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APPENDICES

APPENDIX A
REAGENTS FOR LABORATORY EXPERIMENTS

REAGENTS FOR LABORATORY EXPERIMENTS

1. 0.85 % NSS

NaCl	8.5	g
Distilled water	1,000	ml

2. Pepsin A solution

Pepsin A powder (0.025%)	2.5	g
NaCl (0.085%)	8.5	g
HCl	15	ml
Distilled water	1,000	ml

3. 70 % ethanol

Absolute ethanol	70	ml
Add distilled water to a final volume of	100	ml

4. TE buffer, pH 8.0

10 mM Tris-HCl, pH 7.4-8.0	1 ml	of 1 M
1 mM EDTA, pH 8.0	200 μ l	of 0.5 M
Add distilled water to a final volume of	100	ml
Autoclave before used		

5. Lysis solution (10% SDS)

SDS	10	g
Add distilled water to a final volume of	100	ml

6. 0.2 mg/ml Proteinase K (stock 20 mg/ml)

Proteinase K	1	μ l
Distilled water	99	μ l

7. Phenol

Phenol (saturated in TE)	100	ml
8-hydroxyquinoline (C ₂ H ₇ NO)	100	μl

8. 3 M Sodium acetate pH 5.2

Sodium acetate·3H ₂ O	408.3	g
Distilled water	800	ml
Adjust the pH to at 5.2 with glacial acetic acid		
Add distilled water to a final volume of	1000	ml

9. Extraction buffer, pH 8.3

20 mM Tris-HCl	2 ml of 1 M
20 mM EDTA	4 ml of 1.5 M
300 mM NaCl	30 ml of 1 M
Add distilled water to a final volume of	100 ml
Autoclave before used	

10. 7.5 M Ammonium acetate

Ammonium acetate	38.5	g
Add distilled water to a final volume of	200	ml

11. 0.5 M EDTA (Ethylene diaminetetraacetic acid)

Na ₂ EDTA·2H ₂ O (M.W=372.2 g/mole)	186.1	g
NaOH (M.W=40 g/mole)	20	g
Add distilled water to a final volume of	1,000	ml
Autoclave before used		

12. 3 M NaAC, pH 5.2

NaAC (Sodium Acetate Trihydrate,	408.1	g
CH ₃ COONaH ₂ O=136.06 g/mole)	1,000	ml
Add distilled water to a final volume of		

13. 10xTBE buffer

Tris base	108	g
Boric acid	55	g
0.5 M EDTA, pH 8.0	40	ml
Add distilled water to a final volume of	1,000	ml

14. 20xLoading buffer

Bromophenol Blue	0.125	g
Glycerol	15	ml
10xTBE buffer	30	ml
Add distilled water to a final volume of	50	ml

15. Staining solution

1x TBE buffer	100	ml
Ethidium bromide (5 mg/ml)	10	μl

16. 1.5% Agarose gel

Agarose powder	1.5	g
Add TBE buffer, pH 8.0 to a final volume of	100	ml

17. 10% formalin

100% formaldehyde	10	ml
Add distilled water to a final volume of	100	ml

APPENDIX B
RESEARCH PUBLICATION & PRESENTATIONS



RESEARCH PUBLICATION

Kulthida Kopolrat, Nonglak Laoprom and Paiboon Sithithaworn (2011) Freshwater snails as a potential source of fish-borne zoonotic trematodes in fish farm. Proceeding in the 96 Years of Opisthorchiasis: Past, Present and Future International Congress of Liver Flukes. Khon kaen, Thailand. March 2011.

RESEARCH PRESENTATIONS

Kulthida Kopolrat, Nonglak Laoprom and Paiboon Sithithaworn . Roles of snails as source of fish-borne trematode in aquaculture farm. RGJ Seminar Series LXXV: Biomedical Sciences: Research for Healthy Society. Faculty of Medicine, Khon kaen University, Khon kaen, thailand. 2010 (*Poster presentation*) October 11.

