



PREVALENCE OF TREMATODE IN WILD FISH FROM FISHING IN HO CHI MINH CITY

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ABSTRACT

*A research on the prevalence of fishborne zoonotic trematodes (FZT), metacercariae stage, in wild fish from fishing in Nhieu Loc – Thi Nghe, Tau Hu – Ben Nghe and Thay Cai – An Ha canals was conducted in Ho Chi Minh City in the period of May 2017 and April 2018. The results showed that no metacercariae were found in fish in Nhieu Loc – Thi Nghe and Tau Hu – Ben Nghe canals. However, wild fish in Thay Cai – An Ha canal were infected with *Haplorchis pumilio* and *Centrocestus formosanus*. Metacercariae were infected in silver barb (80%), climbing perch (11.7%), snakehead (7.5%), and walking catfish (1.7%). The FZT prevalence in the wet season is significantly higher than in the dry season ($P < 0.05$).*

Keywords: prevalence, trematode, metacercariae, wild fish.

1. Introduction

Foodborne trematodiasis is an emerging public health problem around the world (Chai and Lee, 2002), particularly in Southeast Asia and the western Pacific region (Keiser and Utzinger, 2005). Humans and domestic animals are commonly infected by helminth parasites transmitted from fish, especially in Asia (WHO, 1995). Fish is a major source of protein for people in Southeast Asia, especially for the ones living in rural areas, where cultural habits and preferences for raw fish or other types of inadequately prepared fish dishes are popular. However, such eating practices are associated with high risks of acquiring infections with fishborne zoonotic trematodes (WHO, 2004; Chai et al. 2005). In 1995, the World Health Organization estimated that the number of people infected with FZTs exceeded 18 million, and one tenth of the world population is at risk of infection (WHO, 1995). People living in areas along rivers were found to have a higher risk of infection with *C. sinensis* in Korea (Hong and Hong, 2005). Residents living near freshwater bodies were found to have a 2.2-fold higher risk for FZT infection than people living further away from the water (Keiser and Utzinger, 2005).

More than 50 intestinal FZT species are reported from humans worldwide (Sohn et al., 2009) with 31 species belonging to the Heterophyidae family (Nawa et al., 2005) representing nine genera, namely *Haplorchis*, *Centrocestus*, *Stellantchasmus*, *Procevorum*, *Heterophyes*, *Metagonimus*, *Heterophyopsis*, *Pygidiopsis*, and *Stictodora* (Chai and Lee, 2002). Heterophyid infections are found worldwide: *Heterophyes heterophyes* is found in Egypt, Korea, China, Taiwan, Africa, Japan, the Philippines, and

the Mediterranean; *Metagonimus yokogawai* is found in China, Japan, Korea, Russia, Spain, Taiwan, the Balkans, and the Philippines; and various *Haplorchis* species are found in Taiwan, Thailand, and the Philippines (Hawn and Jong, 2001). There are about six million people in China, over five million in Thailand and one and a half million people in Korea infected with either *C. sinensis* or *O. viverrini* (Chai et al. 2005). The oriental liver fluke, *C. sinensis*, is of major socioeconomic importance in China, Japan, Korea, Taiwan, and Vietnam (Lun et al. 2005).

