ẤU TRÙNG SÁN LÁ CÓ NGUỒN GỐC THỦY SẢN Ở VIỆT NAM

Nuôi trồng thủy sản nước ta đóng vai trò quan trọng đảm bảo an ninh lương thực quốc gia, phát triển kinh tế xã hội của đất nước. Do đó, vấn đề an toàn thực phẩm trong nuôi trồng thủy sản cũng nhận được nhiều quan tâm trong xã hội. Tuy nhiên, những hiểu biết về sán lá lây truyền qua cá (Fishborne Zoonotic Trematodes – FZT) và các giai đoạn ấu trùng của chúng đối với an toàn thực phẩm trong nuôi trồng thủy sản còn rất hạn chế. Tại Việt Nam trước năm 2004 có rất ít những nghiên cứu về ấu trùng sán lá nói chung ở trên cá. Trên người và động vật chỉ biết đến sự lưu hành của sán lá gan nhỏ *Clonorchis sinensis* và *Opisthorchis viverrini*. Trong khi đó, hầu như chưa có các nghiên cứu về FZT trong nuôi trồng thủy sản (Phan Thị Vân và Bùi Ngọc Thanh, 2013).

Ấu trùng sán lá trên cá có hình thái tương đối giống nhau vì vậy những phát hiện ấu trùng trên cá thường được mặc định là ấu trùng sán lá gan nhỏ. Chỉ riêng ấu trùng *Centrocestus formosanus* đã được xác định nhưng với quan tâm chủ yếu như tác nhân gây bệnh cho cá hương và cá giống. Từ sau năm 2004 khi dự án FIBOZOPA – Ký sinh trùng gây bệnh có nguồn gốc Thủy sản tại Việt Nam được triển khai thì hàng loạt các ấu trùng sán lá ruột nhỏ được xác nhận nhiễm trên cá. Cho đến nay ít nhất khoảng 40 loài cá nuôi và tự nhiên ở cả nước ngọt và mặn lợ ở cả miền Bắc, Trung và Nam Bộ đã được nghi nhận là nhiễm ấu trùng metacercaria của sán lá có nguồn gốc thủy sản.

Bắc Bộ: Hầu hết các điều tra đều cho thấy cá nhiễm ấu trùng sán với tỷ lệ cao. Cụ thể như nghiên cứu trên cá nước ngọt nuôi thương phẩm tại Nam Định nhiễm ấu trùng sán với tỷ 72% (Phan et al., 2010), cá hương và giống tại Nam Định, Ninh Bình và Bắc Ninh tương ứng nhiễm 14,1-57,8% (Vân Thi Phan, 2010b), cá nuôi và cá tự nhiên tại Nam Định lần lượt nhiễm 64,3-68,9% (Vân Thi Phan, 2010a). Cá nước lợ cũng được xác định tỷ lệ nhiễm là 56% tại Nam Định (Hà, 2007). Ngoài ra một số nghiên cứu khác cũng đều xác nhận nhiều loài cá bao gồm cả cá nuôi và tự nhiên đều nhiễm ấu trùng sán lá có nguồn gốc Thủy sản.

Trung Bộ: Chưa có nhiều nghiên cứu trên cá tại khu vực này nhưng với 2 nghiên cứu điển hình trên cá nước mặn, lợ và cá nước ngọt cũng đã ghi nhận ấu trùng sán lá. Tỷ lệ nhiễm trung bình trên cá thương phẩm là 44,6% và cá giống 43,6% tại Nghệ An (Chi et al., 2008), Cá nước ngọt tại Phú Yên tỷ lệ nhiễm trung bình là 33% (Dung, 2012) và cá nước mặn lợ tại Khánh Hòa cũng nghiễm tới 60-77% (Vo et al., 2008).

Nam Bộ: So với miền Bắc, tỷ lệ nhiễm ấu trùng sán trên cá ở khu vực miền Nam được xem là thấp hơn rất nhiều so với khu vực miền Bắc và miền Trung. Tỷ lệ nhiễm trên cá Tra là 0,7% và cá Lóc tự nhiên là 10,3% tại An Giang (Thu et al., 2007). Tại Cần Thơ và Tiền Giang, cá Tai tượng trong hệ thống nuôi đơn nhiễm ấu trùng 1,7%, cá chép trong hệ thống nuôi ghép là 6,6% và trên cá trong hệ thống VAC là 3,0% (Thien et al., 2007). Cá hương và giống tại các tỉnh Tiền Giang, Cần Thơ, Vĩnh Long và Đồng Tháp cũng được xác định nhiễm FZT từ 1,2-29% (cá rô đồng nhiễm 29,7%, cá sặc rằn 27,8%, cá Tra 1,2%), sự biến động tỷ lệ nhiễm FZT có tính mùa vụ (Thien et al, 2009).

Thông tin về FZT trên cá và các giải pháp ngăn ngừa lây nhiễm rất có ý nghĩa cho việc phát triển nuôi trồng thủy sản bền vững và an toàn thực phẩm cho con người.

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Prevalence of zoonotic trematode parasites in fish fry and juveniles in fish farms of the Mekong Delta, Vietnam

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ABSTRACT

Zoonotic parasites are a significant food safety problem, particularly in Asia. In Vietnam fishborne zoonotic trematodes (FZT) are highly prevalent in fish cultured in grow-out farms. However, FZT infection status of juveniles produced and distributed by hatcheries and nurseries is unknown. Here we report an epidemiological investigation on FZT in fry and juveniles of major cultured freshwater fish species in four provinces of the Mekong Delta of Vietnam. No FZT infections were found in 14 species of fry sampled from hatcheries. In contrast, nursery juveniles of river catfish, hybrid catfish, giant gouramy, climbing perch, common carp, kissing gouramy, silver barb, silver carp, grass carp, Indian carp, pacu, tilapia and snakeskin gouramy were frequently infected with FZT metacercariae (range 1.2–29.7%). Seasonal variation in prevalence was observed: prevalence in river catfish and hybrid catfish were maximal in January, at the end of the flooding season, while the prevalence in juveniles of giant gouramy, climbing perch, common carp, kissing gouramy, silver barb, silver carp grass carp, mrigal and pacu were higher in the wet season, June to November. Overall, FZT prevalence was highest in climbing perch and giant gouramy (29.7% and 27.8%, respectively) and the lowest in river catfish (1.2%). The density of FZT metacercariae in fish varied seasonally only in climbing perch which was maximal in the wet season ($P<0.05$), compared to the dry season (430 vs 28 metacercariae/100 g of fish). These results demonstrate that acquisition of infected seed stock from nurseries is a serious risk for Vietnamese grow-out fish farms, and stress that interventions to control FZT must focus also on these stages of the cultured fish production cycle.