Kulthida Kopolrat, Nonglak Laoprom and Paiboon Sithithaworn . Freshwater snails as a potential source of fish-borne zoonotic trematodes in fish farm. The 96 Years of Opisthorchiasis: Past, Present and Future International Congress of Liver Flukes. Khon kaen, Thailand. 2011. (*Poster presentation*) March 7-8.

P08– Poster presentation

Freshwater snails as a potential source of fish-borne zoonotic trematode in fish farm

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Fish-borne zoonotic trematodes (FZT) comprising both heterophyids and opisthorchiids are of common occurrence in both wild and captured fish in Southeast Asia. These FZTs require specific snails as their first intermediate host to complete the life cycle. Recent rise in production of fresh water aquaculture mainly carps has been implicated as potential source of FZT transmission to human and animals. In order to identify the source of FZT contamination in aquaculture farm, the present study was conducted to examine the link between the presence of cercariae in snails and metacercariae in fish in a nursery farm in Vientiane, Lao PDR. A survey of snails within the farms revealed 3 types of trematode cercariae in *Tarebia granifera* namely xiphidiocercaria, monostome and parapleurolophocercous cercariae. *Melanooides tuberculata* also had infections with parapleurolophocercous cercariae. No cercaria was found in *Filopaludina* sp. The overall prevalence of cercariae in the snail was 3.46%. Concurrent examination FZT in fingerling fish in the farm revealed that 5 out of 7 species of fish harbored *Haplorchis taichui* metacercariae with the average prevalence of 55.02%. The prevalence among infected fish were 85.96% in silver barb or pla tapian (*Barbonymus gonionotus*), 67.14% in silver carp or pla gled lab (*Hypophthalmichthys molitrix*), 47.83% in mrigal or pla nuan chan (*Cirrhinus mrigala*), 45.33% in common carp or pla nai (*Cyprinus carpio*) and 28.85% in grass carp or pla gin ya (*Ctenopharyngodon idllus*). The presence of parapleurolophocercous cercariae in the snails (*M. tuberculata* and *T. granifera*) coincided with *H. taichui* infection in fish suggests the possible roles of snails as a source of FZT contamination in the aquaculture system. Although molecular analysis for identities cercariae in snails and metacercariae in the fish in the farm needed further confirmation, snail control should be integrated into the farm management to prevent FBT contamination in aquaculture farms.

Keywords: Fish-borne zoonotic trematodes, culture fish, snail intermediate hosts, cercariae, *Haplorchis* metacercariae

Proceedings & Abstracts



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Thailand

- P43– Ecological niche model of *Opisthorchis viverrini* in northeast Thailand based on climate and environmental data.** (Apipom Suwannatrai, Thitima Wongsaroj, John B. Malone, Kulwadee Suwannatrai, Jutharat Kulsantiwong, Supawadee Piratae, Chalida Thammasiri, Sattrachai Prasopdee, Panita Khampoosa, Pairat Tarbsripair, Rasamee Suwanwerakamtorn, Thidarat Boonmars, Somsak Sukchan, Jennifer C. McCarroll, Michael T. Kearney and Smarn Tesana)
- P44– Population dynamics of the minute intestinal trematode *Haplorchis pumilio* following experimental infection of young dogs.** (Sofie Nissen, Lan Anh Nguyen, Stig Milan Thamsborg, Anders Dalsgaard and Maria Vang Johansen)
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- Proceeding–2 Freshwater snails as a potential source of fish-borne zoonotic trematodes in fish farm** (Kulthida Kopolrat, Nonglak Laoprom and Paiboon Sithithaworn)

Proceeding-2

Freshwater snails as a potential source of fish-borne zoonotic trematodes in fish farm

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Abstract

Fish-borne zoonotic trematodes (FZT) comprising both heterophyids and opisthorchiids are of common occurrence in both wild and captured fish in Southeast Asia. These FZTs require specific snails as their first intermediate host to complete the life cycle. Recent rise in production of fresh water aquaculture mainly carps has been implicated as potential source of FZT transmission to human and animals. In order to identify the source of FZT contamination in aquaculture farm, the present study was conducted to examine the link between the presence of cercariae in snails and metacercariae in fish in a nursery farm in Vientiane, Lao PDR.

