

TREMATODE LARVAL INFECTIONS IN SNAILS COLLECTED FROM AQUACULTURE PONDS IN HA NOI AND YEN BAI, VIETNAM

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ABSTRACT

The hot-spots of transmission of food-borne zoonotic trematodes in northern Vietnam have been known as the integrated fish-livestock (VAC- Garden, Fish pond, Poultry shed) ponds. A cross-sectional study was conducted in Ha Noi capital and Yen Bai province to investigate the trematode larval infections (cercariae) in freshwater snails in these areas. Snails were collected from 35 VAC ponds, including 25 grow-out and 10 nursery ponds. A total of 13,895 specimens of 12 snail species were examined for cercariae shedding. Six of 12 snail species including *Melanooides tuberculata*, *Tarebia granifera*, *Radix auriculata*, *Gyraulus convexiusculus*, *Bithynia fuchsiana* and *Paraforesarulus manchouricus*, shed cercariae. Seven cercarial types were detected. The amount of snails and the prevalent infection in nursery ponds were 1.14 and 1.3 times higher than in grow-out ponds, respectively. Thus, VAC ponds in northern Vietnam are still the hot-spot for trematode larval infection.

Keywords: Opisthorchiidae, snail hosts, trematode larvae, VAC ponds.

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INTRODUCTION

More than 100 species of flukes (Digenea: Trematoda) can infect mammals including man (Yu & Mott, 1994; Nguyen et al., 2013) and in their life cycle, various snail species play a role as the first intermediate hosts (Keiser & Utzinger, 2009). Cercariae - larvae of flukes, shed from the snail intermediate hosts either encyst on aquatic vegetation such as watercress, water lotus, water morning glory (e.g. *Fasciola* spp., *Fasciolopsis buski*) or penetrate the skin of second intermediate hosts and encyst in the flesh of fish (e.g. *Clonorchis sinensis*, *Opisthorchis viverrini*), freshwater crabs (*Paragonimus* spp.), or frogs, snails, insect larvae (*Echinostoma* spp.). The encysted form of fluke larvae is called metacercariae, and mammalian hosts become infected when they eat raw, pickled, or insufficiently cooked aquatic products harbouring metacercariae or when drinking contaminated water (WHO, 1995). Fish-borne zoonotic trematodes (FZTs) transmitted by fish products pose major health problems worldwide, not only because of their wide geographical distribution, and high prevalence but also the severity of the diseases such as cholangitis, choledocholithiasis, pancreatitis, and cholangiocarcinoma (Chai et al., 2009; Mas-Coma & Bargues, 1997). In addition, FZTs also retard fish growth, reduce the marketability of fish or make them unsuitable for processing (Tucker et al., 2004).

The role of aquaculture in Vietnam is very important, not only because of the supply of high-value protein food sources for local people but also for economic gain through the export of aquatic products. Fish culture systems in Vietnam are very diverse, most freshwater fish species are raised in mono or polyculture and stocked at different levels of intensification (Thien et al., 2009; Phan et al., 2010a, b). Commercial fish culture in the Mekong delta began with the production of river catfish, reared in intensive systems in river cages or earthen ponds, for export (Thien et al., 2009). Other common systems have been developed to mainly supply food for domestic consumption in the whole country,

including giant gourami and hybrid catfish monoculture and in particular integrated VAC systems (Phan et al., 2010b, Bui et al., 2010; Clausen et al., 2012). FZTs transmission is relatively low in the intensive system compared to the family-based production systems, i.e. VAC system (Thien et al., 2009; Clausen et al., 2012). The VAC system is the traditional approach to family food production in the poor, rural regions of Vietnam (Luu, 2001). It is an ecological way of using farm products in natural cycles. The manure from the husbandry is used to fertilize ponds, so as to stimulate algal growth and subsequently fish growth. However, the reuse of waste matter including manure is conducive to the life cycle of FZTs (Bui et al., 2010; Phan et al., 2010a, b). The VAC ponds are commonly associated with rice growing and water can be exchanged between rice fields and ponds, so snails from outside environments can easily get enter and live in these ponds.

