CHAPTER II
LITERATURE REVIEWS

2.1 Fish-borne zoonotic trematodes (FZT)

FZT belonging to the Opisthorchiidae and Heterophyidae families are important emerging and re-emerging pathogens causing liver and intestinal fluke diseases in human (WHO, 2004; Chai et al., 2005a). The life cycle of FZT involves humans and animals such as dog, cat, pig and fish-eating birds as final hosts. The FZT live and develop to adult flukes in the liver or intestines of the final host and produce eggs that are excreted into the environment through faeces. Humans and animals acquire the FZT infection through consumption of raw, inadequately cooked, dried, salted or pickled fish that harbor infective metacercariae stages. The World Health Organization (WHO, 1995; 2004) has estimated that the number of people currently infected in Southeast Asia with FZT exceeds 18 millions. This includes the liver flukes (*Opisthorchis viverrini* and *Clonorchis sinensis*) which are a major public health problem (Sripa et al., 2007) and the 23 species of intestinal flukes which have been reported from man, especially in Southeast Asia (Chai, 2007).

2.2 Biology of the fish-borne zoonotic trematodes

Generally, most of digenetic trematodes are hermaphroditic in which worms have both male and female reproductive organs. After fertilization, the zygote within the egg develops into a ciliated larva, the miracidium within the uterus. Embryonated eggs are released periodically and pass out with host excreta. In aquatic environment, eggs encounter snail intermediate hosts. After ingestion by appropriate snail intermediate hosts of either a freshwater or brackish snail, the miracidium escaped from an egg and transforms into a sporocyst which contains germinal cells. The germinal cells then asexually develop into a new larval type, redia, and later redia will asexually reproduce swimming larvae, cercariae that can leave the molluscan host. After that they shed their propulsive tails and encysted in their second intermediate hosts, the marine or freshwater fish. They are now called metacercariae, the infective stage. Within the cyst, the metacercariae have remained its potential to continue the development which will be completed when the cyst is consumed by their final hosts, fish-eating birds or mammals including humans. It is in the intestines of these definitive hosts that the fluke matures and reproduces eggs which are passed with feces into external environment. The life histories of several species of Heterophyidae have already been developed by different authors (Figure 2.1). The life cycle of heterophyid flukes is parallel with *Opisthorchis* sp., which has different intermediate hosts. Basically, heterophyid life cycle contains three different hosts; the most important first intermediate host is *Melanoides tuberculata* (Pearson and Ow-Yang, 1982; Hernandez et al., 2003; Saenphet, 2007), the other species are *Thiara juncae* (Velasquez, 1973a) and *Tarebia granifera* (Giboda et al., 1991a, 1991b; Kumchoo et al., 2005) the second intermediate hosts are freshwater or brackish water fish, which metacercariae are parasitized in different parts of fish depending on the species of parasites. The metacercariae of Heterophyidae may also encyst in various fish belonging not only to different species but also to different families such as *Cyprinus capio*, *Carassius auratus*, *Zacco platypus*, *Pseudorasbora parva*, *Ophicephalus striatus*, *Anabas testudineus*, *Anchoviella* sp., *Cirrhinus julleine*, *Cyclocheichthyes armatus*, *Cyclocheichthys apogon*, *Hampala dispar*, *Mugil* sp., *Mystacoleucus atridorsalis*, *Prioonobutis koilomotodon*, *Pseudopocryptes lancoelatus*, *Puntius gonionotus*, *Puntius leiacantus*, *Therapon jarbua*, *Rivulus harti* and *Synbranchus marmoratus* (Faust and Nishigori, 1926; Velasquez, 1973b; Hong et al., 1990; Waikagul, 1991, 1998; Srisawangwong et al., 1997; Wongsawad et al., 2000), the definitive hosts are reptiles, piscivorous avians and mammals, including human (Faust and Nishigori, 1926, 1998; Radomyos et al., 1994; Belizario et al., 2004). These phenomena indicate that heterophyid flukes have low specificity to the final host because they may develop partially or completely in both mammals and birds. The natural reservoir hosts of the parasites are, in most cases, dogs, cats and rats (Saenphet, 2007)