A survey of snails within the farms revealed 3 types of trematode cercariae in *Tarebia granifera* namely xiphidiocercaria, monostome and parapleurolophocercous cercariae. *Melanoides tuberculata* also had infections with parapleurolophocercous cercariae. No cercaria was found in *Filopaludina* sp. The overall prevalence of cercariae in the snail was 3.46%. Concurrent examination FZT in fingerling fish in the farm revealed that 5 out of 7 species of fish harbored *Haplorchis taichui* metacercariae with the average prevalence of 55.02%. The prevalence among infected fish were 85.96% in silver barb or pla tapian (*Barbonymus gonionotus*), 67.14% in silver carp or pla gled lab (*Hypophthalmichthys molitrix*), 47.83% in mrigal or pla nuan chan (*Cirrhinus mrigala*), 45.33% in common carp or pla nai (*Cyprinus carpio*) and 28.85% in grass carp or pla gin ya (*Ctenopharyngodon idllus*). The presence of parapleurolophocercous cercariae in the snails (*M. tuberculata* and *T. granifera*) coincided with *H. taichui* infection in fish suggests the possible roles of snails as a source of FZT contamination in the aquaculture system. Although molecular analysis for identities cercariae in snails and metacercariae in the fish in the farm needed further confirmation, snail control should be integrated into the farm management to prevent FBT contamination in aquaculture farms.

Keywords: Fish-borne zoonotic trematodes, culture fish, snail intermediate hosts, cercariae, *Haplorchis* metacercariae

1. Introduction

Fish-borne zoonotic trematodes (FZT) are well-known causes of liver and intestinal trematode (fluke) infection in humans [1, 2]. Because fish is a very important source of protein for people living in rural areas where cultural habits of eating raw or inadequately prepared dishes may pose risk to infection

with FZT. Studies conducted in Vietnam revealed that in the Mekong Delta, 1.2–29.7% of cultured fish were infected with FZT metacercariae [3-5] whereas in the North, prevalence of FZT was 44.6% in Nghe An province and was >50% in Nam Dinh province [6, 7]. A recent study involving the recovery adult of FZT from human and animals revealed a high prevalence in humans (64.9%), cats (70.2%), and dogs (56.9%) in Nam Dinh province, located in the Red River Delta [8]. Distribution and density of the first intermediate hosts, species of freshwater snails, are important in determining transmission patterns of the trematodes [2, 9]. A study on trematode infections in snails in Nam Dinh province, Vietnam reported that many snail species acted as intermediate hosts for FZT [10]. The result revealed that a total of 16 snail species was found and four were widely distributed. Trematode infections were found in eight snail species and among these *Melanooides tuberculata* had the highest overall prevalence of infection (13.28%). Parapleurolophocercous and pleurolophocercous cercariae constituted the most common type of cercariae recovered, contributing 40.6% of all infections.

In Lao PDR, there have been extensive records of FZT in human [11-13]. In addition to *Opisthorchis viverrini*, other FZT with intestinal tropism occur in Lao PDR, the most prominent of which is *Haplorchis* spp. in several species of wild fish [2, 14]. Because of an inadequate supply of wild fish, the government has encouraged aquaculture to meet the demand of consumers. But whether the culture fish pose risk for FZT infection or not has not been investigated. The purpose of this study is to perform a preliminary survey of FBT in an aquaculture farm in Lao PDR particularly the presence of FZT in fingerlings in the nursery farm as well as snails in its vicinity in order to identify the source of FBT contamination in the farm.