The VAC ponds can be divided into the nursery and grow-out ponds. In nursery ponds, fry from hatcheries is kept for a period where they are raised to the juvenile stage before being introduced into grow-out ponds for rearing to market size (Thien et al., 2009). Cercariae can easily penetrate into young fishes because of their thin skin and immature immune status (Lun et al., 2005), so the transmission of FZTs to fish can be very intense in nursery ponds and since nurseries deliver juvenile fishes to a potentially large upland for grow-out, the FZTs can spread to new areas. Hence, priority for FZTs intervention should be given to the nursery system (Clausen et al., 2012).

Several studies on the infection of digenean larvae in snails have been conducted in the Red River delta. Le et al. (1990) reported *Lymnae viridis*, *L. swinhoei*, *Parafossarulus striatulus*, and *Melanoides tuberculata* as intermediate hosts for poultry trematodes. Subsequently, Le et al. (2000) found 5 cercarial types and 7 metacercarial types in *P. striatulus*. Bui et al. (2010) mentioned that the first intermediate host snails of FZT are mainly belonging to two

families Thiaridae and Bithynidae. Recently, Nguyen et al. (2015) and Madsen et al. (2015) reported on the infection status of FZTs larvae in freshwater snails in VAC ponds and associated water bodies in Ninh Binh and Nam Dinh province, respectively.

This study provides new information on freshwater snails and their infection with fluke larvae in VAC ponds from rural districts of Ha Noi capital and Yen Bai province.

MATERIALS AND METHODS

Study areas

The Red River is the second biggest river in Vietnam. This river flows from Yunnan province in Southwest China through northern Vietnam to the Gulf of Tonkin. The upper stretch of the Red River, also called Thao River, Chay River, passes through Yen Bai province (mountainous region). The downstream of the Red River flows past the Ha Noi capital (delta region) before emptying into the Gulf of Tonkin. A total of 35 VAC ponds, including 20 ponds in Ha Noi (Dong Anh and Thuong Tinh districts), and 15 ponds in Yen Bai (Van Yen and Yen Binh districts), were randomly selected for this study. Among them, 25 were grow-out and 10 were nursery ponds.

Snails were collected every four months from April 2018 to April 2019. During the morning hours, snail sampling was conducted by the same person for 20 minutes at 4 sites per pond using scooping (Madsen et al., 1987) and/or hand-picking. Snails were transferred to plastic containers and transported alive to the laboratory where they were identified following the keys of Brandt (1974), Dang (1980), recent documents available (Hayes et al., 2008; Madsen & Nguyen, 2014).

The trematode cercarial infections in snails were examined using the shedding method (Frandsen & Christensen, 1984), small snails were placed individually in small plastic containers (volume 5ml), added 2–3 mL tape water, and left for 12 and 24 hours at room-temperature for shedding. The same procedure

was also used for larger snails except they were kept in glass containers with volumes of 25 or 50 mL. Morphotypes of cercariae were identified using live and unstained cercarial specimens, which were observed under a stereomicroscope Olympus SZX and/or a microscope Olympus CH40. Cercariae were identified according to the keys of Ginetinskaya (1988) and Schell (1985).

Statistical analysis

The presence/absence of trematode cercarial infections in various snail species were analysed using logistic regression. Provinces, time, and the type of VAC pond systems (nursery or grow-out ponds) were used as predictors after adjusting for possible clustering of repeated collecting times. Snail counts were compared among provinces and pond types using count models (Hilbe, 2008). All analyses were done using statistical analysis software (STATA/IC 12; StataCorp LP, College Station, TX, USA) and the differences with a probability value of less than 0.05 were considered significant.

RESULTS

Diversity and distribution of snails in VAC ponds

A total of 13,895 snail specimens, representing 12 species of 6 families, were collected from VAC ponds (Table 1). Among them, *Melanoides tuberculata*, *Pomacea canaliculata*, *Angulyagra polyzonata*, *Sinotaia aeruginosa* were the common species, they are present in almost all of the studied ponds. Other species such as *Tarebia granifera*, and *Filopaludina sumatrensis* were collected in large numbers but they were found in few sites. Three pulmonate snails, *Austropeplea viridis*, *Radix auriculata* and *Gyraulus convexiusculus*, were found in the root of floating aquatic plants and tended to be more common in rice fields and small canals (running water) than in other habitats. Similarly, two bithynid snails, *Bithynia fuchsiana* and *Parafosarulus manchouricus* were found in ponds that exchanged water with outside environments, i.e. small canals or rice fields nearby.