In Vietnam, recent investigations on the diversity and prevalence of FZT in Vietnam have shown that the parasites are common in humans and domestic animals (De et al., 2003; Dung et al., 2007; Anh et al., 2009) as well as in fish juveniles, grow-out fish and wild-caught fish (Thu et al., 2007; Hop et al., 2007; Thien et al., 2007; Chi et al., 2008; Thien et al., 2009; Thuy et al., 2010; Van et al., 2010). In general, the FZT prevalence of fish was high in Nghe An, Nam Dinh, and Ninh Binh provinces in the North of Vietnam with 43.6% of juvenile fish infected in nurseries (ranging from 7.4-62.8%) and 44.6% of fish in grow-out farms infected (ranging from 12.5-61.0%) in Nghe An province (Chi et al., 2008). Fish juveniles from Nam Dinh and Ninh Binh had a prevalence of 48.6% after four weeks of pond culture and reached 57.8% after the juveniles overwintered in the ponds. The FZT prevalence of grow-out farmed fish, wild fish and wastewater-fed in Nam Dinh were 75.5%, 68.6% (Van, 2010), and 5.7% (Hop et al. 2007), respectively. The FZT prevalence of fish in the Mekong Delta seems lower with the FZT prevalence in wild fish found to be 10.3% and 0.7% in cultured river catfish (Thu et al., 2007). The most common FZT species found in fish in Vietnam are *H. pumilio* (Thu et al., 2007; Chi et al., 2008; Thuy et al., 2010; Van et al., 2010) and other intestinal flukes such as *H. taichui*, *Centrocestus formosanus* (Chi et al., 2008; Thuy et al., 2010; Van et al., 2010), *H. yokogawai* (Chi et al., 2008; Van et al., 2010), *Procerovum* sp. (Thu et al., 2007; Thuy et al., 2010; Van et al., 2010), *Stellantchasmus falcatus* and *Echinochasmus japonicas* (Chi et al., 2008). Only 0.1% of cultured silver carp was infected with *C. sinensis* in Nam Dinh province (Van et al., 2010) in the North and 1.9% wild fish infected with *O. viverrini* in An Giang province (Thu et al., 2007). Thanh et al. (2016) found *Clonorchis sinensis* infected very high prevalence of 31.1-76.7% in wild fish in Thac Ba reservoir, Yen Bai province, Vietnam.

The results showed that there were a lot of studies on FZT in cultured and wild fish in Vietnam and other countries. However, there was no information about the FZT in wild fish in Ho Chi Minh City, so a research on FZT in wild fish from fishing in canals in Ho Chi Minh City, Vietnam was implemented.

2. Materials and methods

• Study areas

According to the report of Sub-Department of Quality management and Fisheries resources protection of Ho Chi Minh City in 2016, wild fish were fished mostly in three

canals including Nhieu Loc – Thi Nghe, Tau Hu – Ben Nghe, and Thay Cai – An Ha. Therefore, wild fish from these three canals were chosen for research from May 2017 to April 2018.

- *Sampling of fish*

Two cross-sectional studies of FZT prevalence in wild fish fishing from canals were carried out in the wet and the dry seasons in the period of May 2017 to April 2018. Each fish species was collected randomly 10 fish/ canal/ season by fishermen to analyze FZT. Snakehead and tilapia could not sample in Tau Hu – Ben Nghe canal. Common carp could be found only in Nhieu Loc – Thi Nghe canal and silver barb was collected only in Thay Cai – An Ha canal (Table 1). All the fish samples were brought to the laboratory of Ho Chi Minh City University of Education for FZT examination.

Table 1. Species of wild fish collected from fishing in three canals in Ho Chi Minh City

No	Fish species	Total fish		Samples in each canal		
		Wet season*	Dry season*	Nhieu Loc – Thi Nghe	Tau Hu – Ben Nghe	Thay Cai – An Ha
1	Walking catfish (<i>Clarias batrachus</i>)	30	30	20	20	20
2	Hybrid catfish (<i>Clarias</i> sp.)	30	30	20	20	20
3	River catfish (<i>Pangasanodon hypophthalmus</i>)	30	30	20	20	20
4	Climbing perch (<i>Anabas testudineus</i>)	30	30	20	20	20
5	Tilapia (<i>Oreochromis niloticus</i>)	20	20	20		20
6	Snakehead (<i>Channa</i> sp.)	20	20	20		20
7	Common carp (<i>Cyprinus carpio</i>)	10	10	20		
8	Silver barb (<i>Puntius gonionotus</i>)	10	10			20

* Wet season is from May to October; dry season is from November to April.