1. Introduction

Fish are a very important source of protein for people living in rural areas of Southeast Asia, where cultural habits of eating raw or inadequately prepared dishes often lead to infection with fishborne zoonotic trematodes (FZT) (Chai et al., 2005). These zoonotic species of trematodes ("flukes") can be transmitted as larvae (metacercariae) to a wide range of mammals and fish-eating bird hosts through the eating of raw or improperly cooked fish. In these hosts, the trematodes become sexually mature, produce eggs which are voided in the feces, and if these reach bodies of water containing suitable snail hosts, infect them and reproduce asexually. Eventually the snail sheds numerous swimming trematode larvae, called cercariae, which can infect many species of fish, in which they encyst and develop to the metacercarial stage, completing the life cycle. Although Vietnam has a high risk of FZT in humans (De et al., 2003; Dung et al., 2007), the information on the epidemiology of these infections in the country's fish is limited. The World Health Organization (1995, 2004) has estimated that the number of people currently infected in south-east Asia with FZT exceeds 18 millions. This includes the liver flukes (*Opisthorchis viverini*, and *Clonorchis sinensis*) which are a major public health problem (Sripa et al., 2003), and the twenty-three species of intestinal flukes which have been reported from man, especially in south-east Asia (Chai, 2007). Recently, infections with zoonotic trematodes in Vietnamese cultured and wild-caught fish from the Mekong Delta have been discovered (Thu et al., 2007; Thien et al., 2007), raising food safety concerns.

In Vietnam, as elsewhere, fry from hatcheries are distributed to nurseries which grow the fish to the juvenile stage. Fry in hatcheries are grown in a protected and highly controlled environment while the nurseries are typically earthen ponds with high exposure to various external environmental factors, i.e. faecal contamination from various animals and human activities. The juveniles are marketed widely to farms which in grow-out ponds produce the end product, market fish for consumers. The fry can be produced year round although the main

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spawning season is from April to September in the Mekong Delta region. Juveniles are produced throughout the year.

An important need for the development of effective control interventions and preventive management practices for aquaculture is greater knowledge of the epidemiology of FZT in the fish production chain. To contribute to this goal, a series of investigations are being carried out in the important Mekong Delta production areas to assess the status of FZT in cultured fish and to identify important risk factors. The initial investigations on grow-out fish have been reported by Thien et al., (2007). Chi et al. (2008) also reported a high prevalence of FZT in juveniles in cultured fish in the northern province of Nghe An. This study was conducted to examine further this problem in the production of fry and juveniles in the Mekong Delta. The results of this investigation are reported here.

2. Materials and methods

2.1. Survey areas and study design

The study was carried out in Tien Giang, Can Tho, Vinh Long and Dong Thap provinces in the Mekong Delta of Vietnam where fish fry and juvenile production in aquaculture are highly developed (Fig. 1). Typically fish fry are produced in hatcheries and then transferred to nurseries when approximately 2 days of age (river catfish), 1 week (giant gouramy) or 3 days old for the other fish species included in this study. The juveniles are grown to market size over a period of 4–12 weeks, depending on the stocking density and the fish species, and are then widely distributed geographically to grow-out farms where they are fed to commercial market size. The hatcheries and nurseries studied were selected randomly from a list provided by the Departments of Agriculture and Rural Development in each of the four provinces.

Table 1
Species of fish fry and juveniles collected from hatcheries and nursery ponds located in Tien Giang, Vinh Long, Can Tho and Dong Thap provinces in the Mekong Delta, Vietnam (2007–2008).

Fish species	Number of hatcheries[a]	Number of nurseries[a]
Climbing perch/*Anabas testudineus*	22	32
Common carp/*Cyprinus carpio*	18	41
Giant gouramy/*Osphronemus gourami*	14	18
Grass carp[b]/*Ctenopharyngodon idellus*	18	41
Hybrid catfish/*Clarias* sp.	17	33
Kissing gouramy/*Helostoma temmincki*	21	32
Mrigal/*Cirrhinus mrigala*	18	41
Pacu[b]/*Colossoma macroponmum*	21	32
Red tilapia/*Oreochromis* sp.	31	42
River catfish/*Pangasianodon hypophthalmus*	34	82
Silver barb/*Puntius gonionotus*	19	32
Silver carp/*Hypophthalmichthys molitrix*	18	41
Snakeskin gouramy/*Trichogaster pectoralis*	21	32
Tilapia/*Oreochromis niloticus*	30	40

[a] An individual hatchery or nursery often produced more than 1 fish species.
[b] Fry of grass carp and pacu were produced outside the 4 provinces, but was transported to nurseries in 1 or more of the 4 study provinces.

A cross-sectional study for FZT infection in fry was performed with a single sampling of fry of 14 fish species (total of 302 samples, Table 1) from the Tien Giang, Can Tho, Vinh Long, and Dong Thap provinces during the period of May to October 2007, the season for spawning of the major freshwater fish in southern Vietnam.

Fig. 1. The map shows the location of the four study provinces of Tien Giang, Can Tho, Vinh Long and Dong Thap in the Mekong Delta.

Table 2
The prevalence and density of *Haplorchis pumilio* metacercariae in catfish juveniles.

Sampling period	Total number fish infected/number examined (%)	Mean density (metacercariae/100 g) (SD)
River catfish		
June–November 2007	0/82 (0)	0
January 2008	3/82 (3.7)	0.3 (0.1)
March 2008	1/82 (1.2)	0.2 (0.02)
May 2008	0/82 (0)	0
Hybrid catfish		
June–November 2007	0/33 (0)	0
January 2008	4/33 (12.1)	2.7 (1.6)
March–May 2008	2/33 (6.1)	0.9 (0.2)

The number of nurseries sampled for each of the 14 species of juvenile fish is listed in Table 1. Prevalence surveys for FZT in these juveniles were conducted in 2 or more of the 4 provinces during both the wet season (June 2007 to January 2008) and the dry season (March to May 2008); sampling dates are indicated in Table 2. For hybrid catfish juveniles (*Clarias* sp. = *Clarias macrocephalus* × *C. gariepinus*), 3 cross-sectional surveys were carried out in Vinh Long, Can Tho and Tien Giang provinces; river catfish (*Pangasianodon hypophthalmus*) juveniles were sampled 4 times in all four provinces. The remaining 12 fish species from the 4 provinces were sampled once in the wet season and a second sampling was made during the dry season (Table 3). During the time interval between the first and second sampling, some of the original 152 farms producing juveniles terminated their operations (31.7%), therefore, new farm replacements were randomly selected from the list of the total farms as described above. It should be noted that a single nursery commonly produced more than one fish species, however, each fish species was always cultured in separate, individual ponds.

2.2. Description of hatcheries

Fish broodstock used for fry production are kept in earthen ponds and subjected to induced breeding techniques, except for tilapia that are naturally breeding in earth ponds. A single hatchery may produce more than one fish species. Fertilized fish eggs are then incubated in water tanks with running water and occasional aeration. Tanks are typically cleaned and disinfected between fry production cycles. The water used is obtained from river, canal or wells, and stored in cisterns before use. When properly managed, snails and other aquatic animals or vegetation are not introduced into the tanks. Eggs of tilapia and red tilapia are collected and kept in plastic trays for hatching. After hatching (2–7 days, depending upon species) the fry are transferred to earthen ponds for growing to the juvenile size. In the case of giant gouramy, nursing ponds may be lined with plastic sheets.

2.3. Description of nursery ponds

The earthen nursing ponds for all fish species investigated were typically located very near the sources of water supplies, i.e. canals, rivers or wells. Most nursery ponds have surface areas of 300–3000 m^2. Prior to stocking of fry, the ponds are drained, limed normally with 7–12 kg $CaCO_3$/100 m^2, and dried for 4–7 days, depending on the pond condition, to kill wild fish, snails and other aquatic animals prior to restocking with fry. Liming also helps to disinfect the pond and maintain an alkaline pH that facilitates growth of plankton, an important food item for the fry. Water is normally pumped through fine mesh filters into the ponds to a depth of 1.0–1.5 m to prevent wild fish, aquatic plants and other organic substances from entering the ponds. The ponds are subjected to vegetation removal at pond preparation and during the nursing period.