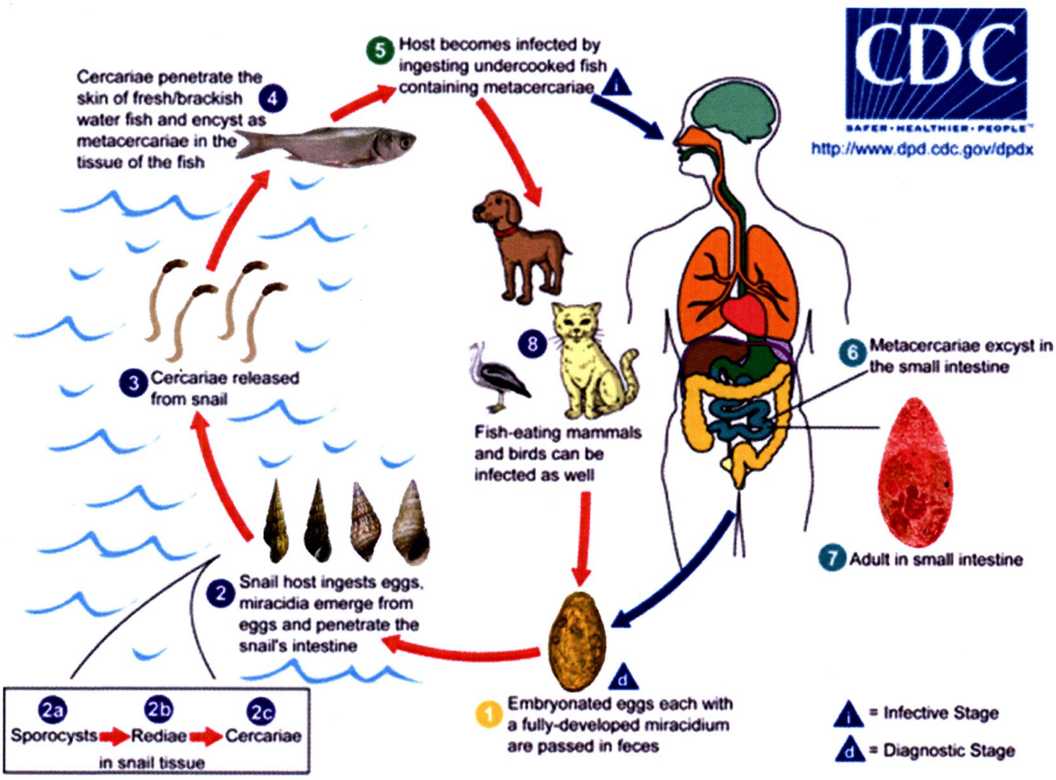

Figure 2.1 Life cycle of fish-borne zoonotic trematodes (minute intestinal fluke) (modified from Centers for Disease Control and Prevention, 2011)

2.3 Epidemiology of fish-borne zoonotic trematodes

2.3.1 Liver flukes

The liver flukes are a closely related group of trematodes belonging to the family Opisthorchiidae, and have similar life cycles and epidemiology (Table 2.1). Liver flukes have long been known to cause serious disease in Southeast Asia i.e. Thailand, Lao PDR, Cambodia and Vietnam. Cholangitis, cholelithiasis, pancreatitis, and cholangiocarcinoma are the major clinical problems, associated with the long chronic pattern of these infections. Although the extent of the problem is difficult to assess due to the lack of comprehensive epidemiological studies, particularly in Southeast Asia, there is evidence that the greatest risk factor for humans, the consumption of raw or improperly cooked or processed fish, is increasing in some regions, facilitated partly by population migrations and partly by commercial provision of these products (WHO, 2004). The causative agents of human infections include *C. sinensis* (in East and Southeast Asia), *O. viverrini* (in Southeast Asia), *Opisthorchis felineus* (in Russia and Eastern Europe), and *Metorchis conjunctus* (in North America) (Table 2.1). A total of 17 million people around the world are estimated to be infected with these liver flukes (WHO, 1995).

