Emerg Infect Dis* 2007; 13: 1828-1833.
4. Chai JY. Human Intestinal Flukes. From Discovery to Treatment and Control. GX Dordrecht, the Netherlands. Springer Nature BC. 2019, pp 1-520.
5. Sohn WM. Fish-borne zoonotic trematode metacercariae in the Republic of Korea. *Korean J Parasitol* 2009; 47(suppl): 1-10.
6. Sohn WM. Trematodes: Platyhelminthes: Trematoda: Strigeidida, Echinostomida, Plagiorchiida, Posithorchiida. Incheon, Korea. The National Institute of Biological Resources. 2013, pp 1-125.
7. Sohn WM, Eom KS, Min DY, Rim HJ, Hoang EH, Yang Y, Li X. Fishborne trematode metacercariae in freshwater fish from Guangxi Zhuang Autonomous Region, China. *Korean J Parasitol* 2009; 47: 249-257.
8. Chai JY, De NV, Sohn WM. Foodborne trematode metacercariae in fish from northern Vietnam and their adults recovered from experimental hamsters. *Korean J Parasitol* 2012; 50: 317-325.
9. Chai JY, Sohn WM, Na BK, Yong TS, Eom KS, Yoon CH, Hoang EH, Jeoung HG, Socheat D. Zoonotic trematode metacercariae in fish from Phnom Penh and Pursat, Cambodia. *Korean J Parasitol* 2014; 52: 35-40.
10. Chai JY, Sohn WM, Na BK, Park JB, Jeoung HG, Hoang EH, Htoon TT, Tin HH. Zoonotic trematode metacercariae in fish from Yangon, Myanmar and their adults recovered from experimental animals. *Korean J Parasitol* 2017; 55: 631-641.
11. Chai JY, Lee SH, Rim HJ, Sohn WM, Phommasack B. Infection status with zoonotic trematode metacercariae in fish from Lao PDR. *Acta Trop* 2019; 199: 105-100.
12. Thu ND, Loan LTT, Dalsgaard A, Murrell KD. Survey for zoonotic liver and intestinal trematode metacercariae in cultured and wild fish in An Giang Province, Vietnam. *Korean J Parasitol* 2007; 45: 45-54.
13. Thien PC, Dalsgaard A, Bui NT, Olsen A, Murrell KD. Prevalence of fishborne zoonotic parasites in important cultured fish species in the Mekong Delta, Vietnam. *Parasitol Res* 2007; 101: 1277-1284.
14. Hop NT, De NV, Murrell KD, Dalsgaard A. Occurrence and species distribution of fishborne zoonotic trematodes in wastewater-fed aquaculture in northern Vietnam. *Trop Med Int Health* 2007; 12: 66-72.
15. Chi TT, Dalsgaard A, Turnbull JE, Tuan PA, Murrell KD. Prevalence of zoonotic trematodes in fish from a Vietnamese fish farming community. *J Parasitol* 2008; 94: 423-428.
16. Vo DT, Murrell D, Dalsgaard A, Bristow G, Nguyen DH, Bui TN, Vo DT. Prevalence of zoonotic metacercariae in two species of grouper, *Epinephelus coioides* and *Epinephelus bleekeri*, and flat-head mullet, *Mugil cephalus*, in Vietnam. *Korean J Parasitol* 2008; 46: 77-82.
17. Tran TKC, Dalsgaard A, Turnbull JE, Tuan PA, Murrell KD. Prevalence of zoonotic trematodes in fish from a Vietnamese fish-farming community. *J Parasitol* 2008; 94: 423-428.
18. Phan VT, Ersbøll AK, Nguyen KV, Madsen H, Dalsgaard A. Farm-level risk factors for fishborne zoonotic trematode infection in integrated small-scale fish farms in northern Vietnam. *PLoS Negl Trop Dis* 2010; 4: 742-750.
19. Phan TV, Ersbøll AK, Bui TQ, Nguyen HT, Murrell D, Dalsgaard A. Fishborne zoonotic trematodes in cultured and wild-caught freshwater fish from Red River delta, Vietnam. *Vector Borne Zoonotic Dis* 2010; 10: 861-866.
20. Bui TD, Madsen H, Dang TT. Distribution of freshwater snails in family-based VAC ponds and associated water bodies with special reference to intermediate hosts of fishborne zoonotic trematodes in Nam Dinh province, Vietnam. *Acta Trop* 2010; 116: 15-23.
21. De NV, Le TH, Murrell KD. Prevalence and intensity of fishborne zoonotic trematodes in cultured freshwater fish from rural and urban areas of northern Vietnam. *J Parasitol* 2012; 98: 1023-1025.
22. Hung NM, Dung DT, Anh NTL, Van PT, Thanh BN, Ha NV, Hien

- HV, Canh LX. Current status of fishborne zoonotic trematode infections in Gia Vien district, Ninh Binh province, Vietnam. *Parasit Vectors* 2015; 8: 21-30.
23. Information on Yen Bai province, Vietnam [Internet]; Available from: <http://en.wikipedia.org>.
24. Identification of fish species (Search fishbase) [Internet]; Available from: <https://www.fishbase.se/search.php>
25. Doanh PN, Nawa Y. *Clonorchis sinensis* and *Opisthorchis* spp. in Vietnam: current status and prospects. *Trans R Soc Trop Med Hyg* 2016; 110: 13-20.