The economically important fish species, catfish and giant gouramy, are nursed in somewhat different pond systems. Giant gouramy nurseries normally maintain the fry for 3 weeks in ponds lined with plastic, after which they are transferred to earthen ponds and grown to a length of about 6 cm. The size of these nursing ponds varies from 200 to 1000 m^2. In contrast, river catfish nurseries vary from 500 to 10,000 m^2 in area, although most are larger than 2000 m^2. The majority of catfish nurseries produce juveniles with a size of 10 to 12 cm, although this may vary depending upon production times, which can range from 3 weeks to 12 weeks.

2.4. Collection and examination of fry and juveniles

In each hatchery, a 65-ml volume cup was used to sample the fry; this volume collected between 12,000 and 150,000 fry, depending on fish size. The fry were poured into a nylon bag and oxygen added before transport to the laboratory. Fry weights were recorded as the pooled weight of one cup (volume) of fry. The fry were kept in the plastic bag for a maximum of 2 days, with new oxygen added if required, before being subjected to pepsin digestion to recover metacercariae.

For collection of juveniles, a total of 30 juveniles were collected by cast net and/or hand net from a nursery pond at each sampling. If the farm raised more than one fish species, a similar number of fish was collected for each species. The species juveniles were kept separately in a nylon bag and oxygen added before transport to the laboratory. Juvenile weights were recorded as the pooled weight of 30 juveniles. The juveniles were kept in the plastic bag or bucket for a maximum of 2 days with new oxygen added or new water exchanged if required before being subjected to pepsin digestion to recover metacercariae as described by Thien et al. (2007).

The fry were ground using pestle and mortar, and the juveniles were ground by using an electric grinder. After digestion and washing, the sediment was examined for the presence of metacercariae and enumerated. The number of metacercariae from each pool was recorded and the density expressed as number of metacercariae/100 g of fry or juveniles.

Encysted metacercariae, which could not be readily identified morphologically were excysted by either physical pressure (pressing on the coverslip) or by placing them in trypsin digestion fluid (0.4% sodium bicarbonate and 1.0% trypsin in 0.85% NaCl, pH 7.2) (Komiya, 1965) for 20 to 30 min at room temperature until they emerged from the cyst. The metacercariae were examined under a compound microscope and identified using morphological criteria described in Pearson and Ow-Yang (1982), Scholtz et al. (1991) and Yamaguti

Table 3
The prevalence and density of FZT metacercariae in juveniles of non-catfish species sampled in wet and dry seasons (2007–2008).

Juvenile species	2007 (wet season)			2008 (dry season)	
	Nurseries sampled	Nurseries infected (%)	Mean density[a] (SD)	Nurseries infected (%)	Mean density[a] (SD)
Climbing perch	32	11 (34.4)	429.8 (381.6)[b]	8 (25.0)	27.9 (17.0)[a]
Common carp	41	7 (17.1)	20.0 (12.6)	2 (4.9)	0.9 (0.2)
Giant gouramy	18	6 (33.3)	47.3 (34.8)	4 (22.2)	0.9 (0.4)
Grass carp	41	5 (12.2)	63.6 (24.7)	1 (2.4)	0.5 (0.1)
Kissing gouramy	32	6 (18.8)	13.7 (11.4)	4 (12.5)	1.7 (0.6)
Mrigal	41	3 (7.3)	32.0 (8.9)	2 (4.9)	1.8 (0.4)
Pacu	32	2 (6.3)	19.7 (6.4)	1 (3.1)	1.8 (0.3)
Red tilapia	42	2 (4.8)	1.7 (0.4)	2 (4.8)	0.6 (0.1)
Silver barb	32	6 (18.8)	15.6 (8.9)	3 (9.4)	4.2 (1.7)
Silver carp	41	6 (14.6)	46.6 (21.2)	2 (4.9)	5.9 (1.6)
Snakeskin gouramy	32	1 (3.1)	2.9 (0.5)	1 (3.1)	2.1 (0.4)
Tilapia	40	1 (2.5)	2.6 (0.4)	1 (2.5)	0.6 (0.1)

[a] Number of metacercaria/100 g.
[b] Significant seasonal differences in density of fishborne zoonotic metacercariae within same fish species (<0.05).

(1971). The work described in this paper has been conducted in accordance with the World Medical Association International Code of Medical Ethics (revised October 2006) (*http://www.wma.net/e/policy/c8.htm*).

2.5. Data analyses

Microsoft Office Excel 2007 and SPSS software (Statistical Package for Social Sciences version 15; SPSS Inc., Chicago, Illinois) were used for data entry and statistical analyses.

The Chi-square test was used to compare the difference of FZT prevalence between seasons. The comparison of FZT densities between 2 seasons and among the fish species was analyzed by one-way analysis of variance (ANOVA). A value of $P<0.05$ was considered significant.

3. Results

3.1. Prevalence and density of FZT metacercariae in hatchery and nursery fish

Metacercariae were not found in any of the 302 fry samples representing 14 different fish species in the 4 provinces in the Mekong Delta (Table 1). Only 4 nurseries producing river catfish juveniles were infected and prevalences ranged from 1.2 (March 2008) to 3.7% (January 2008) (Table 2). A similar infection pattern was seen in hybrid catfish nurseries for the January (12.1%) and March–May (6.1%) samplings (Table 2).

The FZT prevalence in nurseries culturing the non-catfish species was not significantly higher in the wet season than in the dry season (Table 3), except for red tilapia, tilapia and snakeskin gouramy producers, which did not exhibit a significant seasonal change in FZT prevalence. The combined seasonal nursery FZT prevalence showed that overall, climbing perch and giant gouramy juvenile producers had the highest prevalence (29.7% and 27.8%, respectively), while those producing river catfish had the lowest prevalence (1.2%) (Tables 2 and 4).

The diversity of FZT species found in fish from infected nurseries is shown in Table 4. *Haplorchis pumilio* occurred in all juvenile species, while *H. taichui* occurred only in giant gouramy and kissing gouramy, and *Stellantchasmus falcatus* was only found in giant gouramy (Table 4). River and hybrid catfish were infected only with *H. pumilio*. It should be noted that the metacercariae of the liver flukes, *O. viverrini* and *C. sinensis*, were not found.

Table 4
The prevalence of FZT metacercariae species in the non-catfish juvenile species collected in the wet and dry seasons.