Table 2.1 Species of liver flukes reported from humans

Species	Molluscan and piscine hosts	Other definitive hosts	Geographic distribution
Clonorchis sinensis	Freshwater snails and fish	Dogs, cats, rats, pigs, badgers, weasels, camels, buffaloes	Korea, China, Taiwan, Vietnam, Russia
Opisthorchis viverrini	Freshwater snails and fish	Dogs, cats, rats, pigs	Thailand, Laos, Cambodia, Vietnam
Opisthorchis felineus	Freshwater snails and fish	Dogs, foxes, cats, rats, pigs, rabbits, seals, lions, wolverines, martens, polecats	Spain, Italy, Albania, Greece, France, Macedonia, Switzerland, Germany, Poland, Russia, Turkey, Caucasus
Metorchis conjunctus	Freshwater snails and fish	Dogs, cats, wolves, foxes, coyotes, raccoons, muskrats, minks, fishers	Canada, USA

(Chai et al., 2005a)

Prevalence and distribution

C. sinensis, the Chinese liver fluke, is the most important species of FZT in East Asia (Rim, 1990; Chen et al., 1994; Hong et al., 2003). It is widely distributed in this region (Table 2.1). In 1947, the estimated number of infected people worldwide was about 19 million (Stoll, 1947), but more recently it has been estimated to be about 7–10 million (WHO, 1995; Crompton, 1999) and recently 35 million (Lun et al., 2005). In Japan, this parasite was formerly prevalent, but has been successfully controlled since the

1960 s (Chen et al., 1994; Hong et al., 2003). Current endemic areas of clonorchiasis include South Korea, China (except northwestern parts), Taiwan, northern Vietnam, and the far eastern part of Russia (Tables 2.1 and 2.2).

In the Republic of Korea, national surveys in 1971 and 2004 revealed 1.4–4.6% egg positive rates the number of infected people currently in Korea is estimated at about 1.5 million. In China, clonorchiasis is distributed in a total of 24 provinces, municipalities and autonomous regions (Chen et al., 1994). Guangdong Province (including Hong Kong) and Guangxi Zhuang Autonomous Region, Heilongjiang, Jilin and Liaoning provinces are the areas with most reported infections (Yu et al., 2003). In a nationwide survey, the prevalence of *C. sinensis* was 0.4% among almost 1.5 million people examined (Xu et al., 1995). Based on these data, the number of infected people in China is believed to be around 15 million (Lun et al., 2005). In Hong Kong, the prevalence has decreased owing to control measures (Chen et al., 1994). In Taiwan, clonorchiasis was formerly endemic in three areas, Mei- Nung in the south, Sun-Moon Lake in the center and Miao- Li in the north (Cross, 1984), but the current status is unknown. In Vietnam, clonorchiasis has been endemic mainly in the north, especially along the Red River Delta including Haiphong and Hanoi (Rim et al., 1982; De et al., 2003). In southern parts of Vietnam, *C. sinensis* is not reported, although opisthorchiasis is reported to be endemic (De et al., 2003). Cases of *C. sinensis* infection have been reported in the Amur River territory, the far eastern part of Russia (Chen et al., 1994).
O. viverrini is a particularly serious liver fluke and is highly prevalent in Southeast Asia including Thailand, Lao PDR, Cambodia and southern Vietnam; about 9 million people are estimated to be infected globally (Sithithaworn and Haswell-Elkins, 2003; Andrews et al., 2008). In Thailand, it is widespread in the northern and northeastern regions. The number of infected people in the northeastern region alone was estimated in the 1960 to be over 3.5 million (Wykoff et al., 1965), and this figure seems to have changed little; the estimated number of infected people is currently about 6 million (Sripa et al., 2003). In Lao PDR, the Mekong River basin is the most heavily infected area (Chai et al., 2005a).

In Vietnam, several southern provinces such as Phu Yen have reported infections, with prevalences above 10% (De et al., 2003).

2.3.2 The intestinal flukes

Heterophyids

These minute intestinal flukes of the family Heterophyidae are parasites of birds and mammals (Table 2.2). A large number of species has been reported from humans, among which *Metagonimus yokogawai* and *Heterophyes heterophyes* are generally considered the most important species (Yu and Mott, 1994). However, because an extraordinary number of heterophyid species are zoonotic (about 35 species) and have very similar transmission patterns, this group is a very significant food safety and quality problem, but one has not attracted the interest of international agencies until recently. The importance of these flukes is being increasingly recognized through recent studies from the Philippines (Belizario et al., 2001), from Thailand on *Haplorchis taichui* (Waikagul, 1991; Sukontason et al., 2001) and from Korea on several species including *Heterophyes nocens* and *Metagonimus* spp. (Chai and Lee, 1991; 2002).