2. Materials and methods

2.1 Study area

In the aquaculture practice, fish fry are produced from hatcheries and distributed to nurseries where the fish are grown to the juvenile stage (fingerling) and subsequently supplied to farmers. Typically, in the hatcheries fish are grown in a protected and highly controlled environment while in the nurseries they are typically reared in opened earthen ponds with high exposure to various external environmental factors, i.e. fecal contamination from various animals and human activities. In this study, a cross-sectional survey of fingerlings in a nursery farm in Vientiane province known to have FZT was performed. The main species stocked in the farm are carps such as silver barb, grass carp, silver carp, common carp, rohu, Nile tilapia, bighead carp, red – finned peccu and mrigal.

2.2 Snails and cercariae

Snails were collected from fish farms by handpicking from objects and solid surfaces and by dredging the sediment with a scoop. Snail samples were *M. tuberculata*, *Tarebia granifera* and *Filopaludina* sp. The snail samples were sorted by species, cleaned and examined for trematode infection by cercarial shedding. The cercarial shedding were done by placing individual snails in a small plastic container half-filled with dechlorinated water and exposed to a 60 W light for 5 hours at room temperature. The emerged cercariae were examined and identified by morphological criteria [15].

2.3 Fish and metacercariae

Seven species of fish available in the farm were sampled for metacercariae examination. The samples included silver barb (*Barbonymus gonionotus*), grass carp (*Ctenopharyngodon idellus*), silver

carp (*Hypophthalmichthys molitrix*), common carp (*Cyprinus carpio*), rohu (*Labeo rohita*), Nile tilapia (*Oreochromis niloticus*), bighead carp (*Aristichthys nobilis*), red – finned pecc (*Piaractus brachypomus*) and Mrigal (*Cirrhinus mrigala*). At least 25 fish /species were randomly selected for the analysis. After collection, the fish were placed on ice and transported to the laboratory. Fish were held at 4 °C in a refrigerator until processed, within 1 week of collection. Individual fish were examined for metacercarial infections by using standard pepsin digestion technique [16]. The fish were individually minced by electric blender and digested with 1% pepsin A (BDH). The ratio of pepsin per fish-homogenate was 4:1 and the mixture solution was incubated at 37 °C in shaking water bath for 1 hour. Thereafter, the mixture was strained only once through the 250-micrometer apertures. The final filtrate was sedimented in conical jars with several changes of 0.85% saline until the supernatant was clear and examined using a dissecting microscope. Identification of the metacercariae was assisted by use of morphological criteria detailed with published keys [17, 18]

3. Results

3.1 Trematode cercariae in snails

The samples consisted of 3 species of snails. The majority being *Tarebia granifera* (n=1288) while the other two species were fewer in numbers (n=30-39) namely *M. tuberculata* and *Filopaludina* sp. As detailed in Table 1, 3 types of cercariae namely parapleurolophocercous cercariae (2.48%), xiphidiocercaria (0.85%) and monostome cercariae (0.16%) were detected in *T. granifera*. Although with relatively smaller sample size (n=30), the parapleurolophocercous cercariae were found in *M. tuberculata* with the prevalence of 6.67%. No cercariae were found in *Filopaludina* sp. The overall prevalence of cercariae within the snail sample was 3.46%. *Bithynia siamensis goniomphalos* was not present in the farm during the study.