Juvenile species	Nurseries sampled	Nurseries infected (%)	Species of FZT metacercariae detected in the wet and dry seasons							
			H. p[a]		*H. t*[b]		*C. f*[c]		*S. f*[d]	
			Wet	Dry	Wet	Dry	Wet	Dry	Wet	Dry
Climbing perch	64	19 (29.7)	+	+	−	−	+	+	−	−
Common carp	82	9 (11.0)	+	+	−	−	+	−	−	−
Giant gouramy	36	10 (27.8)	+	+	+	+	−	−	+	+
Grass carp	82	6 (7.3)	+	+	−	−	+	−	−	−
Kissing gouramy	64	10 (15.6)	+	+	+	+	+	−	−	−
Mrigal	82	5 (6.1)	+	+	−	−	+	+	−	−
Pacu	64	3 (4.7)	+	+	−	−	−	−	−	−
Red tilapia	84	4 (4.8)	+	+	−	−	−	−	−	−
Silver barb	64	9 (14.1)	+	+	−	−	+	−	−	−
Silver carp	82	8 (9.8)	+	+	−	−	+	−	−	−
Snakeskin gouramy	64	2 (3.1)	+	+	−	−	−	−	−	−
Tilapia	80	2 (2.5)	+	+	−	−	−	−	−	−

[a] *H. p*: *Haplorchis pumilio*.
[b] *H. t*: *Haplorchis taichui*.
[c] *C. f*: *Centrocestus formosanus*.
[d] *S. f*: *Stellantchasmus falcatus*.

The mean density of FZT metacercariae in all fish species was low, and only exceeded 100/100 g in climbing perch (Table 3). The density of FZT metacercariae in climbing perch juveniles was significantly higher in the wet season than in the dry season (429.8/100 g vs 27.9/100 g of fish, $P<0.05$). The density of FZT metacercariae in juveniles of the other fish species did not vary significantly with season.

4. Discussion

The results from this investigation confirm and extend the observations reported by Chi et al. (2008) that juveniles produced in the nursery stage of aquaculture in Vietnam are highly infected with fishborne zoonotic parasites (FZT). These findings are of great importance because of the wide dissemination of juveniles to fish farms that grow the fish to commercial size. The acquisition of infected juveniles by grow-out operators can negate efforts to implement good farming practices aimed at producing high quality and safe fish for consumption. Our findings will also affect the strategy of control interventions to prevent FZT infection in aquaculture as previous attempts to implement control programs in fresh water fish production have focused on the grow-out stage of the production chain (Khamboonraung et al., 1997; Guoqing et al., 2001).

The absence of infection in fish fry is not surprising since the eggs are hatched for the most part in cement or composite tanks in water that is filtered, which would exclude snails. Further, the fry are held in these tanks for only 2 to 7 days, depending upon species, which would constitute a relatively short exposure time. Juveniles, however, are grown in earthen ponds over a normally 1 to 3 month period, which is an interval sufficient to allow populations of snails to increase and become infected through exposure to trematode eggs shed into the ponds either directly or through surface water run-off. The major snail vector for heterophyid FTZ in Vietnam is probably *Melanoides tuberculata*, but this needs further confirmation (De et al., 2003; Chai, 2007).

The lower prevalence of FZT in catfish compared to other species of fish in the present study is in accordance with other studies carried out in the Mekong Delta (Thu et al., 2007; Thien et al., 2007) and may be due to the better management practices associated with commercial catfish production. The large nursery and grow-out ponds used for catfish production are subjected to frequent vegetation removal, which reduces snail habitat and may affect snail populations important for FZT transmission. Another factor may be that water entering the ponds is normally passed through screens which may exclude snails (e.g., *M. tuberculata*) although this is not yet confirmed. In comparison, the presence of vegetation is markedly greater in the nursery production of other fish species such as the giant gouramy. Further studies are needed to determine the importance of vegetation removal and water screening in the transmission of FZT.

The possibility that host-specific factors influence either susceptibility or resistance to FZT infection in fish (e.g. catfish) deserves study. In Vietnam, differences in FZT prevalence among different fish species, reared under similar production systems have been observed (Thu et al., 2007; Thien et al., 2007; Dung et al., 2008). However, there have been few experimental studies under controlled conditions to compare fish species for their innate differences in susceptibility to cercarial infection. Alternatively, these differences in fish species prevalence may be the result of either management practices or ecological behaviours of the fish, as suggested by Thu et al. (2007).

The seasonality of FZT prevalence observed in this study is in agreement with the data reported earlier (Thien et al., 2007) on the influence of wet and dry seasons on the FZT prevalence in grow-out systems in the Mekong Delta. How season influences snail and fish infections is unknown, but it is likely that the generally higher prevalence of FZT in the wet season is the result of a combination of factors. Increased rain and flooding lead to an increase of snail populations, the reduction in trematode egg mortality due to desiccation and an increase in the contamination of water bodies with host faeces (i.e. dogs, cats, pigs and humans) (Khamboonraung et al., 1997; Sithithaworn and Haswell-Elkins, 2003). Understanding seasonality and its causes will enhance the design of effective control strategies, as has been proposed for control of liver flukes in humans (Hinz et al., 1994; Khamboonraung et al., 1997).

Reservoir hosts such as dog, cats and pigs are also important risk factors in Vietnamese aquaculture (Anh et al., 2009). However, because of the infrequent presence of such animals at giant gouramy nurseries, and the considerable effort needed to determine the environmental FZT egg contamination that can be attributed to these hosts (Anh et al., 2009), their role was not included in this study. Follow up studies will need to be conducted to determine this before control strategies can be developed (WHO, 1995; Sen-Hai and Long-Qi, 2005; Long-Qi et al., 2005).

5. Conclusion

The investigation of the prevalence of fishborne zoonotic trematodes in the production chain for catfish and giant gouramy in the Mekong Delta demonstrated that a major risk of infection to juveniles occurs in the nurseries. The identification of the nursery stage in the fish production system as a "hot spot" for FZT transmission will assist in the development of intervention schemes to prevent FZT infection during the fish production cycle in both the Mekong Delta, and in aquaculture, generally. Among the interventions that have the potential to eliminate or reduce FZT transmission in nurseries are drug treatments of humans and reservoir hosts associated with the nursery to reduce environmental contamination with trematode eggs; adequate treatment of faeces if used for fertilizer for ponds and crop fields to kill trematode eggs; redirecting drainage from pig pens and human latrines away from ponds and canals to help prevent eggs from contaminating water bodies; reduction of snail populations in the ponds through vegetation and mud drying/removal after ponds are drained between production cycles, and the filtering of water to reduce immigration of snails and intake of eggs and cercariae. These approaches to control FZT transmission in aquaculture are now under going evaluation by the authors in Vietnam.

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ORIGINAL PAPER

Prevalence of fishborne zoonotic parasites in important cultured fish species in the Mekong Delta, Vietnam

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Abstract A seasonal investigation on the occurrence of fishborne zoonotic trematodes (FZT) in economically important mono-cultured hybrid catfish and giant gouramy was conducted in the Mekong Delta of Vietnam. Fish from carp poly-culture and intensive small-scale integrated vegetable–aquaculture–animal husbandry farming (VAC) systems were also examined. No FZT metacercariae were found in any mono-cultured hybrid catfish. FZT metacercariae were common, however, in fish from the other three systems: All metacercariae belonged to the *Heterophyidae* family of trematodes, *Haplorchis pumilio*, *H. taichui*, *Centrocestus formosanus* and *Stellantchasmus falcatus*. The FZT prevalence was 1.7% in mono-cultured giant gouramy, 6.6% in carp from the poly-culture and 3.0% in fish raised in the VAC system. *H. pumilio* was the most common FZT species constituting more than 58.0% of all metacercariae recovered. The prevalence of infection was significantly higher in the flooding season compared to the non-flooding season for both giant gouramy and fish reared in the VAC system. FZT intensity was greatest in fish from carp poly-culture, particularly in the flooding season. The results indicate that certain fish production systems are at risk for FZT, and control approaches will benefit from understanding these risk factors.