Table 1. The distribution of snails in study sites

Study sites	Ha Noi capital		Yen Bai province	
	Grow-out pond (n = 15)	Nursery pond (n = 5)	Grow-out ponds (n = 10)	Nursery pond (n = 5)
Family Ampullariidae				
<i>Pomacea canaliculata</i>	843	244	248	98
Family Bithynidae				
<i>Bithynia fuchsiana</i>	711	217	398	287
<i>Parafosarulus manchouricus</i>	295	134	237	85
Family Lymnaeidae				
<i>Austropelea viridis</i>	95	42	80	54
<i>Radix auriculata</i>	87	38	55	27
Family Planorbidae				
<i>Gyraulus convexiusculus</i>	32	22	18	4
Family Thiariidae				
<i>Melanoides tuberculata</i>	1,013	584	575	453
<i>Tarebia granifera</i>	258	41	66	26
<i>Thiara scabra</i>	18	0	0	0
Family Viviparidae				
<i>Angulyagra polyzonata</i>	1,491	983	1,193	694
<i>Filopaludina sumatrensis</i>	0	0	571	355
<i>Sinotaia aeruginosa</i>	690	295	160	78
Total	5,533	2,600	3,601	2,161

The amount of collected snails from VAC ponds in Ha Noi was 1.14 times higher than in Yen Bai but this difference has no statistically significance ($p = 0.161$). On the contrary, a significant difference was observed between the two VAC pond types. The number of snail specimens in the nursery ponds was 1.3 times greater than in the grow-out ponds ($p = 0.037$).

The infection rates and cercarial types in snails

Cercariae-infected snails were found in 17 out of 35 ponds, 11 of which were grow-out ponds, and 6 were nursery ponds. Six out of 12 snail species shed the cercariae invitro test (Table 2). The prevalence of infected snails from Yen Bai province was 1.33 times higher than in Ha Noi ($p = 0.001$). Similarly, the infection rate of snails from nursery ponds was 1.3 times higher than in grow-out ponds ($p = 0.002$). The highest infection was in *M. tuberculata* with a prevalence of 14.4%. The

prevalent infection of other thiarid snails, *T. granifera*, was 4.26%. Two bithynid snails, *P. manchouricus* and *B. fuchsiana*, had prevalences of 8.39% and 7.89%, respectively. Other species, *R. auriculata* and *G. convexiusculus*, had very low infection rates, the prevalent infection of *R. auriculata* was 0.97% and *G. convexiusculus* was 1.32%.

Seven types of cercariae were identified (Fig. 1, Table 3). Three species, *M. tuberculata*, *B. fuchsiana*, and *P. manchouricus*, emitted 5 cercarial types, *T. granifera* shedded 2 types, while *R. auriculata* and *G. convexiusculus* had only 1 type. Among cercarial types, Pleurolophocercous type 2 was the most common cercariae constituting 30.63% of all cercariae recorded followed by Xiphidiocercariae type 2 (25.53%), Xiphidiocercariae type 1 (23.94%), Echinostome cercariae (15.49%), and other types (4.41 %).