Table 1. Prevalence rates of different trematode cercariae in snail samples from the studied farm in Lao PDR

Species of snail	Sample size	Type of cercariae	Prevalence (%)
<i>Tarebia granifera</i>	1288	Parapleurolophocercous -Heterophyidae	2.48
		Xiphidiocercariae -Virgulate	0.85
		Monostome	0.16
<i>Melanooides tuberculata</i>	30	Parapleurolophocercous -Heterophyidae	6.67
<i>Filopaludina</i> sp.	39	-	-

3.2 FZT metacercariae in fish

Among 7 species of fingerling fish, 5 species (71.4%) harbored known trematode metacercariae, *H. taichui*. As shown in Table 2, the analyses of individual fish separated by species revealed that the silver barb (*B. gonionotus*) had the highest prevalence of 85.9% followed by the silver carp (*H. molitrix*) with the prevalence of 67.1%. The other three groups of fish namely Mrigal or Pla Nuan Chan (*C. mrigala*),

common carp or pla nai (*C. carpio*) and grass carp or pla ginya (*C. idllus*) exhibited lower prevalence ranged from 28.8-45.3%. The intensity of metacercariae was 12.2 cyst/fish in the silver barb and those for other fish were relatively similar ranged from 3.7-3.9 cysts/fish. The density of infection per weight of fish showed similar pattern to the intensity of infection. No *H.taichui* metacercariae were seen in the red-finned pecu and the tilapia.

Table 2. Prevalence, intensity and density of *Haplorchis taichui* metacercariae in fish samples obtained from the fish farm in Lao PDR.

The species of fish	Sample size	Prevalence (%)	Intensity (cyst/fish)	Density (cyst/g)
Mrigal	69	47.83	3.91	2.91
Common carp	75	45.33	3.79	15.05
Silver barb	57	85.96	12.22	40.31
Red – finned pecu	40	-	-	-
Silver carp	70	67.14	4.38	2.61
Grass carp	52	28.85	3.73	2.39
Tilapia	39	-	-	-

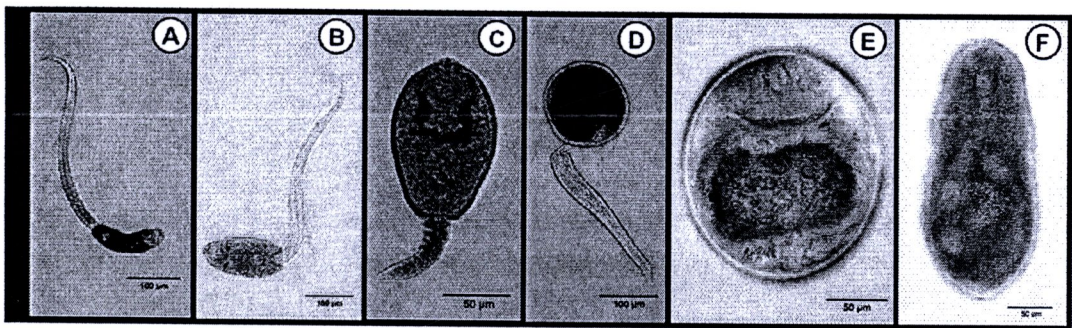


Figure 1. Images of trematode cercariae from snails and *Haplorchis taichui* metacercariae from fish observed in this study. A: Parapleurolophocercous cercariae (heterophyidae type 1) ; B: Parapleurolophocercous cercariae (heterophyidae type 2) (A and B are human and mammal trematodes) ; C: Xiphidiocercariae (Virgulate type) (trematode of invertebrate, amphibians and reptile); D: Monostome (trematode of cattle, horse or frog); E: *Haplorchis taichui* metacercariae (trematode of human and domestic animals); F: *Haplorchis taichui* excysted metacercariae.

4. Discussion

High prevalence of FZT including *O. viverrini* and several species of minute intestinal fluke (MIF) in human reported in Lao PDR indicated high transmission of these trematodes in this country [11, 19, 20]. The highest prevalence rates in human were found in central and southern parts, reaching levels of up to 60% [21, 22]. In addition wild fish collected from the NumNgum reservoir and other areas in Vientiane were also reported to harbor several types of trematode metacercariae, including *O. viverrini* [14, 23]. Recently, metacercariae of *O. viverrini*, *H. taichui*, *H. yokogawai* and *C. formosanus* were detected in the fish from Savannakhet and Vientiane, Lao PDR [24].