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Introduction

Humans and domestic animals are commonly infected by helminth parasites transmitted from fish, especially in Asia (WHO 1995, 2004; Chai et al. 2005). Cultural food behaviours, such as preferences for raw fish, are important risk factors for human infection with fishborne trematodes (FZT; WHO 2004; Chai et al. 2005). The liver fluke *Clonorchis sinensis* may infect more than 20 million people with an estimated 600 million people at risk (Keiser and Utzinger 2005). Human infections with *Opisthorchis viverrini* in Thailand, Laos and Vietnam are undetermined but are estimated to be in the millions (Sripa et al. 2003). Additionally, human prevalence of intestinal flukes belonging mainly to the Heterophyidae and Echinostomatidae are probably even more prevalent in Southeast Asia, but reliable estimates are not available (Chai et al. 2005). However, more than 50 intestinal FZT species are reported from humans worldwide and at least 13 species from man in Southeast Asia (Waikagul 1991; Chai et al. 2005). Of great significance for this parasite zoonosis is the fact that worldwide fish are increasingly produced in aquaculture, particularly in Southeast Asia where aquaculture is expanding rapidly and fish are a major source of protein.

Current information on the prevalence and species diversity of FZT suggests that these parasites are important public health problems in Vietnam. A recent review of available data revealed that FZT are widespread in Vietnam (De et al. 2003). *C. sinensis* has been reported from nine Northern provinces with human infection rates ranging from 0.2 to 26.0%, whereas *O. viverrini* is reported to occur in at least three Southern provinces. Reports from Vietnam of intestinal FZT in humans are lacking, although they are highly prevalent elsewhere in Southeast Asia (De et al. 2003); this may be due, at least in part, to the difficulty in distinguishing between the eggs of liver and heterophyid intestinal flukes in human faecal surveys (Ditrich et al. 1990). This raises the question of what is the precise distribution and prevalence of different FZT species in Vietnam.

The study reported here had, then, as its genesis the interest in assessing the risk from FZT in Vietnam's major aquaculture systems because of their importance to the country's food supply and economy. Freshwater fish production in Vietnam has increased from 41,750 tons in 1962 to 390,000 tons in 2005 a 9.3-fold increase (Keiser and Utzinger 2005). Therefore, reliable data on FZT prevalence, distribution and species diversity are needed to develop an accurate assessment of the food safety risk and economic impact of FZT on the nation's aquaculture sector.

Before this investigation, only one other research study on FZT in Vietnamese cultured fish had been reported (Thu et al. 2007), which demonstrated that FZT are common in fish from the Mekong Delta, a major aquaculture region. The occurrence of FZT in giant gouramy (*Osphronemus gourami*) and hybrid catfish (*Clarias* sp.) is of special concern because of their economic importance to the domestic fish supply. Seasonal prevalence studies for FZT in these cultured fish species and in other common species were therefore carried out in various aquaculture systems in Tien Giang province and in Can Tho city district, in the Mekong Delta, in 2005 and 2006. These investigations included aquaculture systems that varied in intensity of production, mono- vs poly-culture, and utilization of animal manures for fish feed and pond fertilization.

Materials and methods

Sites and aquaculture systems studied

The study sites of Tien Giang province and Can Tho city are centrally located in the Mekong Delta of Vietnam where fish production in aquaculture is highly developed for both domestic as well as export markets. Two cross-sectional studies on FZT prevalence in freshwater fish were carried out in these two provinces during the Mekong River flooding season of September 2005 to February 2006 and the non-flooding season of February to August 2006. In the flooding season, caused by overflow of the Mekong River and its tributaries, water availability in the delta for aquaculture is increased. In contrast, in the non-flooding season, because of a shortage of water supply in some areas, e.g. in Cho Gao district of Tien Giang province, water in the ponds become stagnant, so most of the fish are harvested at the end of the flooding season. Consequently, the acreage devoted to ponds is greater in the flooding season and is lowest in the first 2 months of the non-flooding season. The flooding season is also conducive to immigration of wild fish and also other aquatic animals, e.g. snails into fish ponds from the river, rice fields and canals in the Mekong Delta. The influence of these seasonally dependent events on FZT epidemiology is unknown.

Fish from the following major aquaculture pond production systems were studied: (a) mono-culture systems producing hybrid catfish (*Clarias* sp.) located in Can Tho city, (b) mono-culture farms producing giant gouramy (*O. gourami*) located in Tien Giang province, (c) carp poly-culture (multiple species) ponds located in Tien Giang province, which included culture of silver carp (*Hypophthalmichthys molitrix*), grass carp (*Ctenopharyngodon idellus*), common carp (*Cyprinus carpio*) and Indian carp (*Mrigal* and *Labeo rohita*) and (d) VAC systems, which are small-scale integrated vegetable–aquaculture–animal husbandry farming systems (Vuon=garden, Ao=pond, Chuong=pigsty) located in Tien Giang province; in this system, 13 different fish species are cultured: river catfish (*Pangasianodon hypophthalmus*), hybrid catfish, tilapia (*Oreochromis niloticus*), kissing gouramy (*Helostoma temmincki*), giant gouramy, pacu (*Colossoma macroponmum*), three spot gouramy (*Trichogaster trichopterus*), climbing perch (*Anabas testudineus*), silver barb (*Puntius gonionotus*), common carp, silver carp and snakeskin gouramy (*Trichogaster pectoralis*). The mono-culture fish production systems represent major commercial systems for production of high-value hybrid catfish and giant gouramy, whereas the carp poly-culture and the VAC systems are important household-based production systems that provide both local income and a supply of affordable protein mainly for local consumption. Hybrid catfish are typically fed with a raw fish by-product blended with rice bran. Giant gouramy culture is based on feeding by-products of fresh vegetables and commercial pelleted fish feed.

Sampling of fish

For the first sampling (flooding season), 16 carp poly-culture farms and 60 farms of each of the other three culture systems (196 farms in total) were selected from a list of farms provided by the Department of Agriculture and Rural Development (Can Tho city) and by the Department of Fisheries (Tien Giang province). Farms were numbered and selected using a random table. Fingerlings were stocked only once at each farm, mainly in April. The grow-out period was about 4 months for hybrid catfish mono-culture, 12 months for carps and fish raised in the VAC systems and at least 18 months for giant gouramy. Ten fish were collected from each farm culturing giant gouramy and hybrid catfish by cast nets yielding a total of 600 fish from each of these two culture systems (Table 1). Ten fish were also collected from each carp poly-culture and VAC poly-culture systems with fish collected representing the diversity of species cultured (Tables 2 and 3). The second sampling (non-flooding season) was carried out from February to August 2006 and included to detect any seasonal differences in FZT transmission. To achieve this, the farms included in the first sampling (flooding season, 2005) were again visited and fish collected. During the time interval between the first and second sampling, a number of farms had terminated fish culture and, therefore, new farms were randomly selected from the previously described list of farms. The new farms selected for the second sampling included 29 giant gouramy, 35 hybrid catfish, 31 VAC and ten carp farms.