Although generally not considered of significant clinical importance relative to the liver flukes, several heterophyid species, including *Stellantchasmus falcatus*, *Haplorchis* spp., and *Procerovum* spp., can cause significant pathology, often fatal, in the heart, brain, and spinal cord of humans (WHO, 1995). The exact mechanisms of pathogenesis responsible are not clear but may be related to invasion of the circulatory system by worm eggs. Disease is usually related to worm burdens, which in some cases can be very heavy (MacLean et al., 1999).

Another very important issue related to heterophyids is the difficulty of differentiating the eggs from those of the liver flukes in human fecal examinations, which may cause inaccurate estimates of the prevalence of both trematode groups (Lee, 1984; Chai and Lee, 1991; 2002; Ditrich et al., 1992). New diagnostic techniques including PCR are needed to improve specific diagnosis of these flukes. The discussion which follows focuses on the more common of these intestinal flukes, and refers to the other less frequently encountered species. Each species is discussed separately because of individual variation, but it should be noted that it is more common to encounter multiple species infections rather than infections with single species, which compounds the problem of diagnosis by fecal examinations (Lee, 1984; Giboda et al., 1991a, 1991b; Kaewkes et al., 1991).

***Haplorchis* spp.**

The genus *Haplorchis* is characterized by the presence of only one testis and a ventro-genital-sucker complex armed with gonotyl and chitinous spines (Yamaguti, 1958). Five species, namely *H. taichui*, *Haplorchis pumilio*, *Haplorchis yokogawai*, *Haplorchis pleurolophocerca*, and *Haplorchis vanissimus*, are responsible for human infections (Yu and Mott, 1994); the first three are the most important (Table 2.2). *H. taichui* was first described from birds and mammals from central Taiwan (Faust and Nishigori, 1926). Human infections are now commonly found throughout Asia (Velasquez, 1982). *H. pumilio* was originally described from birds and mammals in Egypt; it is also now known to be distributed in Asia (Velasquez, 1982; Yu and Mott, 1994). *H. yokogawai* was described from dogs and cats fed with metacercariae from mullet in Taiwan; human infections have now been reported from many Asian countries, Australia, and Egypt (Velasquez, 1982; Yu and Mott, 1994). Recent studies in Vietnam as well as Thailand revealed that *H. taichui* are common in both culture and wild capture fish (Kumchoo et al., 2005; Thien et al., 2007; Chi et al., 2008; Nithikathkul and Wongsawad, 2008; Skov et al., 2009; Van et al., 2009; Phan et al., 2010a, 2010b, 2010c; Wongsawad and Wongsawad, 2011).

Table 2.2 Important heterophyid species reported from humans

Species	**Molluscan and piscine hosts**	**Other definitive host**	**Geographic distribution**
Metagonimus yokogawai	Freshwater snails and fish	Dogs, cats, rats	Korea, China, Taiwan, Japan, Russia, Indonesia, Israel, Spain
Metagonimus takahashii	Freshwater snails and fish	(experimentally) mice, dogs	Korea, Japan
Metagonimus miyatai	Freshwater snails and fish	(experimentally) mice, rats, hamsters, dogs	Korea, Japan
Heterophyes heterophyes	Brackish water snails and fish	Cats, dogs, foxes, Wolves, pelicans	Egypt, Sudan, Palestine, Brazil, Spain, Turkey, Iran, India, Russia
Heterophyes nocens	Brackish water snails and fish	Cats	Korea, Japan, China
Haplorchis taichui	Freshwater snails and fish	Cats, dogs, foxes egret	Taiwan, Philippines, Bangladesh, India, Palestine, Egypt, Malaysia, Thailand, Laos, Vietnam, China
Haplorchis pumilio	Freshwater snails and fish	Cats, dogs, foxes, Wolves, pelicans	Thailand, Laos, China
Haplorchis yokogawai	Freshwater snails and fish	Cats, dogs, egret	Taiwan, Philippines, China, Malaysia, Indonesia, Thailand, Laos, India, Australia, Egypt
Pygidiopsis summa	Brackish water snails and fish	Cats	Korea, Japan