In this study, *H. taichui* metacercariae were highly common in most species of culture fingerling fish in the nursery farm with the average prevalence of 55.02%. In particular, the silver barb which is a native species in Southeast Asian countries including Thailand and Lao PDR seems to be susceptible to not only infections with *H. taichui* but also other FZT such as *O. viverrini* (Sithithaworn, unpublished). There were reports that cercariae of *H. taichui* and *O. viverrini* cercariae can be found in *T. granifera* (Thiaridae) and *B.s. goniomphalos*, respectively [12]. Our study found that parapleurolophocercous cercariae were detected in two species of snail (*M. tuberculata* and *T. granifera*) in parallel with *H. taichui* infection in fish. This finding clearly signified the fundamental roles of snails as a source of FZT contamination in the aquaculture system. Although identification of cercariae by morphology alone is not conclusive and a confirmation of species by molecular method is needed, it is likely the 2 species of snail (*M. tuberculata* and *T. granifera*) are the likely source of FZT (*H. taichui*) in fish. Alternatively, cercariae may be contaminated with water source in the fish pond and create infection in the fish.

It is important to note that the fish examined in this study are fingerlings which are distributed to farmers and thus may spread FZT to other areas. If the snails are present in the culture ponds, they can then propagate and may complete the life cycle of FZT. Therefore there is need to impose control measure particularly the nursery farm to be aware of the FZT problem and improvement of the farm management should be encouraged such that parasite-free fingerlings are produced for farmer. This approach may help for FZT control at large since there is limited action to apply similar measure to the wild fish.

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RGJ Seminar Series LXXV:
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- Poster-16:** Inhibitory effect of aspirin on pathological changes in experimental cholangiocarcinogenesis
Pakkayanee Sudsarn, Amornrat Juasook, Orasa Wonkchalee, Sirintip Boonjaruspinyo, Wipaporn Ruangjirachuporn, Thidarut Boonmars
- Poster-17:** Opisthorchiasis cause biliary cirrhosis in gerbil
Orasa Wonkchalee, Sasithron Kaewkes, Yaoyaluk Chamgramol, Amornrat Juasook, Pakkayanee Sudsarn, Sirintip Boonjaruspinyo, Thidarut Boonmars
- Poster-18:** Gemcitabine induced cytotoxic and adaptive responses in cholangiocarcinoma cells
Sarinya Kongpetch, Veerapol Kukongviriyapan, Auemduan Prawan, Laddawan Senggunprai, Upa Kukongviriyapan
- Poster-19:** Proteomics of cholangiocarcinoma for early marker discovery in the serum
Jarinya Khoontawad, Sittiruk Roytrakul, Pornip Pinlaor, Chaisiri Wongkham, Puangrat Yongvanit, Thidarut Boonmars, Somchai Pinlaor
- Poster-20:** Molecular cloning and partial sequencing of hemoglobin beta chain gene from crocodile (*Crocodylus siamensis*)
Thai Kabbua, Atcha boonmee, Sompong Thammasirirak
- Poster-21:** Antibacterial activity of leukocyte extracts from crocodile (*Crocodylus siamensis*)
Nualyai Yaraksa, Sakda Daduang, Sompong Thammasirirak
- Poster-22:** Low cure rate of praziquantel treatment in opisthorchiasis Assessed by PCR-BASED diagnosis
Kunyarat Duengjai, Thidarut Boonmars, Jiraporn Sithithaworn, Paiboon Sithithaworn
- Poster-23:** Evidence of *Opisthorchis viverrini* species complex in Thailand and Lao PDR: host-parasite relationship in snail intermediate host, *Bithynia siamensis goniomphalos*
Nadda Kiatsopit, Thidarut Boonmars, Smarn Tesana, Paiboon Sithithaworn
- Poster-24:** Detection and identification of *Opisthorchis viverrini* and fish borne trematode in cultured and captured fish
Opal Pitaksakulrat, Nongluk Laoprom, Thewarach Laha, Paiboon Sithithaworn
- Poster-25:** Roles of snails as source of fish borne trematode in aquaculture farm
Kulthida Kopolrat, Nonglak Laoprom, Paiboon Sithithaworn
- Poster-26:** Phenolic acids and fatty acids composition in Thai underutilized fruits
Jittawan Kubola, Sirithon Siriamornpun
- Poster-27:** Fatty acids composition in Thai herbs and spices
Pornpimol Raksakantong, Sirithon Siriamornpun
- Poster-28:** Anti-proliferative effect of extracts from northeastern Thai vegetables against human cancer cells
Onanong Kaisoon, Sirithon Siriamornpun, Izabela Konczak
- Poster-29:** Effect of adding Ling-zhi (*Ganoderma lucidum*) on antioxidant activity and oxidative stability of smoked fish sausage
Wannasiri Wannasupchue, Kannikar Huaisan, Jirapun Huaisan, Naret Meeso, Sirithon Siriamornpun
- Poster-30:** Loop-mediated isothermal amplification (LAMP) method as a new diagnosis tool for diagnosis of *Opisthorchis viverrini* infection
Yuji Arimatsu, Banchob Sripa
- Poster-31:** Interleukin-6 expression in cholangiocarcinoma: a clinicopathological correlation study
Pokpong Pra-yong, Suwit Balthaisong, Chawalit Pairojkul, Vajrabhongsas Bhudhisawasdi, Yaowalux Chamgramol, Banchob Sripa
- Poster-32:** Cytokine profiles in *Opisthorchis viverrini*-stimulated PBMCs patients with cholangiocarcinoma
Arpa Surapaitoon, Thewarach Laha, Chawalit Pairojkul, Vajrabhongsas Bhudhisawasdi, Banchob Sripa