Examination of fish for metacecariae of FZT

The collected fish were transported alive and kept at cold storage (4°C) in the laboratory until they were examined for metacercariae, normally within 2 days of collection. The length and weight of fish were recorded in the laboratory. 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 (e.g. giant gouramy) were divided into five sub-samples for digestion. After digestion and washing, the sediment was examined for the presence of metacercariae. Encysted metacercariae, which could not easily be identified morphologically, were excysted by either physical pressure (pressing on the coverslip) or by placing them in trypsin digestion fluid (0.4% sodium bicarbonate and 1.0% trypsin in 0.85% NaCl, pH 7.2; Komiya 1965) for 20 to 30 min at room temperature until they emerged from the cyst. The metacercariae were examined under a compound microscope and identified using morphological criteria described in (Kaewkes 2003), Pearson and Ow-Yang 1982, Schell 1970, Scholtz et al. 1991, Velasquez 1973 and Yamaguti 1971.

Data analysis

The Microsoft Office Excel 2003 and SPSS software (Statistical Package for Social Sciences version 10; SPSS, Chicago, IL) for Windows 11.5 were used for data entry and statistical analyses. Linear regression was used to investigate the association between weight or length and the intensity of metacercariae infection in individual fish species. The one-way analysis of variance was used to compare the difference of intensity between seasons and among species. The Chi-squared test was used to compare the difference of FZT prevalence between aquaculture systems, seasons and fish sizes. A value of $P<0.05$ was considered significant.

Results

Prevalence and intensity of FZT metacercariae in mono-culture hybrid catfish and giant gouramy

Metacercariae were not found in any of the 1,200 mono-cultured hybrid catfish collected and examined in both the flooding and non-flooding seasons. FZT were observed in giant gouramy, and the overall prevalence of metacercariae (combining data from the two seasons) was 1.7% (Table 1). The prevalence of infection was not associated with fish size ($P>0.05$), but a significantly higher prevalence was observed in fish collected during the flooding season (3.0%) compared to the non-flooding season (0.3%; $P<0.001$; Table 1). *Haplorchis pumilio* was the most common FZT species found, comprising 91.0% of the total meta-

Table 1 Prevalence of FZT infection in fish from different aquaculture systems during the flooding and non-flooding season in the Mekong Delta, Vietnam

Aquaculture systems	Number of ponds examined/season	Number of fish/season	FZT prevalence (%)		
			Flooding season (Sept 05–Feb 06)	Non-flooding season (Feb–Aug 06)	Combined prevalence data
Hybrid catfish mono-culture	60	600	0 (0.0)	0 (0.0)	0 (0.0)
Giant gouramy mono-culture	60	600	18 (3.0^{a})	2 (0.3^{b})	20 (1.7^{c})
VAC poly-culture	60	600	33 (5.5^{a})	3 (0.5^{b})	36 (3.0^{d})
Carp poly-culture	16	160	13 (8.1)	8 (5.0)	21 (6.6^{e})

^{a,b} Indicates significant differences ($P<0.001$)
$^{c,d \text{ and } d,e}$ Indicates significant differences ($P<0.05$)

Table 2 Species of FZT metacercariae in fish from integrated fish-livestock (VAC) systems

Fish name	Number of fish F/NF	Infected fish: F/NF	Number of fish infected with different FZT species in F and NF season				
			Hp[a]: F/NF	*Ht:* F/NF	*Cf:* F/NF	*Sf*: F/NF	U: F/NF
River catfish (*Pangasianodon hypophthalmus*)	205/209	7/0	3/0	0/0	1/0	0/0	6/0
Hybrid catfish (*Clarias* sp.)	111/171	12/0	11/0	0/0	0/0	0/0	9/0
Tilapia (*Oreochromis niloticus*)	113/95	0/0	0/0	0/0	0/0	0/0	0/0
Kissing gouramy (*Helostoma temmincki*)	71/24	5/0	0/0	1/0	0/0	0/0	4/0
Giant gouramy (*Osphronemus gourami*)	34/28	2/0	1/0	0/0	0/0	1/0	0/0
Red tilapia (*Oreochromis* sp.)	27/34	0/1	0/ 1	0/0	0/0	0/0	0/1
Pacu (*Colossoma macroponmum*)	10/23	0/0	0/0	0/0	0/0	0/0	0/0
Three-spot gouramy (*Trichogaster trichopterus*)	12/7	2/ 1	2/0	0/0	0/0	0/0	2/1
Climbing perch (*Anabas testudineus*)	13/0	5/0	5/0	0/0	1/0	0/0	3/0
Silver barb (*Puntius gonionotus*)	2/4	0/0	0/0	0/0	0/0	0/0	0/0
Common carp (*Cyprinus carpio*)	0/5	0/1	0/1	0/0	0/0	0/0	0/0
Silver carp (*Hypophthalmichthys molitrix*)	1/0	0/0	0/0	0/0	0/0	0/0	0/0
Snakeskin gouramy (*Trichogaster pectoralis*)	1/0	0/0	0/0	0/0	0/0	0/0	0/0
Total	600/600	33/3	22/2	1/0	2/0	1/0	24/2

[a] *Hp*, *Haplorchis pumilio*, *Ht H. taichui*, *Cf Centrocestus formosanus*, *Sf Stellantchasmus falcatus*, *U* unknown, *F* flooding, *NF* non-flooding

cercariae recovered and were found in most infected fish (15 of 20). *Stellantchasmus falcatus composed* 4.7% and *H. taichui* 4.3% of the metacercariae recovered and occurred in only a few of the fish. Multiple infections occurred in only two fish, one with both *H. pumilio* and *S. falcatus* and the second with both *H. taichui* and *S. falcatus*. The intensity of infection with metacercariae was not affected by the weight and length of the fish ($P>0.05$). Although the average FZT intensity for all metacercariae species in infected giant gouramy appeared to be greater in fish sampled in the flooding season (21.8±28.3) compared to the non-flooding season (9.3±8.5), the difference was not significant ($P>0.05$).

The prevalence and intensity of FZT in the integrated vegetable–aquaculture–animal husbandry system

The overall FZT prevalence in the VAC-cultured fish was 3.0%, which was significantly higher than the prevalence observed in fish raised in mono-culture systems (e.g. giant gourmay, Table 1). The prevalence of metacercariae was significantly higher in the flooding season compared with non-flooding season (5.5 vs 0.5%, $P<0.001$; Table 1). River catfish, hybrid catfish, kissing gouramy, giant gouramy and climbing perch were only found infected in the flooding season (Table 2).