Table 2. Number of infected snails in different pond types

Snail species	Number of infected snails (%)			
	Ha Noi capital		Yen Bai province	
	Grow-out pond	Nursery pond	Grow-out pond	Nursery pond
<i>Bithynia fuchsiana</i>	41 (5.77)	26 (11.98)	29 (7.29)	17 (5.92)
<i>Parafosarulus manchouricus</i>	23 (7.80)	13 (9.70)	19 (8.02)	8 (9.41)
<i>Radix auriculata</i>	0	2 (5.26)	0	0
<i>Gyraulus convexiusculus</i>	0	1 (4.55)	0	0
<i>Melanoides tuberculata</i>	124 (12.24)	68 (11.64)	102 (17.74)	84 (18.54)
<i>Tarebia granifera</i>	7 (2.71)	4 (9.76)	0	0

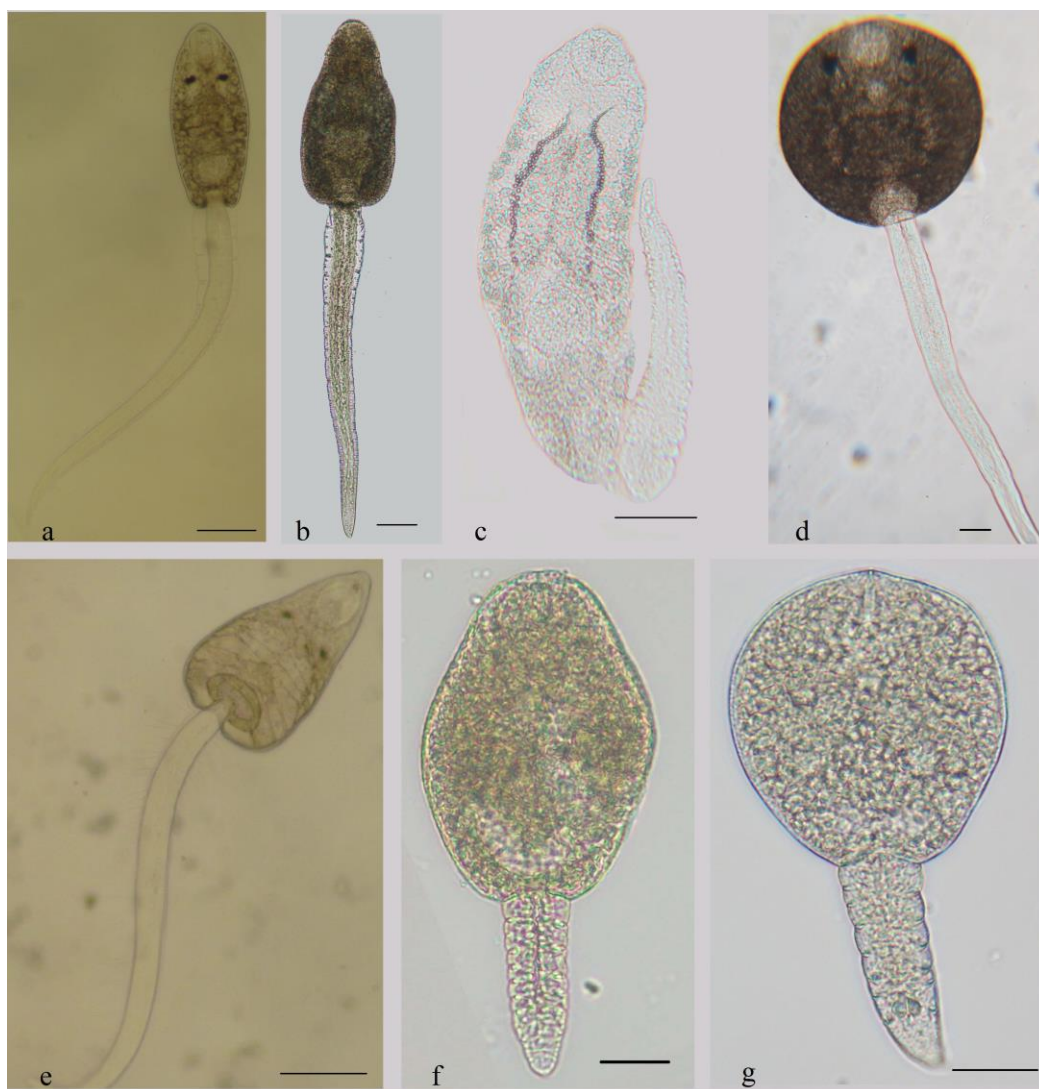


Figure 1. Cercariae found in freshwater snails collected in VAC ponds in Ha Noi capital and Yen Bai province. a) Pleurolophocercous type 1; b) Echinostomatata; c) Gymnocephalous; d) Amphistomata; e) Pleurolophocercous type 2; f) Xiphidiocercariae type 1; g) Xiphidiocercariae type 2. Scale bar: a–e = 50 μ m; f–g = 20 μ m