Poster-25**Roles of snails as source of fish borne trematode in aquaculture farm**

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Fish-borne trematodes (FBT) comprising both heterophyids and opisthorchiids are of common occurrence in both wild and captured fish in Southeast Asia. Recent surge in production of fresh water aquaculture mainly carps raises concern of their roles as potential source of FBT transmission in human and animals. To identify source of FBT contamination in fish farm, this study was undertaken to characterize and identify cercariae in snails and fish-borne metacercariae from selected nursery ponds in Lao PDR. A survey of snails within the farms revealed two types of trematode cercariae in *Bithynia siamensis goniomphalos* namely monostome and xiphidiocercaria. *Melanoides tuberculata* shed two types of cercariae namely xiphidiocercaria and parapleurolophocercous cercariae. *Tarebia granifera* shed three types of cercariae namely xiphidiocercaria, monostome and parapleurolophocercous cercariae. *Filopaludina sp* harbor no trematode cercaria. A concurrent survey of FBT in culture fish (fingerlings) revealed that 4 out of 8 species of carps harbored *Haplorchis taichui* metacercariae with the average prevalence of 30.7%. The prevalence were 40% in silver carp or pla gled lab (*Hypophthalmichthys molitrix*), 39.2% in silver barb or pla tapian (*Barbonymus gonionotus*), 28.5% in migral or pla nuan chan (*Cirrhinus mrigala*) and 8% in grass carp or pla gin ya (*Ctenopharyngodon idllus*). The presence of parapleurolophocercous cercariae in two species of snail (*Melanoides tuberculata* and *Tarebia granifera*) in parallel with *H. taichui* infection in fish signified the fundamental roles of snails as a source of FBT contamination in the aquaculture system. Although the association between trematodes metacercariae other than *H. taichui* in fish and cercariae other than parapleurolophocercous cercariae in snails deserved further study, the presence of *H. taichui* in snails and fish in the culture system is verified. Thus, snail control should be one of the vital options that needed integration into the farm management to prevent FBT infection in aquaculture farms.



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