- *Examination of fish for metaceariae of FZT*

Metacercariae were isolated and recovered using the standard pepsin digestion procedure described in Annex 6 of WHO (1995) and in Thu et al. (2007). The entire fish was minced and digested for fish weighing less than 50 g (the whole digestion) and larger

fish were divided into five sub-samples for digestion. After digestion and washing, the sediment was examined for the presence of metacercariae. The metacercariae were examined under a compound microscope and identified using morphological criteria described in Kaewkes (2003) and in Pearson and Ow-Yang (1982).

- *Data analysis*

Microsoft Excel 2010 and SPSS (Statistical Package for Social Sciences version 20; SPSS Inc., Chicago, Illinois) were used for data entry and data analysis. The Chi-squared test was used to compare the difference of FZT prevalence between seasons. A value of $P < 0.05$ was considered significant.

3. Results

3.1. Prevalence of FZT metacercariae in wild fish from fishing

There was no fish in Nhieu Loc – Thi Nghe and Tau Hu – Ben Nghe canals infected with metacercariae. However, fish from fishing in Thay Cai – An Ha canal were infected with FZT including walking catfish, climbing perch, snakehead, and silver barb (Table 2). Of all these species, silver barb had the highest prevalence of 80% (Table 3) with 16 infected fish/ total 20 sampled fish (Table 2).

Table 2. Prevalence of FZT metacercariae in wild fish from fishing

No	Fish species	Infected fish/ Total fish		Infected fish/ total sampled fish		
		Wet season	Dry season	Nhieu Loc – Thi Nghe	Tau Hu – Ben Nghe	Thay Cai – An Ha
1	Walking catfish	1/30	0/30	0/20	0/20	1/20
2	Hybrid catfish	0/30	0/30	0/20	0/20	0/20
3	River catfish	0/30	0/30	0/20	0/20	0/20
4	Climbing perch	5/30	2/30	0/20	0/20	7/20
5	Tilapia	0/20	0/20	0/20		0/20
6	Snakehead	3/20	0/20	0/20		3/20
7	Common carp	0/10	0/10	0/20		0/20
8	Silver barb	10/10	6/10			16/20

3.2. Species of FZT metacercariae infected in wild fish from fishing

Metacercariae of two intestinal flukes including *Haplorchis pumilio* and *Centrocestus formosanus* infected in fish. In which, walking catfish, sneakehead, and silver barb infected with *Haplorchis pumilio* whereas climbing perch infected with both *Haplorchis pumilio* and *Centrocestus formosanus*. No liver lukes were found in the researched fish (Table 4).

The morphology of *Haplorchis pumilio* metacercariae was oval, tegumental spines and oral sucker were visible, ventral sucker armed with structures appeared in 1-2 rows of 36-42 deer horn-like minute spines around ventrogenital complex, excretory bladder O-shaped and occupying large portion of posterior body. It had single testis. Excretory

bladder rounded and contained small and dark granules. Characteristics of *Centrocestus formosanus* are the presence of 32-36 circumoral spines arranged in 2 alternative rows around the oral sucker, and the oral sucker was located at the anterior terminal. The ventral sucker, about a half size of the oral sucker, was located in the middle portion of the body. It had a small number of the intrauterine eggs, the median location of the ovary, excretory bladder X-shaped and the side-by side location of two testes including the right and the left testis.

Table 4. FZT species infected in wild fish from fishing

No	Fish species	Infected fish / Total samples	Prevalence (%)	FZT species
1	Walking catfish	1/60	1.7	<i>Haplorchis pumilio</i>
2	Hybrid catfish	0/60	0	-
3	River catfish	0/60	0	-
4	Climbing perch	7/60	11.7	<i>Haplorchis pumilio</i> , <i>Centrocestus formosanus</i>
5	Tilapia	0/40	0	-
6	Sneakehead	3/40	7.5	<i>Haplorchis pumilio</i>
7	Common carp	0/20	0	-
8	Silver barb	16/20	80.0	<i>Haplorchis pumilio</i>

3.3. Impact of seasons to FZT prevalence

In the research result, only climbing perch and silver barb infected with FZT in the dry season whereas walking catfish, snakehead, climbing perch, and silver barb infected with FZT in the wet season. In general (N=360), the FZT prevalence in the wet season (N=180) was significantly higher than in the dry season (N=180) (P<0.05) (Table 5).