(Chai et al., 2005a)

Echinostomes

Trematodes of the family Echinostomatidae are intestinal parasites of birds and mammals. At least 30 genera and more than 200 species are known; about 15 species infect humans (Huffman and Fried, 1990). There are 11 reported fish-borne echinostome species of which *Echinostoma hortense* and *Echinochasmus japonicus* are the most important (Yu and Mott, 1994; Chai and Lee, 2002). Most human echinostome infections have been reported from Asia and the Western Pacific, but infections probably occur also in Africa (Yu and Mott, 1994). The disease is generally mild, but ulcerations and bleeding in the stomach or duodenum may occur, as in *E. hortense* infection (Chai et al., 1994).

2.4 Aquaculture in Asia

Aquaculture, whether it occurs in backyard ponds, family farms or industrial systems, has become an important source of much needed food security. Overall, finfish farms 16% of the animal protein in the world's food consumption and 24% of the animal protein in the diet of Asian people. Food and Agriculture Organization (FAO) estimates that during 1999 the global production of aquatic products (animal and plants combined) amounted to 42.77 million metric tons (mmt) valued at US$ 47.87 billion. Over 90% of the world's aquaculture production during 1999 was based in Asia (38.89 mmt) with China contributing 30.04 mmt of aquaculture products to the total. FAO also calculates that some 45% of the global aquaculture is centered on freshwater yielding about 19.4 mmt of finfish; again almost all this fish originates in Asia where FZT infections are endemic (WHO, 2004).

In addition to the year 2000 a projected world population of 6.1 billion people will, according to 1980 levels of consumption, require an additional 19 million metric tons of fish. If one adds to this, income growths of 2.8 percent in industrial countries and 4.5 percent in developing countries, the increased demand for fish by the year 2000 would be about 30 million metric tons (Shilo and Sarig, 1989). In the past increased demand was met through increased harvests from the oceans, and from inland capture fisheries, particularly in Asia. However, the capture fisheries have leveled off, and in some countries, even declined. Future fish landings from capture fisheries are likely to remain constant or decline, creating the need for an alternative source of supply such as aquaculture.

2.5 Culture fish species in Lao PDR

Since the thesis was conducted in fish farms in Lao PDR, review of aquaculture in Lao PDR is addressed here. Fish species generally produced in Lao PDR are *Cyprinus carpio*, *Puntius* spp., and *Tilapia* spp. The state hatcheries produce a wider range of species including the above and also Chinese carps and Indian carps and some indigenous species

One of the problems confronting the hatcheries is the maintenance of broodstock. Since the hatcheries attempt to hold as many species as possible they often are left with insufficient numbers of any species to produce significant numbers of fry. This shortage of broodstock prevents several spawning cycles of a single species during the appropriate season. The limitation of the number of species held by each hatchery should be promoted and emphasis placed on species that are easy to produce in large numbers (and preferably indigenous species). There are several *Puntius* species indigenous to Lao PDR and preliminary rearing trials by the Department of Fisheries and Livestock (DLF) suggest fast growth rates in ponds. *Puntius* sp. is attractive for mass production due to its extreme ease of spawning and maturation at a small size (> 250g). These fish are also acceptable to local people who can also catch them occasionally in the rivers.

Previously, Chinese and Indian carp have been promoted in Lao PDR as suitable candidates for aquaculture. These species have advantages in that the production techniques for these species were well established and the fry are relatively easy to nurse. It is because of this that many of the state as well as some private hatcheries still try and produces these species. The problems associated with these carp species is that they mature at a large size (ideal broodstock size is at least 2 kg) and thus they must be maintained for long periods at the hatchery before they can be matured and spawned.

Many of the state hatcheries failures to produce fry from these species have been due to attempting to spawn these fish before they are fully mature. The long holding time for the fish has also resulted in poor nutrition due to lack of appropriate feeding at the hatcheries.

Common carp (*C. carpio*) are very suitable to both small-scale farmers and also the government hatcheries. This species is very easy to produce and often forms the bulk of fry produced by many operators. It has a relatively lower market value and the fact that small farmers are able to produce these species results in government hatcheries avoiding mass production since they would be competing against the small farmers. It is probable that the state hatcheries could not produce common carp fry for a price as low as that of private enterprises.