Table 3. Cercarial groups found in the first intermediate snail hosts

Cercarial type	Number of snails emitted cercariae						Total
	<i>B. fuchsiana</i>	<i>P. manchouricus</i>	<i>R. auriculata</i>	<i>G. convexiusculus</i>	<i>M. tuberculata</i>	<i>T. granifera</i>	
Pleurolophocercous type 1		9					9
Pleurolophocercous type 2					167	7	174
Xiphidiocercarial type 1	41	18			73	4	136
Xiphidiocercarial type 2	38	25			82		145
Echinostomatata	21	11			56		88
Gymnocephalous			2				2
Amphistomata	13			1			14

DISCUSSION

All of the important FZTs parasitize either the liver or in intestines of the final hosts. Among the human liver flukes of Opisthorchiidae in Vietnam, the most prevalent is *Clonorchis sinensis* in Northern provinces and *Opisthorchis viverrini* in South Central provinces (Nguyen et al., 2013). The fish-borne intestinal trematodes are also quite common and are comprised of a very diverse group of trematodes, including representatives from the Heterophyidae and Echinostomatidae (Dung et al., 2007; Nguyen et al., 2013). Regarding cercarial larvae, pleurolophocercariae type 1 is known as the larvae of opisthorchiids, this type was only found in Yen Bai province and only *P. manchouricus* shedded this cercarial type. In previous studies, Kino et al. (1998), as well as Nguyen et al. (2003) reported that *M. tuberculata* and *T. granifera* have been served as first intermediate hosts of the liver fluke *C. sinensis*; however, their role as true first intermediate hosts has been excluded by Nguyen et al. (2021). Pleurolophocercariae type 2 could be larvae of heterophyids e.g. *Haplorchis* spp., *Heterophyes* spp., *Metagonimus* spp., *Stellantchasmus* spp., *Procerovum* spp., *Centrocestus* spp., cryptogonimids or/and other opisthochiids, e.g. *Metorchis* spp. (Doanh & Le 2005). Similarly, echinostomatata is the larvae of echinostomatids, e.g. *Echinochasmus* spp., *Echinostoma* spp. These adult trematodes are not only found in humans/mammals (Dung et

al. 2007; Nguyen et al. 2009), the metacercariae are also found in freshwater fishes (Phan et al., 2010a, b).

Cercariae of amphistomata type belong to Paraphistomatidae and Diplodiscidae (Doanh & Le 2005). Almost amphistomata cercariae were emitted from pulmonate snails. Besprozvannykh et al. (2013), however, found this cercarial type from prosobranchs snails (*Bithynia fuchsiana*) which were collected in Nam Dinh province. In the present study, amphistomata cercariae which were emitted from pulmonate snail *G. convexiusculus*, did not encyst on the walls of plastic containers and after a short period of swimming, their died.

The cercarial type of two families Fasciolidae and Psilostomatidae are gymnocephalous (Doanh & Le 2005). In Vietnam, 4 snail species, including *Radix auriculata* (syn. *Lymnae swinhoi*) have been found to emit this cercaria (Doanh & Le, 2005; Bui et al., 2010; Besprozvannykh et al., 2013; Dung et al., 2013). Up to present, the gymnocephalous cercariae of three species i.e. *Fasciola gigantica*, *Sphaeridiotrema monorchis* and *S. pseudoglobulus* have been identified by the experimental infection or molecular analyses (Besprozvannykh et al., 2013; Doanh et al. 2019). Regarding the snail host, *Radix auriculata* is of great parasitological importance as it includes several intermediate hosts of trematodes which infect man, mammals, e.g. large liver flukes *Fasciola* spp., and avians e.g. intestinal

flukes *Echinoparyphium recurvatum*, *Hypoderma conoideum*, blood fluke *Trichobilharzia anatina* (Doanh & Le, 2005).