Table 5. Using Chi-square tests to analyse seasons as risk factors associated with the occurrence of FZT prevalence in wild fish from fishing in Ho Chi Minh City

Chi-Square Tests

	Value	df	Asymp. Sig. (2-sided)	Exact Sig. (2-sided)	Exact Sig. (1-sided)
Pearson Chi-Square	4.845 ^a	1	.028		
Continuity Correction ^b	4.004	1	.045		
Likelihood Ratio	4.978	1	.026		
Fisher's Exact Test				.044	.022
Linear-by-Linear Association	4.831	1	.028		
N of Valid Cases	360				

a. 0 cells (0.0%) have expected count less than 5. The minimum expected count is 13.50.

b. Computed only for a 2x2 table

4. Discussion

The research result provides more information about the FZT prevalence in wild fish in Vietnam. No metacecariae were found in the fish from fishing in Nhieu Loc – Thi Nghe and Tau Hu - Ben Nghe canals. The reason why no metacercariae were found in these fish sources is that most the fish originally came from fish farm, they were often set free into these two canals each month and they had metacercariae free at the time of releasing into canals. Moreover, the water depth in these two canals were more than 3 meters deep, so the intermediate host like snails could not survive and had less chance for cercariae to penetrate the fish when these fish species lived in the two canals.

Hybrid catfish, river catfish, tilapia and common carp were found infected in grow-out and nursing ponds (Thien et al., 2007; Thien et al., 2009); however, no metacercariae were found in these wild fish species from fishing in this research. The first reason is that the juveniles were not infected with metacercariae when they were set free into the canals. One more reason is that the catfish like hybrid catfish and river catfish have slippery skin which may reduce the chance for the cercariae to attach and subsequently, penetrate the skin. In farmed condition, these two catfish species also had the lowest prevalence comparing to finfish (Thu et al., 2007; Thien et al., 2009; Thuy et al., 2010). Although catfish can be infected with FZT (WHO, 1995; Sen-Hai and Long-Qi, 2005), they may not be as highly susceptible to FZT infection as other fish. Tilapia is also a special case, the previous research (Thien et al., 2007; Thien et al., 2009; Thien et al., 2019) found that the farmed tilapia had the low FZT prevalence. Thu et al. (2007) also found wild tilapia had no metacercariae infection, the possible explanation is that the living space of tilapia is far from the place where snails prefer living. The FZT prevalence of common carp is a question. Hong and Hong (2005) said that cyprinid (carp) fish are an important source of infection for clonorchiasis, common carp also infected at the nursing stage (Thien et al., 2009) and growing stage (Thien et al., 2007). Thanh et al. (2016) stated that wild common carp in Thac Ba reservoir had the high prevalence of 65.9%. The common carp in this study was not infected with metacercariae because they might live far from the cercariae area, also the number of samples was still too small with only 20 fish.

Silver barb, climbing perch, snakehead and walking catfish in the research were found infected with FZT. The high prevalence was in silver barb (80.0%) and climbing perch (11.7%). The interview with the fisherman at the canal showed that silver barb's habitat was mainly in the rice fields and the ditches besides the fields where snails were available, so this may be the reason silver barb had the highest FZT prevalence. For wild climbing perch, the prevalence was still lower than the ones in An Giang province with 25.7% (Thu et al., 2007) and 100% in Thac Ba reservoir (Thanh et al., 2016). The prevalence of FZT seems to depend on species and ecological circumstances. The FZT species found in this study are similar to the previous result in Mekong Delta and in Northern Vietnam

including *Haplorchis pumilio* and *Centrocestus formosanus* from wild fish (Thu et al., 2007; Thanh et al., 2016) and farmed fish (Thien et al., 2007; Thien et al., 2009; Thuy et al., 2010). However, only climbing perch found to be infected with both *Haplorchis pumilio* and *Centrocestus formosanus* whereas the others only infected with *Haplorchis pumilio*.