Tilapia spp. is often held at hatcheries since they can be produced almost all year round when water is available. This species is not really suitable for the state hatcheries since the fry production techniques require large numbers of net cages and this uses valuable space at the hatcheries. Whilst fry can be produced all year round, the seasonal demand for fish fry that occurs during the early part of the monsoon season cannot be fulfilled by the *Tilapia* cage rearing technology.

In addition to local hatcheries, fry can be imported from Thailand through boarder provinces such as Nongkhai and Ubon Rachathani. This cross broader trade facilitates transmission of FZT to fish farms between countries.

2.6 Fish-borne zoonotic trematodes in aquaculture

The persistence and spread of FZT infections depends on habitats of the species of susceptible snail which require freshwater or, in some cases, mildly brackish water for their development. Exposure of susceptible snails to trematode eggs when fresh faces from infected humans and animals are introduced into water almost guarantees the continuation of the infections. When freshwater fish and snails are farmed in the same contaminated water, human health is put at risk.

The prevalence of FZT metacercariae was investigated in fish farmed by rural households in Nghe An Province, located in northern Vietnam. In total, 716 fish, including nile tilapia (*Oreochromis niloticus*) and 6 carp species, i.e., grass carp (*Ctenopharyngodon idellus*), bighead carp (*Aristichthys nobilis*), mrigal (*Cirrhinus mrigala*), common carp (*C. carpio*), silver carp (*Hypophthalmychthys molitrix*), and rohu (*Labeo rohita*), collected from 53 fish farms were examined. The overall prevalence of FZT metacercariae was 44.6%, ranging from 12.5% to 61.0% in fish species collected from grow-out ponds, which are the production system for growing fish from fingerling size to market size. The overall prevalence was 43.6% in fingerlings cultured in nurseries, ranging from 7.4% to 62.8% for different fish species. The FZT species recovered were heterophyids and echinostomatids and included *H. pumilio*, *H. taichui*, *H. yokogawai*, *Centrocestus formosanus*, *S. falcatus*, and *E. japonicus*, all of which are intestinal flukes in humans, other mammals, and birds (Tran et al., 2008). A subsequent study on a total of 852 cultured fish from 4 districts were collected and examined by pepsin digestion to determine their FZT infection status. In Tra catfish, the prevalence of all types of metacercariae was 2.6 %. (Thu et al., 2007) 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 (Thien et al., 2007; Thu et al., 2007). 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 behaviors 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) (Sithithaworn and Haswell-Elkins, 2003). Reservoir hosts such as dog, cats and pigs are also important risk factors in aquaculture (Anh et al., 2009). Recently, the prevalence and species composition of FZT infections in dogs, cats, and pigs in a fish-farming community in Nghe An Province, Vietnam. Feces from 35 cats, 80 dogs, and 114 pigs contained small trematode eggs at 48.6%, 35.0%, and 14.4%, respectively; 7 species of adult FZT were recovered from these hosts. The study in Nghia Hung district, Nam Dinh province Vietnam, the prevalence of FZT varied significantly between cats (70.2%), dogs (56.9%) and pigs (7.7%). Forty-nine of the egg-positive animals (25 dogs, 20 cats and 4 pigs) were necropsied to obtain adult trematodes for identification. The liver fluke, *C. sinensis,* and 11 species of intestinal flukes including *Haplorchis*, *Stellantchasmus*, *Stictodora* and *Centrocestus* were recovered from the infected animals. (Anh et al., 2009)

Distribution and density of the first intermediate hosts, species of freshwater snails, are important in determining transmission patterns of the trematodes (WHO, 1995; Chai et al., 2005a). A study on trematode infections in snails in Nam Dinh province, Vietnam reported that many snail species acted as intermediate hosts for FZT (Dung et al., 2010). 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 *M. 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.

Similar data in other Southeast Asian countries, i.e. Thailand and Lao PDR are limited and thus more studies are needed. It is anticipated that, given similar ecology and levels of infection in fish and human, FZT should be prevalent in wild as well as culture fisheries. Therefore there is need to have current FZT data such that programs for control could be tested in the farm conditions.