The xiphidio cercariae belong to the families Plagiochiidae, Telorchidae, Microphallidae and Lecithodendriidae (Doanh & Le, 2005). This group has many cercarial types and the identification of trematode based on the morphological characteristics of xiphidio-cercaria is impossible. In my personal data, at least 20 snail species emit xiphidio cercariae. Among them, *Triculinae* spp. and *Oncomelania* spp. serve as the first intermediate hosts of lung flukes *Paragonimus* spp. and both snails and lung flukes are present in Yen Bai province (Doanh et al., 2002).

Concerning to snail count, Bui et al. (2010) found that the average number of snails in nursery ponds was nearly 3 times higher than those in grow-out ponds (132.1 vs 37.7). The present study revealed a similar result when the number of snails in nursery ponds was 1.3 times greater than those in grow-out ponds (476.1 vs 365.4). The difference of amount snails in the two pond types could be explained by the presence of mollusc eating animals (duck, black carp) in grow-out ponds while they are absent in nursery ponds. The farmers do not release duck, black carp into the nursery ponds, the reason is not only mollusk eating animals can consume fish fries, but also their disturbances to the pond environment might affect fry survival; for example suspension of bottom sediment in the water due to its feeding at the bottoms (Hung et al., 2013). In addition, the difference in the number of snails per pond between the present study and Bui et al. (2010) could be explained by the snail sampling. Snails were conducted for 20 minutes at 4 sites per pond in the present study while only 30 minutes per pond in Bui et al. (2010).

Despite fewer snails, the infection rate of snails with trematodes in grow-out ponds is higher than that of snails in nursery ponds. Nguyen et al. (2018) also found the high prevalent infections in grow-out ponds than

those in nursery ponds in Thanh Hoa, Ninh Binh, Nam Dinh, and Thai Binh provinces. This can be explained by the accumulation of infected snails. The cycle of grow-out ponds normally lasts for 1–2.5 years, and the farmers do not dredge the pond bed during this time period, so that snails can remain there a long time and have more chances to get in touch with trematode's eggs in these ponds. Compared with the results of Bui et al. (2010), the infection rate of 4 remaining snails, *M. tuberculata*, *T. granifera*, *B. fuchsiana* and *P. manhouricus*, in this study were more than 3–5 times higher than their results. The difference can be explained, at least in part, by the range of studies. Bui et al. (2010) surveyed two communes of Nam Dinh province, and they collected snails in various environments i.e. ponds, canals, ricefields, rivers. In contrast, the snail samples in the present study were only collected from VAC ponds in two provinces.

In Vietnam, the common floating aquatic plants e.g. duckweed, water hyacinth, water morning glory, and many other kinds of grass are collected from outside and released into VAC ponds by the owners as food for fish, i.e. grass carp (*Ctenopharyngodon idella*). These plants also can cover the surface of the pond to reduce the water temperature in summer and create a living environment for zooplanktons. The transportation of aquatic plants from outside to VAC ponds contributes to an increase in the number of snails, not only because of the carry-over of attached snails with plants but also by creating a suitable environment for preexisting snails in these ponds. In addition, farmers also collect snails (mainly *A. polyzonata*, *S. aeruginosa*, *F. sumatrensis*, and *P. canaliculata*) from canals, rivers, etc. and release them into their ponds to supply the food for black carp (*Mylopharyngodon piceus*) and ducks (*Anas platyrhynchos dom.*) in these ponds (Nguyen, 2016), and this was identified as a risk factor the foodborne trematode transmission because these snails can be the second intermediate hosts of trematodes (Le et al., 1990) or other

parasites, e.g. *Angiostrongylus cantonensis* (Shan et al., 2018).

CONCLUSIONS

The infection rates of snails with trematode larvae in VAC ponds still remain high. Any strategies to control trematodiasis must pay attention to the existence of freshwater snails - the first intermediate hosts of trematodes in VAC ponds and also manage ponds to reduce snails and their habitats, and prevent faecal contamination (eggs of trematodes) from outside into the ponds.

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