The FZT prevalence in wild fish from fishing in Ho Chi Minh City in the wet season was higher than in the dry season ($P < 0.05$). The seasonality of FZT prevalence observed in this study is in agreement with the data reported earlier on the influence of rain on the FZT prevalence in fish culture in Mekong Delta of Vietnam (Thien et al., 2007; Thien et al., 2009; Thuy et al., 2010, Thien et al., 2018). The FZT prevalence is seasonal not only in Vietnam, but throughout Asia, especially in the transmission of liver flukes (Hinz et al., 1994; Rim, 1990; Sithithaworn et al., 1997). Snail populations are typically more abundant in the rainy season (Brockelman et al., 1986) which provides good conditions for the multiplication of snails e.g. *Melanoides tuberculatus*, an important first intermediate host (Sommerville, 1982; Scholz and Salgado-Maldonado, 1999) for intestinal flukes. How season more specifically influences snail and fish infections is not known in detail, but it is likely that the generally higher prevalence of FZT in the wet season is the result of a combination of factors. That run-off water carries waste substances into the canals during the rainy season might be the first factor. In addition, increasing rain and flooding lead to an increase of snail populations and FZT eggs (i.e., dogs, cats, pigs, and humans) in fecal contaminated water bodies (Khamboonraung et al., 1997; Sithithaworn and Haswell-Elkins, 2003; Thuy et al., 2010), so the FZT prevalence was high in the wet season, especially in Thay Cai – An Ha canal in this study.

5. Conclusions

No metacercariae were found in wild fish in Nhieu Loc – Thi Nghe and Tau Hu – Ben Nghe canals. *Haplorchis pumilio* and *Centrocestus formosanus* metacercariae were infected in fish in Thay Cai – An Ha canal. Four species were infected with FZT including silver barb (80%), climbing perch (11.7%), snakehead (7.5%), and walking catfish (1.7%). The FZT prevalence in the wet season is significantly higher than in the dry season ($P < 0.05$).

❖ **Conflict of Interest:** Authors have no conflict of interest to declare.

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TỈ LỆ NHIỄM SÁN LÁ SONG CHỦ TRÊN CÁ TỰ NHIÊN TỪ HOẠT ĐỘNG CÂU CÁ Ở THÀNH PHỐ HỒ CHÍ MINH

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TÓM TẮT

*Nghiên cứu tỉ lệ nhiễm sán lá song chủ (giai đoạn metacercariae) trên cá câu từ kênh Nhiêu Lộc – Thị Nghè, kênh Tàu Hủ - Bến Nghé và kênh Thầy Cai - An Hạ, Thành phố Hồ Chí Minh được thực hiện từ tháng 05/2017-04/2018. Kết quả cho thấy không có cá tự nhiên nhiễm metacercariae trên cá câu từ kênh Nhiêu Lộc – Thị Nghè và kênh Tàu Hủ - Bến Nghé. Tuy nhiên, cá câu từ kênh Thầy Cai – An Hạ bị nhiễm hai loài sán lá ruột nhỏ là *Haplorchis pumilio* và *Centrocestus formosanus*. Các loài cá bị nhiễm gồm có mè vinh (80,0%), rô đồng (11,7%), lóc đồng (7,5%) và trê trắng (1,7%). Tỉ lệ nhiễm metacercariae trên cá tự nhiên trong mùa mưa cao hơn mùa khô ($P < 0,05$).*

Từ khóa: *tỉ lệ nhiễm, sán lá song chủ, metacercariae, cá tự nhiên.*