The prevalence of infection varied greatly between fish species raised in the VAC system (Table 2). Combining both seasons, climbing perch had a higher prevalence (5 of 13, $P<0.05$), than hybrid catfish (12 of 282), kissing gouramy (5 of 95), red tilapia (1 of 61), giant gouramy (2 of 62) and river catfish (7 of 414). No metacercariae were found in tilapia (*O. niloticus*; $n=208$), pacu (*C. macroponmum*; $n=33$), silver barb (*P. gonionotus*; $n=6$), silver carp ($n=1$) and snakeskin gouramy (*T. pectoralis*; $n=1$). *H. pumilio* was the most common FZP species found in VAC fish (75.2% of the total metacercariae recovered), followed by *Centrocestus formosanus* (3.1%), *H. taichui* (0.3%) and

Table 3 Species of FZT metacercariae in fish from the carp poly culture system

Common name	Number of fish F/NF	Infected fish: F/NF	Number of fish infected with different FZT species recovered in F and NF season				
			Hp[a]: F/NF	*Ht:* F/NF	*Cf:* F/NF	Sf: F/NF	U: F/NF
Grass carp (*Ctenopharyngodon idellus*)	39/65	0/4	0/4	0/0	0/1	0/0	0/0
Silver carp (*Hypophthalmichthys molitrix*)	51/51	6/0	6/0	1/0	0/0	0/0	5/0
Common carp (*Cyprinus carpio*)	41/40	4/4	2/2	1/0	4/3	0/0	4/0
Indian carps (*Mrigal* and *Labeo rohita*)	29/4	3/0	3/0	0/0	0/0	0/0	2/0
Total	160/160	13/8	11/6	2/0	4/4	0/0	11/0

[a] *Hp Haplorchis pumilio*, *Ht H. taichui*, *Cf Centrocestus formosanus*, *Sf Stellantchasmus falcatus*, *U* unknown, *F* flooding, *NF* non-flooding

S. falcatus (0.1%). An unidentified metacercariae was found in 21.3% of fish examined and is designated MD-1 (Fig. 1). The morphological characteristics of MD-1 were similar to those of *H. pumilio*, but hooklets on the ventral sucker could not be identified. In the VAC system, for all fish species combined, smaller fish (less than 100 g) had a higher prevalence of infection compared to larger fish (≥100 g; 6.4 vs 0.9%; $P<0.05$). However, when comparisons were made within individual fish species, the difference was significant only between small river catfish and larger river catfish (5.9 vs 0.9%; $P<0.05$), and the small hybrid catfish and large hybrid catfish (9.4 vs 0.6%; $P<0.001$). Fish infection intensity in the VAC system was not significantly affected by weight and length of fish species ($P>0.05$). The overall mean FZT intensity (±SD) of all fish species was 55.6 (±91.8) metacercariae/100 g in the flooding season ($n=33$) and 19.7(±12.8) metacercariae/100 g in the non-flooding season ($n=3$; $P>0.05$). The comparison among fish species collected in the flooding season showed that the intensities of all FZT species in climbing perch were significantly greater in comparison to hybrid catfish (135.1 vs 24.7, $P<0.05$). For individual FZT species, *H. pumilio* intensity in river catfish (139.9) was significantly higher than that for hybrid catfish in the flooding season (20.7; $P<0.05$). Overall, there were no significant differences among FZT species prevalence and intensity between flooding and non-flooding seasons.

The prevalence and intensity of FZT in carp poly culture system

The seasonally combined prevalence of FZT for the poly culture system was 6.6%, which was significantly higher than the prevalence for fish collected from the VAC systems and mono-culture systems ($P<0.05$, Table 1). The differences in FZT prevalence between individual fish species in carp poly-culture were not significant, however. The dominant species of metacercariae in the carp poly-culture was *H. pumilio*, representing 58.6% of the total metacercariae recovered, followed by *C. formosanus* (12.0%), *H. taichui* (1.2%) and MD-1 (28.1%; Fig. 1). In the carp poly-culture system, the occurrence of metacercariae varied according to fish size. Small fish (<100 g) of all four carp species combined had a higher prevalence compared to the larger fish of those species (≥100 g) (12.5 vs 3.7%, $P<0.05$). For the individual fish species, only small grass carp had a higher prevalence compared to the larger grass carp (12.5 vs 1.3%, $P<0.05$) Metacercariae in silver carp (11.8%) and Indian carp (10.3%), were present only in the flooding season, whereas common carp were infected in both flooding and non-flooding seasons (prevalence 9.8 and 10.0%, respectively). Grass carp were found infected only in the non-flooding season (6.2% prevalence; Table 3). However, season did not affect the prevalence of metacercariae for all fish species overall ($P<0.05$, Table 1). The intensity was not influenced by fish length and weight, except for the weight of Indian carp ($P<0.05$). The mean intensity (±SD) overall in carp poly-culture was 90.8 metacercariae/100 g of fish, which were significantly greater than that for giant gouramy from mono-culture (20.6 metacercariae/100 g of fish, $P<0.01$). The overall mean intensity in carp poly-culture (for all fish species) was significant higher in the flooding season (138.1/100 g) than in the non-flooding season (13.8/100 g; $P<0.01$). The intensity of *H. pumilio* was also significantly higher in the flooding season than in the non-flooding season (96.0 vs 10.3 metacercariae/100 g of fish; $P<0.05$; Fig. 2). The intensity of FZT in common carp, the only fish species that was found infected in both seasons, was not significantly different between flooding and non-flooding seasons.

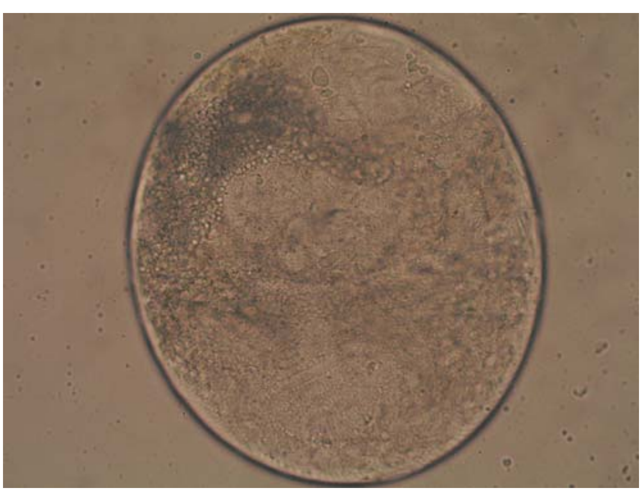

Fig. 1 Photograph of unidentified metacercariae (MD-1)

Discussion

In the present study, heterophyid zoonotic trematodes were found in all three infected fish culture systems. *H. pumilio* was considerably more common (58% of all metacercariae recovered) than the other FZT species found. *H. pumilio* is also commonly found in freshwater fish and humans in Thailand, Laos and China (Chai et al. 2005) and has been reported from river catfish, climbing perch and snake head (*Channa strita*) in An Giang province, Mekong Delta (Thu et al. 2007). It remains to be determined if *H. pumilio* is also the dominant FZT causing human infections. The prominence of this heterophyid trematode in freshwater fish is interesting and may be related to the availability of definitive hosts for this species. According to Pearson and

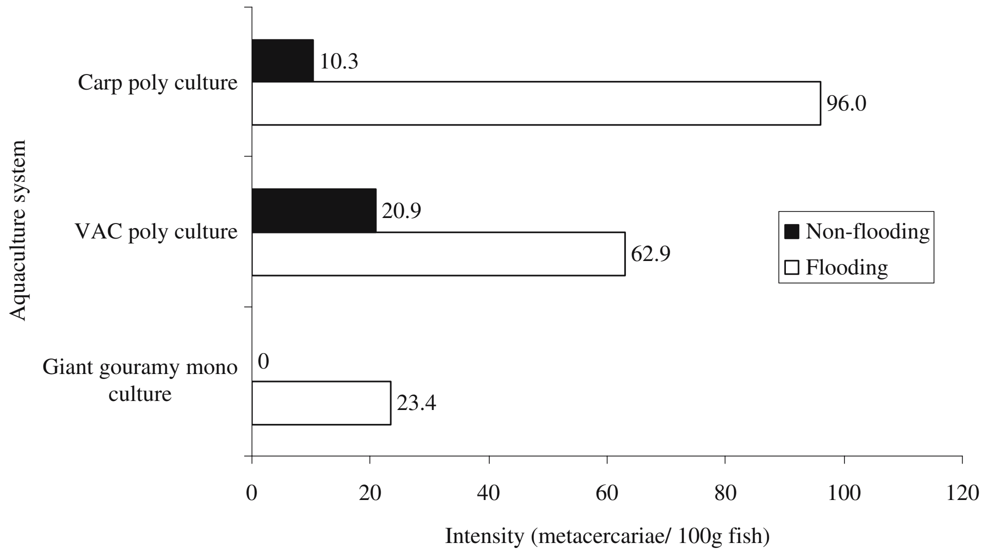

Fig. 2 Seasonal intensity of *Haplorchis pumilio* (per 100 g fish) in the three aquaculture systems

Ow-Yang 1982, fish-eating birds, in addition to a wide variety of mammals, are suitable hosts for *H. pumilio*. No comprehensive studies on the host range for this parasite in Southeast Asia have appeared, but the presence of herons, egrets and other fish-eating birds are commonly encountered in fish farm areas. Other possible hosts are domestic animals such as dogs, cats and pigs. The importance of these reservoir hosts in the epidemiology of FZT needs to be carefully assessed (Hinz et al. 1994).

The FZT prevalence in freshwater fish varied among the culture systems in these Mekong Delta districts. The influence of animal and human faecal waste utilization in fish farming seems clearly indicated because this practice is most frequent in the VAC and carp-poly-culture production systems which exhibited the highest FZT prevalence. This management practice has been identified by others as an important risk factor for FZT transmission (Naegel 1990; WHO 2006). The intensive mono-culture of hybrid catfish, which relies on commercial feed, rice bran and by-products of raw fish from fish processing factories, did not have FZT infections, and intensive giant gouramy mono-culture, which also utilizes commercial feed and vegetables rather than manure for fish feed and fertilizer, had a relatively low FZT prevalence compared to the VAC and carp production systems. These results strongly suggest that management practices are an important determinant of FZT transmission in aquaculture systems. However, this absence of infection could have other determinants, particularly differences between fish species in susceptibility to FZT. Arizono et al. (2005) failed to find metacercariae in *Clarias fuscus*, and in our study, no FZT were found in intensively mono-cultured hybrid catfish; therefore, catfish may not be as highly susceptible to FZT infection as other fish species such as carps. Catfish is listed as a host in China for *C. formosanus* and *Haplorchis yokogawai* by Yu and Xu 2005, and in this study, hybrid catfish from the VAC systems had an FZT prevalence of 4.3%, evidence that genetically based resistance is less a factor in infection than risk factors associated with pond management. It remains for further risk assessment research to determine what factors are important in FZT epidemiology in aquaculture.

The fish species in the carp poly-culture, which belong to the family of *Cyprinidae*, are considered the most suitable intermediate hosts for FZT (WHO 1995; Sukontason et al. 1999). Of the four fish species examined from the carp poly-culture system, common carp was the most commonly infected host species, and grass carp was the least infected. This finding is in agreement with the observations reported by Lun et al. 2005, except that grass carp also had a high infection rate.

In the present study, the most frequently infected fish species in the VAC poly-culture system was climbing perch (*A. testudineus*). The reasons for its high infection prevalence are unknown, but are currently being investigated. The reasons for this deserve greater attention because its risk of infection may be determined by critical ecological aspects of this fish's behaviour. The physical association of this fish with the preferred pond sites of the vector snail may be a key factor because small fish such as *A. testudineus* may more frequently locate in shallow water where infected snails are more abundant and, therefore, be more exposed to cercariae, in contrast to larger fish, which are reported to prefer deeper water depths in ponds (Li 1982). An understanding of these aspects could provide ideas for designing interventions for prevention and control of FZT. Other potential determinants of infection deserving closer scrutiny is higher susceptibility to cercarial infection as a result of skin features, such as thinness, difference in scale structure, wormicidal activity of skin mucus and fish immune status (Chun 1964; Rhee et al. 1988; Lun et al. 2005). An influence by the latter condition could encourage research on immunization as a control tool.

Although *Tilapia nilotica* (*O. niloticus*) was found infected with FZT in Thailand, Laos and China (Chai et al. 2005) and infected *Tilapia mossambica* were found in Northern Vietnam (De et al. 2003), none the 208 tilapia from the VAC system examined in this study were infected. The reasons for this are unknown, but some of these fish species-specific factors may be important, and their elucidation could provide clues to the ecological and management factors that determine FZT transmission.

FZT intensity can vary widely among fish species, and hundreds to thousands of metacercariae can be present in a single fish, depending on species and ecological circumstances (Lun et al. 2005). However, the relationship of intensity to fish size, sex, age or ecology appears to be little explored. In the current study, a relationship to fish size was detected only in Indian carp; however, this was based on the examination of only three fish. Although we saw indications in all fish size classes of an association between metacercarial numbers and fish size, the relationship was not statistically significant. This may be because the production system allows only 4 to 18 months for the grow-out phase before harvest, yielding a fish population with too little age variation. Investigations into this aspect of the ecology of FZT transmission may be more fruitful if conducted on wild fish populations, which probably have a broader age range.

The marked seasonal variation in FZT prevalence between the fall (non-flooding) season and the spring (flooding) season is somewhat typical throughout Asia, especially in the transmission of liver flukes (Hinz et al. 1994; Rim 1990; Sithithaworn et al. 1997). According to (Sithithaworn and Haswell-Elkins 2003), fish have their highest metacercarial burden in winter because snail populations and fluke transmissions are low during the earlier winter dry season, and with the resumption of rain and high temperature in the May to September period, snail populations increase, and fluke eggs shed into the environment are more likely to be washed into water bodies (Long-Qi et al. 2004). These events lead to increasing snail infections and cercarial production, which results in the accumulation of fish metacercariae at about the time the rainy season ends in the late fall and winter (approximately November to March). Although there is no true "winter" in the Mekong Delta of Vietnam, the temperature is usually lowest during the flooding season, which is associated with heavy rainfall. The prevalence of metacercariae in all the systems studied here was lower in the period of February to August and higher in the period just after the end of the rainy season. The flooding season is also a very suitable period for the development of snails, e.g. *Melanoides tuberculatus*, an important first intermediate host (Sommerville 1982; Scholz 1999) for intestinal flukes and very much involved in the transmission of FZT such as *H. pumilio* and *Centrocestus* sp. This fits with a pattern in which rising snail populations and increased trematode egg contamination of ponds, stimulated by rising temperature and increased rainfall, results in cercariae being shed into the fish environment by late summer to early fall.

The results from this study extend our understanding on the geographic distribution of FZT in Mekong Delta of Vietnam. They also indicate that there is a low risk (if any) of acquiring FZT infection from consumption of monocultured hybrid catfish, while the other culture systems present varying levels of food safety risks. Further research on the ecology and epidemiology of these FZT in Mekong Delta is needed to understand the major risk factors of FZT transmission in important aquaculture fish species in Mekong Delta.

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